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XV ISAH Congress 2011

Proceedings of the XVth International Congress of the
International Society for Animal Hygiene

"Animal Hygiene and Sustainable Livestock Production"

Innovations in Hygiene, Nutrition and Housing
for Healthy Food from Healthy Animals

International Society for Animal Hygiene

University of Veterinary Medicine, Vienna
Austrian Agency for Health and Food Safety
Austrian Federal Ministry of Health

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Preface

More than 40 years after the foundation of the International Society for Animal Hygiene, ISAH, in Hungary 1970, and 31 years after the IIIrd ISAH International Congress in Vienna, 1980, it is our great honor and pleasure – and challenge – to welcome you to participate in the XVth International Congress on Animal Hygiene ISAH 2011, again in the lovely capital of Austria, Vienna. It is also our great pleasure to honour this time one of the founding members of ISAH and former congress president of III ISAH 1980, late Professor Dr. Hermann Willinger, with a special memorial lecture during the opening ceremony of this congress.

The motto of XV ISAH 2011 is "Animal Hygiene and Sustainable Livestock Production" and puts the focus of the congress right into the centre of the three principle domains of ISAH, namely preserving animal health, human health and the health of the environment. The congress therefore gives emphasis to all recent, novel and innovative research on animal hygiene, animal health and welfare and sustainable livestock production. Special focus of XV ISAH 2011 lies on the interaction of animal hygiene and veterinary public health. Particular attention will be paid to prevention strategies against the development and spread of diseases and pathogens in animals including those that pose a risk to human health (zoonoses). Other important topics of the conference are environmental implications of livestock production as well as all other related impact on natural resources, in particular water, air, and soil resources. Animal by-products and waste management and the associated issues of microbiological safety, round up the major conference themes.

The International Society for Animal Hygiene (ISAH) is an association of veterinarians and other professional scientists, practitioners and students working in the field of animal health and welfare, animal hygiene, biosecurity, safety of food of animal origin, environmental protection in relation to animal production and related areas. ISAH is a highly international organisation with members in 51 countries all over the world.

This two volume book of the XV ISAH Congress 2011 proceedings presents papers of lectures from invited speakers, oral and poster presentations held in 27 parallel sessions, 2 special sessions and a joint OIE/FAO - ISAH symposium.

The realisation of such a congress requires the help and input of many people, and we hereby would like to express our deep thanks to all who contributed to make XV ISAH 2011 a memorable event. Our most sincere gratitude goes to the XV ISAH 2011 Organising Committee, the Scientific Committee and the Executive Board of ISAH.

Our special thanks are reserved to our host, the University of Veterinary Medicine Vienna, namely Rector Dr. Sonja Hammerschmid and her fellow rectorate and the involved university services. Our gratitude also goes to Minister of Health Alois Stöger and the Austrian Ministry of Health, and to AGES Chief Executives Dr. Bernhard Url and Dr. Heinz Frühauf, and the Austrian Agency for Health and Food Safety for functioning as co-hosts and co-organisers of this congress. We also thank them and Mayor Dr. Michael Häupl and the City of Vienna, specifically its Vienna Convention Bureau, for their generous support and sponsorship. Big thanks go also to all our other supporters from industry and business and all those that opened their premises for our technical tours. An excellent job was done by Tribun EU s.r.o., Brno, again printing these proceedings in high quality.

We would also like to thank all the many helping hands, most prominently the competent experts of Austropa Interconvention, Vice-Director Alfred Kerschenbauer and PCO Claudia Stelzer and the AGES Teams AKAD around Dr. Friedrich Polesny and Mag. Christoph Unger, and COM around Dr. Klaus Hasler and Sylvia Stepanek for invaluable help and indefatigable support. Thanks also to Mag. Ulla Winkler, Dr. Sabine Wanda and Dr. Friederike Hilbert for their help on the way. Special appreciation and big thanks go also to the office of Prof. Hartung at TiHo Hannover, Ms. Petra Sommer, Ms. Ebru Jackson and Ms. Dipl. biol. Annette Clauß who contributed ideas and organisational skills.

Last not least, it is our privilege to thank all participants, contributors, chairpersons, organisational and technical assistance for their considerable efforts and inputs. Special thanks also to Prof. Martin Tielen and the Professor Tielen Foundation for their generosity enabling indigenous students from Overseas to attend this conference.

We do hope the congress will provide to you all a unique opportunity to present recent research results, to meet and get together with international experts and professionals, to discuss interesting results and ponder new problems in a stimulating intellectual atmosphere and last not least to enjoy the charms of a world famous capital and its beautiful surroundings

Prof Josef Köfer,
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Part I

Hermann Willinger Memorial Lecture

FUNCTIONAL MOLECULAR INFECTION EPIDEMIOLOGY OF *E. COLI* – CURRENT CONCEPTS

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SUMMARY

The bacterial species *Escherichia* (*E.*) *coli* has been the focus of intensive research since the 1940s, starting off as a model organism for bacterial physiology. Nowadays we distinguish three principal subtypes of *E. coli*, namely non-pathogenic commensals, intestinal pathogenic (InPEC) and extraintestinal pathogenic (ExPEC) types. Although this distinction is clinically and epidemiologically relevant, with some exceptions laboratory based diagnostic techniques are still not able to group each and every isolate into one of these types without anamnestic records of the isolates. Therefore one still has to elaborate Henle-Koch´s postulates to prove the pathogenicity of distinct isolates, which in effect is no suitable approach. However, based on the concept of habitat specific evolution, it should principally be possible to define specific subtypes by comparative analyses of genomic DNA-sequences. In this paper I will address recent concepts we are now pursuing to pave the way for the optimal identification of pathogenic subtypes. By polyphasic approaches based on initial analyses of core genome genes by Multi-locus sequence typing (MLST), defining virulence gene patterns and their respective alleles, as well as in vivo-virulence testing, in the long run we will try to pin down biological behaviour to single DNA-sequence motifs. The paper will address our current concepts, correlating biological function with molecular infection epidemiology, which is Functional Molecular Infection Epidemiology.

The *E. coli* enigma

The bacterial species *Escherichia* (*E.*) *coli* has been researched on since the 1940s, be it as a work horse of molecular biology experiments or as the paradigm of evolution from a commensal to a highly diverse pathogen. One logical consequence was the unravelling of an *E. coli* K-12 genome already in 1997 (3), being the second bacterial genome ever sequenced. However, basic questions like proper diagnostic methods or a clear definition of the virulence potential of various pathogenic subtypes are still not answered. Initially strains were typed for the expression of antigens, utilizing the renowned serotyping scheme of Kaufmann and White, a concept suggesting that identical serotypes belong to one single clone. Today we know that this concept is shortcoming, as various *E. coli* serotypes are polyphyletic. Furthermore, not every *E. coli* strain can be serotyped due to the high diversity of the respective antigens expressed. Beginning in the 1980s, typing of *E. coli* then moved towards the identification of specific virulence factors, leading to the identification of a growing number of pathotypes (7). Today we distinguish three basic subtypes of *E. coli*, namely non-pathogenic commensal, intestinal (InPEC) as well as extraintestinal pathogenic (ExPEC) types (10). Although theoretically this distinction is appropriate, laboratory based diagnostic techniques still are not able to group each and every isolate into one of these types without anamnestic records of the isolates. With the era of genomics, new insights have been gained into this versatile bacterial species. Basically, by generating whole genome sequences the phylogeny of various *E. coli* subtypes can be determined, and this should finally lead to a clear typing of each strain. However, apart from the fact that this is not feasible for routine diagnostic purposes due to the lack of financial and bioinformatic resources, the data unravelled an unexpected fact: the currently known *E. coli* genomes vary largely between 4.1 Mbp (ExPEC strain IHE3034, serotype O18:K1:H7) and 5.9 Mbp (EHEC strain 11368, serotype O16:H11), which leads to a currently defined core genome of roughly 1,500 gene families, but a pan genome of more than 13,000 gene families (9). This would basically mean that the core genome gets further reduced with every newly sequenced strain.

Being aware of this fact, since the beginning of this millennium we have concentrated our initial typing efforts on Multi-locus sequence typing (MLST), developed by the group of Mark Achtman (11). Using a universal language and the unambiguous sequencing method, each single strain can be easily typed into a sequence type (ST), and all data can be entered, via the Internet, into a single, curated global database (www.mlst.net). It is because of these features that MLST is now the "Gold standard" for long-term epidemiology and conservative bacterial typing. In addition, the generated sequence data can be used for phylotyping, thus unravelling the microevolution of certain phylogenetic lineages. By further characterizing strains of distinctive phylogenetic lineages, we try to find specific markers for virulence. In this paper I will address the

recent concepts on the microevolution of *E. coli*, concentrating on ExPEC.

ExPEC constitute a diverse group of pathogens. We and others could show, that ExPEC have evolved several times independently from each other (1, 4-6, 8). Basically, this microevolution led to the development of several subtypes with different biological features. Having this in mind, the research questions we now address are (i) do ExPEC strains have different virulence potentials, (ii) which of the known ExPEC subtypes have zoonotic potential, and (iii) can we identify unique markers that could serve as potential ExPEC-specific diagnostic markers. Most of the ExPEC strains, regardless of whether they have been isolated from humans or animals, cluster in a limited number of sequence type complexes (STCs), and it is these types, which we currently focus on. To give one example: the most prominent STC 95 almost exclusively harbours ExPEC isolated from diseased humans and poultry, and it is therefore tempting to speculate that strains in this STC could potentially be zoonotic pathogens To address this, we therefore comparatively type distinctive strains via multiplex-PCR, microarray and whole-genome sequencing, as well as test these strains in a chicken infection model (2) to be able to correlate genomic features with biological behaviour.

CONCLUSION

The complex phylogeny and the highly diversifying microevolution of *Escherichia coli* poses an ongoing challenge for research on *E. coli*. Strategies for an adequate virulence definition and defining the pathogenic mechanisms for ExPEC pursued in our laboratory are discussed.

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Part II

OIE / FAO – ISAH Symposium

THE WORLD ORGANISATION FOR ANIMAL HEALTH (OIE) AND THE GLOBAL CONTROL OF EPIZOOTIC DISEASES

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SUMMARY

The OIE is the main global organization dedicated to improving animal health and welfare globally with clear mandates and missions to enhance knowledge on animal diseases, including zoonoses, and ensure transparency on the part of its 178 Member countries. OIE Members have the obligation to submit information on their animal disease situation to the OIE in a timely and transparent manner. To accomplish its mandate, the OIE manages the World Animal Health Information System (WAHIS) and its WAHID interface, which provides public access to data validated by the OIE. The OIE also establishes international standards for the prevention, diagnosis, reporting, control and management of listed animal diseases. The standards, which are published in the Terrestrial Animal Health Code and related Codes and Manuals, provide a scientific basis for the control of animal diseases and zoonoses and the prevention of disease spread via international trade in animals and their products. Other activities and programs including the ones developed with partners are described particularly in the field of capacity building through training of Veterinary Services in order to support their key roles to insure good governance in preventing and controlling diseases and in contributing to public health and food safety. Overviews of international and regional control or eradication programs for three selected diseases (foot and mouth disease, rinderpest and highly pathogenic avian influenza) are presented to illustrate the OIE's role and activities in supporting its Members in the global control of epizootic diseases.

INTRODUCTION

Animal and animal products play a major role in food security in providing high nutritional value proteins. In addition animal energy and manure increase crop production and animal production provides key revenues to people including millions of poor small holders in developing countries.

Animal diseases are recognized to be one of the main causes for reduced productivity of domestic animals and they are also at the origin of many serious human diseases, mainly due to direct transmission of animal pathogens to humans or through food. Therefore prevention and control strategies against the spread of diseases and pathogens in animals are of crucial importance for the prevention of human zoonotic diseases, safety of food of animal origin as well as food security, animal welfare and the environmental implications of livestock production.

A recent review on the role of veterinary activities in supporting global food security was presented at the 79th General Session of the OIE[i] in May 2011 (1).

Due to increased movements of animals, animal products and humans, particularly related to globalization of trade and development of tourism, pathogens can move from one region to another very rapidly and over long distances. Global changes, including climate, natural and cultivated land management systems, wildlife, vector ecosystems, are among the driving factors for the emergence or re-emergence of diseases and international crises. The examples have multiplied during the past ten years with eg. foot and mouth disease (FMD) in Europe, Nipah in Asia, H5N1 highly pathogenic avian influenza, bluetongue… and it becomes more and more obvious that there is a need to consolidate or to improve the capabilities of the veterinary services and their partner stakeholders, including the private sector, in order to implement effective prevention and control strategies. These strategies to control transboundary and/or zoonotic diseases which are at the origin of important losses in animal production and of major national or international crises are considered to be public goods; decision makers and donors should invest more in this field.

The OIE had played a significant role since its establishment in 1924 and today it has become the leading international organization supporting the global control of epizootic diseases at the service of its Member Countries.

ROLE OF THE OIE IN THE GLOBAL CONTROL OF EPIZOOTIC DISEASES

The OIE was created in 1924 as an intergovernmental organization based in Paris. As at May 2011, there are five Regional Representations and 6 Sub-Regional Representations. 178 countries and territories are members of the OIE.

The mandate of the OIE is now become wider than when it was initially established. It has broadened its mandate from 'preventing the spread of diseases throughout the world' to 'the improvement of animal health and welfare, veterinary public health and consolidation of the animal's role worldwide'.

The missions and objectives of the OIE are described in its 5th Strategic Plan (2011-2015) (27). They address transparency of the global animal disease situation, dissemination of veterinary scientific information, international solidarity in the control of animal diseases, safeguarding world trade by publishing health standards, supporting the improvement of national Veterinary Services and providing better guarantees for food and animal welfare. The final objectives and activities regarding prevention and control of diseases are based on a chain of basic essential tools and methods providing for good surveillance, early detection of pathogen incursions and warning , emergency response to new outbreaks having the potential to become epizootics and better long term control of enzootic diseases.

Due to the increased risk of emergence of new pathogens and the multiplicity of factors involved, OIE promotes a more holistic approach and the development of multidisciplinary and multisectoral collaborations between animal and human health sectors, wildlife and ecologists, hunters, fishermen, as well as socio-economists and faming system specialists. This cross-sectoral cooperation and strong partnership represents the basis of the "One Health" vision for managing risk at the animal-human interface ecosystems. In April 2010 OIE, FAO[ii] and WHO[iii] published an official concept note setting out this important tripartite agreement to share responsibilities and to coordinate global activities. (30)

It is important to point out that, through the publication of norms and guidelines and through direct interaction with its Member Countries, where capacity building programs are currently being implemented for national policy makers, the OIE gives them an important support to prepare strategies and meet all generic and specific conditions to prevent and control major transboundary diseases.

Enhance knowledge and elaborate standards

The network of OIE Collaborating Centers and Reference Laboratories constitutes the core of the organisation's global scientific expertise. The OIE Specialist Commissions (Terrestrial Animal Health Standards Commission, Scientific Commission for Animal Diseases, Biological Standards Commission, Aquatic Animal Health Standards Commission) use the best current scientific information to address all issue related to prevention and control of animal diseases and to develop standards and guidelines. All documents from the OIE Specialist Commissions are published as well as the reports from relevant Working and ad hoc Groups, making possible comments from partner organization and most importantly from all the 178 national OIE Delegates. All standards have to be adopted by the OIE World Assembly, which meets every year in May at the OIE General Assembly in Paris.

The OIE publishes two Codes (Terrestrial Animal Health Code and Aquatic Animal health Code) (12, 21)to assure the sanitary safety of international trade in live animals and their products. The Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (17) and of Diagnostic Tests for Aquatic Animals (13)describe internationally agreed laboratory diagnostic techniques and vaccines.

By making available to Member Countries all the scientific information, standards and guidelines published by the OIE, the Organization helps them to better define and implement methods and strategies in order to prevent and control animal diseases and to secure sanitary safety of international trade of animals and animal products.

The OIE scientific network is composed of expert centers for animal diseases: 190 Reference Laboratories covering 101 diseases or topics (19) and 37 Collaborating Centers covering 35 horizontal topics.(16)

Disease information

To accomplish its mandate, the OIE manages the World Animal Health Information System (WAHIS) (25) and its World Animal Health Information Database (WAHID) (26) interface which provides public access to data validated by the OIE.

Comprehensive, reliable and transparent sanitary information represents an absolute basis for an effective animal disease prevention and control system. A full range of information is available from immediate notifications and follow-up reports submitted by Member Countries in response to exceptional events; from six-monthly reports on the national disease situation regarding the OIE listed diseases, and from other reports including specific data regarding diseases such as avian influenza or bovine spongiform encephalopathy (BSE). Once they have been received and validated immediate notifications are published under the heading 'alert' open to all Delegates and other interested parties (22). OIE also has the mandate to officially recognize the animal disease status for BSE, contagious bovine pleuropneumonia (CBPP), FMD and rinderpest (RP). This allows countries to gain the trust of trading partners, neighboring countries and the international community. The specific mandate of OIE in the field of disease information dissemination and recognition of national's disease statuses for trade purposes is linked with on the WTO[iv] Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement 1995) (33) and on binding commitment of OIE Members through their official membership to the OIE.The global legal basis for notification of animal diseases is described on the OIE website. This a an important issue because a country's credibility is based on transparent notification of outbreaks and sharing of information relevant to disease risks. It is an unconditional duty of all OIE Members to disclose all relevant information, even if notification of diseases may have negative impacts on trade, particularly with regard to export markets. These obligations are set out in the OIE Organic Statutes.

The WAHIS allows all members to be on line electronically with a server located in the OIE headquarters which gives the capacity to relay and make immediately public worldwide sanitary information.

In addition to information provided by OIE National Delegate, other information can come from other credible sources such as OIE Reference Laboratories. Unofficial but reliable information is also used by the OIE in conducting further investigation and confirmation of information

provided by the veterinary authority. This rumor tracking system, managed by OIE specialists, proves to be very effective and it strongly complements the official declaration system.
The analysis of all this sanitary information, with reference to the epidemiological context, allows an assessment to be made of the evolution of the animal health situation and the publication of alert messages. The OIE, FAO and WHO have set up a Global Early Warning System (GLEWS) (8) to synergistically address the complexity of disease epidemiology and improve global early warning as well as transparency among countries.
As already said about scientific knowledge and standards, disease information dissemination represents a crucial tool to help non infected countries preparing themselves to better prevent the introduction of pathogens, to define emergency plans and, in case of any new outbreak occurrence, to better respond to eliminate the pathogen and avoid disease spread.

GOVERNANCE, EDUCATION AND CAPACITY BUILDING FOR VETERINARY SERVICES

Good veterinary governance relies on efficient national Veterinary Services (VS), complying with the OIE quality standards. National VS represent the corner stone of any effective animal health system at the national, regional or international levels. Effective VS need suitable legislation and its efficient implementation, a well-defined national chain of command and relevant diagnostic capacities for surveillance and detection, and response support to disease outbreaks. Alliances also need to be developed between the public and private sectors (farmers, private veterinarians, traders, consumers…).
With regard to veterinary education, the OIE is working to improve the quality of veterinary education globally and to support international recognition of veterinary qualifications and greater mobility of professionals. Recognising the global need to adaptat veterinary education curricula to the evolving global risks and societal expectations, the OIE held a 1st Global Conference on this topic in October 2009 (14). The conference was attended by deans of veterinary education establishments from all around the world. The OIE is now undertaking important work to follow-up the conference recommendations, including by the development of recommendations on minimum 'day 1' competencies for veterinary graduates, to enable veterinary services (private and public components) to meet the OIE quality standards.
One of the most important OIE objectives is to strengthen the capacity of members' Veterinary Services to participate in the development of international standards and guidelines and to implement them, thereby achieving the improvement of animal health and veterinary public health globally. OIE makes all necessary efforts towards capacity building through implementing appropriate training activities directed to the Delegates and their national key competent teams of thematic focal points.

Economic studies

Economic studies on the prevention and control of animal diseases worldwide have been conducted by the OIE. Economic studies on the cost of prevention versus outbreak management and on the cost of national prevention systems for animal health and zoonosis in developing and 'in transition' countries generally showed that prevention is less expensive than control of epizootics (2, 11, 31). The OIE will continue to assess the cost-benefit of prevention and/or control programs against major diseases. Such studies are indispensable for effective advocacy in favor of investing in prevention and control of diseases.

Collaboration with partners

OIE does not work in isolation and it has signed collaboration agreements with many partners, among them are public international (FAO, WHO, Codex Alimentarius, IPPC[v], WB[vi]…) and regional organizations (EU[vii], AU-IBAR[viii], PAHO[ix], SADC[x], ASEAN[xi]…) as well as bodies representing the private sector (SSAFE[xii], IFAH[xiii], WVA[xiv], IMS[xv], IDF[xvi], FEI[xvii]…).
A specific agreement has been signed with FAO, the Global Framework for the progressive control of Transboundary Animal Diseases (GF-TADs) which serves as an institutional basis to develop joint activities. Other specific joint tools have been established with FAO such as the OIE-FAO Network of Influenza virus Laboratories (OFFLU) (29), the FAO-OIE-WHO Global Early Warning System (GLEWS) (12), several Regional Animal Health Centers or the Crisis Management Center for Animal Health (CMC-AH).

Support to Member Countries

OIE provides support to its Member Countries for the improvement of animal health through the use of several tools and projects.
The importance of providing scientific information, standards and guidelines or disease information has already been mentioned, as well as education and training activities for improvement of Veterinary Services for good governance.
Other specific tools and programs can be mentioned, notably the **Performance of Veterinary Services (PVS) Pathway** (23) which is a global program for the sustainable improvement of Veterinary Services' compliance with OIE quality standards. Two chapters of the Terrestrial Animal Health Code are dedicated to the quality of Veterinary Services. To support these goals, appropriate legislation is also needed. The OIE PVS Pathway starts with the PVS Evaluation (which assess the

compliance of veterinary services using the indicators set out in the PVS Tool). This initial assessment may be followed by several steps, including the PVS Gap Analysis (which addresses the needed investments to support compliance with the OIE quality standards, according to the country's national priorities). Other activities include missions to assess the quality of the national veterinary legislation, laboratory support (see below) and follow-up missions.

Laboratory Twinning (15) is another program of the OIE which aims at improving laboratory diagnostic capacities and building specialized expertise at the national and regional levels with the objective of improving the North-South balance. Each twinning project links an existing OIE Reference Laboratory or Collaborating Center with a selected laboratory in a developing country.

OIE has also developed a concept of **vaccine banks** (24) which create virtual rolling stocks. This enables the rapid direct supply from the private company provider of emergency stocks of vaccines when urgently needed. Vaccines remain with the supplier and this concept has been applied to avian influenza, FMD and rabies.

OVERVIEW OF CONTROL PROGRAMMES FOR THREE SELECTED DISEASES

The OIE has implemented a number of programs to control major transboundary diseases, most of the time in collaboration with other international and regional organizations and with donor's support. Several could be mentioned here, either programmes for the development of tools (e.g. GLEWS, PVS, Global laboratory Networks such as the OIE/FAO Network of expertise on animal influenzas (OFFLU), CMC-AH), generic initiatives (e.g. the "One Health" vision) or programs addressing the control of specific diseases (e.g. BSE, rabies, H1N1 Influenza …). Three examples will be presented to illustrate how the OIE has contributed to the support national, regional and international activities to fight against FMD, RP and H5N1 AI. The control strategies and tools against these three diseases are typically classed as global public goods since they benefit all countries, or several groups of countries, and all populations and future generations, and these benefits extend beyond national borders and not just the productivity of livestock populations (the fight against poverty and food insecurity, contribution to public health and food safety notably in developing countries). Moreover, a single country failing to control the disease can have adverse consequences for neighbouring or even distant countries

Foot and Mouth Disease (FMD)

FMD is one of the most contagious animal diseases and its transboundary nature is accentuated by the rapid development of international trade in animals and animal products. Due to the economic losses it causes, FMD is one of the major diseases affecting production and trade of food of animal origin. Currently, out of the 178 OIE Member Countries, 96 do not have an FMD free status, 66 countries are officially recognised as FMD free (65 without vaccination and 1 with vaccination) and 16 countries have one or more zones officially recognised as FMD free (10 without vaccination and 6 with vaccination) (25, 26). The possibility for countries or zones to be officially recognized as free by the OIE represents a strong incentive for many of them and consistence towards global control objectives. The evolution of the FMD situation worldwide is well documented by the OIE, which continuously collects data on outbreaks notified by countries and publishes them, notably in the form of geo-referenced maps (WAHID and WAHIS systems). OIE and FAO Reference Laboratories for FMD, in particular the Institute for Animal health (IAH) which the FMD Reference Laboratory at Pirbright (United Kingdom), monitor and publish details of the virus strains circulating in infected countries (10). The epidemiological situation is also analysed and published by the joint FAO/OIE/WHO platform GLEWS and by a number of regional bodies, such as the FAO's EuFMD Commission[xviii] (3), AU-IBAR, EU–DG SANCO[xix] and PAHO/Panaftosa. In view of the global situation the OIE, with partners such as FAO and regional organisations, is mobilising to encourage Member Countries and donors to increase their efforts aimed at better control of the disease. Ever since its creation, the OIE has backed up and supported scientific and technical advances by developing standards and guidelines applicable to FMD control and international trade, surveillance and diagnostic methods and tools, and vaccines, published in the *Terrestrial Code* and the *Terrestrial Manual*.

In South-East Asia, the OIE and the member countries of ASEAN have, since the end of the 1990s, developed a programme for the progressive control of FMD within the region, called SEAFMD[xx] (32). Coordination plays an important part and all aspects of the programme are continuously monitored and evaluated. Positive results have been obtained, such as OIE recognition of countries, or zones within countries, as being FMD free, either with or without vaccination (Indonesia, Brunei, Philippines, Malaysia). The programme includes the establishment of buffer zones between infected zones and of priority control zones such as those of Myanmar, the Lower Mekong, the Red River Delta and the Upper Mekong. This chronological, sequential approach, based on epidemiological characteristics and benefiting from strong political involvement on the part of ASEAN member countries and sound governance, is a good example of what can be achieved collectively at a regional level for the benefit of each partner country. China, Brunei and Singapore became recently members and the name of the program changed as SEACFMD. The case of South America and its Southern Cone region is worth looking as the results achieved are very positive and can also serve as a model. An agreement called PAMA[xxi], signed by the regional body Mercosur and its member countries and specific agreement between the OIE and the Mercosur PVC, signed in March 2007, provided for the setting up and monitoring of activities in border zones, known as "high surveillance" zones where surveillance and disease prevention operations have been considerably strengthened (18).

However, the time has come to take a new step forward and, building on previous advances, embark on a phase involving the development and implementation of a global control programme, with particular emphasis on regions of the world where the disease remains enzootic and which represent an increasingly serious threat to FMD free countries. Defining a global strategy and convincing governments and donors to make a proactive commitment are among the conclusions and recommendations of the OIE/FAO Global Conference on FMD, held in Asunción, Paraguay, in June 2009 (28). In line with the conclusions of the OIE/FAO Global Conference on FMD held in Asunción in June 2009 and its recommendations, a Global FMD Working Group, reporting to the GF-TADs Global Steering Committee, was set up, associating the OIE and FAO. The Working Group is tasked with proposing a draft global strategy, in collaboration with the regional bodies and relevant experts and by analysing the results of strategies currently being implemented in Member Countries, particularly in regions where positive results have been achieved.

Several international conferences have helped to advance knowledge of FMD, both in terms of the situation in the various countries and in terms of the development of new tools such as the PCP document (5) prepared by FAO and the OIE, with contributions from numerous experts. The document is available on the websites of both FAO and the OIE. It will be an essential tool for implementing and monitoring the global strategy. It describes a set of activities, divided into different stages, which can be used to evaluate the stage of advancement of a country or region in their FMD control and eradication programmes.

Another major advance in the OIE's involvement in the implementation of a global strategy is the preparation of a new article for Chapter 8.5. of the *Terrestrial Code* which provides for the OIE to endorse national FMD control programmes submitted to it by countries involved in the PCP pathway and that are not FMD free. The new control programme being submitted to the OIE will mark the country's entry into the pathway to eradication. This programme will be accompanied by a list of documents demonstrating that the country is in a position to implement it successfully particularly with regards to effectiveness of the Veterinary Services, knowledge of the FMD situation in the country, a major reduction in the impact of the disease, the existence of suitable legislation, effective surveillance and diagnostic systems, the existence of contingency plans, etc…

The OIE's policy on support for FMD control is reflected in a series of decisions and actions designed to help countries control the disease and to organise the necessary level of worldwide coordination to implement a global strategy. Several regions have managed to achieve lasting eradication, though they still face the risk of virus reintroduction and must therefore maintain constant vigilance. In contrast, numerous developing countries are experiencing more difficult conditions and help needs to be mobilised to assist them in their efforts, which will of course have direct favourable consequences by reducing the risk of re-infection for FMD free countries. It is therefore in the interests of FMD free countries to help infected countries eradicate the disease.

Rinderpest

Rinderpest was the most devastating disease of cattle. It is believed to have originated in Asia later spreading to other continents including Africa through the transport of cattle. Rinderpest is an infectious viral disease of cattle, domestic buffalo and some species of wildlife. Death rates during outbreaks were usually extremely high, approaching 100% in immunologically naïve populations.

Rinderpest is a very special disease for the OIE since its creation was decided in 1924, following a new incursion of the virus in Europe. Nearly 90 years later, the initial recommendations to promote a coordinated international effort for the control of rinderpest and other epizootics listed at that time (foot and mouth disease, anthrax, sheep and goat pox, rabies, glanders, dourine, classical swine fever) laid the groundwork for what were to become OIE-recommended international health policies.

The OIE's first steps in rinderpest control consisted in the establishment of scientific cooperation with existing national research institutes in order to detect the most efficient methods for fighting the spread of rinderpest, including the production and standardisation of safe and effective vaccines, and to achieve a strategic consensus on the scientific bases of the organisation's actions aiming at controlling and preventing rinderpest in the Member Countries.

Mass vaccination campaigns which started in the years 1960s led to an important decline in the disease. But it reappeared on the African continent in the 1980s. The

international response was once again supported by the OIE's action, in particular the publication of recommended standards for the establishment of rinderpest epidemiological surveillance systems. This contained what was called the "OIE Pathway" for eligible Member Countries to be officially recognised as enjoying rinderpest-free status, which initially set out three steps that each infected country had to take in order to obtain such recognition by the OIE (20).

The FAO started in the 1990s coordinating the GREP – Global Rinderpest Eradication Programme (4) – in collaboration with the OIE and the UN International Atomic Energy Agency (IAEA)[xxii] and with massive support to eligible countries from donors such as the European Union, with the aim of obtaining, by 2011 at the very latest, an official declaration of world rinderpest eradication.

During the past years, countries have successively been recognized as rinderpest-free by the OIE, with permanent support from the FAO. These national statuses were approved by the OIE General Assembly yearly sessions after being recommended by the OIE Scientific Commission for Animal Diseases on the basis of the analysis of the dossiers presented to expert's members of a RP ad hoc OIE Group. The experts systematically verified the absence of rinderpest viral circulation in all countries concerned.

This procedure represented an important example of major improvement for the policies of cooperation and coordination amongst international organizations and between those and the international community as a whole. It is also a success for veterinary services and the entire veterinary profession.

In 2011, the official proclamation by the FAO and the OIE of planetary rinderpest eradication is a cause for celebration, and coincides with the 250th anniversary of the official creation of the veterinary profession. This is the first time an animal disease has been eradicated in the world.

One last challenge remains during the post-eradication phase. Although the rinderpest virus no longer circulates amongst live animals, it is still present in certain laboratories. International coordination and cooperation will once again prove crucial in order to define acceptable conditions for the possession and use of the virus in a limited number of highly bio secure laboratories, to be used for research and production of vaccines in the event the disease was to reappear due to an accident or an act of bioterrorism (Rinderpest was one of more than a dozen agents that the United States researched as potential biological weapons before suspending its biological weapons program).

Highly Pathogenic Avian Influenza due to H5N1

Highly Pathogenic Avian Influenza due to H5N1 was an unprecedented crisis. The importance of the crises was due to dramatic destruction of assets (over 300 million poultry have died), Market shocks (fears of consumers drive down demand, import bans, poultry prices increased or decreased, global trade changes with winners and losers). Internationally, in 2004-05, there was a 8% decline in global poultry trade and the national impact was also related to the Livelihoods impacts of the disease and control programs: killing birds meant compensation needed, restriction of movement and sales meant smallholders recovered slowly and lost market share. Without forgetting to mention loss of income for food, education of children and other household expenses..., as well as changes to the structure of poultry market chains, and the gender issue (poultry is often owned and managed by women).

On top of these socio economic impacts, the importance of the HPAI crises was directly related to the major human risk of an international pandemic.

HPAI has a complex epidemiology and there are multiple factors to be considered as risk factors such as weak economies and animal health services, poultry production systems, movements (local, regional, international trade, legal, illegal), cultural practices, wild birds migrations…

The OIE has started to respond to the crises as soon as it appeared end of 2003. The information system, through official declarations to the OIE and dissemination of the information was a crucial way of mobilizing the governments and donors as well as the national veterinary services and regional and international organizations and donors. WHO collected the human health information and FAO started to analyze the epidemiology evolutions.

A joint Global Strategy was published by OIE and FAO and revised every year (6) and the available tools were promoted and used in strong collaboration between OIE, FAO and WHO (surveillance and disease intelligence, stamping out, biosecurity, movement control, vaccination…).

Some specific tools and methods were developed and tested through pilot studies or wider programs such as the use of Community-Based Disease Surveillance, particular disease intelligence to address the emergence or re emergence of new pathogens with regard to global changes, hot spots identification. Culling compensation studies were carried out and FAO, IFPRI[xxiii], OIE and World Bank published a document in 2008 (9) addressing issues and practices as well as management of compensation. FAO, OIE, and WB also published a Report on Biosecurity: Issues and options, in August 2008 (7).

Regarding vaccination strategies, FAO and OIE worked together intensively. FAO supported vaccination programs in several countries and OIE established an ad hoc group who published a guideline on vaccination which addressed issues such as the various options for vaccination strategies, the need for quality controlled vaccines (OIE Standards), post-vaccination monitoring, the DIVA[xxiv] approach and the necessary exit strategy.

The Cooperation between OIE and FAO was developed in various fields. The FAO - OIE GF TADS agreement signed in 2004 proved to be an excellent mechanism to develop these collaborations. Many tools, already mentioned above were established or strengthened such as the network of reference laboratories and centers (OFFLU), the Global Early Warning System (GLEWS), the Crises Management Center for Animal Health (CMC-AH), FAO OIE Regional Animal Health Centers... FAO, in collaboration with the

OIE, established several Regional Networks on epidemiology, diagnostic and research laboratories, socio economics or communication.
The intersectoral cooperation was also an important area of collaboration particularly between human and animal health systems and with other sectors (wildlife, environment, trade, tourism, police, medias, land management…). As a matter of fact the HPAI crises showed how important these interactions are and it allowed to identify many areas for improvement. The work done on HPAI represented a very stimulation starting point to develop the "One Health Strategy" together with FAO and WHO in association with UNICEF[xxv], UNSIC[xxvi], WB, EU and many other partners.
The global results are that the situation improvement dramatically compared with 2006. There were more transparency, more awareness and preparedness, strengthened Veterinary Services, more sensibilisation and commitment. Improved knowledge of the disease, of its epidemiology and of root causes of emergence and spread were obtained and socio economic impacts are better known.
But the virus is still present in a few countries (around 5 to 6) and recurrent introduction or reintroduction of the virus in countries or regions can be seen. Understanding roots of disease emergence and develop long term global approaches are necessary and remaining gaps are to be filled through research in areas such as virology, epidemiology, trade routes, animal-human transmission, vaccines, wildlire or socio economics.

A number of lessons were learnt in many fields such as the need to be ready to respond quickly to emergencies in order to stop the outbreaks before they spread and become a crisis through emergency preparedness and short term improved capabilities including the need for financial compensation for poultry owners , the need to better address the socio economic issues (economic analysis inputs to disease epidemiology to support risk assessments, socio economic impacts of HPAI, costs and cost-effectiveness of prevention and control measures, long term restructuring and socio economic impacts on small holders including mitigation options…) and impacts on biodiversity.
Other lessons were that there is a need to develop public-private partnership at all levels as well as more participatory approaches with regard to small holders-villagers and more focus on disease drivers and not only on disease events.
And on top of that capacity building through training, particularly directed to the public and private components of Veterinary Services, as well as communication and information should be strongly supported.
The credo of the OIE, in collaboration with its partners, to better prevent and control such great crises could be summarized by "effective surveillance, early detection, early warning and rapid response". This needs more investment, strong government political commitment to implement and enforce the prevention and control measures, more private-public partnership and good governance based on Strong Veterinary Services complying with OIE standards.

CONCLUSIONS

The OIE is the main global organization dedicated to improving animal health and welfare globally with clear mandates and missions to enhance knowledge on animal diseases, including zoonoses and ensure transparency on the part of its 178 Member countries.
Enhance scientific and technical knowledge represents one of its major objective. OIE makes available to its Member Countries (MCs) the best scientific information to help those preparing efficient prevention and control strategies. This knowledge also constitutes the basis to prepare OIE standards and guidelines which are officially adopted by the 178 MCs. These standards and guidelines provide very detailed and comprehensive background to MCs to develop and implement programs to
prevent and control diseases which are at the origin of important losses in animal production and of major national or international crises and which are consequently considered to be public goods.
Another crucial role is the collection and validation of animal sanitary information. The OIE disease information, based on a reliable and transparent system (WAHIS/WAHID) provides immediate and detailed reports on outbreak events and it represents one of the key OIE mandate in support to its MCs. These activities are based on various methods and on the unconditional duty of OIE Members to disclose all relevant information.
Several other OIE activities related to global control of epizootic diseases are mentioned in the article and capacity building is certainly one of the most important OIE strongly develops. Trainings directed to the Delegates, Chiefs of the Veterinary Services and their teams (composed of focal points responsible for specific domains), are organized all over the world. These programs, which include private components of the veterinary services, build solid and indispensable foundations to support their key roles to insure good governance in preventing and controlling diseases and in contributing to public health and food safety. They also allow better interactions between the national veterinary services themselves and with the entire OIE community.
An overview of international and regional control or eradication programs for three selected diseases (foot and mouth disease, rinderpest and highly pathogenic avian influenza) have illustrated the OIE's role and activities in supporting its Members in the fight against diseases. Several lessons were learnt and some generic ones are considered to be prerequisites if the objective of improving animal health is to be reached. The constant challenge to better prevent devastating sanitary crises is based on effective surveillance, early detection, early warning and rapid response and it needs strong government political commitment and more national and international investment to implement and enforce the prevention and control measures, more private-public partnership and good governance based on strong Veterinary Services complying with OIE standards. The need for more intersectoral cooperation between human and animal health systems and with other sectors

(wildlife, environment, trade, tourism, police, medias, land management…) was also an important lesson learnt from the HPAI crises and OIE has taken a very active position to develop the "One Health Strategy" together with FAO and WHO in association with many other partners.

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A GLANCE INTO THE FUTURE OF THE VETERINARY PUBLIC HEALTH PROFESSIONAL IN AN INCREASINGLY THREATENED WORLD

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SUMMARY

This paper addresses current global developments with regards to animal production and health and looks into the new developing areas of work for veterinarians specialized in veterinary public health[1] in an increasingly threatened world. The paper begins by providing a brief overview of veterinary public health and its role in society. This is followed by paragraphs on veterinary education, and an elucidation of the One Health approach and the excitingly new opportunities for veterinary professionals. Lastly, our collective reflections are summarized in a conclusions section.

INTRODUCTION

The livestock subsector is one of the fastest growing parts of the agricultural economy, contributing to roughly 40 percent of the global value of agricultural production. Livestock provides income, high-quality protein-based foods, fuel, draught power, building materials, and manure as organic fertilizer, and thus contributes to food and income security, soil improvement, and nutrition for adults and children. For many small-scale farmers, livestock also provides an important safety net in times of need. When emergencies strike, as they often do, animals are often sold for cash or traded for other food items. Investments in agricultural research and more robust governance are required to ensure that the world's livestock sector responds to a growing demand for animal products and at the same time contributes to poverty reduction, hunger mitigation, environmental sustainability, and human health.

The principal driving forces behind the growing demand for meat and meat products in middle income and developing countries include population growth, rapid urbanization and the phenomenon of globalization. In order to meet this rapidly rising demand, FAO estimates that global annual meat production will need to expand from the current 228 million tonnes to 463 million tonnes by 2050 with the cattle population estimated to grow from 1.5 billion to 2.6 billion and that of goats and sheep from 1.7 billion to 2.7 billion.[2] This will require more efficient natural-resource use in the sector and measures to reduce the environmental footprint of livestock production.[3]

The challenges presented by higher demand of meat and meat products, coupled with climatic changes and the rapidly evolving agro-ecological and land use patterns will invariably impact on the underlying drivers of disease and associated ecological factors. This could result in increased incursions of disease agents and pests in environmental niches shared by animals and humans. It is at this nexus that most of the future changes for veterinary public health professional will lie. Not only will he/she need to deal with the classical dimensions of the veterinary public health (VPH) that are fairly well known, but also with novel public health challenges that may involve new pathogens, new non-infectious diseases, new clinical signs, new hosts, and evolving disease dynamics. These dynamics will require veterinarians to look at new challenges with a different lens, one that brings contemporary realities of a warmer, more crowded, and more interconnected world into focus. While the task is daunting and indeed largely uncertain, we believe that now is the time to explore and to develop the more demanding requirements VPH professionals will need to deploy so that the societal impacts are minimized to the greatest extent possible.

MATERIALS AND METHODS

This paper draws from a number of sources. A brief literature review of books, reports, discussion papers, essays, and scholarly articles was undertaken to gather the range of viewpoints opinions and research findings. Our thoughts, borne out of our experience in tracking developments in the VPH field, supplemented the information from the literature review. The headings were chosen to guide readers through the different issues explored, while also seeking to make linkages to multiple health domains.

VETERINARY PUBLIC HEALTH AND ITS ROLE IN SOCIETY

It is widely acknowledged that the range of emerging health threats, mostly of animal origin, have increased over the years. These include threats from both infectious and non-infectious agents.

In recent years, the world has witnessed outbreaks of H5N1 highly pathogenic avian influenza (H5N1 HPAI) in Asia, Africa and Europe. The international effort to control and prevent H5N1 HPAI has been truly unprecedented and by highlighting the increasing risk of emergence of pandemic threats has served to increase public awareness and the need for more multi-disciplinary and multi-sectoral collaboration to prevent or rapidly detect and respond to such threats. However, whilst the attention to HPAI has served to galvanise a global response, diseases such as brucellosis, rabies, cysticercosis, echinococcosis, leishmaniasis, and many others that continue to cause illness, death and impose serious burdens, often in the poorest communities, are for the most part left on the margins of health programmes. These neglected zoonotic diseases need to be brought back to the mainstream of public health, particularly in the context of poverty alleviation, food security and global public health. Therefore there is need for more political commitment and financial resources to be allocated for their control. Moreover, most people would agree that the complex and rapid development of international trade, coupled with increasing societal demands for the production of abundant and inexpensive food that is safe and has been raised in a humane and environmentally friendly manner, requires immediate attention from all relevant stakeholders in the veterinary community.[4]

The emergence of bovine spongiform encephalopathy (BSE) and *Escherichia coli* O157:H7 also created more awareness among the general population about food safety issues. In addition the recent dioxin contamination of pork in Ireland and Germany and the adulteration of milk with melamine in China further illustrate the need for more robust and better coordinated national, regional, and international monitoring, surveillance and regulation. For these supervisory tasks to be successful, it will require investments in national policy, legislative and regulatory frameworks, and animal health and food safety infrastructures to minimize risks to animal, humans, and economies.

Humans, animals, and animal products now move rapidly around the world through air, land, and sea transport. Pathogens are adapting, finding new niches, and jumping across species into new hosts. Professionals trained in veterinary sciences are often the most qualified individuals to deal with these public health issues. Those with training in VPH must be able to develop, implement, and execute public and private health programs designed to prevent and control zoonotic diseases in both animal and human populations. There is an increasing societal need for public health professionals with the competencies, knowledge, and skills to address the multidimensional problems of zoonotic and food-borne diseases. [5]

VETERINARY EDUCATION

The history of veterinary medicine is intimately intertwined with duties to public health. This has remained true for 250 years and is becoming even more important with new, significant threats to public health.[6] As an educational imperative, the work of VPH and veterinary education needs to be placed within the framework of a trade-oriented and interconnected world, linking the evolving realities of developed, transitional and developing countries. This is because the disease landscape has radically changed in the past 30 years, and how we address hazards and threats has also had to evolve in order to incorporate technological advances and new communication and information tools to make the work of veterinary professionals and public health officials much more efficient.

Veterinary professionals coming out of educational institutions need a good grasp of the overlap between transboundary animal diseases and veterinary public health and that is not limited to zoonotic pathogens; but also encompasses insidious animal diseases and non-infectious health risks that affect people's livelihoods, social resilience, and food security. Furthermore, veterinarians need to be acquainted with socioeconomic aspects of animal production and health policies, existing national and international regulations, legislation, new concerns regarding animal welfare, and environmental protection, etcetera. There is a need for the integration of new themes/topics in the curricula of graduate and post-graduate veterinary education programs, with specific emphasis on practical epidemiological training, outbreak investigations, enhancing communication and leadership skills, development of cultural sensitivity, the ability to acquire local knowledge in a variety of socio-cultural settings, and an ability to be engaged in multifaceted and multidisciplinary teamwork.

It is for the abovementioned reasons that veterinary training must be creative and flexible to be able to graduate veterinarians who are capable of addressing rapidly changing needs.[7] Veterinarians' educational background in basic biomedical and clinical sciences is very similar to that of physicians; however, veterinarians must possess a profound knowledge of health and disease in multiple species. Primary veterinary training emphasizes comparative medicine. The veterinary profession has always focused on protecting and improving both animal health and human health.[8] Veterinarians are trained in preventive medicine, population health, parasitology, zoonotic disease transmission and epidemiology. This prepares them well for careers in public health.

In veterinary education, the curriculum themes and topics related to VPH are very often only partly addressed within the context of a limited number of subjects such as infectious diseases, meat inspection, and food safety/hygiene. In fact, until recently, only a small number of veterinary faculties had a specific subject dealing with all the relevant aspects of VPH. Current accreditation requirements for veterinary schools and colleges are still vague with regard to the requirements for public health education. This leaves each college or school of veterinary medicine to implement its own programme of veterinary public health education, often based on prevailing

perceptions of need. This has led to the veterinary profession failing to meet the increasing needs for competent and well-rounded veterinarians trained in population medicine and public and environmental health and with understanding of local, national, regional, and international contexts. Veterinary professionals need to know about international organizations involved with animal and human health, and the internationally-led health initiatives in place. For instance, we note that the roles of the FAO/WHO Codex Alimentarius, the Terrestrial Animal Health codes of the World Organisation for Animal Health (OIE), the International Health Regulations (IHR) by WHO, and more recent developments like the One Health concept are not always well understood by today's students.

THE ONE HEALTH APPROACH AND THE NEW OPPORTUNITIES FOR VETERINARY PUBLIC HEALTH PROFESSIONALS

The One Health concept describes a holistic approach to address health risks at the animal, human and environmental interface in order to enhance human and animal wellbeing and welfare, and sustainable management of the environment. The concept promotes a holistic view and fosters cooperation, communication and coordination among sectors. A One Health joint strategic document was presented by FAO, OIE, WHO, the United Nations System Influenza Coordination (UNSIC), the United Nations Children's Fund (UNICEF), and the World Bank during the Sharm-el-Sheikh International Ministerial Conference on Animal and Pandemic Influenza (IMCAPI) held in October 2008 in Egypt.[9] As a follow up to this document, FAO, OIE, and WHO elaborated a Tripartite Concept Note with a vision of a "world capable of preventing, detecting, containing, eliminating, and responding to animal and public health risks attributable to zoonoses and animal diseases with an impact on food security through multi-sectoral cooperation and strong partnerships."[10] Other, related initiatives have also helped to promote the One Health concept. It is essential for new veterinary professionals to understand principles of One Health and its application in addressing health risks emerging at the interface shared by animals, humans, and the natural environment.

The recognized importance of addressing animal, human, and environmental health and wellbeing has created a need for veterinarians with a level of knowledge and skills beyond those gained during their professional education. Needs and opportunities for veterinarians are expanding rapidly in organizations ranging from public and private agencies dealing with animal and human health, to agencies and corporations charged with safeguarding food safety, consumer protection and food security. The demand is increasing for veterinarians with additional education in food safety, food and animal production, zoonotic diseases, bio-security, research methods, administration, and public policy. The veterinary degree alone is not enough to prepare veterinarians to meet these challenges and opportunities. Veterinarians are the only health professionals trained in 'multi-species' comparative medicine and the profession links agriculture, medicine, and even health issues at the household level through their involvement with companion animals.[11] In addition, practicing veterinarians are the first line of defence of newly introduced diseases and will very likely be at the front line in detecting terrorist-engineered epidemics.

The USDA Food Safety Inspection Service (USDA-FSIS) is the single largest employer of veterinarians in the United States and possibly the world. This one agency estimates it will need 500 new veterinarians in the next five years.[12] Other opportunities for service exist at the state, municipal, county, and university level. Masters in veterinary preventive medicine or masters of public health is necessary for many of these career opportunities.[11] In Europe, for example, veterinarians find employment with national animal health departments or veterinary services, food safety/standards authorities, international technical agencies, the European Commission, and with the private sector. In Asia, Australia, Africa and Latin America, similarly, veterinarians find remunerative employment with a wide array of agencies and bodies that do not exclusively deal with traditional aspects of animal health and production.

Global veterinary leadership is needed to reduce the global threat of infectious diseases of major food animal and public health importance.[13] New inspection and certification systems are needed as risks and transmission patterns change. Classical macroscopic meat inspection is insufficient to detect emerging risks from salmonella, campylobacter, *E. coli*, and various residues, which require new and more risk-based interventions. The farm-to-fork approach requires interlinked prevention, control and inspection services and enhanced communication, coordination, and information exchange. In fact, this becomes crucial during crisis situations when various services and sectors need to respond in a coordinated manner.

Moreover, the creation of food safety agencies at national and regional levels, such as the European Food Safety Authority (EFSA), comes at a time when governments are more responsive to the demands of better informed citizens as risk assessors and regulators of food and feed safety. For example, EFSA works closely with national authorities in the European Union and provides independent risk assessment, and scientific advice on existing and emerging risks and threats. Responsibility for the management and communication of these risks and threats are still largely with competent authorities in each country, which are usually embedded within the ministries of health and ministries of agriculture but are in some cases delivered by specialised agencies. The unique exception is Italy, where the entire veterinary services and food inspection are both located within the ministry of health. The key point here is that veterinary professionals are often hired by these specialized entities to conduct assessments and management of risks that fall outside the

immediate realm of classical veterinary medicine. The scope of practice of veterinarians has thus broadened with time, in response to changing needs and this needs to be reflected in dynamic and evolving academic curricula as well as in the provision of continued professional development programs.

Gradually, in many different countries—especially in Latin America—zoonoses centres are becoming established at regional, national, and municipal levels. Although generally embedded as part of the ministry of health, they keep close links with the official veterinary services and those entities dealing with national parks, ecological systems, and environments as well as various veterinary and medical faculties and health professionals at large. Their establishment, mandate, responsibilities and funding mechanisms are normally determined within a legislative framework and their areas of work generally relate to zoonotic diseases in urban, suburban, and periurban areas. These centres especially deal with diseases transmitted by pets, wildlife and synanthropic animals which are normally not sufficiently addressed by veterinary service teams that are commonly engaged with economically important livestock production and health.

The involvement of veterinary public health professionals in integrated national surveillance systems for zoonotic diseases is pivotal, and this has created many new opportunities. This is because these systems should ideally include surveillance of diseases in various animal species and humans, as well as the strengthening of the diagnostic capacities, the timely exchange of information on animal and human diseases and risk factors, coupled with the development of outbreak investigation and response capacities across sectors. For instance, in several Latin American countries, such as Argentina, Brazil and Colombia the ministries of health are in charge of the control of dog rabies and the provision of human rabies post-exposure prophylaxis. This could have contributed to the successful control of dog rabies in these countries as rabies in dogs in other parts of the world is generally neglected when the responsibility is placed with the veterinary services embedded in ministries of agriculture. The zoonoses centres in Latin America are established at the municipal level and zoonoses departments at the national level. Besides rabies, these centres are also addressing a wider range of diseases and issues at the human-animal-ecosystems interface.

CONCLUSIONS

Veterinarians with specialisations and experiences in (veterinary) public health are in a unique position to strengthen and contribute to the expanding work within the ministries of health and agriculture, public and private institutions and industries with regard to preventing and controlling zoonotic diseases and other health-related risks that originate from animals, their products, and their living environment. This assertion is supported by the increasingly visible cases of food and feed contamination, pathogens exhibiting antimicrobial resistance, and of the much higher awareness of consumers to food safety issues throughout the value chain. Also, in the tropics there are still some diseases that continue to plague local populations even though others have eliminated them completely. The veterinary public health professional stands at a privileged place to witness, and carefully examine the many links that continue to evolve between animals, humans and the environment. The ability to understand complex interactions, working in multidisciplinary teams, and fully embrace the One Health approach will further make the veterinarian a key player in enhancing global health and wellbeing.

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NEW OBJECTIVES FOR THE AGRICULTURAL SECTOR AND THEIR APPLICATION IN AUSTRIA

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KEY CHALLENGES

In view of the enormous global challenges, the current reform of the Common Agricultural Policy (CAP) is under great pressure to succeed. Food security for a growing population, the partial downturn in productivity growth, progressive climate change, diminishing biodiversity, volatile markets as well as the high dependency on the oil price are making the supply of food and the survival of rural farms worldwide more and more difficult. As the largest exporter and importer of agricultural products, Europe plays an important role in the design of farming in the future. In the face of the current problems, it is even more vital that those who are calling for sustainable and high quality food production triumph in the discussion on the future shape of the CAP. Food security and environmental security must be considered together in the future. Both have to be guaranteed and supported by the suitable design of the CAP.

The Challenge of Global Food Security with Rising Demand

Securing food in the future for 9 to 10 billion people (2050) will be immensely difficult. Nearly one billion people are already suffering from hunger or nutritional deficiency. More than two thirds of those affected live in rural areas, many of whom are farming families [1]. At the same time, the demand for meat products in emerging markets such as China is growing with the establishment of a wealthy middle class. According to FAO, meat production has more than trebled in the last four decades. Demand will double again by 2050 [2]. Also, in many parts of the world there has been a decline in income growth in the past few years with the effect that currently, for example, the demand for wheat or rice is growing notably faster than yields per hectare. This trend is one of the main reasons for the rising prices of staple foods and can only be kept at bay by an increase in research and development which aims to bring about the sustainable intensification of agriculture.

The increased use of agricultural products for fuel and power generation, which in part is also being greatly promoted, is having an increasing impact on the demand for agricultural commodities. As there are not any binding ecological and social standards for the production of biofuels globally, this has already led to competition in some countries between food and fuel. This hits poorer people in particular and is also leading to conflicts about access to farmland. Consequently the gap between supply and demand for foodstuffs is widening.

In developing countries dependency on food imports is increasing further. According to FAO, food imports have risen by 60 % since 1980 [3]. Particularly countries south of the Sahara as well as a number of countries in Asia and Latin America are affected by hunger [4]. The neglect of agriculture over the past few decades is the main reason for insufficient development in developing countries. At present, yields per hectare in Africa are merely half as large as in other parts of the world [5]. Positive developments have almost exclusively been made in areas intended for export such as cacao and cotton. At the same time around 1.5 bn people are dependent on the yield of their small farms [6]. It is here that help must be given if we want to meet Millennium Development Goal Number One.

First and foremost, smallholder farmers need better access to knowledge, seeds, fertilisers, irrigation, micro loans, plant protection and much more. The World Bank has shown that growth in agriculture can be much more effective in reducing poverty than growth in other sectors. Agricultural development is therefore the basis for poverty and hunger reduction [7]. This can only happen, however, when we succeed in building up a multifunctional agricultural sector that sustains the environment and resources and we avoid the mistakes made in industrialised agriculture.

The Challenge of Climate Change

Supplying the world population with food is weather dependent like no other area. Global warming of more than 2 degrees Celsius will in all probability have extremely negative impacts on food security worldwide. Extreme weather conditions such as floods or droughts and water shortages caused by climate change will affect the whole farming industry, although according to the estimates of climate experts there will be above average incidence and intensity in those regions which are already prone to extreme weather conditions. Particularly vulnerable are those farmers that do not have sufficient means to adapt their holdings to climate change und have poor access to information. Current calculations show that without adaptation measures, declines in yields in agriculture can be expected, particularly in Africa, Australia and parts of Central and South America, the USA

and in South Asia. Although the changes will not tend to have negative effects in the other regions, weather extremes could still exacerbate the farming situation here as well [8]. Therefore, if we do not steer away from climate change the number of people suffering from hunger will just continue to rise.

Farming is particularly affected by the effects of climate change, water shortages or the loss of biodiversity, on the other hand it is also a causer of these things. As a result of soil cultivation, livestock farming and manuring, agriculture generates around 10-14% of global greenhouse gases [9]. The large-scale deforestation of tropical rainforests claims an even greater share of the greenhouse gas emissions. Therefore, in the face of advancing climate change, it is particularly important that we anchor ecologically sustainable agriculture in the future CAP.

The Challenge of Price and Market Volatility

According to World Bank estimates (Food Price Watch April 2011) global food prices rose by an average of 36 % over one year. Maize is now 74 % more expensive on average than one year ago, the prices for wheat have risen by 69 %, soybeans by 36 % and sugar by 21 %. Such price spikes were last seen in 2008. The painful consequences: Since June last year an additional 44 million people have fallen below the poverty line which means that they now have less than $1.25-a-day to survive on [10]. The reasons for the rising price levels and volatile markets are not just attributable to harvest shortfalls resulting from natural disasters, the high oil prices and the strong demand for biofuels but also, and above all, due to the massive market speculation on agricultural primary product derivatives. The volume of these speculations is now 15 times greater than the value of agricultural production itself. That this price bubble will burst is foreseeable. Producers and consumers in developing countries, but also in Europe, will be the ones to suffer from the unstable prices.

While food has become considerably more expensive for consumers, the farming industry has not profited from the price rises to the same degree. This is why global rules for the financial industry have to be drawn up urgently and highly speculative derivative transactions on agricultural commodities have to be disciplined. The introduction of a financial transaction tax would be a further building block in reducing volatility. Even a tax rate as low as 0.1 pro mille on all financial transactions could lead to more price stability as well as to greater transparency on the financial markets [11]. The global adoption of a financial transactions tax has to be the ultimate goal but implementation Europe-wide would also be prudent as a first step.

To stabilise the agricultural commodities market, a European stock exchange for agricultural products would make sense as a counterbalance to the stock exchanges in Chicago and New York.

Goals and reform of the cap

Agriculture is the most integrated sector in the EU. The CAP is no longer a price support instrument as it was in its early years but determines the common goals for agriculture in all Member States and the criteria according to which the sector is promoted. The next reform of the CAP, which is currently under discussion, should accomplish several tasks: It should base itself on the economic and social model found in the Lisbon Treaty, i.e. achieve a sustainable balance between ecology, economy and social responsibility, and make agriculture a central part of the knowledge based economy with more green jobs than ever before. It should offer approaches to solve the problems of climate change and volatile prices. It should bring the CAP in line with the new EU expenditure structure. It is planned that the reform of the CAP will be agreed for the first time mid 2013 in the so-called co-decision procedure of the Council and European Parliament.

Alongside the question of the budget for agriculture and its distribution within the EU, particular focus is being put on the fundamental question of how we can safeguard the European perception of agriculture in the future. Phasing out the CAP or financial cutbacks would lead to a complete de-industrialisation of this sector, the variety of products would decrease and concentrate on favoured areas. In the less-favoured areas, on the other hand, more and more acreage would fall into disuse. However, we want precisely the opposite in Europe, namely a farming industry which safeguards the supply of food as well as provides sufficient public goods and services which are essential for all of us. These include caring for and keeping water, air and soil clean as well as ensuring that we have an environment worth living in, unspoilt habitats and attractive landscapes. As long as these public goods cannot be marketed, payment for these services must be guaranteed by public funding. How an ecosocial economic activity in agriculture should be remunerated within the EU in the future, is the crucial question in the discussions on reform and the future CAP budget.

In Europe around €50 billion is being channelled at present into subsidies [12] for more than 13m farmers [13]. In comparison: the USA pays out a higher amount for its less than 2m farmers. The proportion of public expenditure for agriculture in the EU (EU funds and national budgets) is less than half a percent of GDP [14]. Nevertheless, the EU agricultural system is still in need of improvement.

The reform concept of the EU Agriculture Commissioner, Dacian Ciolos, of last autumn shows a clear commitment to achieving the aims of the Lisbon Treaty and providing public goods through agriculture. Pursuant to the new challenges, he wants to tie funding in the future even more closely to environmental and climate protection – i.e. make agriculture even more ecological. We must be careful though that the planned greening of the CAP does not lead to more bureaucracy in the administration of agriculture.

The attempt to give the Member States the opportunity to develop meaningful instruments in the future that stabilise the market is a positive step. The growing volatility of prices and markets harms farmers, producers, the retail market and consumers. Ciolos has also proposed creating more social balance by placing an upper ceiling on support payments and harmonising the level of payments to agricultural producers and Member States. It remains to be seen, however, whether we will succeed this time in implementing the proposals which a number of large Member States are fighting against.

The Austrian approach

The basic principles of the EU reform agenda known so far such as greening, the promotion of public goods or the payment ceiling are acceptable to Austria's small scale farming industry. Austria's funding framework is more performance related than the European average. Alongside Finland, Austria is the only EU Member State which uses more than 60 % of EU funding to compensate environmental services, promote farmers in mountain areas and organic farmers, to invest in renewable raw materials or to modernise rural areas [15]. The EU average is a mere quarter of funding that goes into these areas. The second pillar of the CAP is therefore far more important than the Single Payment Scheme. In Austria too, a lot of catching up has to be done on informing citizens about the activities and achievements of farmers in order to satisfy the demands of society.

What the EU Commissioner's concept ultimately means in practice can only then be judged when the legislative proposals and the budget figures are presented.

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THE MANY FACES OF THE *CHLAMYDIAE*: FROM SYMBIONTS OF AMOEBAE TO VETERINARY AND HUMAN PATHOGENS

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The *Chlamydiae*, originally considered a small group of closely related bacteria infecting humans and animals, are much more diverse than recognized previously. To date eight families are known, with a broad range of eukaryotic hosts including protozoa and members of most animal lineages. In this presentation I will summarize recent advances regarding our understanding of the diversity of the *Chlamydiae*, and I will highlight how the analysis of amoeba-associated chlamydiae has contributed to our knowledge about the evolution of this unique group of bacteria and their intracellular life style. I will specifically focus on 'Candidatus *Clavochlamydia salmonicola'*, a so far uncultured causative agent of epitheliocystis only distantly related to known chlamydial pathogens. To gain first insights into the biology of 'Ca. *C. salmonicola'* we purified these bacteria from infected salmon gill tissue and extracted genomic DNA which was then subjected to pyrosequencing. From the resulting metagenome, a nearly complete genome sequence of 'Ca. *C. salmonicola'* was reconstructed (1.1 Mb). A high degree of genomic synteny with the *Chlamydiaceae* was observed, and several genes so far being regarded as *Chlamydiaceae*-specific were identified. Remarkably, key metabolic pathways present in all known *Chlamydiae* are absent in 'Ca. *C. salmonicola'* suggesting that this fish pathogen reveals the highest host-dependency amongst known *Chlamydiae*. The cultivation-independent sequencing approach presented here provided first insights into the genomic capacities of a not yet cultivated chlamydial pathogen, illustrating exemplarily the need to further explore the diversity and occurrence of the *Chlamydiae* in nature, their biology and their pathogenic potential.

Part III

Oral Presentations

Block 1

ANIMAL HYGIENE AS INTEGRAL PART OF ANIMAL HUSBANDRY OR THE GROWING POWER OF HYGEIA

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SUMMARY

The paper gives tries to give a general definition of animal health in its broadest sense, which, particularly in the context of producing food with and from animals, has been modulated over time by a whole set of societal determinants. The most drastic changes in the societal understanding of and demands for animal health have occurred in those countries and societies that have intensified their food production beyond the point where a highly intensified food production of food and an almost unlimited access to food from all over the world has led to a stable food security for their populations and hunger or shortenings in the food supply are totally unknown especially to the younger generation. The paper argues that animal hygiene, as defined today by the International Society for Animal Hygiene, a) meets the demands of modern societies and of affluent consumers by being an interdisciplinary set of interacting measures to assure the highest possible status of health and wellbeing for our food animal populations, and b) is not just another discipline of the many disciplines of veterinary medicine (as suggested by the structure of the undergraduate curricula of veterinary medicine) that may "also" be applied when consulting farmers and animal caretakers, but that nowadays is fully integrated in modern animal husbandry systems both at the single farm and at a regional, national and international level. All in all: animal hygiene has left its initial niche and has become a mainstream prerequisite for an ethically accepted and sustainable production of food from animals.

Definition of animal health over time

Animal health has definitively been in the very early focus of the first human tribes that started, after turning the wolf from being their enemies and its kinship to their partner, to domesticate animals for their food supply. Experience told them that any impairment of the health of these animals shortened their availability to feed their tribe. It can be assumed that even the earliest animal sacrifices to try to get the support of those gods and goddesses that were thought to have the power to protect humans against harm und disease, were also meant to ask for protecting the health of their food supplying animals as well. From the Old Testament we know that the animals selected for the animal sacrifices were always to be the strongest and with this the healthiest, since unhealthy animals would have been an offence to those that were asked for luck and protection. Some of the still today valid religious rules for the spiritually right way to eat such as the prohibition of pork or the early rule to withdraw from eating animals that were not healthy demonstrate that it was quite early understood that not only diseases of single animal diseases, but various health impairments that applied to the entire flock or herd of animals. Early concepts of population health may have been developed not only by observing health and disease in human, but also in animal populations.

The Greeks' fondness of inventing very specialized gods and goddesses sowed that they understood that for being able to live a long and healthy life needs several not only a physician. Among the full gods, Apollon was "The Physician", but he was, except for "Healing", also responsible for the light, the liberal arts like poetry and singing, the moral purity of human life and even for the spring. Too much to do for one god, thus, the Greek came up with powerful helpers for Apollon, the half-god Ascleipios and their daughters the half-goddess Hygeia and their sister the half-goddess Panakeia. In contrast to Ascleipios, with his responsibility for all physicians and pharmacists, and Panakeia, being responsible for medicine and miracles, who both very much concentrated on dealing with disease, Hygeia's job was to deal with health. Hygeia's tools, healthy food, a healthy living style, cleanliness and keeping away from especially contagious diseases are the proof of a quite early understanding of population medicine.

A big boost for understanding Hygeia's messages was triggered by the milestone discoveries by Koch, Pasteur, Semmelweis and others that made us comprehend the nature of infection and the mechanisms of the contagiousness of diseases. Applying the basic principles of cleanliness and separation of diseased from healthy individuals were also in veterinary medicine the major reasons for historical successes of Hygeia such as the eradication of human endemic diseases like the Plague and Cholera and of animal diseases like the Rinderpest and Contagious Bovine Pleuropneumonia from Europe even before the development of vaccines and the discovery of Penicillin.

Ironically enough, this overwhelming proof for Hygeia's power, the growing availability of cheaper and cheaper antimicrobial substances also for animals and their steeply growing use even for "prophylactic" and "metaphylactic" reasons in whole animal groups of herds and flocks of food animals has slowed down the awareness for Hygeia

(with a clear difference between the animal production systems in the "West" and the "East" before the "Iron Wall" broke down). The result of this development is that until recently the major focus of farmers and their consulting veterinarians has been the fast and low-cost curing of clinical diseases, which was not really questioned by consumers and the society as long as there was a societal consensus that an increase of the supply of low-cost animal protein food is appreciated. Veterinary medicine and animal husbandry sciences were production-driven and the production performance per animal has to be increased.

In the industrialised countries, however, where the younger generations do luckily not have any understanding of hunger and food shortage, the societal values concerning animal production and food of animal origin are changing: ethical concerns and animal welfare, sophisticated food safety and food quality criteria beyond a good taste and nice packaging come to the fore, i.e. "…the changing culture of Western society is now embracing values beyond cheap food" (Hodges, 2006). Especially the growing demand for an ethical production of food from and with animals will result in a next big boost for the understanding of Hygeia's messages, since offering the animals husbandry systems that are adapted to the needs of our food animals and that do not demand for impairments of the integrity of animals (beak trimming, tail cutting etc.) and guaranteeing a preventive animal health care and the humane handling of the animals are as much in the core of animal hygiene as the principles of a hygienic waste management focussing on re-cycling and re-use of the by-products of animal production as well as minimising the routine use of antibiotic subjects of animal hygiene. These major areas make animal hygiene a multifaceted tool that addresses the economical, the ecological and the societal concerns with raising animals for producing food, which means that animal hygiene becomes more and more one of the preconditions for the sustainability of the animal husbandry systems of today and the future.

Animal Hygiene as defined by ISAH

The description of the history of ISAH on the society's homepage of starts with: "The beginnings of friendly contacts and scientific communication between animal hygienists of western and eastern European countries date back to the sixties [of the last century]. They were initiated most of all by the developing animal production and the intensification-oriented changes in animal husbandry and the associated health risk. The requirements on the exchange of information and scientific collaboration increased and culminated in the statutory meeting of the International Society for Animal Hygiene (ISAH) held in Budapest…". As stated, the beginning of the intensification with its new challenges to animal health and soon to the environment due to massive by-products, was the major trigger of a slowly growing number of veterinary universities to found institutes for animal hygiene. However, this new discipline was, compared to the well-established clinical and para-clinical veterinary disciplines, a tiny newcomer that mostly was hardly recognized and often not appreciated by their older and at this time much mightier sisters such as internal veterinary medicine, surgery and all other disease-oriented disciplines of veterinary medicine. Most "animal hygienists" felt pretty much isolated in their own universities and looked for contacts with colleagues that were worked in the same area of interest and were in need of cooperation and information exchange. In the beginning of ISAH, Hygeia's power was limited, but fortunately, her priests, the animal hygienists, worked hard to remind veterinary medicine of her existence.

However, with the growing intensification of food animal production also more and more western countries, with incidents like BSE and the increase of Salmonella outbreaks due to Salmonella Serovars associated with poultry and pork, and with more zoonoses threatening human health, the understanding of Hygeia's messages started to grow.

Since the 2005 General Assembly Meeting during the International Congress of Animal Hygiene in Warsaw, the ISAH declares the following missions:

- Improve the knowledge on risk factors and on measures which prevent the development and spread of diseases and pathogens in animals including those that pose a risk to human health (zoonoses and food safety relevant infections and contaminations such as residues in food derived from animals).
- Improve the knowledge on measures which will optimise animal welfare.
- Improve the knowledge on measures to minimize the potential adverse effects of animal production on the environment including those that pose a direct and/or indirect risk to human health.
- Promote the creation of interdisciplinary networks of scientists working in the field of animal hygiene and related areas.
- Transfer the "cutting-edge" knowledge and information on animal hygiene to veterinarians, animal scientists, animal producers, physicians and public health professionals as well as to decision makers in agribusiness and politics.

With this mission, "Animal Hygiene" is the discipline of veterinary medicine that is not focussed on animal disease, but on animal health, which means: strengthening disease prevention and continuously adapting the tools of prevention to the changing conditions (Blaha, 2005). In recent decades, especially in the framework of the International Society for Animal Hygiene (ISAH), the scope of "animal hygiene" has been broadened from "just" animal disease prevention to:

- **animal health and animal wellbeing** in the widest possible sense (freedom from disease, freedom from suffering and pain, freedom from pathogens harmful to animals and humans);

- **food safety** at herd level (no microbiological, chemical or physical contamination of meat, milk and eggs, and minimisation of bacterial resistance); and
- **environmental protection** in all areas that are affected by animal production (waste management, protection of soil and ground water and minimisation of emissions from animal husbandry).

Implementing these components of "Animal Hygiene" as integral parts of Good Agricultural Practices and Good Veterinary Practices into the daily production procedures in livestock and into the veterinary service, the production of food of animal origin will continue to change from single animal care actions to cure diseased animals by therapeutic efforts to flock and herd health improvements for maximising the economic output of the livestock operations on to a transparent and socially acceptable production of wholesome, healthy and safe food produced under sustainable production conditions (Blaha and Koefer, 2009).

The updated mission statement if ISAH illustrates that animal hygiene is addressing the core elements of the new European Animal Health Strategy 2007-2013 with its motto "Prevention is better than cure", and the One-health Concept that is embraced by organisations and associations such as WHO, FAO, FVE and AVMA.

In other words: Hygeia has gained full power in the framework of animal health and animal welfare, and Animal Hygiene has become an interdisciplinary mainstream tool that is fully integrated in and indispensible for any ethically accepted and sustainable production of food of animal origin.

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THE CURRENT STATUS OF VETERINARY HERD HEALTH MANAGEMENT IN THE NETHERLANDS: AN OUTLINE

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SUMMARY

This paper describes a study on veterinary herd health management (VHHM), focussing on the current execution of VHHM, the satisfaction of farmers with VHHM and some possibilities for improvement of VHHM. Two questionnaires were sent to 800 randomly selected Dutch dairy farmers, one questionnaire focusing on participants of VHHM and one focusing on non-participants of VHHM. Both questionnaires contained questions about descriptive farm data and opinions on VHHM. The questionnaire for participants contained additional questions about structure, content and satisfaction of VHHM. Farmers who were participating in VHHM had better farm performance than those not participating. They were satisfied with the way VHHM is executed on their farm. However, some areas of attention were recognizable. Goal setting and evaluation were still not regular parts of VHHM, even though it is said to be effective in literature. Time spent on VHHM not visible to the farmer was often not charged or not clearly specified on the bill.

INTRODUCTION

Over the past decades several changes have taken place in dairy farming. Industrialization and increasing (international) competition have led to selective breeding of high producing cows. A shift of curative treatment on a cow level towards preventive treatment on herd level, and the increasing importance of quality assurance and food safety has taken place. Over the past few years livestock sustainability was raised as a new topic of change, since it combined health, welfare and environment. This topic was placed high on the European agenda. Given their broad knowledge on dairy cattle, nutrition, prevention and disease, veterinarians are key figures in the implementation of livestock sustainability on the farm. Advice on prevention of disease, together with a specialized on farm treatment program, can increase both life span and welfare of the cows. To support veterinarians in this role a study was conducted in 2007 in the Netherlands, focusing on veterinary herd health management (VHHM), a form of veterinary advice which integrates animal health, welfare, prevention and quality assurance. Aim of this study was to describe the current execution of VHHM, to measure the satisfaction of farmers with VHHM and to determine the possibilities for improvement of VHHM.

MATERIAL AND METHODS

The two questionnaires were sent to eight hundred randomly selected Dutch dairy farmers, one questionnaire focusing on participants of VHHM and one focusing on non-participants of VHHM. Both questionnaires contained questions about descriptive farm data and experiences with VHHM. The questionnaire for participants contained additional questions about structure, content and satisfaction of VHHM. An accompanying letter asked farmers who participated in VHHM to fill in the questionnaire for participants, and farmers who did not participate in VHHM to fill in the questionnaire for non-participants. No definition of VHHM was given. The returned questionnaires were analyzed and inferential statistics were applied.

RESULTS

Response rate of this questionnaire was 31.75%. Of the 254 returned questionnaires 169 (67 %) were filled in by participating farmers and 86 (33 %) by non-participating farmers. Both participating and non-participating farmers were asked to fill in their general farm data (table 1), after which they were compared:

Table 1: General farm data divided in participants in VHHM (VHHM) and non-participants in VHHM (NVHHM), followed by the P-value of the difference in means.

Variable	category	n	mean	sd	min	max	P-value
Number of cows	VHHM	166	86	39	38	380	0,0283
	NVHHM	85	76	27	34	155	
Somatic cell count	VHHM	160	182	45	60	300	0,3562
(*1000)	NVHHM	85	188	57	60	325	

305-day production	VHHM	159	8.850	1.029	3.500	11.000	0,0072
	NVHHM	83	8.473	1.031	6.458	11.993	
Calving interval	VHHM	152	409	18	357	460	0,5503
	NVHHM	80	403	17	368	453	
BSK	VHHM	163	42,4	4,3	26	52	0,0033
	NVHHM	80	40,5	4,9	31	55	
Quotum (*1000)	VHHM	163	722	327	215	3.000	0,0098
	NVHHM	83	616	236	233	1.320	

The most common interval of farm visits concerning VHHM was every four weeks (59%). Thirty-four percent had an interval of every six weeks. Seven percent of the farmers indicated VHHM was done irregularly on their farm, or together with the obliged 3-monthly farm visits.

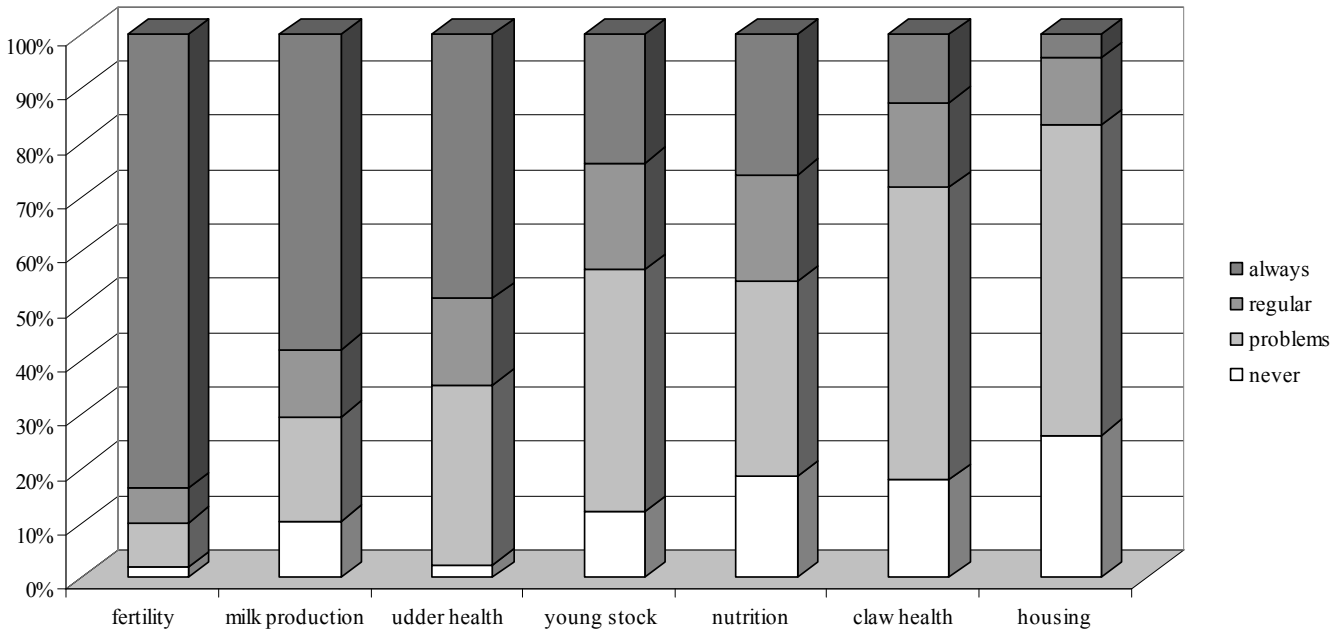

Figure 1: Importance of different items of VHHM in terms of the frequency they are discussed during farm visits

Fertility was one of the major components of VHHM (figure 1), followed by udder health and milk production. Nutrition and claw health were mostly discussed only when problems arose; most farmers received advise on these topics from other parties, like the feed advisor (65%) or the claw trimmer (32%).

On 50% of the farms, according to the farmer the goals of the farm were clear to the veterinarian and they were used in VHHM. Twenty-three percent of the farmers indicated that the goals were known to the veterinarian but were ignored during VHHM. The remaining 27% did not discuss the farm goals with their veterinarian; 13% had goals but these were not known to the veterinarian, 14% had no goals. During VHHM, the selection of topics to discuss was based on former farm visits in 16% of the cases.

Farmers were asked about the (perceived) advantages and disadvantages of VHHM (table 2).

Table 2: Difference in the perceived (dis-)advantages of VHHM between participants (VHHM) and non-participants (NVHHM) in VHHM, rated on a five-point Likert scale

Advantages	category	n	mean	sd	P-value
Increased milk production	VHHM	160	2,78	0,96	0,2826
	NVHHM	82	2,63	0,99	
External support of the farm	VHHM	166	4,00	0,71	<0,0001
	NVHHM	82	3,55	0,88	
Regular check production values	VHHM	159	3,21	0,98	0,0032
	NVHHM	81	2,83	1,03	
Prevent organisational blindness	VHHM	161	3,85	0,82	0,4546
	NVHHM	81	3,74	0,91	
Awareness veterinary developments	VHHM	160	3,54	1,01	0,0165
	NVHHM	82	3,20	1,09	
Structural problem solving	VHHM	160	3,98	0,78	0,0005
	NVHHM	80	3,51	1,03	
Disadvantages	category	n	mean	sd	P-value
(High) costs	VHHM	166	3,31	1,14	<0,0001
	NVHHM	80	4,08	0,93	
Too time-consuming	VHHM	162	2,70	0,97	<0,0001
	NVHHM	80	3,48	0,99	
Often an inconvenient moment	VHHM	161	2,12	0,91	<0,0001
	NVHHM	80	2,75	1,04	
Hard to gather information	VHHM	160	1,86	0,82	<0,0001
	NVHHM	79	2,38	0,97	
Hard to follow up advice	VHHM	161	2,43	0,89	0,1107
	NVHHM	80	2,61	0,89	
Veterinarian intervenes too much	VHHM	159	2,03	0,91	<0,0001
with my farm management	NVHHM	80	2,69	1,07	

Most practices charge for VHHM per hour, including performed acts (69% of the farms). Charging per hour with a separate charge for perfomed acts was also often used (28%). Other possible ways of charging were a fixed fee per cow per year or packages, like an udder health package. Fifty-six percent of the farmers received a bill with a specified call out fee, while preparation time was specified on the bill of only 8% of the farmers.

DISCUSSION

In this study farmers participating in VHHM had larger farms and better farm performance. In large farms, herd management is important. VHHM can help support farmers to make managerial decisions; therefore the larger farms might be predisposed for participation in VHHM. With the differences in farm performance, the question remains whether better farms are more likely to join VHHM, or whether VHHM improves the farm performance (egg or chicken).

The ideal execution of VHHM, according to literature, includes goal setting, planning, execution and evaluation (1). On 50% of the farms in this study, farm goals were not integrated in VHHM. Also, only in 16% of the cases the chosen topics during VHHM was based on former farm visits. This shows that there is room for improvement at this point. When veterinary advice does not fit to the farmers' wishes, the compliance to the advices might decrease. Also, when advices are not evaluated properly, the farmer has no way to provide positive or negative feedback on previous actions, which might be a drawback for the effectiveness of advising.

Participating and non-participating farmers have different views on the advantages and disadvantages of VHHM. The veterinarian might consider providing more information about VHHM to non-participants, including the experiences of participating farmers. Another possibility could be that the participants and non-participants of VHHM show different farming characteristics. Previous studies have shown that farmers can be divided in subgroups (2).

Time spent on VHHM invisible to the farmer (e.g. preparation time) in this study was often not specified on the bill. If veterinarians want to expand the advising in their practice, it might be recommendable to improve transparency on the bill. In that way farmers know what they are paying for and gain better understanding of the costs involved in VHHM.

CONCLUSIONS

Farmers who were participating in VHHM had better farm performance than those not participating. Participating farmers were satisfied with the way VHHM was executed on their farm. However, there were some areas of attention. Goal setting and evaluation were not regular parts of VHHM, even though it is said to be effective in literature. Invisible time spent on VHHM (e.g. preparation of the farm visit) was often not charged or not clearly specified on the bill.

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PREVALENCE OF PODODERMATITIS IN BROILER CHICKENS KEPT ACCORDING TO DIRECTIVE 2007/43/EC STOCKING DENSITIES

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SUMMARY

Since 2007 rearing of broiler chickens is regulated by the Council Directive 2007/43/EC (EU-CD) allowing stocking densities (SD) up to 42 kg/m² at the point of slaughter under certain keeping conditions. However, there are doubts whether such high SD still can provide a living environment which can meet animal health and welfare requirements. The aim of this paper is to show the prevalence of foot pad dermatitis (Pododermatitis, FPD) in broiler chickens kept at the three stocking densities (33 kg/m², 39 kg/m², 42 kg/m²) given in the EU-CD in relation to commonly used fattening times.

The investigations were carried out under practical conditions for a total of 10 fattening periods (Ross 308) in two identical barns on the same farm simultaneously. The three stocking densities given in the EU-CD and the target weights at the point of slaughter were 1.5 kg (short fattening, 28 days), 2.0 kg (medium fattening, 34 days) and 2.5 kg (long fattening, 40 days). For each trial both barns were stocked at the same time with two different SD and depopulated after the same fattening period. The birds were kept on wood shavings which were supplied once at the start of the fattening period. The condition of the foot pads were evaluated at slaughter (n= 400 foot pads per fattening period and barn). Overall, between 58% and 100% of the investigated feet at slaughter showed mild, moderate or severe forms of FPD with moderate and severe lesions dominating. FPD occurred most frequently with prevalence between 98% and 100% in the short fattening periods of all three SD in cold season. In general within the same fattening time with increasing SD the flocks often showed a higher frequency of FPD (1% and up to 20%) and more severe lesions.

INTRODUCTION

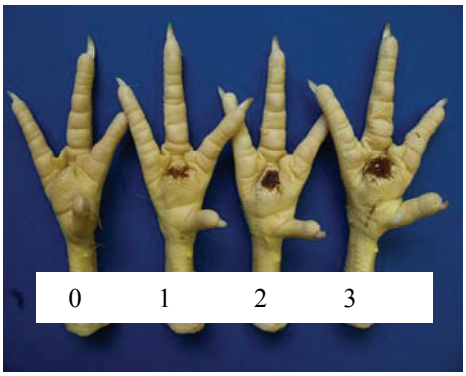
Since 2007 rearing of broiler chickens is regulated by the Council Directive 2007/43/EC (EU-CD) allowing stocking densities (SD) of 33 kg/m². When specific requirements for husbandry and management are complied 39 kg/m² and up to 42 kg/m² at the point of slaughter is permissible resulting in stocking densities of 13 and up to 28 broilers/m² depending on the production target weight (fattening time). However, there are doubts whether the highest SD still provides conditions which meet animal health and welfare requirements. The aim of this paper is to show the prevalence of pododermatitis (FPD) in broiler chickens kept at the three stocking densities given in the EU-CD in relation to commonly used fattening times.

ANIMALS, MATERIAL AND METHODS

For the investigations a total of 10 broiler fattening periods (Ross 308) in a course of nearly two years were considered. The broilers were fattened under practical conditions in a confined building with two identical broiler barns, each with an area of 472 m². The two barns were equipped with separate forced ventilation systems and separate feeding. Stocking densities were 33 kg/m², 39 kg/m² and 42 kg/m². The target weights at the point of slaughter were 1.5 kg (short fattening, 28 days), 2.0 kg (medium fattening, 34 days) and 2.5 kg (long fattening, 40 days), each combination with one repetition per stocking density and fattening time. The number of broiler birds, stocking density and length of fattening time are given in Table 1. Between 13 and 28 broilers/m² were housed. For each trial both barns were stocked at the same time with two different SD and depopulated after the same fattening period. The birds were kept on wood shavings. A refresh during fattening did not occur. The condition of the foot pads were evaluated at slaughter (n= 400 foot pads per fattening period and barn). Figure 1 and Table 2 show the used scoring system.

Tab. 1: Number of housed broiler chickens per m² depending on stocking density (kg/m²) and target weights at the point of slaughter (fattening time)

Fattening period	Stocking density 33 kg/m²	Stocking density 39 kg/m²	Stocking density 42 kg/m²
Short fattening, 30 days (1.5 kg target weight)	22 broiler chickens	26 broiler chickens	28 broiler chickens
Medium fattening , 34 days (2.0 kg target weight)	17 broiler chickens	20 broiler chickens	21 broiler chickens
Long fattening, 40 days (2.5 kg target weight)	13 broiler chickens	16 broiler chickens	17 broiler chickens



Scoring	Macroscopic finding class
0	No macroscopic findings
1	Slight FPD: Up to 1/3 of the Metatarsal foot pad affected
2	Moderate FPD: Up to ½ of the Metatarsal foot pad affected
3	Severe FPD: More than ½ of the Metatarsal foot pad affected

Fig. 1 and Tab. 2: Scoring scheme of foot pad dermatitis (Pododermatitis of the metatarsal foot pad, FPD)

RESULTS

Table 3 shows the frequency (in %) and degree (Class 0-3) of pododermatitis (FPD) at the end of the investigated fattening periods (Mean of two or three flocks and standard deviation). Overall ,on average at least 71% and nearly 100% of the investigated feet at slaughter showed FPD. Moderate (Class 2) and severe (Class 3) lesions dominated. FPD occurred most frequently with prevalence between 98% and 99% in the short fattening periods (30 days and 1.5 kg target weight) of all three stocking densities. In medium fattening period (34 days, 2.0 kg target weight) FPD are observed less frequently per flock with around 82% to 94%. In long fattening period (40 days 2.5 kg target weight) up to 29% of observed foot pads per flock showed no macroscopically findings and a maximum of 91% had FPD. A considerable seasonal influence was observed.

When comparing the three stocking densities, tested within the same fattening time, a clear influence of stocking density on the incidence of FPD often can be found. Lowest lesion scores were seen in all three tested fattening periods at the lowest density of 33 kg/m², most commonly foot pads were without lesions. At a stocking density of 39 kg/m² the prevalence of FPD was at least 1% (short fattening), 3% (medium fattening) and up to 20% (long fattening) higher, most commonly with moderate foot pad lesions. A further increase of the stocking density to 42 kg/m² led to a further increase of FPD to WHAT? (Exception: Long fattening period) could be shown (Increase: 1% and 9%).

Tab. 3: Frequency (% of investigated feets) of Pododermatitis (FPD) in broiler chickens at point of slaughter after three different fattening times (short, medium and long) and stocking densities (33 kg/m², 39 kg/m², 42 kg/m²). n= 2 or 3 flocks per fattening time and SD; Mean and Standard deviation.

Frequency of Pododermatitis (%) Mean (Standard deviation)									
Fattening time	Short (30 days)			Medium (34 days)			Long (40 days)		
Stocking Density	33 kg/m²	39 kg/m²	42 kg/m²	33 kg/m²	39 kg/m²	42 kg/m²	33 kg/m²	39 kg/m²	42 kg/m²
Number of feet	1206	801	1199	804	787	803	808	803	802
Class 0	1.33 (1.12)	0.12 (0.17)	0.25 (0.43)	17.63 (17.28)	14.61 (14.69)	5.57 (1.61)	28.71 (18.65)	8.72 (1.42)	22.32 (3.70)
Class 1	4.72 (6.08)	0.50 (0.00)	0.50 (0.49)	14.64 (10.25)	9.93 (8.06)	3.97 (1.43)	6.33 (3.39)	4.73 (0.70)	7.61 (3.35)
Class 2	36.19 (8.91)	25.74 (0.18)	20.47 (15.65)	47.36 (12.10)	44.49 (5.58)	38.52 (8.15)	36.39 (10.55)	39.98 (10.81)	34.16 (2.47)
Class 3	57.43 (13.78)	72.78 (0.86)	78.13 (14.52)	19.87 (14.74)	31.09 (28.50)	51.33 (9.97)	28.70 (11.31)	46.57 (11.54)	35.91 (2.12)
FPD in total	98.34 (0.88)	99.01 (1.04)	99.10 (1.16)	81.87 (16.58)	85.51 (14.86)	93.81 (0.39)	71.42 (18.48)	91.28 (1.42)	77.68 (3.70)

DISCUSSION

The results indicate that there seems to be a major problem with FPD in broiler chickens when raised at high animal densities. The incidence rate of FPD can reach up to 100% of the whole flock. The higher the stocking density (kg/m²) and the number of birds per m² at the end of the fattening period the higher the number of moderate and severe lesions. The main reason for FPD seems to be poor litter condition which can support massive development of contact dermatitis, hock burns and foot pad dermatitis (FPD) (e.g. MARTLAND 1985). The visual inspection of the litter structure showed already in the middle of fattening period partly caked dry and wet litter (moisture content above 30%). Special problems are dripping nipple drinkers without cups independent of stocking density and fattening time (SPINDLER and HARTUNG 2009). The litter was not refreshed during fattening resulting partly in sticky and sludgy litter at the end of fattening. The findings underline the importance of carefully selected litter materials for broilers with high sorption and adsorption capacity for moisture. In addition the amount of litter supplied, feed composition, ventilation and indoor climate control and stocking density seem to have a considerable influence on the development of FPD.
Also the calculated weights at the point of slaughter (fattening period) seem to have an influence in combination with the stocking density. A short fattening period and high stocking densities result in a higher prevalence of FPD. The reason may be the high stocking density of up to 28 broilers/m² which results in a high excreation of faeces increasing the moisture content in the litter with increasing ammonia formation and release. This promotes the formation of FPD (SØRENSEN et al., 2000; DOZIER et al., 2005). When interpreting the presented findings of this field study it has to be taken into account that the investigation of the short fattening periods happened mainly in the wet-cold season (except fattening period X) which may have worsen the results.

CONCLUSIONS

This orientating field study including 10 Fattening periods (20 herds) shows that FPD appears as a frequent problem in broiler production. Overall, between 71% and 100% of the examined feet showed forms of FPD. There is a tendency that with increasing stocking density the incidence of FPD increases. A short fattening time and a target weight at the end of fattening of 39 kg/m² and 42 kg/m² results in a 100% prevalence of FPD in wintertime. This is usually associated with poor litter quality. Most important husbandry measures in order to reduce FPD are high litter quality and good ventilation to keep the litter as dry as possible. Also refreshing the litter during the fattening period may be a measure to reduce FPD.

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ECONOMICS OF THE CONTROL OF LIVESTOCK EPIDEMICS BY INCLUDING VACCINATION

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SUMMARY

Outbreaks of contagious animal disease have detrimental effects on the Dutch livestock sector as well as on Dutch society as a whole. It is the task of the responsible authorities in case of an outbreak to act in a quick and adequate way. This task is becoming more complicated recently given the different and sometimes contrary objectives that (international) society and agricultural sector have. In future outbreaks vaccination can be part of the control strategies. This has consequences on the economic effects of the outbreak. This paper summarizes the effects of control strategies for FMD, CSF that include vaccination. Results show that vaccination in a radius of 2 km is as effective as culling in a 1 km radius, whereas the economic and social effects are substantially smaller. The livestock industry suffers a lot from an epidemic of CSF or FMD. Although vaccination can limit the costs of an epidemic it also introduces the potential problem of reduced market access for products of vaccinated animals. Acceptance by the trade partners of products originating from vaccinated animals might cushion the economic effects of an epidemic.

INTRODUCTION

Outbreaks of contagious animal disease have detrimental effects on the Dutch livestock sector as well as on Dutch society as a whole. It is the task of the responsible authorities in case of an outbreak to act in a quick and adequate way. This task is becoming more complicated recently given the different and sometimes contrary objectives that (international) society and agricultural sector have. In the past especially (macro)economic considerations had a big impact on the eradication strategy of choice. Nowadays other considerations as animal welfare, or the public opinion towards large scale culling of animals influence the decision on the preferred strategy as well. Since DIVA vaccines for FMD and CSF became available and it is possible to distinguish vaccinated from infected animals, vaccination can be part of the control strategies for future outbreaks.

Using a vaccination-to-live strategy has consequences for the economic effects of the outbreak. The objective of this paper is to compare the socio-economic effects of implementing a vaccination-to-live strategy with a strategy in which animals around infected farms are preventively culled.

MATERIAL AND METHODS

Given the high livestock density in the Netherlands just applying the EU strategy (culling infected farms and implementing a surveillance and a movement restriction zone) does not guarantee a timely and adequate eradication of the infection. Therefore additional measures need to be implemented. In the past they consisted of preventive culling of animals in a circle round infected farms, now vaccination is also a possible strategy.

The following vaccination strategies are proposed:

- FMD: a single vaccination of all susceptible animals on farms in a radius around infected farms.
- CSF: a single vaccination of all not pregnant pigs on farms in a radius around infected farms.
- Infected farms are always culled irrespective the farms were vaccinated or not.

Epidemiological models (Bergevoet, 2007, Backer , 2010) simulated the effects of different control strategies e.g. number of infected farms, number of culled farms, number of vaccinated animals and duration of the outbreak in case of an outbreak of FMD and CSF. These data were used to get insight into the consequences of different control strategies. When evaluating the costs of an epidemic only those components that differ significantly between the different strategies were evaluated. For this the method of Partial Budgeting was used (Dijkhuizen and Morris 1997). The following costs were calculated:

- Operational costs (crisis centers, tracking and tracing, clinical examination and clinical inspection and the costs of police involvement)
- Costs related to culled animals and destructed feed and milk
- Costs of culling and disinfection culled farms
- Costs of empty housing in culled farms
- Costs of repopulation culled farms
- Costs of vaccinating

- Value loss of vaccinated animals
- Value loss of milk from vaccinated animals and costs for logistic processes of milk from vaccinated animals
- Costs of logistic processing of vaccinated animals
- Costs of transportation prohibition of non-infected farms
- Costs of empty houses and repopulation of non-infected farms in infected compartments

RESULTS

Epidemiological and economic results of the different vaccination strategies are presented. For FMD the results were

Table 1 Total costs of the different strategies against CSF in which at the start of the eradication already between 11-20 farms are infected

	cull_1km		Vac _1km		Vac_2km	
	Mean	95%	Mean	95%	Mean	95%
Eradication and control costs	61	101	44	55	42	50
Logistic processing of meat and vaccinated			5	13	9	16
Storage costs	51	276	68	442	34	167
Reduced revenues of piglets	73	189	88	257	54	128
Total in Million €	185	566	204	767	140	362

Based on Bergevoet et al 2007.

Table 2 Table with the total costs of the different strategies FMD for two different areas

	Number of culled farms			Last week of detection			Total cost in million €		
	Percentile			Percentile			Percentile		
	50	5	95	50	5	95	50	5	95
Friesland start in cattle farm									
1 km ring culling	56	2	295	3	1	8	61	48	109
2 km ring vaccination	30	2	117	3	1	8	61	48	108
5 km ring vaccination	30	2	113	3	1	6	65	48	121
Gelderse Valley start in cattle farm									
1 km ring culling	971	206	3217	9	4	15	236	94	615
2 km ring vaccination	260	70	707	10	5	17	227	99	526
5 km ring vaccination	230	68	571	6	4	11	228	106	504

Based on Backer et al. 2009

DISCUSSION

Although vaccination can limit the costs of an epidemic it also introduces the potential problem of reduced market access for products of vaccinated animals.

For the pig industry in applying the vaccination-to-live strategy in case of an outbreak of CS of FMD, the highest costs originate from the reduced acceptance of animals and their products from infected compartments by (international) trade partners.

In case of applying the vaccination –to-live strategy in case of an outbreak of FMD For the dairy industry a large part of the costs originate from the inability to create value from side products of the processing of milk of vaccinated cows.

For the veal calve industry the highest costs originate from animals getting older than eight months at slaughter during an epidemic, so that their meat cannot be sold as white veal but have to be sold as low value minced meat.
A coordinated action between the relevant stakeholders during an epidemic can reduce the value loss of milk from vaccinated dairy cows. Logistic cooperation between dairy companies can reduce the logistic costs and limit the number of locations where the milk from vaccinated animals has to be processed.

Initiate consultations with the trade partners on the acceptance of products from vaccinated animals.

CONCLUSIONS

- Culling strategy is the economically preferred strategy in SPLAs.
- Vaccination is the economically preferred strategy in DPLAs.
- In DPLAs with very high densities of livestock vaccination in 5 km around detected farms results in the lowest costs whereas in other DPLAs vaccination in 2 km around detected farms results in the lowest costs.
- Vaccination in a radius of 2 km in case of an outbreak of FMD or CSF is as effective as culling in a 1 km radius, whereas the economic and social effects are substantially smaller.
- The dairy industry, veal calve industry and the pig industry all suffer a lot from an epidemic of FMD.
- Although vaccination can limit the costs of an epidemic it also introduces the potential problem of reduced market access for products of vaccinated animals.
- Acceptance by the trade partners of products originating from vaccinated animals might cushion the economic effects of an epidemic.

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PREVALENCE OF MACROSCOPIC LUNG LESIONS IN SLAUGHTER PIGS IN FRANCE

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SUMMARY

The aim of the survey was to assess the prevalence of gross lung lesions of slaughter pigs at the National level. The study was carried out in 185 batches of pigs randomly selected from 35 slaughterhouses in France. The number of batches to be selected per slaughterhouse was determined proportionally to the production level. In every batch, lungs from a random sample of 20 pigs were submitted to macroscopic examination at the slaughterhouse. Pneumonia and pleuritis were scored according to the extent of the lesion. Healing following pneumonia, abscesses, nodules and enlargement and congestion of tracheo-bronchial lymph nodes were recorded. Pneumonia and pleuritis were the two most frequent lesions with 50.8% and 13.6% of affected pigs respectively. Healing of pneumonia, enlargement and congestion of lymph nodes were recorded in 14.2%, 15.3% and 16.1% of the pigs respectively. Abscesses and nodules were the less frequently detected lesions, both at the pig and herd levels. For 44.3% of the herds, high pneumonia scores were observed in more than 10% of the pigs. Extended pleuritis was recorded in 29.2% of the batches. Our survey showed that lung lesions are frequently detected at the slaughterhouse in finishing pigs in France, pneumonia and pleuritis being the most prevalent lesions. Despite the wide use of vaccination towards respiratory pathogens, extended lung lesions were detected in a non negligible proportion of herds. Factors related to management, hygiene and housing conditions definitely need to be properly considered when designing control programmes aiming at reducing disease prevalence and severity.

INTRODUCTION

Respiratory diseases are one of the most costly diseases affecting growing-finishing pigs raised under confined conditions in intensive systems worldwide [17]. Lung diseases are associated with economic losses due to lower growth performance, reduced feed efficiency and higher medication costs [2, 17] and have an adverse effect on pig welfare. Even if retrospective evaluation of respiratory disorders by slaughterhouse surveillance is based mainly on chronic lesions, and does not provide information on respiratory illness of pigs throughout the fattening period [15], slaughterhouse inspection is widely used to assess the subclinical respiratory health status of pigs [6, 9, 11, 13]. These data are important to monitor lesion incidence and severity. They can also be used to identify risk factors for lung lesions [7, 13, 16, 14]. This information is extremely useful to further implement adequate control strategies. In France, recently published studies on the prevalence of respiratory lesions are scarce the latest being published in 2005 [11]. Therefore the aim of the present survey was to assess the prevalence of gross lung lesions of slaughter pigs at the national level.

MATERIAL AND METHODS

The study was carried out from May 2008 to December 2009 in 35 slaughterhouses in France representing more than 97% of the National pig production. The number of batches to be selected per slaughterhouse was determined proportionally to the production level (number of tons-carcass slaughtered/year). In total, 185 batches of pigs were randomly selected. A batch was defined as a group of pigs belonging to the same farm that were slaughtered on the same day. In every batch, lungs from a random sample of 20 pigs were submitted to macroscopic examination at the slaughterhouse [5]. The lungs were collected on the slaughter line, just after removal from the carcass, and were removed from the slaughter line for individual macroscopic examination of the lesions. The lungs were palpated and visually assessed for pneumonia and pleuritis according to the method described by Madec and Kobisch [12]. Pneumonic lesions consisted of dark red to grayish purple areas of consolidation in the apical, cardial, accessory and/or diaphragmatic lobes. Pneumonia was scored from 0 to 28 depending on the extent of the lesion of each lobe: point 0 no lesion; 1: lesion affecting <25% of the lobe surface, 2: lesion reaching [25-50[% of the surface, 3: [50-95[% of the surface affected; 4: ≥95% of the surface affected. Pleuritis lesions, *i.e.* inflammation of the visceral and parietal pleura, were graded from 0 to 4: 0 indicating no lesion, 1: one pleural adherence between or at border of lobes, 2: focal lesion with multiple adherence between lobes, 3: extensive parietal adherence with partial adherence to the thoracic wall, 4: adherence of the entire lung to the rib cage. Abscess, nodule and healing following pneumonia and any enlargement or congestion of the tracheo-bronchial lymph nodes were also recorded.

RESULTS

An amount of 3678 lungs were submitted to macroscopical lesions. Pneumonia and pleuritis were the two most frequent lesions with 50.8% (Confidence Interval (C.I.) $_{95\%}$: 49.3; 52.7%) and 13.6% (C.I. $_{95\%}$: 12.8; 15.1%) of affected pigs respectively. Healing of pneumonia, enlargement and congestion of lymph nodes were recorded in 14.2% (C.I. $_{95\%}$:13.0; 15.3%), 15.3% (C.I. $_{95\%}$: 14.1; 16.5%) and 16.1% (C.I. $_{95\%}$: 14.9; 17.3%) of the pigs respectively. Abscesses and nodules were rarely detected (< 1% of the pigs). Pneumonia was observed in 95% of the batches with a mean within herd frequency of 51%. For 44.3% of the herds, high pneumonia scores (≥12/28) were observed in more than 10% of the pigs. Extended pleuritis was recorded in 29.2% of the batches.

DISCUSSION

Our results showed that pneumonia and pleuritis were the two most prevalent lung lesions detected at the slaughterhouse in France. This is in agreement with the results of a previous study carried out in western France where 72.4% and 14.4% of the pigs were found to be affected by pneumonia and pleuritis, respectively [11]. Results of studies in other countries have also shown that lung diseases are widespread in pigs raised under confined and intensive systems with prevalence ranging from 21% to 72% and 14.4% to 62% of the pigs for pneumonia and pleuritis, respectively [1, 4, 7, 8, 11, 19] [14]. Healing following pneumonia was the third most frequent lung alteration which suggests that some recovery of pneumonic lesions had occurred by the time of slaughter [20]. Since healing of pneumonia is a slow process requiring at least eight to ten weeks [10, 18], this observation of healing indicates that pneumonia had developed early in the fattening phase. However, the presence of any healing process should be noted when measuring pneumonia incidence at the slaughterhouse on a herd basis so that the importance of lung problems is not underestimated. Abscesses and nodules were less frequent at both pig and herd levels, as previously reported [3, 8, 11].

CONCLUSIONS

Lung lesions are frequently detected in the lungs of finishing pigs in France, pneumonia and pleuritis being the most prevalent lesions both at the pig and herd level. Despite the wide use of vaccination towards respiratory pathogens, extended lung lesions were detected in a non negligible proportion of herds. Non infectious factors related to herd management, hygiene and housing conditions need to be properly considered in control programmes aiming at reducing the impact of the disease, which in turn would improve pig performance and welfare.

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QUANTIFICATION OF *M. HYOPNEUMONIAE* IN THE AIRWAYS OF FATTENING PIGS USING A RT-PCR ASSAY

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SUMMARY

The aim of the study was to validate the use of a quantitative real-time PCR to assess the amount of *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) in samples taken at different levels of the airways of naturally infected pigs. The study was carried out on a herd chronically affected by respiratory disorders. A sample of 60 pigs was constituted by a random selection from a batch of finishing pigs. Each pig was submitted to 4 samplings: oral-pharyngeal brushing, tracheo-bronchial swabbing, tracheo-bronchial washing and nasal swabbing. *M. hyopneumoniae* DNA was identified by a quantitative Real Time-PCR assay. Differences between sampling methods were compared using a two-part model adapted to paired data. The mean quantities of *M. hyopneumoniae* DNA detected in live pigs by nasal swabbing, oral-pharyngeal brushing, tracheo-bronchial washing and tracheo-bronchial swabbing were $7.0x10^2$ fg/ml, $7.5x10^4$ fg/ml, $4.0x10^6$ fg/ml and $5.0x10^6$ fg/ml, respectively. Significantly higher amounts of *M. hyopneumoniae* DNA were found at the sites of tracheo-bronchial sampling than in the nasal cavities or at the oral-pharyngeal site ($p<0.001$). Our study indicates that tracheo-bronchial swabbing associated with real-time PCR can be an accurate diagnostic tool for assessing infection dynamics and infection pressure in pig herds.

INTRODUCTION

Mycoplasma hyopneumoniae (*M.hyopneumoniae*) is the primary aetiological agent of enzootic pneumonia in pigs a chronic respiratory disease of worldwide distribution [11]. *M. hyopneumoniae*, in association with bacteria and viruses, is also involved in the Porcine Respiratory Disease Complex (PRDC) [10]. These diseases cause major economic losses to the pig industry due to reduced growth rate, increased feed conversion ratio and higher medication costs [5]. Both the infection pattern and the infectious pressure can play a role in disease outcome [1, 2, 9]. Monitoring *M. hyopneumoniae* contamination in live pigs provides useful information on the dynamics of infection within a herd together with insight into the factors influencing the infection pattern and the design of suitably timed preventive and/or control strategies. Little is known about the bacterial load carried by the animals in the field and whether this differs in different parts of the respiratory tract. This information is important when assessing (i) the potential of different sampling techniques to detect infected animals and (ii) the ability of these animals to shed bacteria as high levels are more likely to result in more rapid spreading. Recently, a quantitative real-time PCR assay was developed and validated on samples taken from experimentally infected pigs [6]. The aim of this study was to validate the use of a quantitative real-time PCR to assess the amount of *M. hyopneumoniae* in samples taken at different levels of the airways of naturally infected finishing pigs.

MATERIAL AND METHODS

The study was carried out on a herd chronically affected by respiratory disorders. Coughing was typically expressed during the finishing phase and respiratory disorders were the main reasons for medication. Pigs were vaccinated against *M. hyopneumoniae* at 4 and 7 weeks of age. Pneumonia was regularly observed at the slaughterhouse. A sample of 60 pigs was constituted by a random selection from a batch of finishing pigs. Each pig was submitted to 4 samplings: oral-pharyngeal brushing, tracheo-bronchial swabbing, tracheo-bronchial washing and nasal swabbing. The pig's mouth was held open with a gag to obtain the oral-pharyngeal and tracheo-bronchial samples. Oral-pharyngeal samples were obtained by swabbing the surface of the oral-pharyngeal cavity thoroughly but gently with a brush protected by a catheter (Ori Endometrial BrushTM, Orifice Medical AB, Ystad, Sweden). Tracheo-bronchial swabs were collected with a sterile catheter used for tracheal intubations (Euromedis, Neuilly-sous-Clermont, France). The catheter was deeply inserted into the trachea as the pig inspired, then rotated and moved up-and-down. Tracheo-bronchial washing samples were collected by trans-tracheal aspiration: 10 ml of 0.1 M PBS pH 7.4 containing 0.15M NaCl were introduced into the trachea as deeply as possible with a sterile catheter and immediately aspirated. For nasal sampling, both nasal cavities were swabbed with "CytoBrushs" (VWR International, Fontenay-sous-Bois, France), inserted into the nostrils by rotation to reach deeply into the turbinates. All samples, except the tracheo-bronchial washing fluid, were placed in 2 ml of Buffered Peptone Water Broth. They were individually identified and delivered to the laboratory for processing on the day of collection (Initial Suspension: IS). Samples were prepared for PCR assays as described by Kellog and Kwok [3]. Briefly, 1 ml of each

IS was centrifuged (12,000 xg, 4°C, 20 min) and the pellets were resuspended in 800 ml of lysis solution. Lysates were incubated for 1 h at 60°C, 10 min at 95°C and then kept at -20°C. The RT-PCR assay developed by Marois et al. [6] was used to assess the amount of *M. hyopneumoniae* DNA in each sample. The RT-PCR target defined in the *p102* gene was used in this assay. Briefly, the mixture contained iQsupermix (20 mmol/l Tris–HCl, 50 mmol/l KCl, 3 mmol/l MgCl2 [pH 8.4], 800 µmol/l of each deoxyribonucleoside triphosphate, 0.625 units Taq polymerase and stabilizers) (Bio-Rad, Marnes-La-Coquette, France), 500 µmol/l of each primer, 300 µmol/l of each probe and 5 µl of the DNA template. In the negative control, the DNA template was replaced with double-distilled water. Amplification was performed with the Chromo4 real-time PCR Detection System (Bio-Rad). The reaction procedure consisted of denaturation at 95°C for 3 min then 40 cycles of denaturation at 95°C for 15s and annealing/extension at 60°C for 60s. RT-PCR data were not normally distributed (Kolmogorov Smirnov, $p<0.05$) and showed an excess in zeros. Differences between two sampling methods were therefore compared by a two-part model adapted to paired data [4]. All comparisons were performed using the free software R [8].

RESULTS

M. hyopneumoniae DNA was amplified by RT-PCR assay in 41 of the 60 tested pigs (68.3%). The amounts of *M. hyopneumoniae* DNA detected in samples ranged from 0 to $1.4x10^8$fg/ml whatever the sampling method. The highest amounts of *M. hyopneumoniae* DNA assessed in samples from nasal swabs, oral-pharyngeal brushing, tracheo-bronchial washing and tracheobronchial swabbing were $2.5x10^4$ fg/ml, $1.4x10^6$ fg/ml,$1.3x10^8$ fg/ml and $1.4x10^8$ fg/ml respectively. At least $1.0x10^7$ fg/ml *M. hyopneumoniae* DNA was found in one or more samples from 9 pigs (15%). The mean quantities of *M. hyopneumoniae* DNA detected in live pigs by nasal swabbing, oral-pharyngeal brushing, tracheo-bronchial washing and tracheo-bronchial swabbing are presented Figure 1.

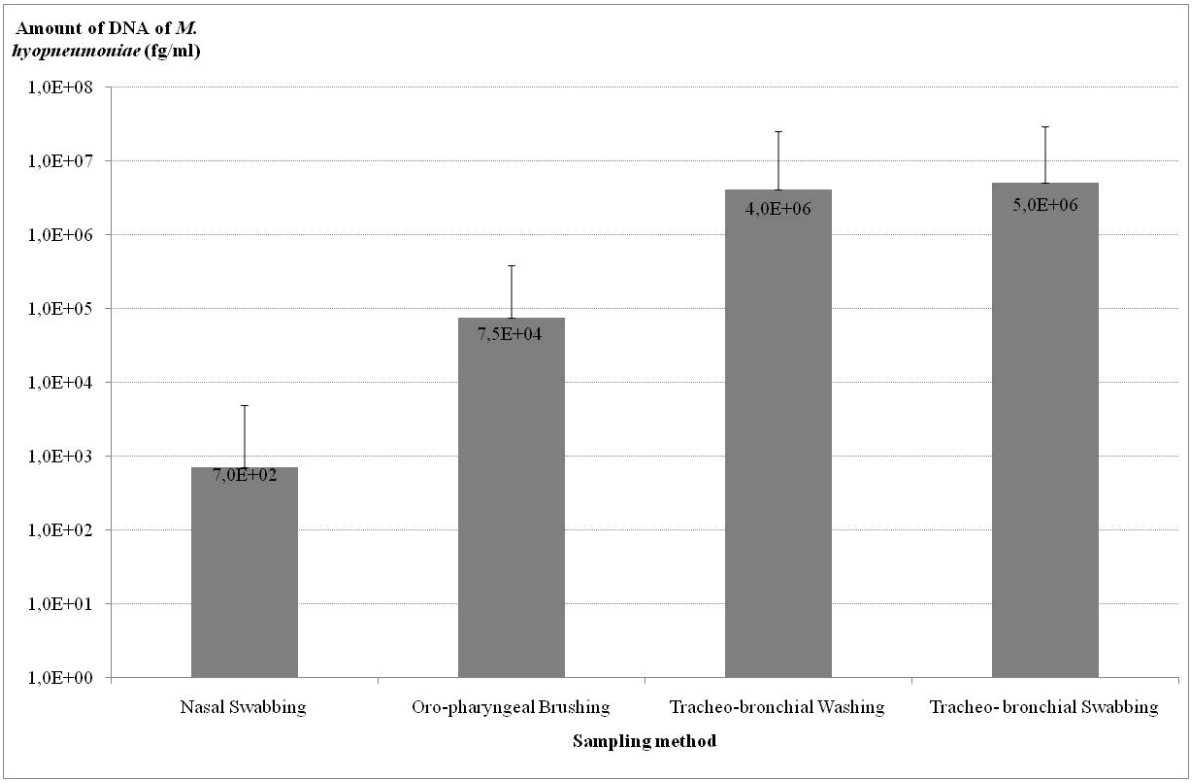

Figure 1: Mean quantities of *M. hyopneumoniae* DNA detected in live pigs by nasal swabbing, oral-pharyngeal brushing, tracheo-bronchial washing and tracheo-bronchial swabbing (60 finishing pigs)

The highest mean amount of *M. hyopneumoniae* DNA was detected by tracheo-bronchial swabbing and the lowest by nasal swabbing with $7.0x10^2$fg/ml and $5.0x10^6$fg/ml, respectively. Significantly higher amounts of *M. hyopneumoniae* DNA were found at the sites of tracheo-bronchial sampling than in the nasal cavities or at the oral-pharyngeal site ($p<0.001$). There was no difference between the tracheo-bronchial washing and the tracheo-bronchial swabbing results ($p>0.05$). The mean amount of *M. hyopneumoniae* DNA recovered from nasal swabs was significantly lower than the amount detected with the other sampling methods ($p<0.001$).

DISCUSSION

Data dealing with the quantification of *M. hyopneumoniae* under field conditions are scarce. Verdin et al. [12], who estimated the number of Mycoplasma cells in tracheo-bronchial washings of 8 finishing pigs by a nested-PCR assay, found titres ranging from 10^4 to 10^8 *M. hyopneumoniae* cells per millilitre of sample. Therefore our study is the first to estimate the number of *M. hyopneumoniae* present in various clinical samples of the airways of naturally infected pigs using a quantitative Real-Time PCR assay. Our results indicated a progressive increase in number of *M. hyopneumoniae* along the respiratory tract of infected pigs, the highest quantity of *M. hyopneumoniae* being in the deeper part. These findings are consistent with the results of a previous experimental study [6]. Marois et al. [6], using a quantitative Real-Time PCR, reported higher numbers of *M. hyopneumoniae* cells in the trachea of experimentally infected pigs than in the nasal cavities and tonsils. The mean quantity of *M. hyopneumoniae* was ten to ten-thousand times higher in the trachea than in the nasal cavities, depending on the infective dose and time after inoculation. Furthermore, similar quantities of the organism were obtained from trachea and lung samples. The results of the present study also showed that within a batch of pigs, while some pigs were either not contaminated with *M. hyopneumoniae* or only at low levels, other pigs shed high numbers of mycoplasma in their airways. Up to 10^8 fg/ml of *M. hyopneumoniae* DNA was detected in the lower part of the respiratory tract of the pigs. Although no distinction between dead and live bacteria could be made by PCR assay, positive nested-PCR pigs were found to be infectious [7]. Therefore we can speculate that pigs with a high *M. hyopneumoniae* DNA load are likely to infect susceptible pigs, by direct contact or contaminated droplets during coughing, sneezing or breathing, which in turn contribute to the persistence of infection.

CONCLUSIONS

In conclusion, the results of the present study indicate that tracheo-bronchial swabbing associated with Real-Time PCR should provide a very useful method for documenting the course of natural *M. hyopneumoniae* infections and studying the dynamics of infection at both pig and herd levels.

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MONITORING ACUTE PHASE PROTEINS IN ORAL FLUID TO ASSESS SUB-CLINICAL DISEASE IN PIGS

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SUMMARY

This study determined levels of acute phase proteins (APP) in oral fluid (OF) from individuals and groups of pigs to assess presence of sub-clinical disease and its relationship to reduced performance. The concentration of APPs in serum and OF was significantly correlated ($P < 0.05$). Concentrations of Haptoglobin (Hp) and C-reactive protein (CRP) in OF were negatively related to the liveweight gain per day of the pigs over the finishing period or lifetime ($P < 0.001$). The concentration of CRP within a pooled OF sample was negatively related to the gain per day during the finishing period ($P < 0.01$).

INTRODUCTION

The presence of sub-clinical disease within livestock systems makes a significant contribution to poor performance, and increases costs of production as a result of reduced growth rates and feed efficiency. For producers wishing to target, manage and control sub-clinical disease, determining the extent of the problem within the farm poses a challenge, as currently there are no objective markers to allow the assessment of sub-clinical disease on farms [1]. Acute phase proteins (APPs) can provide a measure of the extent of immune activation experienced by pigs at different stages of the production system, as their response reflects the magnitude and duration of infection. APPs can now be detected in oral fluid (OF) [3]. OF sampling utilises cheaper collection tools and causes minimal stress to the animals involved, which promotes the ability to conduct diagnostic tests at more frequent intervals. This study determined levels of two APPs, Haptoglobin (Hp) and C-reactive protein (CRP) in OF as a non-invasive alternative to serum, and assessed their usefulness to detect the occurrence of sub-clinical disease in finishing pigs of different ages.

MATERIAL AND METHODS

A total of 80 grower pigs were selected for experiment at ~53kg liveweight, at the point of entry to a fully-slatted, continuous-flow finishing building where sub-clinical disease was thought to be contributing to sub-optimal growth rate. To produce a cross-sectional sample of different ages, 20 pigs of mixed gender were selected per week for 4 consecutive weeks. Pigs were housed in one room of the finisher house in pens of 1.46 x 3.79m, each holding 10 animals, with open bars allowing nose to nose contact between pigs in adjacent pens. The ventilation in the building was by automatically controlled fans, whose speed was adjusted in response to temperature, with the thermostat set at 19˚C. Pigs were fed *ad-libitum* pelleted finisher diet from a feed hopper with five feeding spaces. All feed was weighed into the hoppers. Water was provided via two drinking nipples per pen. Artificial light was provided to the pigs between the hours of 08.00 and 16.30.

Symptoms of disease and treatments administered were recorded daily, cough, diarrhea (scour) and sneeze scores weekly, pig weights and feed intake every two weeks and lung and stomach pathology scores from all pigs at slaughter. As part of a larger study, the weights of these pigs had been recorded periodically since birth. Paired blood and OF samples were collected from each individual pig when the oldest pen groups reached slaughter weight (76kg). At this time, a pooled OF sample was also collected from each pen by extracting OF from chewed lengths of cotton rope which had been suspended in the pen for 1 hour. OF was collected one day prior to serum, to avoid possible confounding effects of blood sampling stress. Serum and OF were extracted, frozen and, within 48 hours, transported at -80ºC to the University of Murcia Veterinary Hospital, for determination of Hp and CRP using time resolved immunofluorometric assays [3].

Statistical analysis

Analysis was performed with the use of Minitab 15.0 and SPSS 17.0 statistical software. To link the APPs to the current productivity of the pigs, the average daily liveweight gain (ADG) of pigs over the 2 week period including the day of sampling was calculated, in addition to growth during the full finishing period and over the whole lifetime of the animal. Data on the health of pigs when alive and at slaughter were collated for each pig. A yes/no score was assigned to each pig, dependent on whether respiratory lesions were present at slaughter. For pen data, the ADG and the feed conversion ratio (FCR) were calculated, together with mean scores for the weekly

pen health, lung and stomach scores. Prior to analysis all data were checked for normality using the Anderson-Darling test. Data of not normal distribution were transformed where possible and an appropriate parametric or non-parametric test selected. Pearson and Spearman's rank correlations were used to examine relationships between the concentrations of APPs in OF and serum, at both individual and pen level. Multiple stepwise regression analysis was performed on individual pig and pen data to determine which measures of pig health and performance were reflected in the levels of APPs.

RESULTS

Correlations between APPs in OF and serum

Significant but weak correlations were found between the concentrations of CRP (r_s = 0.272, P < 0.05) and Hp (r_s = 0.297, P < 0.01) in serum and OF. The concentrations of Hp and CRP within OF were strongly correlated (r_s = 0.734, P < 0.005), as were the concentrations within serum (r_s = 0.524, P < 0.005). The mean of the individual pig OF CRP values in a pen showed a strong positive correlation to the concentration of CRP in the pooled pen OF sample (r_s = 0.786, P < 0.05). However, no correlation found between the mean concentration of OF Hp from individual pigs and the concentration of Hp in the pooled OF pen sample.

Pig health and productivity

The performance of the pigs during the finisher period was sub-optimal at 0.65kg/day yet few clinical disease symptoms were observed (table 1).

Table 1 Productivity and health ailments* of all pigs studied (n = 80, pen mean and range).

	Finisher ADG (kg)	FCR	Total pigs seen coughing	Cough score	Lung score	Scour score	Stomach score
Mean	0.65	3.31	4	1.50	3.30	1.32	3.45
Range	0.59-0.72	2.51-3.93	0-2	0-4.5	0.9-6.5	1-1.8	2.6-1.4

*Cough score: total number of pigs observed coughing within five minutes of observation. Scour score (faeces 1-4): 1 – firm, 2 – soft, spreads slightly, 3 – spreads readily; 4 – water like. Lung score: lobes of lungs graded for area affected by disease on 55 point scale. Stomach score (pars oesophagea region 0-5): 0 – normal, 1 – parakeratosis beginning, 2 – slight parakeratosis, 3- moderate parakeratosis, 4 – severe parakeratosis, 5 – ulcer.

Relationship between APPs, pig health and performance

Relationships between the individual pig health and production factors and the concentrations of APPs are shown in table 2. In the multiple regression analysis, the number of days the pigs had been housed in the finisher building prior to sampling was found to be significantly and negatively related with the concentration of CRP and OF Hp in OF, yet positively related to the serum Hp. The daily gain of pigs in the finisher stage was significantly and negatively related to the concentrations of OF CRP and serum Hp. The lifetime gain per day was significantly and negatively related to the OF and serum concentrations of Hp, but not CRP. The absence of pathological lesions in the lungs at slaughter was positively linked to the concentration of OF Hp. No factors were found to be associated to serum CRP.

Table 2 Relationships between pig production factors and the concentration of APPs in serum and OF from individual pigs (n = 80) and at pen level (n = 8), (regression coefficient and level of significance).

	Individual pig samples				Pooled pen OF samples	
Predictor	OF CRP (log 10)	Serum CRP (log 10)	OF Hp (log 10)	Serum Hp	CRP	Hp
R^2 (adj)	31.10%	no factors significant	28.40%	27.30%	65.30%	No factors significant
Age (weeks)	NS	X	NS	NS	X	X
Days in finisher before sampling	-0.0097***	X	-0.0088***	0.0222**	X	X
Finisher gain/day	-0.52***	X	NS	-1.25*	-176**	X
Gain during sampling	NS	X	NS	X	X	X
Weight at sampling	X	X	NS	X	X	X
Lifetime gain/day	X	X	-0.201***	- 4.5**	X	X
Scour score	X	X	X	X	NS	X
Presence of lung lesion (yes/no)	X	X	0.153**	X	X	X

Significance: *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; NS = not significant. X = term not included in the model as it had not shown a significant effect when running terms individually in a separate model.

At the pen level (table 2) few predictors were significant in the multiple regression model. However, a highly significant negative relationship was found between pooled OF CRP and the pen mean gain per day for the finisher period. No factors were found to relate to the pooled OF Hp.

DISCUSSION

Concentrations of serum Hp and CRP were positively correlated to the OF concentrations, however the correlation was weak. This is not believed to be a result of analytical factors, as the same reagents, assay methodology and laboratory where the test was developed were used for all samples. The weak relationship may result from the fact the OF was collected one day prior to the serum samples, allowing a time period in which the concentrations of the APPs could have changed within the blood. Further studies to clarify salivary APP kinetics could be of interest in the future. There may also be pre-analytical factors, such as feed particles within the OF, although all effort was taken to avoid this. In addition local production of APPs in salivary glands could influence this weak correlation.

The productivity of the pigs (daily gain and FCR) could be considered sub-optimal in comparison to producer targets, and also in comparison to the genetic potential of the pigs. The poor growth and sub-optimal FCR found in this study, with few clinical signs, is suggestive that sub-clinical disease could be contributing to the reduced performance of the pigs. The inverse relationship between the concentration of APPs and ADG of individual pigs has been found elsewhere [2], and supports the hypothesis that the sub-optimal growth rate found in this study could be due to immune system activation, diverting energy away from metabolism and growth. The negative relationship of serum and OF Hp to lifetime ADG of individual pigs suggests that pigs could have been under chronic immune activation. The inverse relationship between the OF APPs and the length of time pigs had been housed within the building could indicate a challenge from new pathogens on entry to the continuous flow building; however, the relationship for serum Hp was in the other direction. The difference in the relationship could in part reflect the difference in kinetics between the two APPs, of which the duration of response can differ [4]. It is also known that Hp in serum, but not OF, can be influenced by disease pathologies causing mild haemolysis or anaemia. A greater number of significant relationships between performance measures and the OF APPs than the serum APPs could indicate that the concentration of APPs in the OF provides a more robust indicator of subclinical disease. However, the results should be interpreted with caution and additional studies with a larger number of animals that incorporate different housing and productive conditions are required. The pooled OF sample reflected the group mean well in the case of CRP, and the inverse relationship of this measure with ADG suggests a potential for use in farm monitoring. However, more work needs to be conducted in different environments to evaluate this, and to explore the lack of significance for Hp.

CONCLUSIONS

Studying APPs in OF could provide a convenient, low stress method to objectively assess the level of immune activation associated with subclinical disease that could be contributing to sub-optimal growth in pigs. This work suggests there is potential for the use of a pooled OF sample to assess APPs which would greatly reduce costs. Further work should be conducted to explore this. Work to assign the degree of lost production relative to a change in APP concentrations would be a further useful step.

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QUANTIFICATION OF BIOSECURITY STATUS IN PIG HERDS USING AN ONLINE SCORING SYSTEM

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SUMMARY

Biosecurity includes all measures implemented to prevent pathogens from entering the herd and to reduce the within-herd spread of pathogens. To quantify the biosecurity status of a pig herd, a scoring system was developed. This system quantifies all aspects of both external and internal biosecurity taking into account their relative importance in infectious disease transmission. This enables to objectivise the biosecurity at a pig herd and to compare herds. It also enables to monitor herds and to motivate farmers to improve the herd biosecurity status. This scoring system was implemented in a website and can be freely filled in online (in Dutch and in English). From December 2008 to February 2011, 270 pig herds from 4 different countries have filled in the questionnaire. The average score for external biosecurity is 66/100 (min 29; max 97) and the average score for internal biosecurity is 51/100 (min 18; max 97). There are big differences between the scores of different herds. On 87% of the herds the score for external biosecurity is higher than the score for internal biosecurity. These results indicate that there is room for improvement in many of the herds, especially with regard to internal biosecurity.

INTRODUCTION

Biosecurity increasingly gains importance for the health management of (future) pig herds. It includes all measures to prevent pathogens from entering the herd and to reduce the spread of pathogens within the herd in order to keep the animals healthy [1]. Biosecurity can be divided into 2 different categories: the external and the internal biosecurity. The external biosecurity deals with measures which prevent pathogens from entering the herd, while the internal biosecurity is related to the reduction of the within-herd spread of pathogens. In order to quantify the biosecurity situation on pig herds a biosecurity scoring system was developed by the Veterinary Epidemiology Unit of the faculty of Veterinary Medicine, Ghent University, and incorporated in a free online application (www.biocheck.ugent.be) [2].

MATERIAL AND METHODS

The scoring system takes both external (preventing pathogens from entering the herd) and internal biosecurity (reducing within herd spread of infection) measures into account. Both parts are divided into 6 subcategories, each consisting of 2 to 13 questions.

The different subcategories for external biosecurity are: 1) purchase of animals and sperm, 2) transport of animals, removal of manure and dead animals, 3) feed, water and equipment supply, 4) personnel and visitors, 5) vermin and bird control, 6) environment and region. Internal biosecurity is divided in: 1) disease management, 2) farrowing and suckling period, 3) nursery unit, 4) fattening unit, 5) measures between compartments and the use of equipment and 6) cleaning and disinfection.

Each question within a subcategory and each subcategory on its own received a weight based on information from literature on pathogen transmission and general knowledge of infection risks [2]. A score between 0 (=total absence of any biosecurity) and 100 (=perfect biosecurity) is obtained for both external and internal biosecurity. The mean of both scores gives the overall biosecurity score. The scoring system is adapted to be appropriate for every type of pig production (fattening herd, breeding herd, mixed herd, etc). The questionnaire is available in Dutch and English.

RESULTS

The online scoring system was launched in December 2008 (first only in Dutch and from June 2010 also in English) and since 270 herds (i.e. 22 breeding herds, 14 fattening herds and 234 mixed herds) from 4 countries (Belgium n=264; Australia n=3, The Netherlands n=2, Denmark n=1) have filled in the questionnaire. On 27% of the herds, other animals were kept for professional use (of which 75% has cattle, and 21% has poultry). The average score for external biosecurity was 66 (min 29; max 97). The score for internal biosecurity was lower in most herds (87%) with an average of 51 (min 18; max 97). Table 1 shows the results for the different subcategories.

Some selected results relating to external biosecurity showed that 78% of the herds purchasing new breeding animals use quarantine facilities for an average period of 44 days and that 80% of these herds performed all-in / all-out in the quarantine stable. Sperm is purchased on 92% of the mixed and breeding herds and 79% knows the health status of the herd of origin. Farm-specific clothing and footwear is provided to visitors in 96% of the herds. Removal of carcasses from dead pigs can be done from the public road on 71% of the herds, but only 57% of the farmers regularly cleans and disinfects the storage place for the dead pig. In 23% of the herds, the farmers never clean or disinfect hands after handling dead pigs or wear gloves when handling carcasses. A sanitary transition zone is not available or used in 18% and only 27% demands visitors to wash and disinfect their hands. Although most farmers are very strict concerning hygienic measures for visitors entering the stables, only in 65% of the herds, the farmer and/or personnel carry out these hygienic measures themselves before entering the stables. On 52% of the herds, cats and dogs are allowed to enter the stables. On 9% of the herds, the transporter of live animals has entrance to the stables and in 50% of these cases, the driver did not wear farm-specific clothing. Loading of the animals is done directly from the stable or central corridor in 80% of the herds. Only 60% of the farmers yearly examine the quality of the drinking water used for the pigs.

Concerning internal biosecurity, all-in / all-out management is practiced in 80% of the herds in the nursery unit and in 66% of the herds in the fattening unit. From all the herds, 60% cleans and disinfects every stable after each production round, but only few (2%) verifies the efficiency of these measures. Only in 42% of the herds diseased animals are housed in separate hospital pens and 47% manipulates the diseased animals after the healthy ones. Suckling piglets are transferred from one litter to another on 98% of the herds, of which 44% does this more than once and 31% keeps on performing this operation after 4 days post partum. In 76%, the farmer never changes clothing and 65% never washes hands between the different age groups. Only 54% of the farmers always work from the younger to the older pigs. In spite of the use of disposable needles, the needle is only changed after 75 animals on average. Although most farmers practice all-in / all-out management, 24% mixes pigs of different ages in order to obtain pens with pigs of similar weight in the nursery and/or fattening unit. On 54 of the herds, a sanitary stand empty period is applied after each production round. Only 32% of the herds have a foot bath with disinfectant at the entry of the herd.

In general there is a positive correlation between the scores for external and internal biosecurity (figure 1).

Table 1. Results per subcategory of the biosecurity scoring system in 270 herds (December 2008 – January 2011)

Subcategory	Average	SD	Min	Max
External biosecurity	**66**	**11**	**29**	**97**
Purchase of animals and sperm	88	13	44	100
Transport of animals, removal of manure and dead animals	67	15	22	100
Feed, water and equipment supply	42	19	0	100
Personnel and visitors	64	18	0	100
Vermin and bird control	60	24	0	100
Environment and region	56	26	0	100
Internal biosecurity	**51**	**15**	**18**	**97**
Disease management	59	30	0	100
Farrowing and suckling period	60	25	0	100
Nursery unit	58	25	0	100
Fattening unit	64	30	0	100
Measures between compartments and the use of equipment	42	20	0	100
Cleaning and disinfection	43	27	0	100
Overall biosecurity	**59**	**12**	**28**	**97**

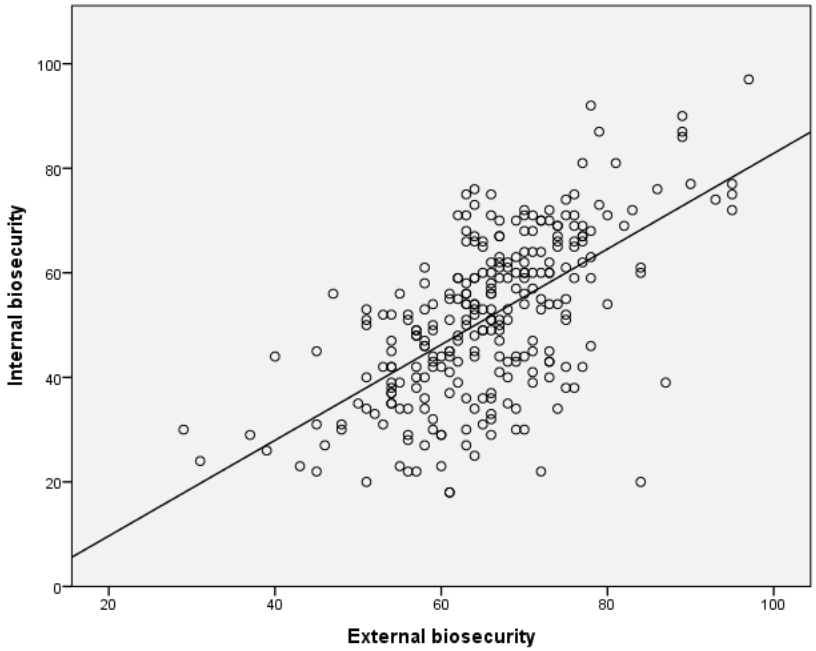

Figure 1: The correlation between the scores for external and internal biosecurity on pig herds.

DISCUSSION

The large differences between the scores of different herds show that there is much room for improvement in many herds. On average, the scores for external biosecurity, which are mainly measures imposed on others (visitors, suppliers, etc) are higher than the scores on internal biosecurity, which are more related to the work and management strategies of the farmers themselves.

As the results show, there are many biosecurity measures that have become common practice for farmers, like providing farm-specific clothing and shoes for visitors to prevent the entry of diseases through visitors. On the other hand, some effective biosecurity measures, like isolation of diseased pigs in a separate hospital pen, should be more frequently practiced. Especially in the internal biosecurity measures still a lot of improvement can be achieved.

Furthermore, some biosecurity measures, like a foot bath with disinfectant at the entry of the herd or a sanitary transition zone, are mandatory in Belgium. But, as the results show, a lot of Belgium herds doesn't implement these measures.

CONCLUSIONS

The Biocheck provides a freely available, risk based and user friendly tool to quantify the biosecurity on a pig herd. It points out strong and weak points of the herd and may help to set priorities for improving and monitoring the biosecurity status. As an objective score is given, it's easier to see improvement over time and to compare with other herds. The latter can motivate farmers to improve or maintain their biosecurity score.

The average biosecurity scores of pig herds are moderate. There are large variations between the scores of different herds, so there is still a lot of improvement possible on many herds.

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PREVALENCE OF POSTPARTUM DYSGALACTIA SYNDROME IN SOWS

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SUMMARY

The aim of this paper is to present recordings for Postpartum Dysgalactia Syndrome (PDS) over the course of two years on three farms. Data assessment took place from July 2008 to July 2010 at three commercial farms (F1, F2, and F3) with similar management conditions, and animal health and hygiene standards. Sows were defined as affected by PDS if they showed a rectal temperature above the threshold of 39.5°C and/or clinical signs of mastitis and/or their piglets' condition or behaviour indicated a lack of milk. Prevalence of PDS was analyzed with respect to the parameters farm, breed and parity. Prevalence differed between the three farms between 10.8%, 6.1% and 6.3%, respectively. The parity-specific prevalence ranged from 2-9% with the third and the eight parity showing the highest mean prevalence (8.9%). Rectal temperature was analyzed with regard to its use as diagnostic tool. Of all diseased sows, 16.6% showed a rectal temperature lower than 39.5°C, and 28.8% had a rectal temperature above 40.0°C. In order to get a clear impression of comparable prevalence data, based on the clinical diagnosis, rectal temperature is still recommended, but additional criteria have to be considered to avoid false negative sows showing no fever above 39.5°C, but being affected by PDS.

INTRODUCTION

Postpartum Dysgalactia Syndrome (PDS), with coliform mastitis as the lead syndrome, is one of the economical most important diseases in sows after parturition. Prevalence of PDS in Belgium was evaluated via questionnaire in a recent study by Papadopoulos et al. [1] with an average prevalence of 6.5% (1–15%) in affected herds. Due to the fact that new scientific information regarding PDS prevalence on the base of individual clinical examinations is lacking, this paper presents recordings for PDS over the course of two years on three farms. Prevalence of PDS was analyzed with respect to the parameters farm, breed and parity.

MATERIAL AND METHODS

Commercial farms with similar management conditions, animal health and hygiene standards were selected to provide maximal comparable environmental factors. The herd owners or the veterinarians of these farms were experienced, and received an additional special training for the sampling procedure for this study. The selected three farms recorded and sampled all PDS-affected sows continuously during the study period of two years. The farm prevalence was estimated weekly based on the total number of sows farrowing in the respective week. Weeks with no sampling due to vacation or other circumstances were excluded.

For this study, PDS was diagnosed via standard rectal temperature control 12 to 48 hours post partum. Sows were defined as affected with a rectal temperature above the threshold of 39.5°C and/or clinical signs of mastitis such as reddening, swelling or hardening of mammary glands and/or changes in piglets' behavior. As a matched sample to the affected sows, healthy half- or fullsib control sows were sampled and recorded in detail, too. In total, 958 affected sows and 897 healthy control sows were evaluated.

Breeds on these farms included pure-bred (landrace (L), large white (LW)) and cross-bred sows (LxLW, LW_Duroc x L, LW_Duroc x LW, L_Duroc x LW, L x L_Duroc). Sows were in their first to tenth parity.

Breed- and parity-specific prevalence and temperature were analysed statistically over all recorded weeks in summary within the R statistical environment.

RESULTS

Prevalence differed between the three farms with 10.8% in average (range from 4.0-17.5%) on F1, 6.1% (1.8-14.5%) on F2 and 6.2% (1.9-20.8%) on F3. Seasonal fluctuation, but no significant trend for an increase or decrease was observed over the two-year-period (Figure 1).

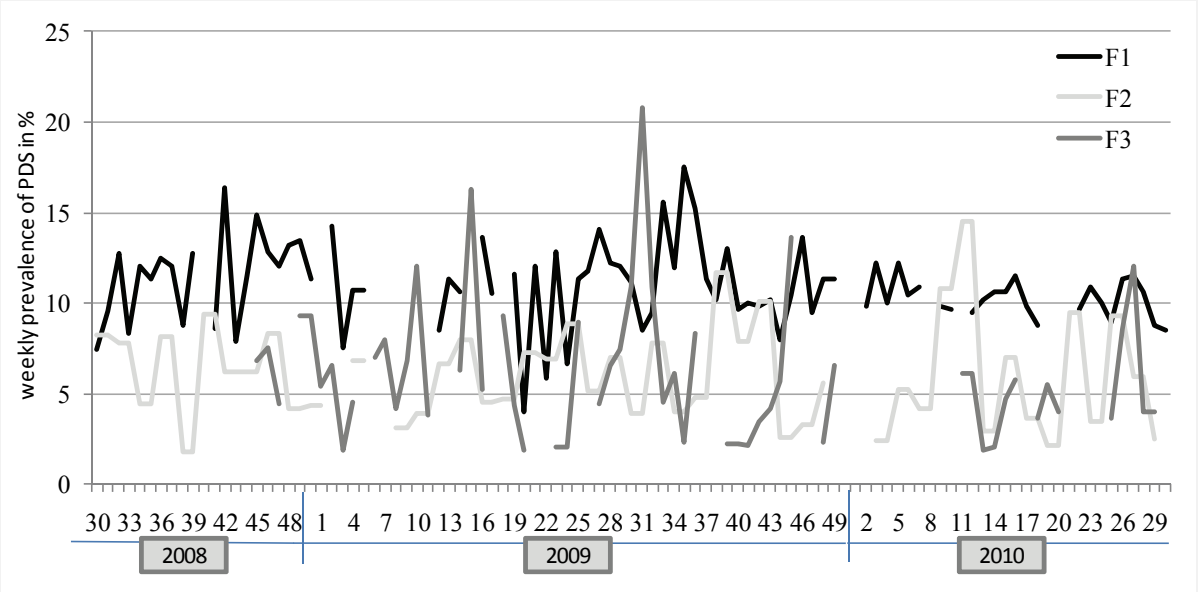

Figure 1: Weekly prevalence of PDS in % from week 30/2008 to week 30/2010 on the three farms F1, F2, F3.

The parity-specific prevalence ranged from 2-9%, whereas the mean prevalence of the third and eight parity number was the highest with 8.9%. While in two farms (F1, F2) crossbred sows showed the highest prevalence with 11.7% (LxLW) and 11.1% (LW_Duroc x L) compared to 6.6% in pure-bred landrace sows on F2, on the other farm (F3) landrace sows had highest prevalence with 12.0%. The prevalence in LW sows differed between the farms from 4.8% (F3) to 6.1% (F2) and 10.7% (F1).

Rectal temperature ranged in affected sows from 38.3°C to 41.6°C (mean = 39.75, median = 39.7, standard deviation (s.d.) = 0.469). In unaffected sows a temperature range from 36.4°C to 39.8°C (mean = 38.72, median = 38.7, s.d. = 0.385) was observed (Figure 2).

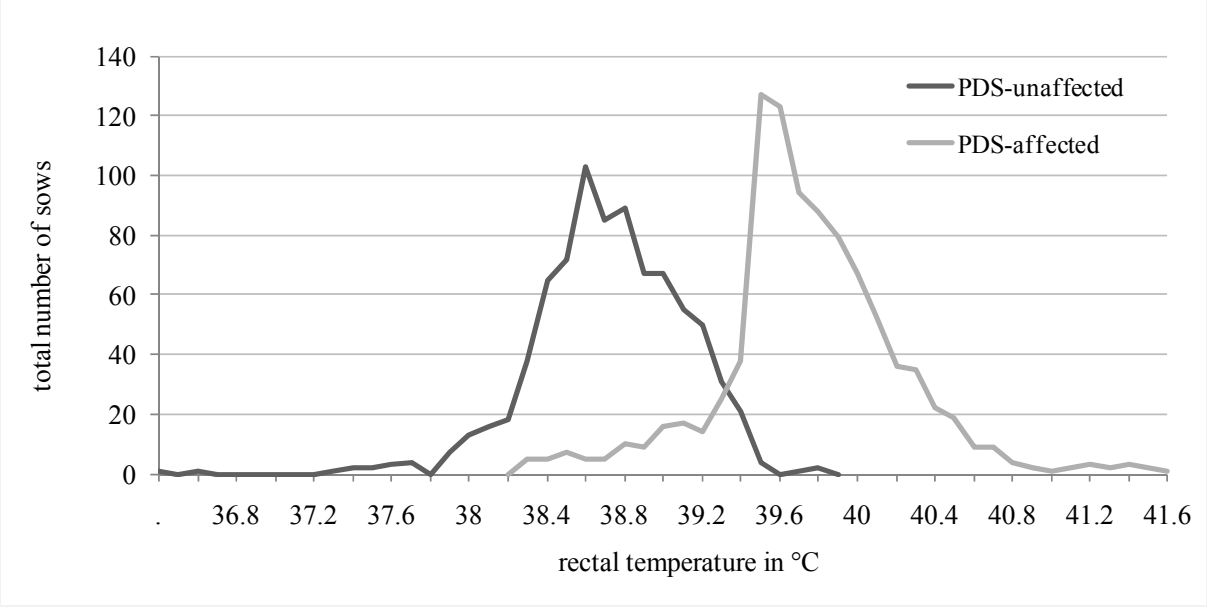

Figure 2: Absolute frequency of PDS-affected and PDS-unaffected sows in relation to rectal temperature.

More than 16.6% of all affected sows had rectal temperatures lower than 39.5°C, and 28.8% had temperatures above 40.0°C. As shown in detail in Figure 3, mainly on F1 several sows were diagnosed without showing rectal temperatures above 39.5°C.

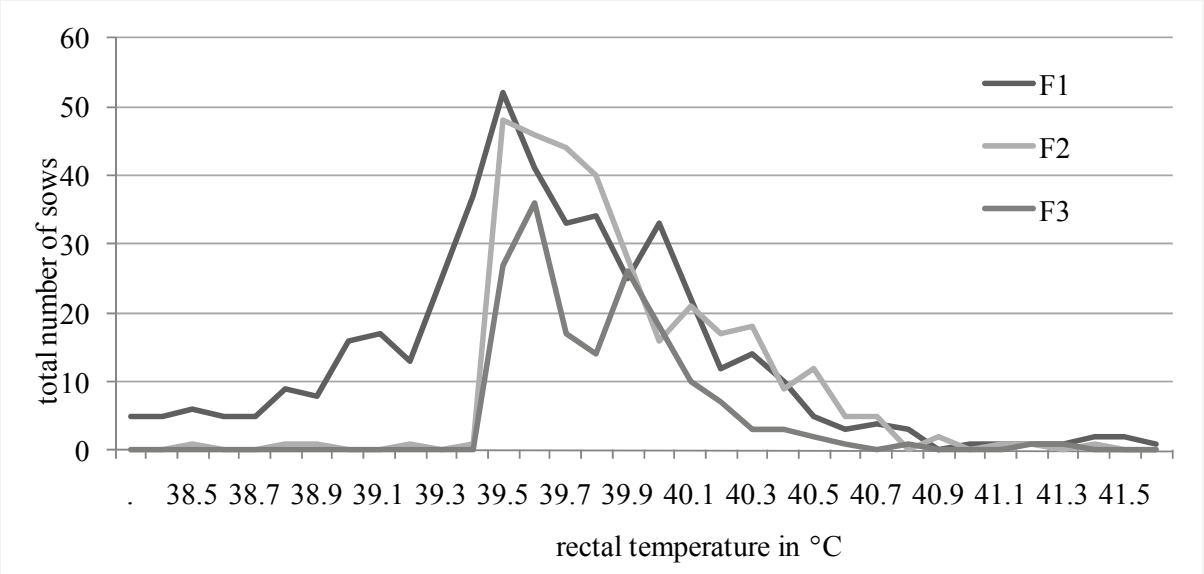

Figure 3: Absolute frequency of PDS-affected sows in relation to rectal temperature on the three examined farms F1, F2, F3.

DISCUSSION

This is - to the authors' knowledge - the first study presenting continuously recorded PDS-frequency over a longer observation period. Prevalence on these farms was - despite the specific aim of PDS-detection - low, and, over the course of two years, no significant trend for a decrease or an increase of prevalence was observed. Due to regular and early control on these farms, sows didn't show severe symptoms of PDS, and only in a few cases piglets were affected.

The significant higher prevalence on F1 might be interpreted as farm-specific fact to the very precise diagnosis by the farm veterinarian, identifying a higher number of recorded affected sows with temperatures lower than 39.5°C.

Because of the strict recording and sampling procedure, the sensitivity to detect PDS-affected sows can be regarded as high. However, the specifity is probably lower due to several factors influencing the postparturient period and other diseases leading to similar clinical signs such as mastitis, fever and hypogalactiae.

A comparison to other studies is difficult to perform, because, the assessed prevalence range depends on the used - and often very various - diagnosis and clinical scores. Therefore, this study emphasizes the importance of long-term recordings of complex disease traits such as PDS on the single farm as a foundation for the selection of animals and for a solid herd health monitoring.

CONCLUSIONS

Over the two examined years, seasonal fluctuations were obvious, but no significant trend for a decrease or an increase of prevalence rate was assessed. In addition to environmental factors, other factors such as genetic susceptibility may influence the outcome of PDS. In order to optimize herd management, a continuous and reproducible disease recording of PDS, and also of other diseases, is recommended. For the diagnosis of PDS, a generally accepted identical clinical score with objective parameters such as rectal temperature, and sows' and piglets' behaviour, is recommended to improve the comparison of the recorded data on different farms.

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BIOLOGICAL PATHWAY ANALYSIS FOR POSTPARTUM DYSGALACTIA SYNDROME IN SOWS VIA A GENOME-WIDE ASSOCIATION STUDY

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SUMMARY

This paper represents the implementation of a genome-wide association study on Postpartum Dysgalactia Syndrome for biological pathway analysis. A family-based study with matched sampling of diseased and healthy control full- or half sib sows was designed. The sows were clinically investigated 12-48 h post partum and were defined as affected when they showed rectal temperatures above 39.5°C and/or clinical signs of mastitis and/or changes in piglets' behaviour. The animals were genotyped on 62,163 genome-wide distributed Single Nucleotid Polymorphisms. Genetic and bioinformatics software tools and databases were used for statistical and functional analysis. Genome-wide significantly associated regions on porcine chromosome 17 indicate and support the important role of neuro-hormonal regulation in pathophysiology. Moderately associated regions on other chromosomes suggest further possible molecular networks for the susceptibility or the resistance to this syndrome. Multiple genes and different path mechanisms are possibly involved.

INTRODUCTION

Postpartum Dysgalactia Syndrome (PDS), with coliform mastitis as the lead syndrome, is one of the economical most important diseases in sows after parturition. Even though most farms have a high standard of hygiene and herd management, this disease still represents a considerable health problem. Therapy with antibiotics and antiphlogistics is feasible, but is only a short term solution. Up to now the detailed pathogenesis is not completely understood. A genetic predisposition for PDS has been discussed [1,2,3,4], but has never been investigated in detail. From the perspective of preventive veterinary medicine, the understanding of biological pathways affecting the clinical course of PDS and a genetic improvement is desirable. In this context, genome-wide association (GWA) analysis offers a deeper insight into the genetic variation. Based on GWA results, gene networks and possible molecular pathways can be evaluated. The aim of this paper is therefore to present methods and first results of biological pathway analysis based on a GWA study on PDS.

MATERIAL AND METHODS

Commercial farms with similar management conditions, animal health and hygiene standards were selected to provide maximal comparable environmental factors. The clinical investigation took place between 12 to 48 h post partum. Sows were defined as affected when they showed rectal temperatures above 39.5°C and/or clinical signs of mastitis such as reddening, swelling or hardening and/or changes in piglets' behavior.

The Illumina PorcineSNP60 BeadChip [5] was used for genotyping on 62,163 Single Nucleotide Polymorphisms (SNPs) and the following quality control criteria were applied: minor allele frequency of at least 0.05, a maximum missingness per SNP of 0.1 and an individual callrate threshold of 0.95. The Hardy-Weinberg-Equilibrium and the raw density plots were checked for each SNP. In total, 585 pigs (314 affected and 271 healthy control sows) and 49.740 SNPs were included in GWA analysis after quality control. A principal components analysis was applied to correct for breed, family structure and other unknown factors. Seventeen principal components and the additional fixed effect birth assistance were used as covariates in an adapted score test. Based on GWA results, possible gene networks were elucidated according to functional and positional information including possible pathway mechanisms. Genetic software tools as for example GenABEL [6], and bioinformatics' databases such as Ensembl (www.ensembl.org), KEGG (www.kegg.com) or PigQTLdb [7] were used for statistical as well as for functional analysis.

RESULTS

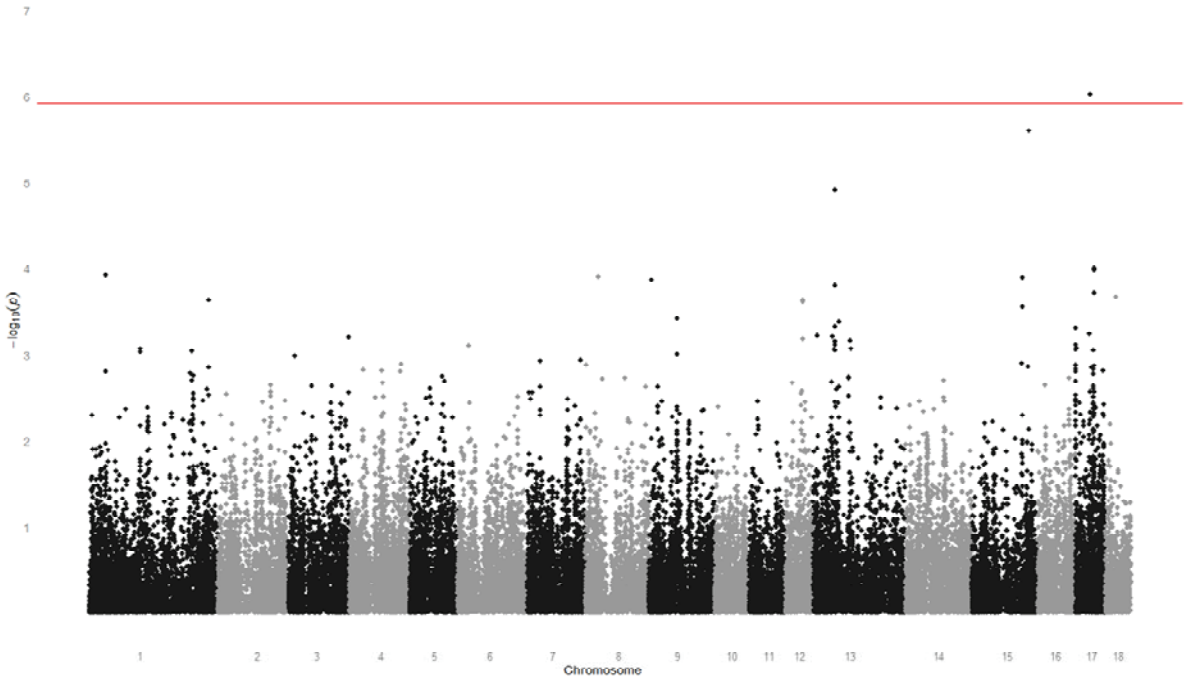

Figure 1: Manhattan-plot of the GWA analysis. Porcine chromosomes are located on x-axis, each dot represents a single SNP. Its respective p-value is found on the y-axis. The horizontal line depicts the strict Bonferroni-corrected genome-wide significance threshold.

Genome-wide significant SNPs were identified via GWA analysis on porcine chromosome (SSC) 15 and SSC17 (Figure 1).

In the associated region on SSC17, several Quantitative Trait Loci (QTL) are located with the following as most interesting: Haptoglobin concentration QTL, Lymphocyte number QTL, and Interferon-gamma to Interleukin-10 ratio QTL. Positional candidate genes in this region are amongst others the oxytocin gene (OXT, ENSSSCG00000007164), and the gene for gonadotropin-releasing hormone 2 (GNRH2, ENSSSCG00000007159).

Several moderate significant variants on SSC1, SSC4, SSC9, and SSC13 were detected for susceptibility to PDS. On SSC13, QTL for nonfunctional nipples, haematocrit and average daily gain (birth-30 kg) as well as age at puberty are described for the moderately associated region. The chromosome-wide significant SNP on SSC13 is located in the possible candidate gene PRICKLE2 (ENSSSCG00000011495). Other neighbouring genes on SSC13 are MUC13, the gene for transmembrane Mucin, which is mentioned for specific resistance mechanisms against enteropathogenic *Escherichia coli* [8].

Up to now, no significant association was detected to potentially functional candidate genes as for example the Toll-like-receptor-gene (TLR4, SSC1), or the prolactin (PRL, SSC7) or the prolactin receptor gene (PRLR, SSC16).

DISCUSSION

Study design, quality control and used statistical analysis corrected for possible stratification and spurious association. However, the sample size allows only finding causative SNP variants with Odds Ratio of at least 1.5, and with a statistical power of 80%. Therefore, additional causative chromosomal regions can be assumed.

The so far analyzed strong associated regions on SSC13 and SSC17 emphasize the importance of genes involved in neuro-hormonal processes and networks. The moderately significant regions on SSC1, SSC4, SSC9 and SSC18 will be examined in further studies.

The Illumina BeadChip with 62,163 genome-wide distributed SNPs allows an optimal whole-genome scan, but there are still a few chromosomal regions with low density or imprecise location. In our study, the significant associated SNP on SSC15 has to be interpreted carefully due to the not assured location in the recently available pig genome sequence (Sscrofa10).

This study put the main emphasis on overall-breed-specific resistance mechanisms, but slightly different results might be observed in breed-specific analysis. Because of the restricted sample size in our study the power of this breed-specific analysis is limited. Due to our results, it can be assumed that there are multiple genes and/or possibly also different path mechanisms leading to various clinical signs subsumed under the term PDS.

CONCLUSIONS

High-throughput genotyping as a modern technique offers the opportunity both for detailed analyses of genetic variation and for subsequent biological analyses of possible disease mechanisms. In this way, this technique provides a useful tool for modern preventive veterinary medicine with a holistic approach, especially for complex diseases such as PDS. In a future perspective, even there is still a long way to go in veterinary as well as in human medicine, a combined interpretation of genomics as wells as proteomics in connection with functional physiology and experimental studies will help to understand complex biological networks leading to disease susceptibility to multifactorial diseases.

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ANTIMICROBIAL RESISTANCE IN SALMONELLA FROM CHICKEN AND BROILER MEAT: 10 YEARS OF SURVEILLANCE

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SUMMARY

This paper analyses antimicrobial resistance of Salmonella isolates from chickens and broiler meat submitted to the National Reference Laboratory for Salmonella between 2000 and 2009. The serovar distribution and the resistance patterns were similar between isolates from animals and food. However, isolates from food tended to be less resistant to antimicrobials than those from animals. While *S.* Enteritidis was mostly susceptible to all antimicrobials investigated, *S.* Typhimurium, the other serovar with a great importance to human medicine, was frequently resistant to several antimicrobials. *S.* Paratyphi B dT+ was the serovar with the highest resistance rates both in animals and in food.

INTRODUCTION

Poultry products have been identified as a major source of salmonellosis in humans. Consequently EU has launched programs to control Salmonella in primary poultry production. In severe cases of human salmonellosis antimicrobial treatment can be essential to avoid casualties. Therefore resistance of Salmonella to antimicrobials used to treat such infections is a major threat to public health. This mainly concerns fluoroquinolones for treatment in adults and 3rd and 4th generation cephalosporins for children. This paper reviews resistance to these substances in Salmonella isolated from chicken and broiler meat between 2000 and 2009.

MATERIAL AND METHODS

A total of 3242 isolates from chicken and 2086 isolates from broiler meat submitted to the National reference laboratory for Salmonella were serotyped and tested for their resistance to antimicrobials using broth microdilution. Resulting MIC were evaluated using epidemiological cut offs as provided by EUCAST. Details have recently been published in a national report (Schroeter and Käsbohrer, 2010).

RESULTS

Most of the isolates from chickens were *S.* Enteritidis (26.7 %), followed by *S.* 4,12:d:- (14.8), *S.* Typhimurium (8.1 %) and *S.* Infantis and *S.* Paratyphi BdT+ (7.2 % each). In broiler meat the same serovars predominated. However, *S.* Paratyphi BdT+ was more frequent than *S.* Typhimurium (9.6 %), *S.* Infantis (7.0 %) and *S.* 4,12:d:- (3.2 %).

The proportions of the different serovars differed substantially between years. Yet there was no clear trend towards or against a specific serovar.

Overall, the proportion of resistant isolates decreased over the years, which could mainly be attributed to *S.* Enteritidis, while trends were less obvious for *S.* Typhimurium and the monophasic serovar *S.* 4,12:d:- and absent for *S.* Paratyphi B dT+. None of the *S.* Paratyphi B dT+ isolates was fully susceptible in any year.

Resistance rate to fluoroquinolones was 10 % in all Salmonella with considerable variability between 3.2 % (2000) and 24.6 % (2005). It was highest in *S.* Paratyphi BdT+ (59.2 %) and *S.* Infantis (30 %) but it was also prevalent in other Salmonella serovars such as *S.* Enteritidis and *S.* Typhimurium with 9.3 and 3 %.

Resistance to 3rd generation cephalosporins was rare in all Salmonella from chicken and broiler meat until 2007 but occurred in *S.* Paratyphi BdT+ from broiler meat in 2007 with a total 12.5 % of resistant isolates in meat and chicken in 2008 and 2009. In 2009, 2/20 isolates (10 %) of *S.* Infantis were also resistant to these cephalosporins. In contrast to *S.* Paratyphi BdT+ *S.* Infantis constantly is among the 10 most frequently recorded salmonellae accounting for 1 % of salmonellosis cases in humans.

DISCUSSION

Salmonella isolates from chicken have two different main sources, i.e. laying hens and broilers. Therefore comparisons between isolates from chickens and broiler meat have to be evaluated cautiously, because layers are less frequently exposed to antimicrobials than broilers and chicken meat is mostly derived from broilers. Information on the precise origin of the isolates from animals was in most cases not available. Despite this cave at, it is obvious

that the serovar pattern and the resistance pattern of the serovars are very similar for isolates from chickens and from broiler meat indicating vertical transmission along the food chain.

Based on Regulation (EC) No. 2160/2003 programs to control Salmonella in flocks of *Gallus gallus* and turkeys have been implemented in recent years. In Germany, these programs were associated with a substantial decrease in the rate of *S.* Enteritidis contamination of broiler meat and in the rate of human salmonellosis cases due to *S.* Entertidis (Tenhagen et al. 2011). However, this reduction so far was not observed for serovars not targeted in the control programs. In terms of antimicrobial resistance this may lead to a relative increase in resistance, as *S.* Enteritidis is among the serovars with the lowest resistance rates.

Resistance to fluoroquinolones was regularly observed and is more frequent than in isolates from pigs or cattle (Schroeter and Käsbohrer, 2010). However, the resistance rate did not show a clear trend indicating the selection pressure is not rapidly evolving.

In contrast, resistance to 3rd and 4th generation cephalosporins is increasing although there is no licensed use of these drugs in poultry. The reasons for this recent development need to be elucidated.

CONCLUSIONS

Results show that resistance to fluoroquinolones is prevalent among isolates from chickens and broiler meat, but the prevalence is more or less stable. In contrast, resistance to 3rd generation cephalosporins is less frequent, but seems to be increasing and therefore warrants further investigations with respect to potential causes.

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BIOFILM BUILDING CAPACITY OF SALMONELLA ENTERICA STRAINS FROM THE POULTRY FARM ENVIRONMENT

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SUMMARY

We have investigated the Biofilm (BF) building capacity of different serotypes of *Salmonella enterica* derived from the poultry farm environment. Starting point for our investigation was the question if farm-isolated Salmonella serotypes with high importance for food safety are capable of forming a BF. Several isolates from different stages of the production cycle were chosen and compared to laboratory grown strains of the same serotype. BF building capacity was analyzed in a 96 well format during a time period of 2 days. Epidemiological methods, such as PFGE and Lysotyping were used to establish a relationship between different isolates. Results indicated that certain farm isolates were capable of forming BF under laboratory conditions. However laboratory grown strains were hardly able to form BF at all. Our investigation showed that the BF building capacity of a monospecies *Salmonella* assay is strongly dependent on the temperature used for incubation and also that considerable differences between different isolates exist. We conclude that the BF building capacity of an isolate might be a function of adaptation to its host environment. Thus the control of BF as a reservoir for Salmonella in the farm environment is of crucial importance for the overall improvement of food safety.

INTRODUCTION

In many natural habitats microbes persist attached to some surface and not as pure cultures of planktonic growth (Costerton et al., 1995; Davey and O´Toole, 2000). A biofilm (BF) can be defined as an assembly of microbes being attached to a surface and furthermore being enclosed in a matrix of extracellular polymeric substances (Donlan, 2002). BFs can be a source of continuous contamination by *Salmonellae* as well as *Campylobacter* jejuni and are often reported to enhance the resistance and virulence of *Salmonellae* (Sheffield et al., 2009).

Starting point for our investigation was the question if farm-isolated *Salmonella* serotypes with high importance for food safety are capable of forming a BF by themselves. We have selected a representative number of strains from several serovars of *Salmonella enterica* of major importance to the poultry industry in Germany and Hungary and examined those for their BF building capacity at RT and at 37°C in a monospecies laboratory assay. Results were linked with epidemiological data.

MATERIAL AND METHODS

59 different isolates of 8 different serotypes from different stages of the poultry production chain were chosen and compared to 8 laboratory adapted strains of a corresponding serotype. 12 selected strains of 2 different serovars were passaged further 10 times in our lab after recovery from animal or environmental sources. 6 live vaccine strains and vaccine precursor strains were also included in the analysis.

For the measurement of BF building capacity, strains were recovered on blood agar at 37+/- 1°C overnight. On the second day they were transferred to reagent tubes containing 5 ml LB Broth and incubated under identical conditions. On the third day 30 µl overnight suspension were transferred to each well in a 96 well polystyrene microtiterplate (Nunc Nunclon; Rosklide, Denmark) containing 100 µl LB broth without NaCl (bacto tryptone 10 g/l, yeast extract 5 g/l) and incubated statically for two days at 20 +/- 1°C or 37 +/- 1°C respectively. All strains were tested in triplicate. Median of triplicate was calculated in order to eliminate potential outliers. OD595 was measured before the plates were gently washed once with water. Then 150 µl 1% crystal violet were added and the mixture was incubated at RT for 30 minutes before the plates were emptied and washed 4 times with 200 µl water to remove all excess dye. Then 150 µl ethanol:acetone (70:30) were added to each well to dissolve any bound dye. OD595 was measured, an OD of 0.5 at 595 nm was defined as the cut-off value for BF production.

Macrorestriction analysis and PFGE was done according to the the PulseNet standardized protocol (www.pulsenet-europe.org). Briefly, agarose gel plugs of isolates were prepared and macrorestriction was carried out using endonucleases XbaI (New England Biolabs, Frankfurt am Main, Germany). The fragments obtained were separated

by pulsed-field gel electrophoresis (PFGE) using the CHEF-DRIII system (Bio-Rad, Munich, Germany).

The band patterns were analyzed using the computer software BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium). Cluster analysis was performed with the Dice product moment correlation method using the unweighted pair group method with arithmetic averages (UPGMA). Position tolerance was set at 1.00 for PFGE. The minimum profiling for each band was set at 5.00%.

RESULTS

Our investigation showed that the BF building capacity of monospecies BF under defined laboratory conditions is strongly dependent on the temperature used for incubation. While different *S*. Saintpaul farm isolates incubated at 37 °C showed a mean BF building capacity as expressed via measured OD levels of 0.0645 the same isolates incubated at RT showed a significantly higher and pronounced BF building capacity resulting in an OD of 0.750. The same observation could be made for isolates of *S*. Paratyphi B, d-tartrate positive and isolates of *S*. Entertidis. Isolates of *S*. Infantis were poor BF producers in general, independent of the incubation temperature. By comparing the overall BF building capacity of different field isolates it became clear that it is strongly dependent on the serovar as well. In our investigation the strongest producers of BF clearly was the serovar *S*. Enteritidis, followed by the serovars *S*. Livingstone, *S*. Paratyphi B, d-tartrate positive, *S*. Virchow, *S*. Saintpaul and *S*. Infantis. Furthermore results indicated that certain farm isolates were capable of forming BF under laboratory conditions, whereas the majority of laboratory adapted strains were hardly able to form BF at all. As an example of this a reference strain of *S*. Paratyphi B, d-tartrate positive obtained from the RKI in Germany had an OD reading of 0.0005 whereas a field isolate of the same serovar displayed an OD reading of 0.982. Those two strains clustered within 94 % similarity in PFGE analyses. *S*. Enteritidis field strains as represented by three isolates obtained from an eggshell sampling showed an OD reading of 1.659 whereas laboratory adapted, vaccine precursor and live-vaccine strains showed a significantly lower BF building capacity at 0.441 OD and 20°C.

DISCUSSION

Naturally occurring BFs are usually multispecies BFs. Starting point for our investigation however was the question if farm-isolated *Salmonella* serotypes with high importance for food safety are capable of forming a BF by themselves. Can any differences be observed between or within different *Salmonella* serovars or within different epidemiological backgrounds in any given serovar using an easy reproducible standard laboratory method?

Few investigations on monospecies BFs exist that describe the capacity of different poultry field isolates across different serovars at different temperatures to build BF. We have found a clear indication that the incubation temperature has a very strong influence on the BF building capacity. The fact that BF is more amply produced at 20 RT than at 37°C might be a reflection of less favorable environmental conditions for *Salmonella* at RT.

Solano *et al.* (2002) found that in an assay that describes pellicle formation at the liquid-air interface 71% of the investigated *S.* Enteritidis isolates were able to build BF at 20°C and 37°C in rich and reduced media. They did not describe the testing of any laboratory-adapted *Salmonella* isolates or any vaccine precursor and live-vaccine strain isolates but finally concluded that BF composition and regulation depended strongly on environmental conditions. Using the above described method we could verify and importantly extend these results to laboratory adapted and live vaccine strains. For *S*. Enteritidis we saw clearly that strains recovered from eggshell surface had a significantly higher BF forming capacity as opposed to strains that had been adapted to the laboratory environment. A similar relation could be observed for *S*. Paratyphi B d-tartrate positive isolates.

Furthermore we obtained two isolates of *S*. Paratyphi B d-tartrate positive over a time frame of 18 months from one clonal origin, as proven by 98% similarity in PFGE. Those isolates were able to produce ample BF on both isolation dates (data not shown). As recently described (Nesse *et al.* 2003; Vestby *et al.* 2009; Habimana *et al.* 2010) for the fish and feed mill environment this has major implications with respect to persistence over several production cycles in selected parts of the poultry production chain.

Importantly live vaccine strains of different suppliers as well as the respective precursor strain of one supplier where also not able to produce any BF. This strongly suggests that BF forming capacity is not only related to the serovar but also to the particular isolate in the genus *Salmonella enterica.*

The fact that *S*. Infantis is overall a rather poor BF producer is interesting as it is the single most dominant serovar in Hungary. However the entire 26 strains obtained from Hungarian broiler farms showed BF below the cut-off value of OD 0.5, whereas 3 isolates from one German farm that cluster together in PFGE analysis (98%) all produce BF well above the set cut-off. It can be hypothesized that their persistence on broiler farms is not due to a BF forming capacity of the tested *S*. Infantis present in Hungary. One could speculate that the high *S*. Infantis presence in Hungary hence could be attributed to its widespread occurrence in the Hungarian broiler production rather than to the persistence of a few extremely hardwearing clones. To highlight this assumption the rather colorful epidemiological picture of *S*. Infantis in Hungary can be used. The 26 Hungarian strains cluster in two large PFGE clusters (74%) and represent 5 different Lysotypes (LT), LT 19 although being

clearly the most predominant that can be found in all integrations tested.

As stated by Solano *et al*, (2002) any single BF formed under specific conditions will be unique to these conditions. As a consequence one has to test the relevance of a monospecies laboratory assay for field conditions. To this end Lohmann Animal Health has designed a testing coupon that will allow direct sampling of BF under practical conditions from the drinker line. This testing device will make field studies on substances or management protocols to prevent or inhibit BF feasible and cost-effective. Patenting is under way.

CONCLUSION

We conclude that the BF building capacity is strongly dependent on incubation temperature. Furthermore BF building capacity might be a function of adaptation to the host environment. Thus the control of BF as a reservoir for *Salmonella* in the farm environment is of crucial importance for the overall improvement of food safety.

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INCIDENCE OF ANTIBIOTIC RESISTANT SALMONELLA SPECIES IN FOOD PRODUCING ANIMALS AND HUMAN CONTACTS

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SUMMARY

The emergence of multiple-antibiotic-resistant salmonellae has become a major concern in recent years. Three hundreds and ten animal samples including 50 fecal (droppings of broiler chickens) and 260 intestinal contents (105 broiler chicken, 50 cattle, 55 buffalo and 50 sheep) were collected from El-Monieb and El-warak abattoirs and different animal and poultry farms at Giza Governorate (Egypt). Also, 48 human fecal samples were collected from the investigated food animal contacts. Bacteriological examination showed that 12.25, 10, and 10.9% of poultry, cattle, and buffalo samples, respectively were positive for *Salmonella* isolation. Serological identification revealed that 40 and 60% of the animal and poultry *Salmonella* isolates were *S. kentucky* and *S. enteritidis*, respectively. Twelve out of the 48 human fecal samples (25%) gave culture for salmonellae and their serological identification revealed that 8 (66.7%) and 4 (33.3%) strains were ***S. enteritidis*** and *S. kentucky*, respectively. The resistance pattern of the bovine (cattle & buffalo) *Salmonella* strains was 60 & 100, 80 & 66.7, 60 & 66.7 and 60 & 50 to nalidixic acid, cephalothin, ampicillin and trimethoprime\sulphamethoxazole, respectively. While, the poultry *Salmonella* strains showed resistance of 84.2% to gentamicin, 79% to cephalothin, 73.7% to tetracycline and nalidixic acid and 68.4% to ofloxacin, cefoxitin and trimethoprime\sulphamethoxazole. Regarding human *Salmonella* strains, they showed resistance of 91.7% to cephalothin and 75% to trimethoprime\sulphamethoxazole and ampicillin. Our results showed high resistance of the isolated animal and poultry *Salmonella* strains to the tested antibiotics which raises the concern that there may be a link among antibiotic use in feeds and the development and presence of antibiotic resistance among bacteria in food producing animals which are consequently transferred to humans through food.

INTRODUCTION

Salmonellosis is a worldwide health problem; *Salmonella* infections are the second leading cause of bacterial foodborne illness in the United States. Approximately 95% of cases of human salmonellosis are associated with the consumption of contaminated products such as meat, poultry, eggs, milk, seafood and fresh produce. *Salmonella* can cause a number of different disease syndromes including gastroenteritis, bacteremia and typhoid fever, with the most common being gastroenteritis, which is often characterized by abdominal pain, nausea, vomiting, diarrhea and headache. Typically the disease is self-limiting; however, with more severe manifestations such as bacteremia, antimicrobial therapy is often administered to treat the infection (Foley and Lynne, 2008).

Animals are the hosts and the principle vectors of zoonotic salmonellosis. Serotypes that are signicantly associated with animal and human disease include Typhimurium, Enteritidis, Newport (Dunkley et al., 2009) and Kentucky (Majtán et al., 2006). Drug resistance in *Salmonella* increases the frequency and severity of infection with this pathogen, limits treatment options, and raise health care costs. These effects may be related to enhance shedding and augmented virulence of resistant strains, increased rates of transmission of this strain and the ineffectiveness of initial regimens of antimicrobial therapy against such strains (Gorbach, 2001).The aim of the present work was to determine the incidence of *Salmonella* species and the distribution of antimicrobial resistant salmonellae in food producing animals and human contacts in Egypt.

MATERIAL AND METHODS

Three hundreds and ten animal samples including 50 fecal (droppings of broiler chickens) and 260 intestinal contents (105 broiler chicken, 50 cattle, 55 buffalo and 50 sheep) were collected from El-Monieb and El-Warak abattoirs and different animal and poultry farms at Giza Governorate during January 2007- March 2008. Also, 48 stool samples were collected from persons in contact with the investigated animals.

Bacteriological analysis of the collected samples: The collected samples were bacteriologically examined and the suspected colonies were identified according to Holt et al. (1994).

Serotyping of Salmonella isolates: Serotyping of the isolated Salmonella strains was carried out according to Popoff and Minor (1997)

Antibiotic sensitivity testing of the isolated Salmonella strains: The agar disk diffusion technique was adopted according to NCCLS (2002).

RESULTS

Results showed that 12.25, 10, and 10.9% of poultry, cattle, and buffalo samples, respectively were positive for *Salmonella* isolation. Serological identification revealed that 40 and 60% of the animal and poultry *Salmonella* isolates were *S. kentucky* and *S. enteritidis*, respectively. 12 out of the 48 human fecal samples (25%) gave culture for salmonellae and their serological identification revealed that 8 (66.7%) and 4 (33.3%) strains were *S. enteritidis* and *S. kentucky*, respectively. The resistance pattern of human, poultry and bovine (cattle & buffalo) *Salmonella* strains is shown in Table 1.

Table 1. Percentages of antibiotic resistant *Salmonella* strains isolated from human, poultry and livestock

	C 30	AK 30	NA 30	KF 30	TE 30	OFX 5	Cip 5	AMC 20/10	AM 10	FOX 30	CN 10	SXT 25
Human Samples (12)	33.3	0	50	91.7	25	8.3	25	33.3	75	50	33.3	75
Poultry Samples (19)	26.3	15.8	73.7	79	73.7	68.4	52.6	42.1	57.9	68.4	84.2	68.4
Cattle Samples (5)	0	20	60	80	40	20	20	0	60	40	40	60
Buffalo Samples (6)	33.3	16.7	100	66.7	16.7	0	0	16.7	66.7	50	50	50

C30= chloramphenicol 30 µg, AK 30= amikacin 30 µg, NA30= nalidixic acid 30 µg, KF 30= cephalothin 30 µg, TE30= tetracycline 30 µg, OFX 5=ofloxacin 5 µg, Cip 5= ciprofloxacin 5 µg, AMC 20/10 = amoxicillin/glavulanic 20/10 µg, AM10=ampicillin 10 µg, FOX30=cefoxitin 30 µg, CN10= gentamicin 10 µg, SXT 25= trimethoprim/sulfamethoxazole 25 µg

DISCUSSION

In the present study, the incidence of *Salmonella* in broiler chickens from intestinal content samples was 11.4%. Higher incidence (17.8%) was recorded by Mohammad et al. (2010). Cattle and buffalo intestinal content samples showed isolation rate of *Salmonella* 10 and 10.9%, respectively. Abouzeed et al. (2000) reported that the prevalence of *Salmonella* in beef cattle intestinal content samples is 4.6%. Molla et al. (2003) recorded incidence of *Salmonella* of 4.2 % in slaughtered cattle. Also, our results showed that 25% of the human fecal samples were positive for *Salmonella*. Molla et al. (2003) recorded incidence of *Salmonella* of 6.0% in slaughterhouse personnel.

In the present work, all the 42 animal (30) and human (12) *Salmonella* isolates were serotyped as 26 *Enteritidis* (61.9%) and 16 *Kentucky* (38.1%). Abouzeed et al. (2000) recorded that the most prevalent serotypes of animal and human salmonellae are serovars *Typhimurium* (44% of all *Salmonella* isolates). While, Molla et al. (2003) stated that *Salmonella typhimurium, S. anatum* and *S. dublin* are isolated in man as well as in food animals and meat product.

Our broiler´ *Salmonella* strains showed high resistance (73.7%) to tetracycline. Lower result was obtained by Bouchrif et al. (2009) (21%). Regarding human, *Salmonella* stool isolates showed resistance of 25% to tetracycline. Lower (13%) and higher (27.3%) results were recorded by Seyfarth et al. (1997) and Soler et al. (2006), respectively.

The resistance rate to ampicillin was 75% among *Salmonella* strains isolated from human stools which is higher than results obtained by Soler et al. (2006) (28.4%). While, the broiler´ *Salmonella* strains showed resistance of 57.9% to ampicillin, lower result (22%) was

recorded by Bouchrif et al. (2009). The highest resistance pattern to nalidixic acid was observed by buffalo *Salmonella* isolates which was 100%, followed by poultry strains 73.7%. While Bouchrif et al. (2009) recorded lower rate (3.8%).

In the current study, 75% of *Salmonella* strains isolated from human stools showed resistance to trimethoprime\ sulfamethoxazole, lower percentage (22.3%) was obtained by Soler et al. (2006). Poultry *Salmonella* isolates exhibited resistance of 68.4% to trimethoprime\ sulfamethoxazole. Higher rate (86.25%) and lower rate (16.5%) were recorded by Cardoso et al. (2006) and Van et al. (2007), respectively.

CONCLUSIONS

In our study, almost all of the human and animal *Salmonella* isolates showed resistance to more than four antibiotics, suggesting that antimicrobial resistance is widespread in both human and livestock. So, surveillance of *Salmonella* strains for resistance to antimicrobial agents at the animal and human levels should be carried on and improved.

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EFFECT OF MOIST FOOD FERMENTED WITH LACTOBACILLUS PLANTARUM ON SALMONELLA TYPHIMURIUM INFECTION IN CHICKENS

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SUMMARY

This paper showed the effect of moist food fermented with Lactobacillus plantarum NCIMB 41607 on Salmonella typhimurium nalrSal 1344 in the digestive tract of broiler chickens. Rifampicin was used as a marker for L.plantarum. In five of the six chicks fed fermented moist food no salmonella were detected in the gut on post mortem. Whereas in all chicks fed the control diet log10 4.05 and 7.34 CFU/ml salmonella were isolated in the ileum and caecum respectively.

INTRODUCTION

Salmonellosis is a zoonotic disease and poultry and poultry products are the main source of the disease in humans [2]. Every year many outbreaks occur around the world. The incidence of salmonella infections in humans may to some extent be controlled by preventing colonization of salmonella in the chicken gut [2]. Probiotics may offer a means of reducing salmonella infections in chickens. The use of probiotics and novel applications such as moist feed fermented with a lactic acid producing probiotic bacteria may provide a way of reducing salmonella in chickens [5]. Moist feed (food1:1.2 water) containing at least 150mmol/l lactic acid with a pH<4.5 and with at least 109 CFU/ml of lactic acid bacteria (LAB) has been shown to reduce contamination of feed by Salmonella [3, 5, 6]. It is consider a biosafe method by which gut and host health can be improved as well as imparting resistance to enteropathogens contamination in feed prior to feeding[3]. FMF can act as a carrier for a high amount of lactic acid (>150mmol/L) and high number of lactic acid bacteria 109 CFU/ml can be delivered to the bird [1].

MATERIAL AND METHODS

A naturally rifampicin resistant strain of Lactobacillus plantarum NCIMB 41607 was isolated using a gradient plate of 0-1 mg/ml rifampicin in MRS agar. Rifampicin resistant colonies were maintained on MRS agar containing 15 microgram/ml rifampicin. This organism was used to produce fermented moist feed for this trial. Twelve chicks were randomly allocated to two dietary treatments of six birds per treatment. Control (CON) birds were fed a commercial chick crumb (BOCM Pauls Ltd, Wherstead, England). Fermented moist food (FMF) birds were fed the same chick crumb fermented at 30oC for 24 h with Lb. plantarum NCIMB 41607. The FMF contained 109 cfu/ml of Lb. plantarum, 175 mmol/l lactic acid and was pH 4.4. Birds were fed the dietary treatments from day of hatch. On day 1, cloacal swabs were taken from all birds and plated onto XLD and Brilliant green agars (BGA) for the presence of Salmonella. On day 3 all birds were given a dose of 105 cfu/ml Salm. typhimurium nalr NCIMB 41607 by oral gavage. Further cloacal swabs were taken on day 5 and day 8 of the trial. On day 14 all the chicks were killed by cervical dislocation. Post mortem digesta from the ileum and caecum of each chick was collected aseptically and examined for the presence of LAB and salmonella. Each intestinal sample was weighed and mixed with phosphate buffered saline to give a dilution of 1:10, further serial dilutions were conducted and 20µl of appropriate dilutions were plated on to XLD agar and BGA for Salmonella and MRS agar for Lactic acid bacteria according to the method of Miles & Misra [4]. All samples incubated at 37ºC for 24 hrs. MRS agar paltes were replicate plated onto Rifampicin-MRS for the detection of rifampicin resistant Lb. plantarum NCIMB 41607

RESULTS

All cloacal swabs taken on day one prior to infection with Salmonella typhimurium nalr were negative for salmonella. All swabs taken on days 5 and 8 in both CON and FMF groups were positive for Salm. typhimurium nalr . One chick died in the control group on day 6 of the trial. All six surviving CON chicks were positive for salmonella with a mean of log10 4.05 CFU/ml ±1.04 (SD) and log10 7.34 CFU/ml ± 0.87(SD) Salm. typhimurium nalr in the ileum and caecum respectively. Only one FMF chick was positive for Salm. typhimurium nalr with log10 4.70 CFU/ml in the caecum. The mean number of LAB in ileum and caecum of control and FMF groups are shown in Figure 1. Numbers of LAB were significantly higher in the caecum compared with the ileum of both groups. The chicks fed FMF had significantly higher numbers of LAB in both the ileum and the caecum compared with CON chicks and the vast majority of these were rifampicin resistant. No rifampicin resistant LAB were detected in the caecum or ileum of CON chicks.

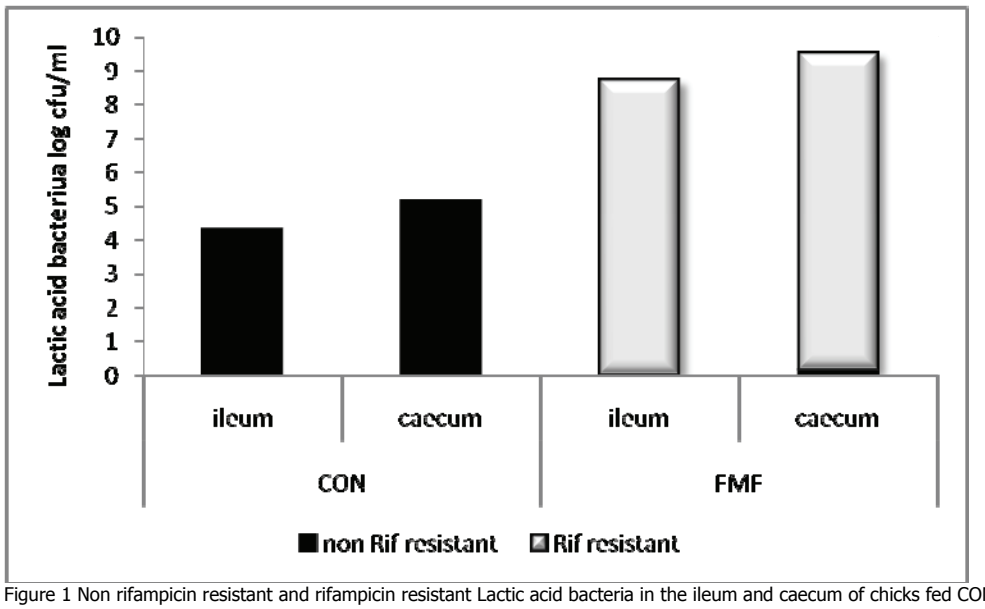

Figure 1 Non rifampicin resistant and rifampicin resistant Lactic acid bacteria in the ileum and caecum of chicks fed CON and FMF diets

DISCUSSION

The results showed that the numbers of Salm. typhimurium nalr in the ileum were significantly less than in the caecum in control group. No Salm. typhimurium nalr were detected in the ileum of chicks fed FMF. While in caecum there were no salmonella detectable in 5 chickens fed FMF and only 1 was positive for Salm. typhimurium nalr. However, all of the FMF chicks had salmonella positive cloacal swabs on the two sampling days after infection with Salm. typhimurium nalr. This suggests that feeding FMF did not prevent infection with salmonella but did aid the chicks in clearing the infection. It has been suggested that probiotics enhance immune function in poultry [5] and this could be the reason for the effects seen in this study.

Rifampicin resistant Lb. plantarum was isolated from the ileum and caecum of birds fed FMF with approximately 80 – 90% of total LAB being rifampicin resistant in this group.. Although it must be acknowledged that naturally occurring rifampicin strains may be already present in the GI tract, the very high numbers occurring in the FMF treated chickens give a good indication that these are likely to be Lb plantarum NCIMB 41607. Therefore, the results of this study indicate that Lactobacillus plantarum NCIMB 41607 survived in the gastro-intestinal tract of chicks fed FMF. This is a very important criterion for any micro-organism to be selected as a probiotic. Using a naturally occurring strain resistant to rifampicin is an inexpensive and convenient way of demonstrating survival of the administered organism in the digestive tract.

CONCLUSIONS

The results of this study indicate that feeding moist feed fermented with Lb. plantarum NCIMB 41607 may be an effective way of reducing salmonella infections in chickens.

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INCIDENCE AND ANTIBIOTIC RESISTANCE OF *SALMONELLA* SPP. ON RAW CHICKEN CARCASSES* (Abstract)

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INTRODUCTION

The current study was carried out to detect *Salmonella* spp. contamination on chicken carcasses and to determine the antibiotic susceptibility profiles and serotype distribution of the isolates.

MATERIALS AND METHODS

A total of 200 packaged fresh raw chicken samples sold at retail in different markets in central Anatolia were analyzed between April 2005 and March 2006. *Salmonella* spp. contamination were determined by conventional culture method and verified by PCR using primers based on ST11 and ST15 gene sequence. The antimicrobial resistance profile and serotype distribution of the isolates were also assessed.

RESULTS

Salmonella spp. was detected in 34% (68/200) of samples using cultural technique and were confirmed by PCR. Ten *Salmonella* serovars were identified; predominant ones included Typhimurium, Infantis and Heidelberg. All of the *Salmonella* spp. isolates tested exhibited resistance to one or more antimicrobial agents used. Resistance to penicillin, oxacillin, clindamycin, vancomycin, erythromycin and ampicillin were evident 100%, 97%, 97%, 92.6%, 89.7% and 85.2%, respectively. Also resistance to tetracycline (67.6%), streptomycin (61.7%), neomycin (55.8%) and cephalothin (52.9%) was observed but a small percentage of the isolates demonstrated resistance to gentamicin (14.7%), chloramphenicol (10.2%), cefotaxime (2.9%) and amikacin (2.9%).

CONCLUSIONS

As a result, high prevalence of *Salmonella* spp. and the relatively high resistance among the bacteria tested could pose public health and therapeutic problems in consumers as potential vehicle of resistant *Salmonella* foodborne infections. To avoid *Salmonella* contamination, hygienic rules of slaughter and poultry meat processing must be rigorously observed and antibiotic use must be controlled by governmental agencies to prevent increased resistance of antibiotics.

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DYNAMICS OF MICROBIAL BIOFILMS ON DIFFERENT MATERIALS IN DRINKING WATER SYSTEMS

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SUMMARY

Biofilms are active communities of microbes attached to surfaces and surrounded by a self-produced matrix of extracellular polymeric substances. The occurrence of biofilms can cause hygiene problems in water distribution systems. A major problem arises when pathogenic or opportunistic pathogenic microbes inhabit these biofilms. The aim of this study was to investigate the attachment of bacteria and the dynamics of microbial biofilm formation on different materials which could be used for drinking water pipeline systems. The experiment was performed using a chlorinated drinking water system and three types of materials: copper, stainless steel and polyvinyl chloride; as test surfaces. Water samples were analyzed after 21 hours of stagnation and 3 hours of continuous flow, over a period of 30 days. Fluorescent microscopy was used for counting surface attached bacteria and confocal laser scanning microscopy was used to measure biofilm thickness. The total number of viable bacteria was higher in stagnant than in flow conditions. Bacterial counts reached $8.76x10^4$ CFU/mL after 21 hours of stagnation. Stainless steel had the highest number of attached bacteria, followed by PVC. The number of bacteria attached to the surfaces ranged between $3.2x10^6$-$1.2x10^7$ bacteria/cm^2 for stainless steel and $6.2x10^6$-$9.2x10^6$ bacteria/cm^2 for PVC. On the copper surface biofilm formation was very weak, consisting of only individually attached bacteria. Microbial biofilms can form in chlorinated drinking water where nutrient levels are low. The biofilms formed faster on stainless steel than on PVC or copper. Minimal biofilm was found to form on the copper surface during the first 30 days of exposure to chlorinated drinking water.

INTRODUCTION

Microbial biofilms are complex structures consisting of an accumulation of microorganisms contained within extracellular products, inorganic and organic debris attached to surface [3].

The development of a biofilm is believed to occur in a sequential process that includes transport of microorganisms to surfaces, initial microbial attachment, formation of microcolonies, production of extracellular polymeric substances (EPS) and biofilm maturation [9].

In drinking water distribution systems, the density of suspended bacteria increases between the treatment plant and the consumers tap as a result of the disinfectant decay, hydraulic residence time, substrate uptake and the presence of corrosion deposits, and it's estimated that 95% of the overall biomass is attached to pipe walls, while only 5% is in the water phase [2, 5]. Therefore, the development of biofilm depends on a variety of factors such as hydrodynamic patterns and surface materials which affect the microbial biofilm cell density through detachment phenomena [10].

Biofilm control strategies employed in drinking water distribution systems currently include the use of residual disinfectants, reduction of organic matter or inorganic electron donors, pipe materials and coatings that reduce biofilm accumulation, frequent flushing of pipelines, and the practice of corrosion control [1].

The influence of materials on biofilm development is still a disputed subject. The materials commonly used for pipeline systems are iron-based, but in recent times they were gradually replaced by copper, polyvinyl chloride (PVC) and polyethylene (PE) which are easier to assemble and handle.

The aim of this study was to investigate the attachment of bacteria and the dynamics of microbial biofilm formation on different materials which could be used for drinking water pipeline systems.

MATERIAL AND METHODS

The experiment was performed using a chlorinated drinking water system and three types of materials (copper, stainless steel and PVC) as test surfaces. The coupons made from stainless steel (W 1.4301) were flat and square (25 x 25 x 1 mm), copper coupons were flat and square (25 x 25 x 0.7 mm) and the PVC coupons were round with 25 mm in diameter and 2 mm thickness. Coupons where cleaned in nitric acid, washed with distillated water and then placed in a modified water filter device, steam sterilized at 121°C, 15 minutes and then connected to an indoor, chlorinated, drinking water pipeline.

Water samples were analyzed after 21 hours of stagnation and three hours of continuous flow (1 L/min) over a period of 30 days. The temperature, pH, chemical oxygen demand (COD), viable cell counts, residual chlorine and copper ions were recorded for each sample.

The formation of microbial biofilm was observed for 30 days by removing test coupons from the device. The coupons were stained with acridine orange (AO) in acetate

buffer solution for 2 minutes at room temperature. Fluorescent microscopy (Leica DM 2500) was used for counting surface attached bacteria and confocal laser scanning microscopy (CLSM) was used to measure biofilm thickness.

RESULTS

The water parameters were relatively constant throughout the experiment and the results are presented in table 1. Differences between stagnant and flow conditions were observed for COD, temperature, residual chlorine and total viable count.

Table 1. Water quality characteristics

Parameter	*Stagnant condition*	*Flow condition*
pH	7.23 (±0.03)	7.09 (±0.02)
Copper	62.31 µg/L	38.85 µg/L
COD	4.9 mg O_2/L	0.5 mg O_2/L
Residual chlorine	0.06 (±0.02) mg/L	0.19 (±0.08)
Temperature	23°C (±0.57)	10.62°C (±0.55)
Total viable count	8.76 ±0.98 x 10^4 CFU/mL	1.6±1.84 CFU/mL

The evolution of the average number of attached bacteria was different, depending on the tested material and experimental day (figure 1). The number of attached bacteria reached 10^6 units/cm^2 on all tested surfaces by the fourth experimental day. On day 16 and 20 the PVC surface had the highest value of attached bacteria ($p<0.05$). On day 24 the number of attached bacteria on PVC had the same value like on stainless steel surface ($p>0.05$) and after 28 and 30 days the highest numbers of attached bacteria were recorded on stainless steel, with more than 10^7 units/cm^2.

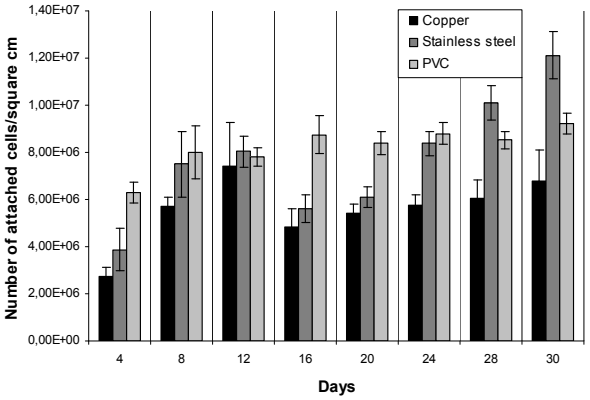

Figure 1. Number of attached cells/cm^2 on copper, stainless steel, PVC

On the 20th day, on the stainless steel coupons, regions with more than two layers of bacteria were identified. These regions were analyzed by CLSM (figure 2) and maximum thickness was determined for days 20, 24 and 30. The values of the maximum thickness on day 20, 24 and 30 were: 3.02, 5.09 and 7.20 µm respectively.

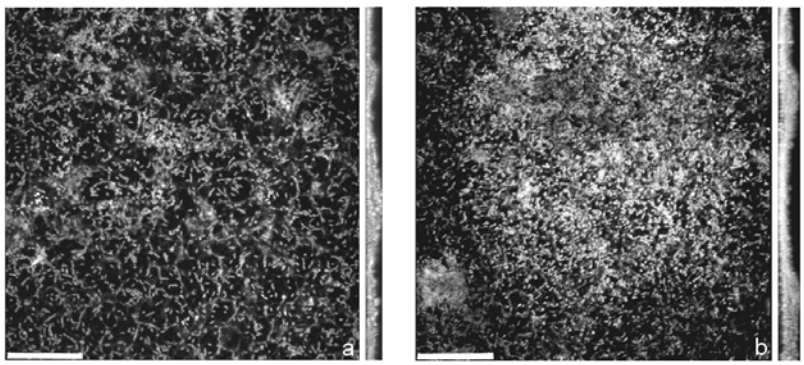

Figure 2. CLSM images representing the thickness of the biofilm on the stainless steel surface; (a) biofilm image after 24 days; (b) biofilm image after 30 days; (scale bar 25µm)

DISCUSSION

Temperature and pH are considered to be two of the most important factors in drinking water systems because it affects the electrostatic interactions between surfaces and microorganisms, enzymatic activity and many other properties. It has been reported that the occurrence of coliform bacteria is increasing when water temperature is higher than 15°C [6]. In this study the increase of bacteria is due to the increase of temperature and decrease of free chlorine concentration which in the stagnant condition was below 0.25 mg/L.

Previous research showed a similar degree of colonization ($4x10^6$-$3x10^7$ bacteria/cm^2) on stainless steel, copper, PVC and polyethylene coupons exposed to non-chlorinated drinking water [11]. Other studies, also demonstrated that the number of attached bacteria is higher on the surface of galvanized steel coupons than on the surface of copper coupons [8].

Overall, the number of attached cells had the lowest values throughout the experiment in the biofilm formed on copper, which was previously proved to have antimicrobial activity and to inhibit the production of extracellular polymeric substances [4, 8].

The data illustrates a marked increase of the biofilm maximum thickness in just six days from 3 to 7 μm, suggesting a thick biofilm which tends to occupy the entire surface of the support. The thickness of the biofilm is influenced by the hydrodynamic conditions. Thus, increased velocities cause greater flux of nutrients to the pipe surface, greater transport of disinfectants, and greater shearing of biofilms from the pipe surface [7].

CONCLUSIONS

Microbial biofilms can form in chlorinated drinking water where nutrient levels are low. Attachment of bacteria on all the surfaces tested in drinking water occured very fast achieving a level of 10^6 bacteria/cm^2 after 4 days.

The biofilms formed faster on stainless steel than on PVC or copper. Minimal biofilm was found to form on the copper surface during the first 30 days of exposure to chlorinated drinking water.

ACKNOWLEDGMENT

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ANNUAL MONITORING OF ENVIRONMENTAL AND HYGIENIC PARAMETERS IN AN INTENSIVE FATTENING RABBIT FARM

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SUMMARY

Environmental and hygienic parameters in intensive fattening rabbit farms have been poorly investigated so far. This paper describes the results arising from one-year monitoring activity carried out in an intensive fattening rabbit farm in Northern Italy. The measured parameters were temperature (T), relative humidity (RH), air speed, airflow, total dust, noxious gases (NH_3, CO_2, CH_4, H_2S), airborne bacteria and fungi. In relation to environmental parameters, T, RH and airflow were subjected to wide and rapid fluctuations during summer, autumn and spring. The highest levels of RH were measured under summer conditions, due to the evaporative cooling system activation. The highest NH_3 and CO_2 concentrations occurred in concurrence with minimum airflow rates (i.e. cold season, night-time, early hours). CH_4 concentrations were highest in summer, whereas H_2S concentrations showed some peaks when manure scrapers were in operation. Total dust levels were higher during autumn and winter, when minimum ventilation levels occurred. As regards hygienic parameters, *P. multocida* seemed influenced by seasonal conditions, probably due to wide and rapid fluctuations in T, RH and airflow as well as to high RH levels, showing an increasing trend from spring to autumn. The dermatophyte *M. canis* showed the highest concentrations in correspondence with minimum airflow rates (i.e. winter). The application of the monitoring protocol developed by the present study might lead to forecast the effects of some structural and management changes on the indoor environmental and hygienic conditions.

INTRODUCTION

The welfare of intensively reared animals depends also on environmental factors related to microclimate and indoor air quality [3, 5]. Moreover, the diseases commonly encountered in farm animals are mostly multi-factorial [7]. The effects of structural and management characteristics of rabbit farms on the environmental and hygienic parameters are still poorly investigated. Notwithstanding, the Council Directive 98/58/EC, which lays down the minimum standards for the protection of animals kept for farming purposes, takes into account aspects related to building, equipment and indoor air. The present study investigates environmental and hygienic parameters aiming to highlight critical situations associated with the season and/or the growing phase of fattening rabbits.

MATERIALS AND METHODS

The rabbit house was 57 m in length, 9 m in width and housed about 6,600 rabbits in dual-purpose-cages assembled in 3 rows of batteries. Air exchange took place by means of a forced ventilation system with longitudinal airflow, completely automated by an electronic unit connected to temperature sensors. Evaporative cooling panels placed on the windows were used under hot conditions. Manure removal was carried out daily by means of scrapers and manure was collected in external storage tanks.
The rabbit farm adopted "all-in all-out" cage management. Does were moved away from the cages when the weaning rabbits reached the age of 38-42 days; rabbits continued the fattening phase in the same cage where they were born until slaughter (age of 78-84 days).

The study period lasted a whole year, during which 4 fattening cycles followed each other, under different seasonal climates (Cycle 1 – Winter, Cycle 2 – Spring, Cycle 3 – Summer, Cycle 4 – Autumn). The survey activity focused on 2 monitoring phases for each cycle, occurring one week after weaning and two weeks before slaughter. During each phase a 7-day continuous monitoring was performed, measuring every 30 minutes T, RH, NH_3, CO_2 and CH_4 in a central position. Moreover, the same parameters, together with H_2S, air speed, airflow, total dust, airborne bacteria and fungi were manually measured during the first and the last day of each monitoring phase in different points of the rabbit house (Tab. 1).

Table 1. Instruments and methods used for environmental and hygienic parameters monitoring.

Parameter	Instrument	Method
Temperature (T)	Econorma mini data-logger FT102, LSI Babuc M	Resistive sensors
Relative Humidity (RH)		Capacitive sensors, wet bulb thermometer
Air speed	LSI Babuc M	Hot wire anemometer
NH_3, CO_2 and CH_4	Innova AirTech 1412	Photo-acoustic infrared field gas monitor
H_2S	KD Engineering Air Quality AirBoxx®	Electrochemical
Total dust	Zambelli ZB2, nitrate cellulose filters 0.8 □m	Gravimetric, after filter standard conditioning
Total Bacteria Count	PBI International SAS Super 100®	Plate Count Agar (PCA)
S. aureus, *P. multocida*		Baird Parker (BP), Sheep Blood Agar (SBA)
Fungi and dermatophyte spores		Sabouraud Chloramphenicol Agar (SCA) and Mycobiotic Agar (MA)

RESULTS

In winter, the weekly mean temperature was 12.8°C. The winter daily temperatures were constant, the maximum daily range being 2.5°C. In spring and autumn daily temperature range was up to 5.5°C. In summer the temperature (27.0°C on average) was very close to the outdoor temperature; daily fluctuations between 6 and 10°C were recorded (Fig. 1). The highest level of RH was recorded in summer (70,5%), whereas the minimum levels were recorded during spring (55.0%) and autumn (58.5%). Unfortunately, RH sensors failed the measurements during winter. The total airflow was approximately 2,760 m^3h^{-1} in winter and about 75,500 $m^3 h^{-1}$ in summer, the latter corresponding to 4.2 $m^3h^{-1}kg^{-1}$ live weight.

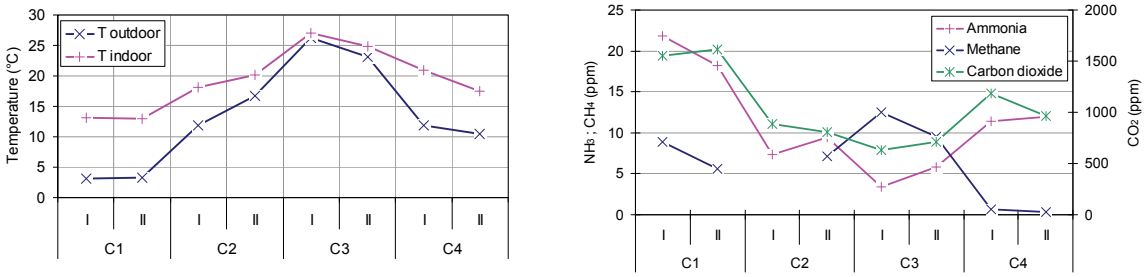


Figure 1 and 2. Mean indoor and outdoor temperatures and mean gas concentrations (C1 = Winter, C2 = Spring, C3 = Summer, C4 = Autumn; I = post-weaning, II = Pre-slaughtering).

The highest NH_3 weekly mean concentrations were recorded in winter, whereas the lowest were recorded in summer, having a weekly average of 21.8 and 3.4 ppm, respectively. During spring, NH_3 weekly mean concentrations were lower, whereas in autumn slightly higher values were recorded (9.4 and 12.0 ppm, respectively) (Fig. 2). The highest weekly mean concentration of CO_2 occurred in winter (1,610 ppm) and the lowest in summer (631 ppm). The highest weekly mean concentration of CH_4 was recorded in summer (12.5 ppm), whereas the lowest were recorded in autumn and spring (in particular during the post-weaning phase), when the concentrations were sometimes below the instrument detection threshold (Fig. 2). The H_2S concentrations rarely exceeded 0.2 ppm, showing maximum values of 5.8 ppm when manure scrapers were in operation. As regards the noxious gas trends during the monitoring periods, gas concentrations (in particular NH_3) had a daily trend in winter, since the values increased during the night-time and decreased during the daytime (Fig. 3). The indoor pattern of NH_3 concentrations showed a longitudinal gradient towards the air outlets (Fig. 4).

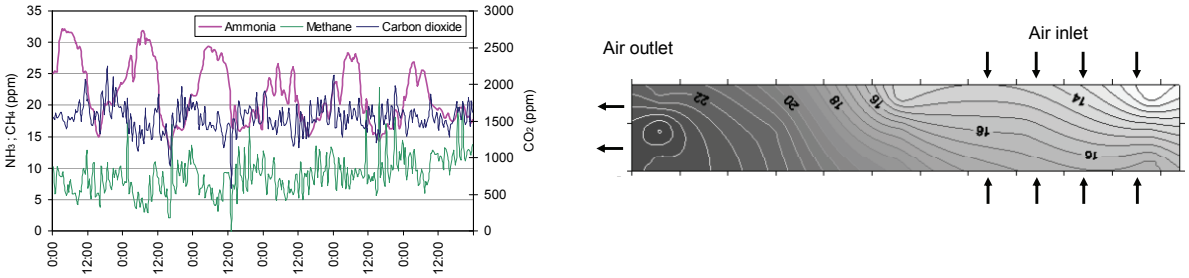
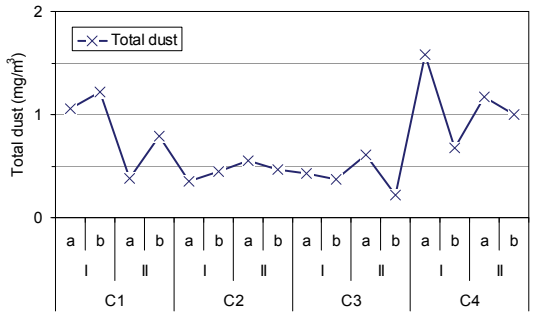


Figure 3 and 4. Hourly gas concentrations and NH_3 pattern during the pre-slaughter phase in winter.

Total dust concentrations were lower than 0.7 mg m^{-3} during spring and summer, whereas they were higher in winter and autumn, although without exceeding 1.6 mg m^{-3} (Fig. 5).
Total bacteria and total fungal spore never exceeded counts of 2,200 and 3,100 CFU m^{-3}, respectively. Among pathogenic bacteria, *P. multocida* showed an increasing trend from spring to autumn (mean 532 CFU m^{-3}), especially in the pre-slaughter phase. *S. aureus* was recovered at daily mean concentration below 80 CFU m^{-3}, except in one day during summer (180 CFU m^{-3}). During all the monitoring periods, the only dermatophyte species recovered was *Microsporum canis*, which reached the maximum concentrations during winter (Fig. 6).



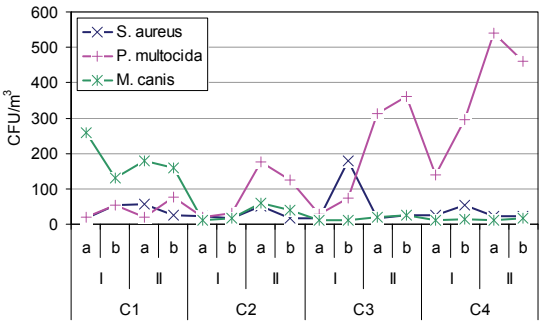


Figure 5 and 6. Total dust concentrations and pathogenic bacteria (*P. multocida* and *S. aureus*) and dermatophyte species (*M. canis*) (C1 = Winter, C2 = Spring, C3 = Summer, C4 = Autumn; I = post-weaning, II = Pre-slaughter; a = Beginning of the monitoring week, b = End of the monitoring week).

DISCUSSION

Under winter conditions, the most critical environmental parameters were the relatively high noxious gas concentrations, which were characterised by a spatial and temporal pattern (the latter being similar to the results reported in [4]), due to the low air flow rates selected on the basis of indoor temperatures. Under summer conditions, the environmental parameter whose critical effects on rabbits should be further investigated, was the high RH level, which was probably determined by the evaporative cooling. As a matter of fact, this climate control system allowed to maintain indoor temperatures similar to the outdoor ones, controlling the extra-heating of the rabbit house due to metabolic heat [7]. Nevertheless, evaporative cooling determined an increase in RH, which probably also contributed to lower the total dust concentrations [2, 6]. The H_2S peaks observed when manure scrapers were in operation have been substantially confirmed by other Authors [1]. The trend of CH_4 concentrations differed from those of NH_3 and CO_2, since CH_4 concentrations showed a peak under summer conditions, thus suggesting that the CH_4 emission rates were fairly high: this point needs to be further investigated. The hygienic parameters (total bacteria and fungi) seemed to be influenced by the prevalence of respiratory disease at farm level rather than by the climate control system, unlike gases and total dust.

CONCLUSIONS

The present study has developed and applied a monitoring protocol based both on continuous measurements and on multiple manual measurements of some environmental and hygienic parameters in the rabbit house. The validation of such a monitoring protocol might lead to forecast the effects of some structural and management changes on the indoor environmental and hygienic conditions, thus being a component of a possible Decision Support System for farmers and technicians dealing with the rabbit sector.

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REAL TIME MONITORING OF FINISHER PIG WATER CONSUMPTION: INVESTIGATION AT PEN LEVEL

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SUMMARY

The daily water consumption of pen groups of 10 pigs was monitored throughout the finisher phase alongside production and health parameters. Mean live weight, daily live weight gain, number of pigs per pen, environmental temperature and drinker type were significant factors affecting mean water consumption per pig per day (P <0.001). In addition, data from a subsample of pens demonstrated significant associations with Food Conversion Ratio (P <0.001). During the first four weeks in the building, the mean quantity of water consumed per pig per day within a given week, differed in relation to the severity of scour observed the following week (P <0.05). This illustrates the potential for automated recording of water consumption to be used as a tool for the early identification of disease.

INTRODUCTION

Automated monitoring systems can provide information on a number of aspects of the livestock environment and of the animals themselves, at relatively little cost and labour input. In pig production systems, recording of water consumption is considered to offer particular benefits for the monitoring and management of health status [3]. The drinking behaviour of pigs has been found to deviate from the consistent patterns seen in healthy pigs upon the arrival of disease, and such changes appear in the sub-clinical stage of infection, before disease symptoms become visually apparent [3]. However, a number of factors are known to affect water intake, and the uptake of systems generating automated alarms requires confidence in their reliability to deliver both sensitivity and specificity for health changes. Work is required to distinguish the changes that occur in healthy pigs (due to environmental variables), stress exposed and pathogen exposed pigs under different conditions of housing, nutrition and management. Whilst whole building or room consumption patterns may be sensitive indicators in all-in, all-out systems [1], the measurement of water consumption in continuous-flow housing systems may present more challenges for disease detection due to the variable age and health status of the pigs, and therefore the limitations of this technique need to be assessed. This study investigated whether monitoring the water consumption at a pen level, rather than for a whole building, shows any deviations in daily water intake patterns that could indicate changes in health and performance.

MATERIAL AND METHODS

Over two replicates, the daily water consumption of 24 pens of mixed gender finishing pigs was monitored. Pigs were selected upon entry to a fully-slatted, continuous-flow finisher building (at 52.6 ± 0.5 kg liveweight), penned in groups of ten (1.46 x 3.79m pens) and monitored until slaughter (85.3 ± 0.6 kg). Water was available *ad libitum* via two nipple drinkers per pen. A water meter was fitted to the water supply of each pen, which logged the flow rate of the water over 5 minute intervals. In addition, temperature sensors logged the internal and external temperatures of the building. The water and temperature meters were linked to a computer software modem (Barn Report, Farmex Ltd, UK), from which the data could be accessed via an internet connection. To determine whether drinker type had an effect on water consumption, two types of commercial drinker nipple were fitted equally across pens. For each pen, clinical disease symptoms and any veterinary treatments administered were recorded daily, cough, diarrhoea (Scour score (faeces): 1 – firm, 2 – spreads slightly, 3 – spreads readily, 4 – water like) and sneeze scores weekly, and pig weights every two weeks. For the second replicate only, pen feed intake was also recorded for each 2 week period between weighing pigs. A diary of staff activity within the finisher building was kept to record any disruption that may have affected the pigs.

Statistical analysis

Data for each pen (daily total water consumption and corresponding building and external temperatures) were downloaded from Barn Report. An estimated daily live weight of the pen was calculated using the start and end weights of each period and incrementing the weight daily by the average daily gain (ADG) over that period. Pen weight was adjusted appropriately if any pigs left the pen during that period. Each day of monitoring was coded with a yes/no score for whether human activity (moving pigs etc) may have disturbed the pigs. For the second

replicate, the average feed consumed per pig per day and feed conversion ratio (FCR) were also calculated for each pen. Prior to analysis, all data were checked for normality using the Anderson-Darling test and where possible transformed.

Using Minitab 15.0 statistical software, multiple regression analysis determined the relationship of various factors with the mean daily water consumption per pig. The initial model included: temperature, number of pigs in the pen, the drinker type, mean live weight and the ADG of the pigs. In this model, the occurrence of human disturbance within the building was also tested as a possible factor. For the subset of pens in the second replicate, feed intake and FCR of the pen were also tested in the model. To determine whether there was an effect of health on the water consumption, and whether the water consumption could be predictive of future health change, a GLM was used to compare the water usage per pig per day within given weeks, against the records for the severity of clinical disease for the corresponding week and for the following week. The average pig weight and environmental temperature were added to the model as covariates.

RESULTS

Pig health and productivity

All pigs except six completed the designated trial period to slaughter. Over the course of the trial, three pigs were removed due to rapid weight loss, one suffered a rectal prolapse, one became lame with an infected limb and one died of unknown causes. In addition, two pigs received veterinary treatment but remained on trial, one for body wounds and one for lameness. The ADG of all pigs was 700g/day. Pigs remained on trial for an average of 6.6 weeks, and overall mean water intake per pig per was 5.8L/day. The mean pen health score gradually increased with the time pigs were in the finisher building, indicating increasing prevalence of ill health.

Factors significantly associated with the variation in daily mean water use per pig for the experimental pens are displayed in table 1. Separate analysis of replicate two, which included the feed intake data for the pens over the weeks on trial, showed an effect of the pen FCR, whilst the drinker type was then no longer a significant factor (table 1). Disruption within the building from human presence had no significant explanatory effect on the daily water consumption.

Table 1 Factors associated with daily mean water use per pig.

Predictors	Replicates 1 & 2 (N=24 pens)	Rep. 2 only¥ (N=12 pens)
R^2 (adj)	31.7%	43.8%
Pig weight	0.0367***	0.0450***
No. Pigs	-0.184***	- 0.203***
Daily live weight gain	1.66***	1.60***
Drinker type	-0.421***	NS
Ext. min. temp.	-0.118***	NS
Ext. max. temp.	0.048***	NS
Room max. temp	NS	- 0.075*
FCR	-	- 0.28*

Where asterisk occur: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$.
¥ = replicate two only included measurement of feed intake.

Analysis of the different symptoms of disease showed that daily mean water use per pig was reduced in pens with pigs suffering from scour (fig. 1). Of relevance to sub-clinical disease detection, pens scored for occurrence of scour in the following week (1 week later), had a reduced water consumption in the current week (fig. 2).

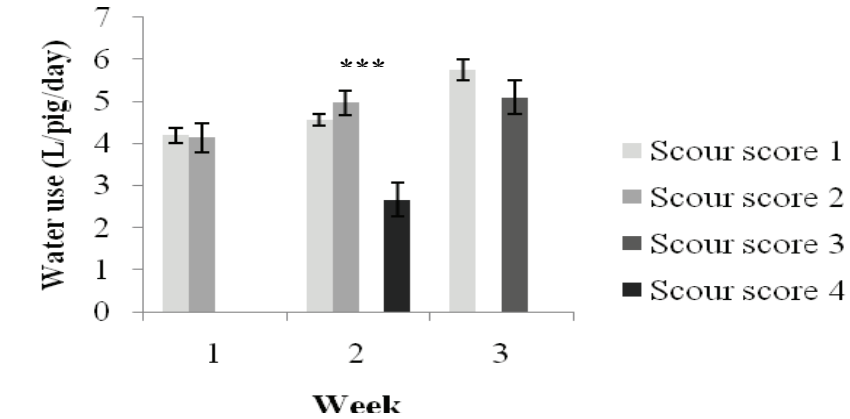

Fig. 1 Water use, adjusted for liveweight and environmental temperature, in relation to severity of scour score observed in that week. *** = $P < 0.001$.

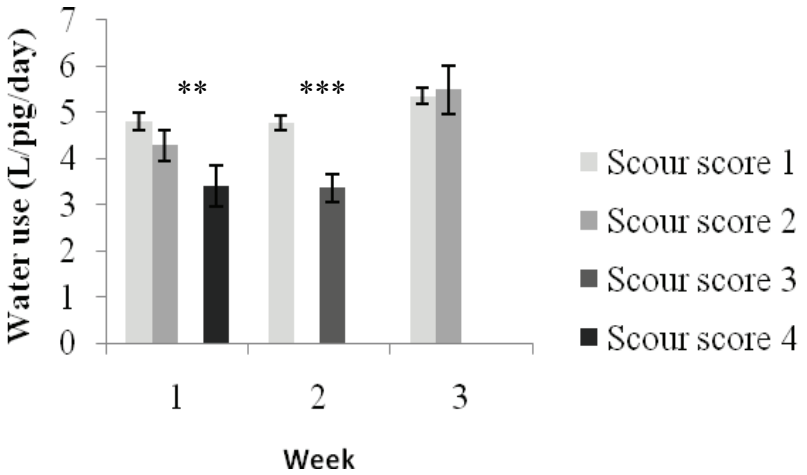

Fig. 2 Water use, adjusted for liveweight and environmental temperature, in relation to severity of scour score observed the following week. *** = $P < 0.001$, ** = $P < 0.01$

DISCUSSION

The weight and number of pigs within the pen and environmental temperature were main factors associated with water usage. The negative relationship with group size suggests this may have affected the ease with which drinkers could be accessed. Whilst the relationship between pig weight and water use is as expected, the negative relationship between minimum daily temperature and water intake might reflect the fact that, during this cold winter period, low temperature stimulated feed intake and entrained water consumption. The positive relationship between water intake and maximum temperatures reached suggests that, as the temperature increased above the set level, pigs would consume more water, perhaps in order to replenish evaporative loss through increased respiration rate. Pigs with a higher daily live weight gain had a larger water intake, requiring water use for the growth of lean tissue. The negative relationship between the FCR and water use could be indicative of feed wastage within the pens, or of different body tissue deposition in the pigs. Pigs depositing a greater proportion of fat utilise less dietary water than those depositing more lean tissue and would also be expected to have increased FCR. The significant reduction in water consumption in relation to severity of scour score could be reflecting the temporary increase in inflammatory cytokines at the onset of disease which characterises "sickness behaviour" in animals [2]. Pigs also consumed significantly less water in any given week if they were seen to scour in the following week, demonstrating a possible link to the occurence of disease at a sub-clinical level.

CONCLUSIONS

The usage of water by pens of pigs was related to a number of animal and environmental factors such as the weight and number of pigs within the pen, and environmental temperature. Modelling of the relationships between water usage and these factors under different conditions could provide a predictive tool enabling deviation from normality to be reliably established as an early warning system for health and performance problems. Differences in the quantity of water consumed one week before scour symptoms are visible, suggest water consumption could be utilised to help detect the occurrence of sub-clinical disease in pigs.

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INCIDENCE OF RECIRCULATION LIQUID ON GAS EMITTED BY PIGGERIES EQUIPPED WITH FLUSHING SYSTEMS

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SUMMARY

During two batches of fattening pigs (B1 and B2), measurements of ammonia and GHG were achieved on the exhaust air of the two identical rooms; only differing in the manure management. In the first room (Reference room), the slurry was stored in the pit during the whole fattening period. In the second one (SR room), a fine layer of water was discharged into the pit before the pigs entered. The day of the feed change, the pit was emptied and an additional layer of water was discharged. No effect of the treatment was observed on animal performance. In the SR room, the N_NH_3 daily emission per pig was reduced by 21% (B1) and 24% (B2) in comparison to emissions from the Reference room. In the treated room, the N_N_2O daily emission per pig was slightly lower than in the Reference room. Odours emitted by the SR room was 25% lower than the Reference room.

INTRODUCTION

In Europe, ammonia emitted by piggeries is regulated by the Industrial Emission Directive (2010/75/UE) for units with over 2 000 places for fattening pigs (+30kg) or 750 places for sows. In the BREF document, a list of Best Available Techniques (BAT) is given as an effective way of reducing ammonia emitted by piggeries. Among the techniques listed, frequent manure removal by scraping or flushing are mainly illustrated. Several studies have already been conducted using these techniques. The efficiency results showed variation, one might assume that this was down to the recirculation liquid used, and the frequency of flushing. The main objective of our study was to determine the incidence of the liquid used to remove fresh slurry and its effects on ammonia and greenhouses gases emitted by the swine buildings.

MATERIAL AND METHODS

A batch of 144 crossbred (PPxLW)x(LWxLD) pigs were fattened at the IFIP's experimental farm from April to December 2010 in three housing conditions which only differed in the management of the slurry. In the first room (reference), the slurry was stored underneath in the pit during the whole fattening period. In the second room (FS room), slurry was removed twice a day (10 a.m and 10 p.m), and the recirculation liquid was the liquid fraction of the slurry produced by pigs kept in this room. In the third room (FW room), slurry was also removed twice a day by a flushing liquid which was only water. For the three rooms, 48 pigs were group-housed in 6 pens on fully slatted floors. The total pen area per animal was 0.7 m^2. Fresh air entered via a ceiling of perforated plastic sheeting and air exhaust was under-floor extraction with chimney.

Pigs were individually weighed at the beginning of the growing period and thereafter every three weeks in order to organize the change of feed and the day before slaughtering. The feed intake was recorded weekly on a pen basis. The water consumption was recorded daily on a pen basis. At slaughtering, carcass characteristics were individually recorded. Temperature and hygrometry were continuously monitored inside and outside the three fattening rooms. The ventilation rate was continuously monitored by measuring the rotation speed of a full-size free-running impeller unit, coupled with the exhaust fan of the room. The set-point temperature was fixed at 24°C during the whole period. For the three rooms, the gas concentrations both in the air of the experimental rooms and outside were measured by photoacoustic infrared absorption spectrometry using a gas analyser (Innova 1412) coupled with a sampler dosimeter (Innova 1303). The gases analysed were NH_3, N_2O, CH_4, CO_2 and water vapour. Slurry samples were achieved in the pit six times during the fattening period. Dry Matter, pH, total nitrogen, ammonium nitrogen and total carbon were analysed on each sample. Emissions factors were validated by the mass balance method. Air samples for odour measurements were achieved and analysed to determine the odour concentrations using dynamic olfactometry in accordance with the European CEN standard.

The mass balance method was applied for nitrogen (N), carbon (C) and water (H_2O) including the calculation of inputs (piglet carcass, feed consumption) and outputs (pig carcass, slurry composition, gaseous emissions). An analysis of variance (SAS 1998, proc GLM) was performed to test the effects of sex (X) and treatment (T) on animal performance.

RESULTS

Growth performance

Table 1 sum up the growth performance of the three rooms involved in this study. For both rooms, the fattening duration was 86 days. All pigs were slaughtered the same day. At slaughtering, pigs reared in the Reference and in FS rooms were significantly heavier than pigs reared in FW rooms. Concerning ADG, the effect of the treatment was clearly identified: pigs kept in the FW room had lower ADG than pigs kept in the Reference and in the FS rooms. For FCR, pigs of the Reference rooms had a worth FCR than pigs of both treated rooms and this during the whole period of fattening. The muscle content of pigs kept in the Reference room was slightly lower than the muscle content of pigs of both treated rooms but without any significant effect of the treatment.

Table 1: Growth performance

Rooms		Reference	FS	FW	RSD	Stat.[1]
Live weight (kg)	At the beginning	34.4±2.0	34.2±2.3	34.2±1.9	2.1	-
	At slaughtering	110.3±7.4a	109.5±5.0a	107.4±5.4b	5.2	S*T***
ADG (g/j)	Growing period	913.6±77.4b	933.3±87.9a	933.2±62.1a	73.6	S***T*
	Finishing period	737.4±106.2a	693.4±84.5b	647.6±85.2c	88.8	T***
	Total	804.5±81.1a	785.2±72.6a	755.8±70.5b	72.9	S**T***
FCR	Growing period	2.64±0.25a	2.35±0.18b	2.33±0.15b	0.18	T**
	Finishing period	3.55±0.29a	3.21±0.24b	3.22±0.22b	0.22	S***T***
	Total	3.15±0.26a	2.82±0.16b	2.81±0.13b	0.17	S*T***
Carcass weight (kg)		88.2±4.1	87.1±3.8	85.9±3.9		
Muscle content (%)		61.9±2.1	62.3±1.8	62.6±1.8	1.8	S***

[1] analysis of variance including sex (X) and treatment (T) as main effects, ***:P<0.001 **:P<0.01 *:P<0.05

Ambient parameters

During the whole fattening period, the average outside temperature was 16.2±7.9°C. The average ambient temperature was 27.0±2.9°C, 27.6±2.6°C and 26.9±2.9°C inside Reference FW and FS rooms, respectively. The average ventilation rate was 1396±577, 13971±577 and 1450±632 m^3 per hour in the Reference, FW and FS rooms, respectively.

Slurry

For both batches, an intermediary emptying was achieved the day of the feed change in the SR room while the slurry was stored during the whole fattening period in the reference room. The lower dry matter of slurry sampled in the SR room is the result of the dilution by the additional water emptied at the beginning of the fattening period and the day of the feed change (table 2). The composition of slurry produced by pigs kept on the reference room was in accordance with literature and previous studies achieved in similar conditions [1, 2].

Input-Output mass balances

For nitrogen, the mass balance deficit per room was 60, 38 and 62% of the input of nitrogen for the Reference, FS and FW rooms, respectively. For carbon, the mass balance deficit was -61, 41 and 66% of the input of carbon for the Reference, FS and FW rooms, respectively.

Gaseous emissions

Nitrogen emissions (NH_3 and N_2O) measured for the Reference, FS and FW rooms explained less than 20% of the nitrogen losses by volatilisation calculated by the input-output mass balances. For the whole period of fattening, ammonia emission was 3.2 g N_NH_3 per pig per day in the Reference room, 3.6 g N_NH_3 per pig for the FS room and 3.8 g N_NH_3 per pig for the FW room. The N_2O emission was 0.6g N_N_2O per pig per day for the Reference room, 0.5g N_N_2O per pig per day for the FS and FW rooms.

Values of the first batch were totally in agreement with data obtained by Philippe et al. (2007) [3].

Carbon emissions (CO_2 and CH_4) measured during the whole fattening period represented 82, 84 and 77% of calculated emissions by the mass balance, for Reference, FS and FW rooms, respectively. For CH_4, emission was 10.1 g C_CH_4 per pig per day in the Reference room and 7.3 g C_CH_4 per pig per day in both FS and FW rooms.. According to Gallman et al. (2003) [4], CH_4 emissions ranged between 6 and 9 g C_CH_4 per pig per day for

animals reared in winter on totally slatted floor with ambient temperatures between 19 and 23°C. For CO_2, for B1, emissions were 583 and 626 g C-CO_2 per pig per day for the Reference and the SR room, respectively. For B2, CO_2 emissions were 706 and 750 g C-CO_2 per pig per day for the Reference and the SR room, respectively. For both batches, our values were lower than those obtained in the literature [3,4] and no effect of the treatment was observed on C_CH_4 and C_CO_2 emissions.

Odours

During the first batch, in both rooms, samples for odour measurements were achieved 26, 83 and 103 days after pigs entered. For the Reference room, the average odour emission was 1.0 10^8±4.5 10^7 vs 7.5 10^7±4.5 10^7 odour units per day for the SR room leading to a reduction of 26% of odour. Value of the Reference room was in accordance with literature data

DISCUSSION

Previous studies concerning slurry removal once during the fattening period have already been achieved, showing no effect on ammonia and an increase in odour emission [5]. The main reason was the sedimentation of the solid fraction of the slurry kept in the bottom of the pit, leading to the volatilization of ammonium. Moreover, some odorous compounds like p-cresol and phenols were present in the solid fraction. The addition of a fine layer of water limits this sedimentation, especially at the beginning of the fattening period, where the slurry volume is very weak and then can be easily deposited into the bottom of the pit. During the first removal, 50 days after the pig's entry, the water layer facilitated the slurry emptying. This pit was cleaner than the reference pit.

CONCLUSIONS

In our study, the addition of water to the pit twice during the fattening period led to a reduction of more than 20% of ammonia and 25 % of odours. This technique can be implemented in all kinds of pig farms without any changes to the building's structure nor in the pig breeding. Nevertheless, the increase in the volume of slurry has to be taken into consideration when managing manure on a farm scale.

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ASSESSMENT OF RESPIRABLE DUST CONCENTRATION IN 144 FRENCH FARROW-TO-FINISH PIG HERDS

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SUMMARY

The aim of the study was to assess respirable dust concentrations in different sections of pig buildings in a sample of 144 farrow-to-finish herds located in western France. In each herd, respirable dust concentration was assessed in three rooms corresponding to different rearing steps: farrowing, post-weaning and finishing phases. The concentration of respirable particles was determined gravimetrically using standard cyclone dust samplers. The sampling devices were placed, in the morning, at 1.40 m height above a selected pen of pigs. The air sampling was done over a 20 hour period starting at 4:00 pm the day of the device placement. Whatever the rearing step, respirable dust concentration ranged from less than 0.01 mg/m^3 to 0.67 mg/m^3. Mean respirable dust concentrations in farrowing, post-weaning and finishing rooms were 0.17 mg/m^3 (σ=0.14), 0.13 mg/m^3 (σ=0.10) and 0.11 mg/m^3 (σ=0.08), respectively. Respirable particle levels were lower than 0.23 mg/m^3 in 76.5%, 87.9% and 91.9% of the farrowing, post-weaning and finishing rooms, respectively. Whatever the rearing step, respirable dust concentration varied from low to high levels. Identification of factors influencing respirable dust concentration should help to adapt building management and to design future livestock buildings avoiding high dust levels.

INTRODUCTION

Airborne particles inside pig buildings mainly consist in animal skin, faeces, bedding material, micro-organisms and feed [16]. Dust particles can transport pollutants like odour molecules, bacteria, endotoxins and viruses, and promote the distribution of these agents within and between livestock buildings [24]. Airborne particles are classified into different sub□classes according to their size. While "Total dust" refers to the whole airborne particles, the fraction containing particles less than 100 µm in aerodynamic diameter is often referred to as "inhalable dust" [4, 18]. The "respirable dust" mainly encompassed particles with an aerodynamic diameter of less than 4 µm [4, 18]. The particle size is highly important since it determines the dust ability to enter and deposit into the respiratory system: the smaller the particle, the greater the likelihood of deep penetration into the respiratory tract [9]. While particles of all sizes may be deposited in the nose and pharyngeal region, only those with aerodynamic diameters <10 µm can enter the trachea-bronchial tree and only those with an aerodynamic diameters <7 µm can reach the alveoli [1, 9, 22]. The fraction of particles that enters the alveoli comprises the respirable fraction of airborne particles. High airborne particle concentrations, especially the respirable fraction one, can potentially affect animal production efficiency and human and animal health [5, 10]. Donham et al. [13], proposed a maximum level of respirable dust of 0.23 mg/m3 to reduce human and animal adverse health effects. However few data related to respirable dust concentration inside buildings of a wide sample of French pig herds have been published. The aim of the present study was to assess respirable dust concentrations in different sections of pig buildings in a sample of 144 herds.

MATERIAL AND METHODS

The study was carried out in 144 farrow-to-finish pig herds located in western France. In each herd, respirable dust concentration was assessed in three rooms corresponding to different rearing steps: farrowing, post-weaning and finishing phases. Concentrations of respirable airborne particles (<4 µm) were determined gravimetrically using cyclone sampler (TSI, Marseille, France). The respirable dust was collected on pre-weighted, 37 mm diameter glass fibre filters fixed in threaded holders (ARELCO, Fontenay-sous-Bois, France). The samplers were connected to air pumps which provided a constant airflow of 1.7 l/min (SP530, TSI, Marseille, France). All exposed filters were subsequently reweighed to the nearest 0.01 mg (AX 105, METTLER-TOLEDO, Viroflay, France) at our laboratory. Before weighing and re-weighing all filters were desiccated for 12 hours. The results were related to air volume that had passed and given as mg/m^3. The sampling devices were placed, in the morning, in a box attached to the ceiling or a beam, using wire cable. The box was lowered at 1.40 m height, without allowing the pigs to interfere with the instruments, above a selected pen representing the average condition of the room. The air sampling was done over a 20 hour period starting at 4:00 pm the day of the device placement.

RESULTS

Out of the 144 herds, 88.2% were located in Brittany. Respirable dust concentrations were assessed in 32 farrowing, 124 post-weaning and 136 finishing rooms from 144 farrow-to-finish herds. On average, the pigs were 15 (σ=6.4), 54 (σ=15) and 165 (σ=13.4) days old in farrowing, post-weaning and finishing rooms, respectively.

Whatever the rearing step, respirable dust concentration ranged from less than 0.01 mg/m^3 to 0.67 mg/m^3. The distributions of the respirable dust concentrations assessed in the three rearing steps are presented Figure 1.

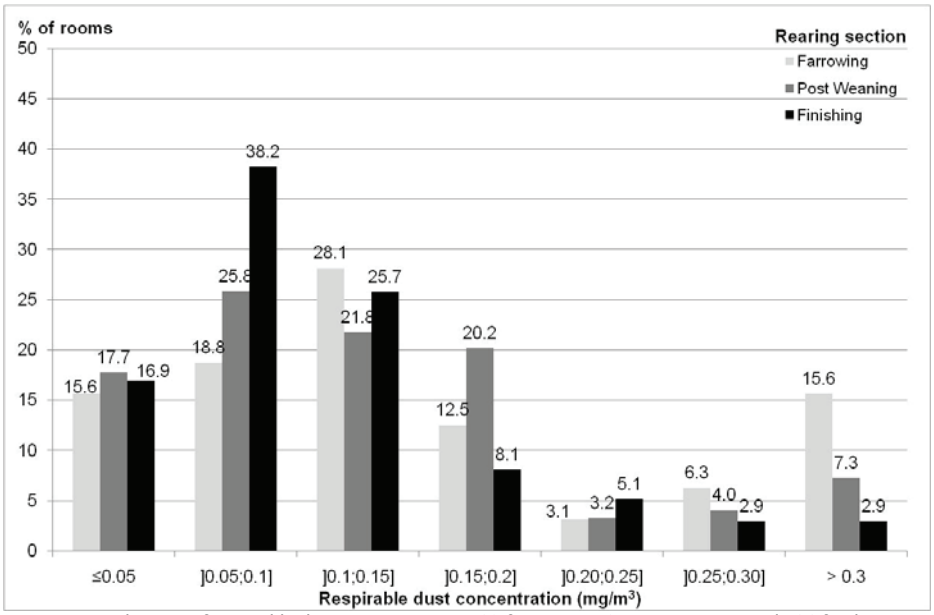

Figure 1: Distributions of respirable dust concentration in 32 farrowing, 124 post-weaning and 136 finishing rooms (144 herds, Western France)

Mean respirable dust concentrations in farrowing, post-weaning and finishing rooms were 0.17 mg/m^3 (σ=0.14), 0.13 mg/m^3 (σ=0.10) and 0.11 mg/m^3 (σ=0.08), respectively. Respirable particle levels were lower than 0.23 mg/m^3 in 76.5%, 87.9% and 91.9% of the farrowing, post-weaning and finishing rooms, respectively.

DISCUSSION

The respirable dust concentrations were assessed by weighing under laboratory conditions airborne particles collected on a fibre filter. This gravimetric so-called method is considered as the gold standard when assessing indoor particles concentrations [4, 14]. The mean respirable dust concentrations found in the present study varied between 0.11 and 0.17 mg/m^3 depending on the rearing stage. Results of previous studies carried out in North America, Australia, Asia and the European Union, obtained with a gravimetric measurement, indicated that the mean respirable dust levels ranged from 0.11 to 0.64 according to the country and the rearing step [2, 7, 8, 11, 12, 24], [6, 19, 21]. To the best of our knowledge few studies included more than 100 herds and different rearing stages. Data related to the farrowing step are scarce, most of the studies focused on the post-weaning and fattening stages. Even if few data concerning husbandry and outdoor climatic conditions near than those encountered in western France are available, mean respirable dust levels assessed in our study seemed to be in the lowest concentrations measured under confined conditions in intensive systems. Indeed, results of an European study involving England, the Netherlands, Denmark and Germany, Takai et al., [24] found mean respirable dust concentrations of 0.43, 0.32, 0.15 et 0.29 mg/m^3 in post-weaning rooms with slatted floors, respectively and of 0.29, 0.24, 0.16 et 0.18 mg/m^3 in fattening rooms with slatted floors, respectively. Several studies showed that the type of herd, the building design and equipments, the building management and outdoor climatic conditions influenced indoor airborne particles concentrations [3, 6, 15, 17, 20, 21, 23]. Those factors are highly susceptible to explain the variations in the respirable dust levels found in different countries.

CONCLUSION

The mean respirable dust concentrations found in this study were in the range of the lowest levels reported in previous studies carried out in confined buildings and intensive systems in Northern Europe and North America. Even if for more than 75% of the herds, respirable dust concentrations were below the recommended level of 0.23 mg/m^3 to reduce animal and human adverse health effects, the lowest levels throughout the pigs' lifetime should be reached to minimize health risks. Whatever the rearing step, respirable dust concentration varied from low

to high levels. Identification of factors influencing respirable dust concentration should help to adapt building management and to design future livestock buildings avoiding high dust levels.

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ANIMAL HYGIENE AND SUSTAINABLE LIVESTOCK PRODUCTION: IMPACT OF GROUND WATER CONTAMINATION WITH ARSENIC

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SUMMARY

There is a growing concern all over the world about contamination of ground water with Arsenic. One of the major repercussions of arsenic contamination is degradation of animal hygiene that ultimately affects sustainable livestock production. The reports suggest that concentration of Arsenic in ground water of twenty one countries is well above the guideline values. Use of such contaminated water for animal husbandry and livestock production compromises with the hygienic value of animal products. Therefore, there is an urgent need to develop low cost treatment technologies for reducing the level of arsenic in ground water to maintain the hygiene and sustainability of livestock production. Most of the traditional treatment technologies are costly and less effective in reducing arsenic concentration to safer limits. Therefore, during present study, an attempt was made to design a low-cost algal adsorbent based filtration unit consisting of polyurethane columns with entrapped algal adsorbents. The column was made of adsorbents of algal origin like agar-agar, alginic acid, calcium alginate and *Spirulina platensis* biomass entrapped in polyurethane foam matrix. The performance of the column was assessed in terms of removal efficiency and the quantity of metal sequestered in unit time interval. The results from the study the show that algal biosorbents and *S. platensis* biomass combination has a capacity to adsorbed arsenic from aqueous solution. The simple design, easy fabrication and no energy requirement for the operation of the filtration unit developed under the present study is suitable to rural areas where arsenic contamination of ground water is adversely affecting the animal hygiene and sustained livestock production.

INTRODUCTION

Environmental Protection Agency and the World Health Organization listed arsenic is a metalloid and ranks 20th in natural abundance, comprising about 0.00005% of the earth's crust, 14th in the seawater and 12th in the human body (Mandal and Suzuki, 2002). High arsenic concentrations have been reported recently from 21 countries including USA, China, Chile, Bangladesh, Taiwan, Mexico, Argentina, Poland, Canada, Hungary, Japan and India (Mohan and Pittman, 2007). An arsenic concentration of 10 µg/l has been recommended by World Health Organization (2008) as a guideline value for drinking water.

Ground water is one of the most important sources of drinking water and contamination of ground water with arsenic is one of the serious problems encountered in India. Arsenite and arsenate compounds are highly toxic to human beings as well as animals (Singh *et al*., 2005). Chronic exposure to arsenic concentrations above 100 µg/l can cause vascular disorders, such as abnormality in dermal pigments (Blackfoot disease) and skin, liver and lung cancer in human beings (Wang *et al*., 2001).
The tolerance level of arsenic varies from animal from animals in age, sex, physiological status, nutritional status, route of exposure and biological availability (Sarder, 2004) The arsenic concentrations in the water could affect human health through milk intake, since the allowable limit for water used to feed cattle is 0.05 mg/ L (USEPA, 1973).
It is clear that arsenic pollution is creating havoc to animal hygiene and sustainable livestock production. There is an emergent need for the removal of arsenic from groundwater and domestic wastewater containing arsenic which has been directly or indirectly used for the sustainable livestock production and their products. Adsorbents of algal origin consist of metals/metalloids binding groups like amino, carboxyl, sulfhydryl etc., which can adsorb the metals/metalloids from aqueous solution. Therefore, the present study aims to develop a low cost and feasible technology for the removal of arsenic using polyurethane blocks loaded with adsorbents of algal origin like agar-agar, alginic acid, calcium alginate, and *S. platensis* dry biomass. Use of these adsorbents in column mode can provide a cost-effective technology for remediation of metals/metalloids including arsenic which is used for animal hygiene and sustainable livestock production.

MATERIALS AND METHODS

Unialgal culture of cyanobacterium, *Spirulina platensis* was obtained from Algal culture laboratory of Central Institute of Fisheries Education (CIFE), Mumbai. The pure culture was sub-cultured in Zarrouk's medium (Zarrouk, 1966) under photoautotrophic conditions. The outdoor mass cultivation was done under natural conditions when solar

radiation reaching the surface of culture was between 2160 and 8450 lux, and temperature ranged from 27 to 34 °C to generate sufficient biomass for the experiment. Designing and preparation of fixed-bed column filtration unit explained in detail in the paper Ranjith *et al*., 2011. The column-bed adsorption study was carried out for 25, 50, 75 and 100 µg/L initial concentrations for an hour. Water samples were digested using a microwave-based closed vessel (Anton Parr, USA) and analyzed by FI-HG-AAS, flow injection-hydride generation atomic absorption spectrometry (AAnalyst 800, Perkin Elmer, USA). The removal efficiency at 30-minute and 60-minute intervals of different column-beds was calculated using Amin *et al*., 2006 equation. The biosorption capacity of the biomass combinations was calculated by using Zhang and Banks, 2006 equation.

RESULT

The observations recorded during the study show that the removal efficiency of Arsenic after 60 minutes treatment time varied from 0.7% to 45% for 25 to 100 µg/l initial concentrations in a cycle of operation with the flow rate of four litres per hour. The best removal efficiency of Arsenic was exhibited by a combination of agar-agar and *S. platensis* biomass which is 27% higher than the agar-agar alone at 25 µg/l initial concentration. The biosorption capacity of Arsenic varied from 108 to 694 µg/g adsorbent for 25 to 100 µg/l initial concentrations and the highest value (694 µg/g) was recorded for agar-agar and *S. platensis* biomass combination.

DISCUSSION

Polyurethane was selected for the present study on the basis of its characteristics like low cost, easy availability of the material in the local market, possibility of up-scaling of the volume of water to be treated, long shelf-life, resistant to heat sterilization, suitable for the entrapment of the algal biomass and its chemical stability in water.

The selection of algal compounds for present study was based upon the earlier reports (Awasthi and Rai, 2006; Bajpai *et al*., 2006). However, in contrast to calcium alginate which is the most commonly used algal compound for immobilization of algae, the reports on the use of alginic acid and agar-agar are very few. Therefore, an attempt was made in this study to assess the biosorption capacities of these compounds along with dehydrated biomass of *S. platensis.*

The alga *S. platensis* used in present study was selected based on the characteristics like fast growing capacity, availability of sufficient base line information about the cultivation techniques and the supply of biomass in required quantity for the column bed preparation and treatment of water will be ensured.

The column bed reactor designed was constructed by low cost materials like polyurethane, PVC pipes, nylon cloth and the average cost of a unit (unit of capacity of 4 liter/hour flow rate.) was calculated to be approximately US $ 11 to 13. The unit can be easily fabricated using household tools and require little technical skills; however the entrapment of the algal compounds and biomass of *S. platensis* requires a small setup with weighing and drying facilities. Thus, it is suggested that PU loaded with appropriate quantity of algal compounds and *S. platensis* biomass can be produced in a separate unit and supplied in the market at reasonable price. The dried *S. platensis* powder is available at a price of US $ 9 to 11 per kg can be used for the column preparation as the cultivation of *S. platensis* is a cumbersome process. Though, this will enhance the cost of construction of the filtration unit but considering the small quantity of the algal compounds and *S. platensis* biomass required for column bed preparation, the overall cost will not vary to a great extent.

CONCLUSION

Few studies have been accomplished on biosorption of arsenic using immobilized algal biomass. Therefore, present work will provide baseline information about the potentialities of algal adsorbents, *S. platensis* biomass immobilized on the PUF matrix at different environmental conditions. So, considering the growing menace of arsenic pollution in various parts of the country the proposed filtration unit would help to reduce arsenic in the water discharged from household after its use in various domestic purposes which adversely affecting the animal hygiene and sustained livestock production.

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LIGHTING SYSTEM FOR LAYING HENS - PRE-TESTING OF NEW TECHNIQUE IN SWEDEN

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SUMMARY

The aims of the project was to record the lighting environments in common types of Swedish hen houses. Furthermore, the HATO® light equipment was tested according to the legal requirements regarding bird health and welfare. A limited study was performed in six commercial farms with laying hens in aviaries and modified cages, with HATO®, fluorescent or incandescent light bulbs, and in one rearing farm. Although lighting environment varied between the different farms, no severe problems of bird health or behaviour in the flocks studied were found, except for occurrence of feather pecking. However, the pattern of feather pecking did not show a clear connection to the housing or light system.

INTRODUCTION

The domestic laying hen is, as its ancestor the red jungle fowl, a day-active gregarious bird. The jungle bird evolved in the equatorial jungle, which has a diurnal rhythm of 12 h of light and 12 h of darkness [3]. Therefore, the lighting environment in egg production is crucial for laying hens and their egg laying. The characteristics of the light differ depending on the source, with respect to both intensity (illuminance) and wavelength (spectrum; colour) of the light. Natural light has an even distribution of the wavelengths between 400 and 700 nm, but the content of the ultra violet A light (UVA, wavelength of 320 to 400 nm) attenuating in natural light as the wavelength is shortening. The incandescent light from e.g. ordinary light bulbs contains more red and less blue wavelengths than natural light.

Compared to humans, that three types of cones which together register electromagnetic radiation between 400-730 nm, birds have a fourth type of cone which allows perception of electromagnetic radiation below 400 nm. Together with a somewhat different structure of the eye, this enables the bird to percept UVA-light [6, 10]. As reviewed by due to that birds can perceive UVA and also have a greater spectral sensitivity than humans between 400 and 480 nm and 580-700 nm respectively, it is very likely that they will perceive light from certain light sources brighter than humans. The degree of brightness will however depend on the type of light source. Since the unit for illuminace (lux) is based upon the sensitivity of the human eye, it may not be suitable to use when describing and adjusting the light intensity in poultry houses. Instead there is an alternative unit termed Gallilux [7].

In Sweden all farm animals should have daylight inlets according to the Swedish animal welfare legislation. Therefore, all poultry houses, in conventional as well as in organic production, should have windows for natural light [12, 13]. However, there are few guidelines in how to arrange these daylight inlets. The law about windows applies for all layer hen houses that are built after 1994 or have made an arrangement in the stable that requires approval from the County administrative board. This means that buildings without proper windows, or no windows at all, are required to have windows. Particularly in layer houses with furnished cages it may be difficult to arrange a suitable daylight inlet.

Birds reared without access to daylight and not given access to natural light until they are adult may have behavioural problems as a consequence of the inability to adapt to the new housing environment [5, 8]. However, inappropriate lighting management may increase the risk for behavioural problems, e.g. cannibalism and feather pecking. Thus, farmers are concerned about how to rebuild old hen houses to satisfy the legal requirements.

Incandescent light has until recently been the most common type of artificial light in commercial hen houses in Sweden, although it can be questioned if incandescent light is an optimal light source for to hens. It has been shown that hens prefer fluorescent tubes over incandescent light from light bulbs, mostly because of its blue wavelength [19] that physical activities of hens might be greater in fluorescent light than in incandescent [2].

There are several differences between natural and incandescent light which might have effects on the behaviour of the chicks. Natural light has a higher level of light (light intensity), it has a complete spectrum compared to incandescent light sources colour spectrum and its characteristics vary a lot more than for incandescent light [11]. Taylor and co-workers [15] found that lower light intensities restricted the movement of birds, when measuring the ability to jump from perch to perch. Brown Leghorn hens have been shown to be willing to work for increased light intensity and they also perceive the opportunity to control their light environment as rewarding [14].

Since the EU ban of opaque incandescent light bulbs in 2009 there is a need for replacement lighting in poultry houses in Sweden. A new lighting equipment (HATO® Agricultural Lighting) has been introduced in Sweden, that is reported to be more similar to natural light with a more even wavelength distribution between 400 and 700 nm, and it contains more of the ultraviolet A light (UVA).

According to Swedish animal welfare legislation, all new technical equipment should be approved before taken into use [13]. Scientific investigations of animal health and welfare of the housing system have to be carried out in order to give a base for making a decision on approval of the equipment. The HATO® was considered to be a new technique in farm building and, therefore, it needed to be tested and evaluated regarding the impact on bird health and welfare before marketed unrestricted in Sweden..

In 2009 a collaboration project was initiated by the Swedish Egg and Poultry Association. The aims of the project was to record the lighting environments in common types of Swedish hen houses. Furthermore, the HATO® was tested according to the legal requirements regarding bird health and welfare.

MATERIAL AND METHODS

As a pre-testing requirement of the Swedish Board of Agriculture, a limited study was performed in six commercial farms with laying hens in aviaries and modified cages, with HATO®, fluorescent or incandescent light bulbs and in one rearing farm (aviary type). Data regarding production, lighting intensity, bird health and qualitative behavioural measurements were recorded. The lighting environment was recorded regarding distribution of light in the compartments, light intensity and spectral distribution. (The detailed light measurements preformed within the project will be reported elsewhere.) Clinical inspections and qualitative behavioural studies were preformed on a random sample of 100 birds in a rearing farm and during the egg laying period in an aviary farm and a farm with enriched cages, both with HATO®. Pullets were inspected at 1, 10 and 14 weeks of age, and the production flocks were inspected at 35, 55 and 70 weeks of age. The scoring was done modified after Gunnarsson and co-workers [5] and Welfare Quality® [1]. Qualitative behavioural records were performed according to methodology used in Welfare Quality® [1] and based on principles developed by Wemelsfelder and co-workers [17, 18].

RESULTS

The median flock size in the production flocks was 15 840 (min 3000; max 31 658) and median mortality during the production period (from delivery until end of lay) was 0.9 % (min 0.8%; max 4.3%). The median laying rate was 93% (min 91%; max 94%) and the median feed consumption was 120g per day and bird (min 114; max 124).

Analysis of the lighting environment showed that the light was evenly distributed in all farms, but the spectral distribution and the intensity varied between farms (median 2.2 lux; min 0.7 lux; max 26 lux). Farms with HATO® or fluorescent light were found to have higher light intensities than those with incandescent light bulbs.

All animals were scored normal during rearing at the rearing farm. In the aviary farms 26% to 64 % of the birds were found to have mild keel bone deviation that increased with age. Aviary birds and birds in modified cages had deteriorating plumage with age and at end of lay almost all birds had featherless areas on neck, wings and breast (96-100%). More birds in aviary farms were featherless on the back than birds in modified cages (97% versus 30%). Other severe clinical remarks were rare.

Qualitative behavioural observations performed by different observers at the same scoring time had a good agreement. No significant difference was found for important parameters, but the scoring of birds in modified cages showed a larger variation compared to aviary birds.

DISCUSSION

The limited project aimed at recording the lighting environments in common types of Swedish hen houses. Furthermore, the HATO® was tested according to the legal requirements regarding possible impairment for bird health and welfare. The limited number of observations is not allowing any extended statistical analysis of the results. The lighting environment varied between the different lighting types as would be expected. No severe problems of bird health in the flocks studied were found except for feather pecking. The pattern of feather pecking did not show a clear connection to the housing or light system. The results will be considered by the Swedish Board of Agriculture in the process of approving the lighting equipment HATO®, in order to analyse if it can be excluded that the equipment has negative effects on bird health and welfare. Feather pecking has previously been reported to be caused by various risk factors related to e.g. genetics, nutrition and rearing environment [16]. It has also been reported that light sources with low wave lengths spectrum and high light intensities is increasing the risk of feather pecking [9]. However, in the present study it was not possible to identify single factors causing the feather pecking.

It has been suggested previously that exposure to natural light would be an ideal solution to many lighting problems and that it increases the welfare of domestic fowl [11]. Further research is also required to reveal which aspects

of natural light are crucial for the behaviour and welfare of adult hens and how to expose the birds optimally to these factors.

CONCLUSIONS

Although lighting environment varied between the different farms, no severe problems of bird health in the flocks studied were found, except for feather pecking. The pattern of feather pecking did not show a clear connection to the housing or light system.

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EFFECT OF FULL SPECTRUM LIGHTING ON PERMORMACE OF FATTENING POULTRY

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SUMMARY

The aim of this study was to determine the influence of full-spectrum light on the fattening of poultry. The influence of full-spectrum lighting was tested in a commercial broiler-rearing facility. Full-spectrum fluorescent lamps were installed in one house (Group E - experimental) and regular fluorescent lamps remained in the other (Group C-control). In total, 6 growing fattening cycles were evaluated in each building. The following parameters were selected as indicators to assess: average live weight at the end of the fattening period, feed consumption per 1 kg of weight gain, and overall mortality during the fattening period. The experimental results with the full-spectrum light in broiler production showed a statistically significant difference ($P < 0.05$) only in the final weight of the animals (2.14 ± 0.21 kg in Group E and 2.03 ± 0.2 kg in Group C). The differences in the other monitored parameters turned out to be statistically insignificant. In conclusion, the final weight of broilers was positively affected by full-spectrum lighting. However, this area needs to be researched more extensively.

INTRODUCTION

Light is an important aspect of animal environment. Illumination with a full spectrum of light is now recommended for humans and animals alike, especially pets. Manufacturers of full-spectrum light sources have claimed a variety of benefits for their products, including better visibility, improved colour rendering, better health, and greater productivity. However, critical reviews of many authors, for example [1-2] and others suggest that the evidence does not show a dramatic effect of full-spectrum lighting on health or behaviour, nor does it support the evolutionary theory. [1] states that some lighting studies fail to provide the basic descriptive statistics such as means and standard deviations.

There are not many scientific publications dealing with the effects of full-spectrum lighting on farm animals and their performance. Therefore, the intent of this study was to determine the influence of full-spectrum light on the fattening of poultry. Poultry was chosen because it is kept in controlled light conditions.

MATERIAL AND METHODS

The influence of full-spectrum lighting was tested in a commercial broiler-rearing facility. Two broiler houses, identical in design and equipment, were selected for the experiment. Full-spectrum fluorescent lamps were installed in one house (Group E - experimental) and regular fluorescent lamps remained in the other (Group C-control).

Each building had a capacity of 17,000 broiler chickens (hybrid ROSS 308), and the fattening period averaged 40 days. In total, 6 growing cycles were evaluated in each building.

The following parameters were selected as indicators to assess the influence of full-spectrum lighting: average live weight at the end of the fattening period, feed consumption per 1 kg of weight gain, and overall mortality during the fattening period.

The data from the two buildings were statistically compared using Statistica.cz (StatSoft, USA) - procedure ANOVA, POST-HOC tests.

RESULTS

The average weight of broilers at the end of the fattening period was 2.14 ± 0.21 kg in Group E and 2.03 ± 0.2 kg in Group C. The average feed consumption per 1 kg of weight gain was the same in both groups, namely 1.99 ± 0.12 kg per kg of live weight. The overall mortality in group E was, on average, 1069 ± 230 pieces as opposed to 935 ± 123 pieces in Group C. A statistically significant difference (at $P<0.05$) was found in the average weight of broilers at the end of the fattening period, while the differences in the other monitored parameters turned out to be statistically insignificant.

DISCUSSION

The experimental results with the full-spectrum light in broiler production showed a statistically significant difference only in the final weight of the animals. However, this difference was only 0.11 kg and cannot be regarded as decisive. The greater numbers of dead animals in the experimental group, although not significant statistically, may be indicative of problems in the organism's physical adaptation to this type of light. As of now, research in this area is lacking, so the results can not be meaningfully compared. Even though the full spectrum of light may be beneficial, for example in a medical treatment [3], it seems more likely [1] that the full-spectrum light will not have a profound effect on live organisms.

CONCLUSIONS

In conclusion, the final weight of broilers was positively affected by full-spectrum lighting. However, this area needs to be researched more extensively.

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MONITORING ENVIRONMENTAL CONDITIONS DURING INCUBATION OF CHICKEN EGGS

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SUMMARY

Differences in environmental conditions during incubation may alter hatching parameters and consequently affect chick quality and growth potential. The objective of this research was to study the effect of high CO_2 level during late stages of incubation. Two small scale custom built incubators, each with a capacity of 300 eggs, (Petersime NV) were employed. The control group experienced a standard high CO_2 concentration program while the test group had a lower CO_2 program during the last 72 hours of incubation. Incubation conditions (air temperature, air humidity, CO_2 concentration and, ventilation rate) were controlled by the incubator controller (IRIS, Petersime™.). Sensors were used to monitor eggshell temperature (OvoScan™ and Tsic 716 sensors), and hatching movement (Synchro-Hatch™ and camera). A standardised method (Petersime™) was used to score day-old chick quality.. Results showed that chickens commenced hatching 11 hours earlier in control group. However there was no difference in hatchability and hatching time between the two groups.

INTRODUCTION

Hormonal balance and CO_2 exchange are of fundamental importance for embryonic development during incubation and may affect survival of the embryo [2, 8]. Previous studies have focused on the effect of high CO_2 concentration during the early and second stages of incubation and suggest that higher levels of CO_2 can have beneficial and persistent effects on embryonic development during incubation by stimulating early hatching and improving hatchability [3, 4, 5, 7, 10]. However, from biological and physiological point of view, it is not known how environmental CO_2 levels influence embryonic development and hatching. This study focused on explaining why high level of CO_2 concentration during the hatcher phase (the last three days of incubation) can induce early piping and advanced hatching. Selected embryonic physiological parameters were compared between two treatments: standard incubation and low CO_2-steady incubation in order to understand possible beneficial effects of high CO_2 concentration on hatching performance.

MATERIAL AND METHODS

588 fertilized Ross 308 eggs were obtained from a local supplier (Henry Stewart & Co. Ltd, Lincolnshire, UK). The eggs were weighed and numbered before setting. Eggs were incubated in two small scale custom built incubators (Petersime NV, Zulte, Belgium). Each incubator was able to set 300 eggs separately in 2 trays. Incubation conditions (machine temperature, humidity, CO_2 concentration, and ventilation rate) were continuously monitored and controlled by the incubator controller (IRIS, Petersime™). CO_2 patterns of control group and test group were programmed and achieved by adjusting ventilation. The control group experienced the standard program with four phases of high CO_2 levels: reaching 0.7% at incubation time 18day18hours (18:18), maintaining 1% 12 hours after onset of IP-step , and reaching 0.8% and 0.7% after 2hours and 6 hours of H (hatch) step initiation, respectively. In the test group CO_2 concentration was maintained at 0.3%. Individual eggshell temperature (EST) of 10 eggs per incubator was measured every 5 minutes by temperature sensors (Tisc™- 716, IST, Switzerland) during the entire incubation process. After transfer to hatcher baskets on day 18, the 10 eggs with temperature sensors were located in a designated area of the upper basket and the hatching process of the entire basket was monitored by camera (CCD digital colour camera, VDC 413) attached to the inside top of the incubator. Images were captureed every minute and saved automatically on a PC. All eggs were candled during transfer and those with evidence of a living embryo were transferred from the turning trays to hatching baskets. After 512h (21:08) of incubation machines were stopped. The number and quality of hatched chicks were recorded and scored by a standard method (Petersime™). Hatchability was determined after hatch. The unhatched eggs were opened and checked. Data were expressed as means ± SE and differences between control and test groups at the same incubation time were analysed using SAS 8.01. The variables of EST were analysed by an analysis of variance (ANOVA) using the GLM procedure (SAS Institute Inc., 1990), with 'egg

categories' as the main effects. A significance level of 0.05 was used. Means were compared using Student-Newman-Keuls multiple range (SNK) test contrasts (SAS Institute Inc., 1990).

RESULTS

1. Hatching parameters

Hatching parameters are summarized in Table 1. Hatchability was similar for both groups. When day-old chick quality scores were taken into account, the percentage of first class chick in control group was significantly higher than that in test group.

Table 1. Fertility, hatchability and first class chick proportion

	Test Group	control Group
Fertility[1] (%)	90.48	94.88
Hatchability[2] (%)	74.18	75.19
First Class Chick[3] (%)	86.19	98.97

[1] Fertility expressed as percentage of eggs set.
[2] Hatchability expressed as percentage of fertile eggs.
[3] First Class Chick expressed as percentage of hatching chicks.

2. Eggshell temperature (EST)

The status of the eggs with attached temperature sensors was determined after hatch. There were three categories of eggs: unfertilized (UN), dead embryos (D), and hatching chicks (H). The EST of 3 categories of eggs showed no significant difference during the first 11 days. However, egg status showed difference in EST at day 18, 19 and 20 (Table 2). The EST of hatching eggs is higher than the EST of eggs with dead embryos and unfertilised eggs ($P \leq 0.05$).

Table 2. Eggshell temperature of three egg categories

Incubation time(day)	H-mean	D-mean	UN-mean
18	37.3±0.12[a]	36.6±0.12[b]	36.43±0.15[b]
19	38.32±0.13[a]	37.64±0.13[b]	37.47±0.16[b]
20	37.7±0.12[a]	37.09±0.12[b]	36.97±0.15[b]

3. Hatching process

The hatching time of first chick in the control group is at day 19 and 11 hours (19.11) compared to 19.22 in the test group (11 hours later (). Most chicks had hatched by 20.17 in the control group compared to 21.06 in the test group. Thus the total length of hatching times (hatch window) for control and test groups are 30 hours and 32 hours, respectively?

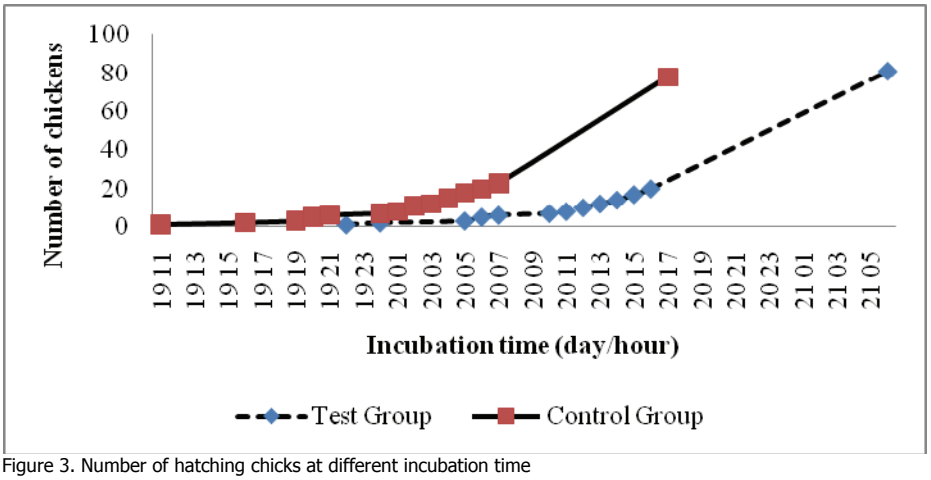

Figure 3. Number of hatching chicks at different incubation time

DISCUSSION

Temperature sensors capture the EST during the complete incubation and show the division of EST of three egg statuses (unfertilised, dead and hatching). Due to an increased metabolism, piping action and hatching movement, the embryo increases heat production at the latter part of incubation which maintains the eggshell temperature during transfer, at day 19 and 20. Thus EST is significant lower in eggs housing dead embryos and

unfertilised eggs at this time. Hatching times within incubators vary greatly, with an average hatching window estimated between 32 and 48 hours [6, 9]. Cameras provided a tool to monitor the hatching process without opening the door of incubator. The end of hatch process is marked by the time when the majority of chickens have hatched and the incubator is stopped. It may also be that incubation management, with regards to ending the incubation at the appropriate time, may optimize hatchability and chick quality. Previous research has reported that an earlier hatch does not result in a smaller duration of the hatching process[1].In this study, the hatching window of control group is only slightly shorter than the test group, even though the hatch started 11 hours early.

CONCLUSIONS

Corticosterone levels increased significantly at the end of incubation and can stimulate the hatch process. High CO_2 level of incubation condition can stimulate corticosterone and this may explain why the chicks in the control group hatched early and have higher quality compared with the test group. Further research is needed into the impact of CO_2 concentrations on hormones levels and hatching windows.

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EFFECTS OF LITTER TYPE/QUALITY AND SPECIFIC DIETARY ADDITIVES ON FOOT PAD DERMATITIS IN GROWING TURKEYS

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SUMMARY

Foot pad dermatitis (FPD) is very common in turkey flocks. As the birds are in direct contact with the litter during their life, the effects of litter type and quality are of special interest in the etiology of FPD. Furthermore, there is a great need to find out preventive measures against FPD. Therefore, two consecutive trials were carried out on 2-week-old female turkeys for 4 weeks. In the first trial, the birds were fed identical commercial diets and housed on different bedding materials: wood shavings, lignocellulose, chopped straw or dried maize silage. In the second trial, the animals were housed on wood shavings and fed on control, high biotin, high Zn or mannan oligosaccharides (MOS) diet. The control diet contained required amounts of biotin (300 µg/kg) and Zn (50 mg/kg), while the high biotin or Zn diet included 2000µg biotin/kg or 150mg Zn/kg. Mannan-oligosaccharides (Bio-Mos®) were added at a level of 1% to the MOS diet (containing identical biotin and Zn amounts to the control diet). In every trial, half of the turkeys in each group were additionally exposed to wet (73% moisture) litter for 8 h/d. Foot pads of the birds were assessed on day 0, 7, 14, 21 and 28 macroscopically and histopathologically. It was found that the severity of FPD was overall much higher (> two-fold) on wet than on dry litter. Lignocellulose showed the lowest severity of FPD, but chopped straw (dry treatment) was associated with higher FPD scores. The high dietary levels of biotin or Zn reduced significantly the severity of FPD on dry litter (25% moisture), but not on wet litter (73% moisture). The obtained results demonstrate that the high litter moisture (for 8 h/d) appears to be the major factor affecting the development of FPD. Lignocellulose as an alternative litter material could reduce the severity of FPD, whereas straw may increase it. High dietary levels of biotin or Zn might be able to lower the development and severity of FPD, but only on dry and not on wet litter.

INTRODUCTION

Foot pad dermatitis is a widespread challenge in turkeys' industry. FPD is a type of contact dermatitis where the lesions appear on the plantar surface of the bird's feet [6]. It was observed that turkeys of almost all ages suffer from FPD and the disease can start at a very early age [14]. The prevalence of FPD in turkeys can be extremely high which can reach up to 98–100% [7]. Since birds are in continuous contact with the litter, the potential impact of litter type and quality on foot pad health is of major concern. The physical structure (hard or soft) as well as the water binding capacity (higher or lower) of the litter can affect the foot pad integrity. The most common bedding materials used for turkeys are wood shavings and/or cereal straw, but there are currently further litter types which can also be used as lignocellulose and dried maize silage. There is a lack in information about the impact of these bedding materials on FPD. Several studies suggested that a strong association between "poor" litter quality and foot pad dermatitis is found [12, 13, 15]. Litter quality is affected by many factors such as drinker design, amount and consistency of excreta (affected by diet), type, depth and moisture content of the litter [15].

There is enormous interest in finding out strategies which can help to reduce FPD. Biotin and zinc are involved in formation, maintenance and healing of the skin through their roles as cofactors for various enzymes in protein synthesis and fatty acid metabolism. There are controversial results about the impact of biotin and Zn in reducing the severity of FPD in turkeys. Buda [5] found that a high level of biotin in the diet had a positive effect, whereas Mayne et al. [16] observed no effect. The inclusion of high levels of Zn in the diet is thought to decrease the development of FPD. Hess et al. [9] found that organic Zn reduced the severity of FPD in broilers but not in birds reared in cold weather. Currently, mannan oligosaccharides (MOS) are widely used for poultry as prebiotic. In most studies on poultry, MOS was used in the diet in small doses (0.05 to 0.20 %), probably for economical reasons. Due to the role of MOS within the intestinal tract (lower pH and ammonia contents) as well as in the immunity, there is an interest to study its potential effects on FPD.

The aim of the present study to assess the impact of the litter type and quality on FPD as well as to develop strategies which can help to reduce the occurrence of FPD through studying the effects of specific dietary additives (biotin, Zn, MOS). All investigated factors were tested under the influence of both dry and wet litter concurrently.

MATERIALS AND METHODS

Two consecutive trials were conducted on turkeys for a period of 4 weeks. A total of 126, day-old, female turkey poults (BUT- Big 6) were used in each trial. These birds were allotted to 4 groups. Before the start of treatments, the birds were housed in floor pens (1.50 m x 1.32 m) which were littered with wood shavings to a depth of approximately 4 cm. The litter was kept clean and dry by removing the top layers of the litter with excreta daily and substituting these with fresh dry clean litter. The experimental treatments were conducted on turkeys at 15 d of age and continued till d 42. The excreta were not removed from dry or wet litter during the experimental period to simulate field conditions. At the beginning of treatments, the number of birds totalled 29 birds in each group. Three birds per group were sacrificed on day 0 for histopathology of foot pads. Experiment 1: The birds were fed ad libitum on identical commercial-turkey diets and housed on different dry litter materials: wood shavings, lignocellulose (SoftCell®), chopped straw (Strohfix®) or dried maize silage. Half of the turkeys (n = 13) in each treatment were additionally exposed to corresponding wet (27% DM) litter, in adjacent separate boxes, daily for 8 h throughout the experimental period. The wetted maize silage had to be changed weekly (not intended) due to the fact that it became mouldy after 5 - 7 days of wetting. For more details: see Youssef et al. [18]. Experiment 2: The turkeys were fed either on a control, high biotin, high Zn or MOS diet. The control diet was formulated to contain the required amounts of biotin (300 µg/kg) and Zn (50 mg/kg), whereas the high biotin and Zn diets were designed to contain about 2000 µg biotin/kg and 150 mg Zn/kg, respectively. Additionally, the MOS diet was formulated to contain mannan oligosaccharides (Bio-Mos®) at a concentration of 1 % of the diet but with the same amounts of biotin and Zn like the control diet. The birds were housed on wood shavings throughout the experiment. As in the previous experiment, half of the turkeys in each treatment were additionally exposed to wet (27% DM) litter for 8 h/d throughout the experiment. For further details: see Youssef et al. [19].

In both trials, the depth of the litter at the start of treatments was identical (approximately 4 cm) in both dry and wet (before application of water) pens. The wet litter was experimentally maintained at a dry matter (DM) content of about 27% by adding water (every 2 or 3 days) as required. The foot pads of all birds in each trial were examined on d 0, 7, 14, 21 and 28 of the treatment and assessed for external lesions according to the scoring system of Mayne et al. [15] which ranged from score 0 (normal skin) to score 7 (> half of foot pad is necrotic). Three birds were selected from each group on d 0, then 6 birds (3 from each litter treatment) per group on d 7, 14 and 28, and 8 birds on day 21, for histopathology of foot pads using scores of Mayne et al. [15]. The results of the last 2 weeks were combined together to give more realistic data at the end of the experiment. Dry matter content of the litter was measured at the start and end of the experiment and once a week throughout the experiment. In experiment 2, biotin and Zn levels were determined in blood plasma of turkeys.

RESULTS

Experiment 1: The severity of FPD was overall much higher (> two-fold) on wet than on dry litter. Lignocellulose showed the lowest severity of FPD, but chopped straw (dry treatment) was associated with higher FPD scores (Table 1 and 2). In spite of identical diets and stocking density, the DM content in the original pens of dry litter treatments was 76.7, 83.2, 68.8 and 75.0% for wood shavings, lignocellulose, chopped straw and dried maize silage, respectively (Table 1). Accordingly, lignocellulose had the lowest moisture content (16.8 %), whereas chopped straw showed the highest (31.2 %) compared to other bedding materials. However, the litter moisture in other bedding materials was similar (about 24 %). The moisture content in the wet litter treatments was identical (about 73 %) as intended due to adding water.

Table 1: DM content (%) of the litter as well as macroscopic scores of FPD severity in turkeys housed on different litter materials over 28 days

Litter			Duration of treatment (day)					
Type	Form	DM (%)	0	7	14	21	28	21/28
			(n = 29)	(n = 13)	(n =10)	(n = 7)	(n = 3)	(n = 7)[1)]
Wood shavings	Dry	76.7 ± 3.09	0.07 ^{aA} ± 0.27	0.38 ^{aB} ± 0.42	0.70 ^{abBC} ± 0.35	1.14 ^{aC} ± 0.24	1.33 ^{a} ± 0.58	1.29 ^{a} ± 0.39
	Wet*	27.8 ± 1.39		3.58 ^{bB} ± 0.45	4.45 ^{cC} ± 0.44	5.07 ^{cC} ± 0.45	5.00 ^{a} ± 0.50	5.14 ^{c} ± 0.48
Ligno-cellulose	Dry	83.2 ± 4.63	0.07 ^{aA} ± 0.22	0.23 ^{aA} ± 0.44	0.39 ^{aA} ± 0.42	0.64 ^{bA} ± 0.48	0.50 ^{a} ± 0.50	0.64 ^{b} ± 0.48
	Wet*	27.5 ± 0.47		3.12 ^{bcB} ± 0.74	3.80 ^{dC} ± 0.54	4.00 ^{dC} ± 0.65	2.83 ^{a} ± 0.76	3.64 ^{d} ± 0.99
Chopped straw	Dry	68.8 ± 9.79	0.03 ^{aA} ± 0.19	0.54 ^{aB} ± 0.43	1.00 ^{bC} ± 0.62	2.00 ^{aC} ± 1.26	1.50 ^{a} ± 0.50	2.07 ^{a} ± 1.21
	Wet*	27.0 ± 0.95		3.46 ^{bB} ± 0.43	4.35 ^{cC} ± 0.47	4.64 ^{cdC} ± 0.38	4.50 ^{a} ± 0.00	4.64 ^{ce} ± 0.24
Dried maize silage	Dry	75.0 ± 9.21	0.07 ^{aA} ± 0.26	0.38 ^{aAB} ± 0.58	0.50 ^{abBC} ± 0.47	1.00 ^{abC} ± 0.58	1.33 ^{a} ± 0.29	1.21 ^{a} ± 0.39
	Wet* (**)	26.6 ± 1.53		2.85 ^{cB} ± 0.77	3.50 ^{dC} ± 1.05	3.86 ^{dD} ± 1.25	2.83 ^{a} ± 1.26	3.71 ^{de} ± 1.25

***** The birds were exposed to wet litter for 8h/d. ** Wet maize silage was changed weekly. [1)] Values of 4 animals (that were selected for histopathology of foot pads) on day 21 and of 3 animals on day 28 were averaged together.

[a,b] Means in the same column with different superscripts are significantly different ($p < 0.05$).
[A,B] Means in the same row with different superscripts are significantly different ($p < 0.05$).

Table 2: Histopathological scores of FPD in turkeys housed on different litter types over 28 days

Litter		Duration of treatment (day)					
Type	Form	0	7	14	21	28	21/28
		(n = 3)	(n = 3)	(n = 3)	(n = 4)	(n = 3)	(n = 7)[1)]
Wood shavings	Dry	1.33 ^{aA} ± 2.31	0.83 ^{aA} ± 0.29	1.67 ^{aA} ± 0.58	1.75 ^{abA} ± 0.96	1.67 ^{aA} ± 0.58	1.71 ^{a} ± 0.76
	Wet*		5.33 ^{aAB} ± 0.58	5.00 ^{aA} ± 0.00	5.75 ^{cB} ± 0.29	5.83 ^{aAB} ± 0.76	5.79 ^{d} ± 0.49
Lignocellulose	Dry	1.83 ^{aA} ± 2.02	0.67 ^{aA} ± 0.29	1.00 ^{aA} ± 0.87	0.63 ^{bA} ± 0.48	0.67 ^{aA} ± 0.58	0.64 ^{b} ± 0.48
	Wet*		4.33 ^{aA} ± 0.29	5.17 ^{aAB} ± 0.29	5.25 ^{cdB} ± 0.29	4.17 ^{aA} ± 0.29	4.79 ^{e} ± 0.64
Chopped straw	Dry	0.50 ^{aAC} ± 0.87	1.67 ^{aABC} ± 2.08	1.50 ^{aA} ± 0.0	3.50 ^{adB} ± 1.29	3.00 ^{aCB} ± 0.00	3.29 ^{c} ± 0.95
	Wet*		5.00 ^{aAB} ± 0.50	5.33 ^{aAB} ± 0.76	5.13 ^{cdB} ± 0.25	5.83 ^{aAB} ± 0.29	5.43 ^{de} ± 0.45
Dried maize silage	Dry	1.33 ^{aA} ± 2.31	1.67 ^{aA} ± 2.08	1.17 ^{aA} ± 0.58	1.88 ^{abA} ± 0.85	2.17 ^{aA} ± 1.61	2.00 ^{ac} ± 1.12
	Wet* (**)		4.83 ^{aAB} ± 0.76	5.67 ^{aAB} ± 0.76	5.38 ^{cdB} ± 0.48	4.50 ^{aAB} ± 1.32	5.00 ^{de} ± 0.96

***** The birds were exposed to wet litter for 8h/d. ** Wet maize silage was changed weekly. [1)] Values of 4 animals on day 21 and of 3 animals on day 28 were averaged together. [a,b] Means in the same column with different superscripts are significantly different. [A,B] Means in the same row with different superscripts are significantly different ($p < 0.05$).

Experiment 2: The high dietary levels of biotin or Zn reduced significantly the severity of FPD on dry litter (25% moisture), but not on wet litter (73% moisture; Table 3 and 4). The DM content of the litter in the original pens was not affected by the diet and the values were mostly identical (about 75%) between the different groups. Also, the DM content of the wet litter was always similar (about 27 %) by adding the water as required. The plasma level of biotin or Zn were significantly higher in birds fed the high biotin or Zn diet, respectively, compared to the others.

Table 3: DM content (%) of the litter as well as macroscopic scores of FPD severity in turkeys housed on dry or wet litter and fed different experimental diets over 28 days

Diet	Litter		Duration of treatment (day)					
			0	7	14	21	28	21/28
	form	DM (%)	(n = 29)	(n = 13)	(n =10)	(n = 7)	(n = 3)	(n = 7)[1)]
Control	Dry	75.2 ± 6.59	0.0 ^{aA} ± 0.0	0.81 ^{aB} ± 0.56	1.60 ^{aC} ± 0.46	2.00 ^{aBC} ± 0.91	1.67 ^{a} ± 0.76	2.00 ^{a} ± 0.91
	Wet*	27.8 ± 0.32		4.15 ^{bB} ± 0.69	5.10 ^{cC} ± 0.39	5.71 ^{cD} ± 0.27	5.83 ^{a} ± 0.29	5.79 ^{c} ± 0.27
Biotin ↑	Dry	75.7 ± 5.17	0.0 ^{aA} ± 0.0	0.69 ^{aB} ± 0.52	1.10 ^{abB} ± 0.66	1.14 ^{abB} ± 0.63	0.50 ^{a} ± 0.50	1.00 ^{b} ± 0.65
	Wet*	27.9 ± 0.16		4.04 ^{bB} ± 0.43	4.75 ^{dC} ± 0.26	5.43 ^{cD} ± 0.45	5.67 ^{a} ± 0.29	5.64 ^{c} ± 0.38
Zinc ↑	Dry	74.4 ± 6.22	0.0 ^{aA} ± 0.0	0.69 ^{aB} ± 0.43	0.85 ^{bB} ± 0.53	1.07 ^{bB} ± 0.35	0.50 ^{a} ± 0.00	0.93 ^{b} ± 0.45
	Wet*	28.1 ± 0.64		4.23 ^{bB} ± 0.60	5.20 ^{cC} ± 0.35	5.36 ^{cC} ± 0.48	5.17 ^{a} ± 0.29	5.43 ^{c} ± 0.45
MOS**	Dry	74.4 ± 5.90	0.0 ^{aA} ± 0.0	0.77 ^{aB} ± 0.53	1.45 ^{aC} ± 0.44	1.57^{abBC} ± 0.73	0.83 ^{a} ± 0.29	1.50 ^{ab} ± 0.82
	Wet*	27.8 ± 0.57		4.08 ^{bB} ± 0.28	5.15 ^{cC} ± 0.24	5.57 ^{cC} ± 0.45	5.33 ^{a} ± 0.58	5.50 ^{c} ± 0.50

***** The birds were exposed to wet litter for 8h/d.** MOS: Mannan oligosaccharides. [1)] Values of 4 animals (that were selected for histopathology of foot pads) on day 21 and of 3 animals on day 28 were averaged together.
[a,b] Means in the same column with different superscripts are significantly different ($p < 0.05$).
[A,B] Means in the same row with different superscripts are significantly different ($p < 0.05$).

Table 4: Histopathological scores of FPD in turkeys fed different experimental diets over 28 days

Diet	Litter	Duration of treatment (day)					
		0	7	14	21	28	21/28
		(n = 3)	(n = 3)	(n = 3)	(n = 4)	(n = 3)	(n = 7)[1)]
Control	Dry	0.17 ^{aA} ± 0.29	0.67 ^{aAB} ± 0.29	2.50 ^{aAB} ± 0.87	3.50 ^{aB} ± 1.08	2.33 ^{aAB} ± 0.58	3.00 ^{a} ± 1.04
	Wet*		5.67 ^{aAB} ± 0.76	5.67 ^{aAB} ± 0.58	6.00 ^{cB} ± 0.41	5.50 ^{aAB} ± 0.50	5.79 ^{c} ± 0.49
High biotin	Dry	0.17 ^{aA} ± 0.29	0.50 ^{aAB} ± 0.50	2.00 ^{aAB} ± 0.87	1.75 ^{abB} ± 0.65	1.33 ^{aAB} ± 0.29	1.57 ^{b} ± 0.53
	Wet*		5.50 ^{aAB} ± 0.50	5.50 ^{aB} ± 0.00	5.63 ^{cB} ± 0.25	6.17 ^{aAB} ± 1.04	5.86 ^{c} ± 0.69
High Zn	Dry	0.17 ^{aA} ± 0.29	1.67 ^{aAB} ± 1.04	2.33 ^{aAB} ± 1.04	1.50 ^{bB} ± 0.41	1.67 ^{aAB} ± 0.29	1.57 ^{b} ± 0.35
	Wet*		6.00 ^{aAB} ± 0.50	5.17 ^{aAB} ± 0.29	6.00 ^{cB} ± 0.71	6.00 ^{aAB} ± 0.50	6.00 ^{c} ± 0.58
MOS	Dry	0.33 ^{aA} ± 0.29	1.83 ^{aAB} ±1.89	2.83 ^{aAB} ± 1.04	3.00 ^{aB} ± 0.82	1.83 ^{aAB} ± 0.29	2.50 ^{a} ± 0.87
	Wet*		5.33 ^{aAB} ± 0.58	5.50 ^{aAB} ± 0.50	5.75 ^{cB} ± 0.50	5.33 ^{aAB} ± 0.29	5.57 ^{c} ± 0.45

***** The birds were exposed to wet litter for 8h/d. [1)] Values of 4 animals on day 21 and of 3 animals on day 28 were averaged together. [a,b] Means in the same column with different superscripts are significantly different ($p < 0.05$).
[A,B] Means in the same row with different superscripts are significantly different ($p < 0.05$).

DISCUSSION

Poor litter quality is clearly associated with the incidence of FPD [12, 13]. In this study, the high litter moisture (for only 8 h/d) had the ability to potentiate the prevalence and severity of FPD. Obviously, the severity of foot pad lesions in both experiments on wet litter was markedly higher (> 2 times) compared to dry litter. This indicates that litter moisture is the major factor resulting in the development of FPD. Similar results were observed in previous experiments [12, 13, 15], but after continuous exposure of birds to wet litter. Moreover, the prevalence of FPD paralleled high litter moisture as also reported by Bilgili et al. [3]. The severity of foot pad dermatitis began to increase when the litter contained more than about 30% moisture as also reported in previous studies [11, 12,

13]. The type of litter had a marked effect on the prevalence of FPD. Of all tested bedding materials, lignocellulose showed the lowest severity of FPD either on dry or wet litter. This could be due to high absorbing capacity as well as a quick release of water. These findings are consistent with the results of Berk [1]. In dry litter treatments (without adding water), chopped straw was associated with higher FPD scores which is probably due to lower water evaporation and caking formation [3], resulting in a higher moisture content in this litter. Also, many studies reported that chopped straw was associated with the highest FPD severity scores in broilers [3] and in turkeys [6]. Based on these results, the ability of litter to bind and/or release water (to air) as quickly as possible seems to be a very important factor in the etiology of FPD. The physical structure of the litter either soft (lignocellulose) or with sharp edges (chopped straw) may also contribute in lowering or increasing the severity of FPD. The FPD scores on wood shavings and dried maize silage were similar on dry treatments. On wet litter treatments, there was no difference in foot pad scores between wood shavings, chopped straw or wet maize silage (histologically only). The external FPD scores on artificially wetted maize silage decreased which could be due to obligatory change of this litter per week (as a result of mould growth) or due to low pH and lactic acid content which might have bactericide effects [4].

High dietary levels of biotin or Zn could help to reduce the incidence of FPD. However, the effects of these nutrients appear to depend largely on DM content of the litter. As observed in this study, supplementing high levels of biotin or Zn reduced the severity of FPD on dry litter, but not on wet litter. It was reported that adding biotin to the diet decreased the severity of FPD in turkey poults raised on dry litter, but not in poults maintained on wet litter [8]. Many studies found a positive effect of biotin supplementation on the prevalence of FPD [5, 7, 17]. In contrast, other authors observed that high dietary biotin levels did not prevent the occurrence of FPD [10, 16]. Concerning the effect of Zn, some studies reported that dietary Zn reduced the incidence and severity of foot pad lesions [2, 9]. However, Hess et al. [9] found also no effect of Zn on the severity of FPD in birds reared in cool weather (4 – 15 ºC), indicating that the impact of Zn appeared to vary with the environmental conditions (which may affect the litter moisture). In the present study, high concentrations of biotin or Zn failed to reduce the severity of FPD on wet litter. It is possible that the effects of these additives on healing of the lesions were retarded by the vigorous effect of high litter moisture. Furthermore, the foot pad lesions on wet litter could be complicated by secondary bacterial contamination which inhibits the healing process induced by biotin or Zn.

CONCLUSIONS

High litter moisture appears to be the major factor influencing the development and severity of FPD. Exposure of birds to wet litter for only 8 h/d was sufficient to cause foot pad lesions. Lignocellulose as an alternative litter material could reduce the severity of FPD, whereas straw may increase it. High dietary levels of biotin or Zn might be able to lower the development and severity of FPD, but only on dry and not on wet litter. MOS tend also to reduce foot pad scores, but on dry litter only.

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EFFECTS OF LITTER TYPE, DIETS AND FLOOR HEATING ON THE DEVELOPMENT OF FOOT PAD DERMATITIS IN YOUNG TURKEYS

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SUMMARY

Foot pad dermatitis (FPD) is a widespread challenge affecting poultry health and welfare. The aetiology of FPD is a complex interaction of different factors. Therefore, this study set out to quantify the effects of litter type and dietary surplus levels of Na and K in young turkeys housed without or with floor heating. Two experiments were performed on 2 weeks old ♀ turkeys all over 3 weeks. **Exp. 1**: All turkeys were fed ad libitum a commercial pelleted diet. The first 2 groups were kept on wood shavings (35 % moisture) without and with floor heating, the other 2 groups on lignocellulose (35 % moisture) without and with floor heating. Half of birds in each group were housed on clean dry litter for 8 h/d in adjacent separate boxes. **Exp. 2**: All birds were housed on wood shavings. Two groups were fed a normal levels of Na and K (1.7 and 8.5 g/kg) without and with floor heating, while the other two groups were fed a surplus of Na and K (3.3 and 15.7 g/kg) without and with floor heating. Half of birds in each group were exposed for 4 h daily to wet wood shavings (35 % water) in adjacent separate boxes. In each experiment, foot pads were assessed weekly macroscopically and at d 35 for histopathological scores. Lignocellulose as litter material resulted in significantly lower histopathological FPD scores (1.4 ± 0.7) compared to wood shavings (1.7 ± 0.8). High dietary Na and K levels increased the severity of FPD (3.6 ± 1) whereas floor heating decreased it significantly. In conclusion using floor heating independent of the litter type (even with wet litter, 35 % moisture) or of high Na and K levels in the diet (despite of forced water intake) resulted in significantly reduced the severity of FPD.

INTRODUCTION

The incidence and severity of foot pad dermatitis (FPD) is of great concern to the poultry industry. FPD is a type of contact dermatitis affecting the plantar region of the feet, with lesions surrounded by a reddening of the foot pads as a first symptom, then discoloration and hyperkeratosis often in combination with erosions and necrosis of the epidermis, with deep ulcers occurring in severe cases [6]. Many factors have been implicated in the prevalence of FPD. Nevertheless, different authors have found positive correlations between good litter quality, particularly low moisture, and the incidence of FPD [6, 9]. Standing on wet litter brings the feet in constant contact with moisture and has been suggested to cause the foot pad to soften and become more prone to damage, predisposing the bird to developing FPD [6]. The first marked increase of FPD lesion was observed after exposure for 4 h/d to 35 % moisture which was nominated as "critical moisture content" [1]. Turkeys spend most of their productive life in close contact with the litter material and hence the type of litter appears to have a marked effect on the incidence of FPD [5]. The effects of litter material on FPD are thought to be due to either the physical structure (hard or soft) or the water-binding capacity (high or low) of the litter. The most common bedding material used for turkeys is wood shavings but there is currently a further litter type which can also be used namely lignocellulose (Soft Cell®). Moreover, the effect of dietary Na and K levels on water intake and excreta moisture is well documented and there is wide agreement among authors that excess of these nutrients in poultry diets increases excreta moisture [8] resulting in "wet litter conditions". In diet formulation it is easy to achieve a low Na content but using normal protein sources often results in K levels >10 g/kg diet. Therefore dietary factors such as the proportion of soybean meal and amounts of oligosaccharides have to be considered. Additionally, an important point of interest is the using floor heating. It has been noted that the prevalence of FPD for floor heating groups was 21.5 % ± 3.7 vs. 45.0 % ± 7.1 for groups not using floor heating [4]. Therefore, this study aimed to test the effects of using floor heating on the development of FPD in relation to litter type as well as to dietary factors.

MATERIALS AND METHODS

Ninety ♀ turkey poults (BUT-Big 6), one-day old, were housed in a floor pen littered with wood shavings, kept dry and clean before beginning of the experiment by daily removing the upper layers of the litter and replacing it with fresh dry litter. At the beginning of the experimental period (d 14) ten birds were sacrificed for foot pads histopathological assessment. The remaining birds 80 in total were then divided into 4 equal groups housed in a floor pen (1.50 m x 1.32 m) over a period of 3 weeks. **Exp. 1**: The first 2 groups were kept on wood shavings (35 % moisture) without and with floor heating. The other 2 groups were housed on lignocellulose (Soft Cell®, Agromed Austria GmbH) of 35 % moisture without and with floor heating. In each group the depth of the litter material was approximately 4 cm over the floor (5 kg/m^2 for wood shavings vs. 10 kg/m^2 for lignocellulose), which was necessary to the experimental design. The wet litter was experimentally maintained by adding water as required. The temperature at litter surface was about 35 °C in the floor heating group vs. 25 °C in the groups without floor heating. Half of the birds in each group (n = 10) were housed daily for 8 h in adjacent separate boxes where the litter was kept as clean and dry as possible during the experimental period. **Exp. 2**: Each floor pen was littered with wood shavings (1 kg/m^2; 86.8 % ± 0.3 DM). Throughout this period, the first 2 groups were fed on normal levels of Na and K (1.6 and 7.8 g/kg diet, respectively) without and with floor heating; two groups were fed on surplus level of Na and K (3.1 and 15.3 g/kg diet, respectively), without and with floor heating. Half of the birds in each group (n=10) were additionally exposed to wet litter (35 % moisture) for 4 h/d in adjacent separate boxes. External assessment of foot pads was done at d 14, 21, 28 and 35. At d 35 all birds were killed for foot pads histopathological scoring. The foot pads scoring in each experiment were recorded externally on a 7-point scale (0 = normal skin; 7 = over half of the foot pad is covered with necrotic scales) and histopathologically on a 7-point scale (0 = normal epidermis; 7 = more than one rupture of the epidermis) according to [7].

RESULTS

The experimental groups were generally healthy and no diseases or mortalities were found throughout the experimental period. All birds were given a coccidiostat in the feed (Exp.1) and in the water (Exp.2). No growth-promoting substances were used in any group, and no birds were otherwise medicated. At the beginning of the experiment (d 14) there were no alterations of foot pads for all birds. **Exp. 1**: Using floor heating resulted in significantly lower external (0.86 ± 0.3) and histopathological (1.1 ± 0.3) FPD scores of litter in comparison to those scores in groups without floor heating (Table 1). Using lignocellulose as a litter material resulted in a trend lower FPD external scores (1.36 ± 0.9) vs. (1.50 ± 0.82) for birds housed on wood shavings (Table 1). Providing dry clean litter in each group 8 h/d resulted in significantly lower FPD scores (1.2 ± 0.6). **Exp. 2**: The "experimental diets" contained about twice the concentrations of Na and K in the control diet. Furthermore, both diets were almost identical in other nutrients (crude ash, protein, amino acids and trace elements) as well as in energy density. Table 2 shows that feeding the high Na and K diet resulted in significantly higher external (3.65 ± 1) and histopathological FPD scores (4.26 ± 1.2) in comparison to those being fed normal Na and K levels. Furthermore, using floor heating resulted in significantly lower external (2.36 ± 0.5) and histopathological (2.76 ± 0.5) FPD scores compared to groups without floor heating.

Table 1. Development of external and histopathological foot pad scores of young turkeys influenced by three factors variance analyses Exp. 1 (Mean ± SD) as observed by [2]

criteria	treatment	day (duration of treatments)/FPD scores			
		external			histopathology
		21 (7) (n=40)	28 (14) (n=40)	35 (21) (n=40)	35 (21) (n=40)
floor heating	-	0.45 ± 0.55	0.85 ± 0.62 ^{A}	2.00 ± 0.87 ^{A}	2.13 ± 0.85 ^{A}
	+	0.20 ± 0.35	0.53 ± 0.43 ^{B}	0.86 ± 0.27 ^{B}	1.10 ± 0.28 ^{B}
litter material	wood shavings	0.40 ± 0.53	0.78 ± 0.59	1.50 ± 0.82	1.76 ± 0.85 ^{A}
	lignocellulose	0.25 ± 0.41	0.60 ± 0.49	1.36 ± 0.89	1.43 ± 0.78 ^{B}
exposure to wet litter (h/d)	16	0.25 ± 0.39	0.58 ± 0.50	1.21 ± 0.62 ^{A}	1.38 ± 0.55 ^{A}
	24	0.40 ± 0.54	0.80 ± 0.58	1.65 ± 1.00^{B}	1.80 ± 0.99 ^{B}

$^{A.B}$ Means in the same column within each criteria with different superscripts are significantly different ($p < 0.05$)

Table 2: Development of external and histopathological foot pad scores of young turkeys influenced by three factors variance analyses Exp. 2 (Mean ± SD) as observed by [3]

criteria	treatment	FPD scores at day (duration of treatments)			
		external			histopathology
		21 (7) (n=40)	28 (14) (n=40)	35 (21) (n=40)	35 (21) (n=40)
Na, K diet	normal	1.31^{A}± 0.61	1.71^{A}± 0.53	2.48^{A}± 0.78	2.90^{A}± 0.74
	high	1.70^{B}± 0.88	2.68^{B}± 1.14	3.65^{B}± 1.03	4.26^{B}± 1.25
floor heating	-	1.91^{A}± 0.81	2.81^{A}± 1.02	3.77^{A}± 1.0	4.40^{A}± 1.16
	+	1.10^{B}± 0.48	1.57^{B}± 0.46	2.36^{B}± 0.58	2.76^{B}± 0.57
exposure to dry/wet (h/d)	24/0	1.43 ± 0.75	2.17 ± 1.15	2.97 ± 1.19	3.46^{A}± 1.42
	20/4	1.58 ± 0.81	2.21 ± 0.85	3.16 ± 0.97	3.70^{B}± 0.99

^{A,B}Means in the same column within each criteria with different superscripts are significantly different ($p < 0.05$)

DISCUSSION

The birds' health and welfare could be influenced by direct contact of foot pads with the litter
and hence the development of FPD. Using lignocellulose resulted in lower FPD scores than wood shavings thus indicating that the physical form of litter either soft (lignocellulose) or sharp edges (wood shavings), may contribute to decreasing or increasing the incidence of FPD. Our findings tally with those of [5]; [9] who observed that turkeys housed on lignocellulose had a lower incidence of FPD than those housed on hard wood shavings which could be attributed to the higher absorbing capacity and also quickly release of water. Providing dry clean litter 8 h/d resulted in markedly decreased the severity of FPD in groups without floor heating. The significant effect of using floor heating on FPD scores could be due to the litter becomes dry as fresh litter or could be due to floor heating leading to "warm foot pad" causing vasodilatation of the blood vessels, increasing the blood flow and promotion healing. On the other hand with the absence of floor heating the litter is quite cool and might leading to blood vessel constrictions in the foot pad with a "cold wet foot pad". The source of warming in turkey houses is hanged from above the pens; so the upper surface of litter will be warm but cold from the bottom of the floor is creeping "upstairs". Thus, FPD can be kept at a minimum if proper litter management is practised. Based on the feed composition, Na and K levels play a major role, due to increased water intake and moisture in the litter predisposing the birds to FPD. Feeding high Na and K diet and the absence of floor heating resulted in significantly higher FPD scores. This could be explained as feeding a high Na and K diet leads to high water intakes and consequently high litter moisture. Our results tally with that of [8] who found that an excess of dietary sodium and potassium leads to wet litter, results in increase the severity of FPD. Despite of forced water intake by feeding high Na and K levels, the litter became drier when floor heating was in use.

CONCLUSIONS

Using floor heating even with wet litter (35 % moisture), independent of the litter type resulted in reduced severity of FPD compared to groups with absence of floor heating. Additionally, using lignocellulose as a litter material resulted in lower FPD compared to wood shavings. Keeping litter dry and "warm" could be achieved by using floor heating which considered as a practical step to enhance animal health and welfare. Despite of forced water intake the litter became dryer when floor heating was in use. Therefore, dietary Na and K levels but also floor heating affected FPD via litter moisture markedly.

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WATER SUPPLY FOR PEKIN DUCKS VIA MODIFIED BELL DRINKERS – EFFECT ON HEALTH AND WATER QUALITY

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SUMMARY

German duck farmers are sceptical about the implementation of the recommendations on Pekin ducks (Anas platyrhynchos) made by the Standing Committee of the European Convention for the Protection of Animals Kept for Farming Purposes (1999). Hygienic and economic considerations can be held responsible for this attitude. Concerning hygiene and health parameters, they either improved or remained unchanged during this study. Although water samples taken from bell drinkers had as well a much higher total germ count as higher count of *Enterobacteriaceae*, a negative impact on the health of the Pekin ducks could not be spotted during the study. Solely the rate of foot pad dermatitis increased as a result of using bell drinkers. It can, however, be assumed that this can be prevented by adequate drainage. Concerning water associated health parameters, the ducks during the test trials had cleaner plumage, less nostril congestions and fewer eye infections than in the control trials with solely nipple drinkers.

The general argument that the use of open water drinking systems is adverse to animal welfare and health cannot be confirmed. From ethological and health viewpoints, the AquaDuc T® bell drinkers are a suitable possibility to offer ducks open water under farm conditions.

INTRODUCTION

In its recommendations concerning Pekin ducks (Anas platyrhynchos), the Standing Committee of the European Convention for the Protection of Animals Kept for Farming Purposes (1999) requires, that ducks with no access to bathing water must be provided with water resources that allow them to take in water with their beaks, to put their heads under water and to splash water over their bodies. Pekin ducks for meat production in Germany are currently held without access to open water, mainly out of practicable and hygienic reasons. Nipple drinkers are the most commonly utilized drinking system for these water birds. The fulfilment of species specific behavior is not possible or mere in a reduced or modified manner. Currently no legally recognized standards exist for the housing of Pekin ducks. Therefore further research is required to design open water drinking systems that preserve the requirements for a hygienic and economic meat production under animal friendly conditions.

Based on previous results on this subject of the Chair of Animal Welfare, Ethology, Animal Hygiene and Animal Housing of the Ludwig-Maximilians-University, Munich and the Bavarian State Research Center for Agriculture (LVFZ) in Kitzingen, the variety with the modified bell drinkers was chosen to be brought into field testing. These drinkers are now distributed commercially under the name "AquaDuc T®" by the company Big Dutchman International GmbH (Vechta, Germany).

The aim of this study was to investigate the suitability of the "modified bell drinker" as a species-appropriate water supply for Pekin ducks under farm condition.

ANIMALS, MATERIAL AND METHODS

For this study Cherry-Valley-Pekin ducks (Wichmann GmbH, Molbergen-Ermke, Deutschland) were housed on three duck farms (7.100 to 13.500 ducks/unit), with a stocking rate of 19.9 to 20.5 kg/qm. For the time period of 37 to 47 days all ducks were fattened on straw bedding in cage-free husbandry. On the incline side of all three duck farm buildings a complete AquaDuc T® system was installed (approx. 250 ducks/drinker). Each bell drinker had a diameter of 45.3 cm and an adjusted water-level between 8-10 cm. This drinking system was offered to the ducks during a daily time period of six hours (in addition to 24 hours offered nipple drinkers), starting at an age of 25 days until slaughter. Five to eight mast periods per trial were examined on each farm, while test trials (plus modified bell drinkers) and control trials (exclusively nipple drinkers) took place alternately. During the mast periods the farms were visited twice in a defined time frame (1st visit during day 28-32, 2nd visit during day 35-39). Per visit 100 randomized ducks underwent an animal health examination primarily concerning the appearance of nostril congestions, eye infections and foot pad dermatitis. Water samples for microbiological analysis were taken from the bell drinkers, the nipple drinkers and if available from dripping pans and wells. In addition the dust concentration and ammonia content in the air was measured on defined points. The study was accompanied

by video observation (six to twelve cameras/farm) to include animal behavior.

RESULTS

The quantitative analysis of the average total germ count and number of *Enterobacteriaceae* showed, that the nipple drinking system revealed the best results, with a total germ count of 10.950 ± 1.583 CFU/ml (n = 226) and an *Enterobacteriaceae* number of 113 ± 30 CFU/ml (n = 187). In the samples of the bell drinkers, a total germ count of 3.955.864 ± 877.640 CFU/ml (n = 40) and an *Enterobacteriaceae* number of 14.763 ± 2.459 CFU/ml (n = 33) was found. The results of the microbiological analysis of the drip pans show, that they were frequently contaminated with feed residues, feathers and dust particles. The total germ count here was revealed to be 5.174.412 ± 564.137 CFU/ml (n = 62) while the *Enterobacteriaceae* number was 47.301 ± 11.057 CFU/ml (n = 44).

Therefore the lowest average results of *Enterobacteriaceae* were found in the samples taken from the nipple drinkers, followed by the bell drinkers and the dripping pans. With regard to the qualitative analysis of the water samples for *Salmonellae*, it was possible to isolate *Salmonellae* out of all offered drinking system varieties. The most frequently found strains were the Serovars *S. choleraesuis* (ten times), *S. arizonae* (three times) and *S. kottbus* (twice).

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Table 1: Farm independent survey over the average Enterobacteriaceae in CFU/ml (Description of the bioburden; n = number of samples; ND = nipple drinkers, DP = drip pans (one farm), BD = bell drinkers, SEM = Standard Error of the Mean)

	ND	**DP**	**BD**
n	187	44	33
Average ± SEM	113 ± 30	47.301 ± 11.057	14.763 ± 2.459
Median	10	26.500	10.075
Min.	0	0	849
Max.	3.500	430.000	658.000
Statistical Significance	ND vs. BD: p<0.001 ND vs. DP: p<0.001 DP vs. BD: p=0.656		

The mean values (± SEM) of the measured dust concentrations varied on the farms between 0.53 ± 0.01 mg/m^3 and 1.08 ± 0.21 mg/m^3. There is a noticeable tendency, that the dust levels descent during the test trials when bell drinkers are offered. The dust levels in poultry (PETERMANN, 2006) and duck husbandry (ZUCKER et al. 2005) were not reached during this study neither in control nor in test trials.

The average ammonia concentrations were measured between 4.33 ± 1.21 ppm and 8.76 ± 0.24 ppm and underlied therefore the recommended 10 ppm in animal husbandry at all times.

The health evaluation revealed a hyperkeratosis rate of the foot pads in over 80 % of the examined ducks on every farm – regardless of the type of trial. On the whole, it was not possible to record a significantly higher or lower hyperkeratosis rate during test trials. On the contrary, the evaluation of foot pad dermatitis (FPD) depended on the type of trial. The likelihood of FPD decreased on all three farms during test runs. A harmful impact could neither be found concerning live weight at the end of the mast (control trial: 3.0 kg/duck; test trial: 2.99 kg/duck; SE: 0,388) nor the total average mortality in percentage (control trial: 5.05 %; test trial: 4.75 %).

During the testing phase, the drinking activity ("drinking" and "cleaning in the drinking area") increased significantly (p<0,001) up to 90 % during the period of access to the bell drinkers, whereas the nipple drinkers were used less during this period and considerably fewer animals rested. During the course of the fattening, drinking behavior increased in all trials. A modified form of bathing behavior could be observed at the bell drinkers where ducks scooped water onto their plumage with head and throat, and then interrupted this routine to clean their plumage.

DISCUSSION

As well the total germ count as the total count of Enterobacteriaceae in CFU/ml were continuously higher in the bell drinker samples than in the nipple drinkers, but at all time lower than in the drip pans. Most of the Salmonella serovars were found in the drip pans. In certain geographical areas with a high content of calcium carbonate in the drinking water, not regularly cleaned drip pans tend to build up a deposit where all kinds of bacteria find a medium. A negative impact on the health of the Pekin ducks could not be spotted during the study.

A time limited access to the bell drinkers can not only result in hygienic but also in economic benefits. The moisture in the straw bedding can therefore be contained, which showed a positive effect on humidity and content of ammonia in the air. A daily import of fresh litter, as realized in this study, and a good adjusted ventilation system are essential to influence the content of air ammonia and dust level in a positive way.

According to MAYNE (2005), the reasons for the appearance of footpad dermatitis are complex. The two

most likely causes are moist litter and a lack of biotin. When installing bell drinkers, therefore, it is essential to ensure that there is a best possible water drainage installed.

Otherwise the ducks with access to bell drinkers almost always scored significantly better ($p<0,05$) concerning water associated health parameters. The ducks in the test trials had cleaner plumage, less nostril congestions and fewer eye infections than in the control trials with solely nipple drinkers.

All results together show that Pekin ducks clearly preferred the modified bell drinkers "AquaDuc T" over the nipple drinkers. They allow the animals to dunk their heads, to drink and strain the water in a species appropriate manner, to groom their plumage with water and to clean their beaks and eyes.

CONCLUSIONS

According to the results, the modified bell drinkers are, from ethological and health viewpoints, a suitable possibility to offer ducks open water under farm conditions. Out of economic reasons, it is important to reduce the daily access to the modified bell drinkers to a limited time span, to lower the costs for water, labor and additional litter. A four to six hour access seems acceptably. An animal-bell drinker ratio of approx. 250:1 can be recommended.

It is technically possible to install the systems in new built farms where it is possible to include adequate drainage and appropriate slurry facilities into the architect's plan. Already existing facilities would have to be remodelled to ensure proper drainage of waste water.

The general argument that the use of open water drinking systems is detrimental to animal welfare and health cannot be confirmed. The AquaDuc T® bell drinker system complies with the requirements of animal-friendly water supplies and offers the possibility to implement in practice the recommendations on Pekin ducks (Anas platyrhynchos) made by the Standing Committee of the European Convention for the Protection of Animals Kept for Farming Purposes (1999).

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Block 2

PRECISION LIVESTOCK FARMING: SCIENTIFIC CONCEPTS AND COMMERCIAL REALITY

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SUMMARY

Precision Livestock Farming (PLF) is potentially one of the most powerful developments amongst a number of interesting new and upcoming technologies that have the potential to revolutionise the livestock farming industry. If properly implemented, PLF or Smart Farming could (1) improve or at least objectively document animal welfare on farms, (2) reduce GHG emission and improve environmental performance of farms, (3) facilitate product segmentation and better marketing of livestock products, (4) reduce illegal trading of livestock products and (5) improve the economic stability of rural areas. However, there are only a few examples of successful commercialisation of PLF technologies introduced by a small number of commercial companies which are actively involved in the PLF commercialisation process. To ensure that the potential of PLF is taken to the industry, we need to: (1) establish a new service industry, (2) verify, demonstrate and publicise the benefits of PLF, (3) better coordinate the efforts of different industry and academic organisations interested in the development and implementation of PLF technologies on farms, and (4) encourage commercial sector to assist with professionally managed product development.

INTRODUCTION

Efficient information management is very much part of profitable livestock production (Thysen, 2000; Lewis, 1998). The main purpose of precision livestock farming (PLF) is to improve the efficiency of production, while increasing animal and human welfare, via applying advanced IT, targeted resource use and precise control of the production process (Chamberlain-Ward, 1998; Cumby and Phillips, 2001). The main purpose of this article is to briefly review the current scientific state of art and, more importantly, the commercialisation aspects of PLF technologies with the view to facilitating more effective technology transfer between scientific and commercial organisations. By doing so, we hope that PLF will not remain simply "the engineers' daydream" but becomes the "animals' friend and the farmers' panacea" (Wathes *et al.*, 2008)

SCIENTIFIC ISSUES

Scientific concepts and principles of PLF

Precision farming through the adoption of electronic data collection, processing and application has the potential to improve production efficiency and reduce costs (Banhazi and Black, 2009; Banhazi *et al.*, 2009b; Banhazi and Lewis, 2009), as well as increase animal and human welfare. There is currently an abundance of information available to livestock managers, but it is not generally structured in a way that can be applied readily. For example, a survey of producers raising beef from pastures in southern Australia showed that over 400 pieces of information could be relevant for their farms. The information comes from many sources including academic organisations, government advisors, producer magazines, newspapers, radio, television and other media sources, company technical advisers and other producers. The information is frequently dispersed and sensationalised and not in a form that can be readily applied on farms. Consequently, farm managers tend to adopt procedures in areas where they have most interest or in which they believe they have most expertise and neglect many other areas that are also essential to drive productivity and profitability.

Furthermore, many producers perceive that adopting highly productive management systems involve increased risk. The perceived risks include the risk of financial failure because of unforseen environmental or market circumstances, damage to the farm infrastructure such as soils and pasture, compromises to animal health and welfare and the risk of increased stress on them from managing an intensified system. These risks are real. Thus, it is important to develop a management system that ensures only the most essential procedures are carried out, they are all carried out correctly and consistently, and in a way that controls risk. Such a system based on the Hazard Analysis Critical Control Point (HACCP) method has been developed for grazing beef enterprises in Australia (Black and Scott, 2002) and forms a model that can be applied to any other animal industry. The principles behind the system are as follows:

(a) Identify those processes which truly have a major effect on productivity, profitability and/or sustainability. These include the actions that if not carried out correctly will substantially reduce the viability of the enterprise. These processes should cover every aspect of the enterprise from strategic planning of the business structure through all aspects of production to sale of the product. It is important to reduce the number of 'essential processes' to only those that will have a major impact on the enterprise if not carried out correctly. The number must be manageable because all are to be consistently applied over time. In the example with grazing beef enterprises in southern Australia, only 29 processes across the entire enterprise were considered to be essential for maximising profitability and sustainability.

(b) Identify, for each essential process, the farm or market variables that must be measured to ensure that each essential process is being carried out correctly. Establish the frequency at which each measurement must be made and set maximum and minimum limits for each measured variable to ensure that the process will continually remain within the optimum range and will not get out of control.

(c) Apply the most profitable pre-determined corrective action whenever measurements are outside these limits. The process of having predetermined actions when the measurement limits are breached substantially reduces the stress level for the manager because the plan of action and when to apply it has already been established and the consequences are known. Partial or whole enterprise budgets are an important tool for selecting the most economically viable corrective action.

(d) Establish Standard Operating Procedures for individual enterprises for each essential process to ensure that, under normal circumstances, the critical measured values will remain within the set limits. Such a process is important so the manager can 'go on leave' knowing that each critical process in the enterprise will be measured and carried out correctly by staff. Both high level (annual calendar and daily actions) and low level (how to do a specific task) procedures are essential.

(e) Provide the tools necessary for making the essential measurements, interpreting the measurements and deciding on the most profitable corrective action. These tools are an essential component of the 'package' and must be provided as part of any adoption package. There is a need also to train staff in these tools.

The fact that humans tend to become lax with the application of repetitive tasks is one of the main reasons for failure of systems like the one outlined above. Recording and checks of measurements and actions by other people is one way to help overcome the problem. The difficulty faced by many rural industries in industrialised countries is obtaining and retaining adequately trained and motivated staff. The lack of good staff frequently contributes to failure of well planned adoption programs.

The major role for Precision Livestock Farming is to simplify this process of collecting processing and analysing data so that the farm manager is presented with solutions, not problems (Berckmans, 2011). Advances in the application of the outlined procedure for adoption of essential enterprise processes will depend more and more on the automated measurement, interpretation and control of these processes. The procedure should include automation of all measurement systems, interpretation of the measurements, identifying when critical measurement limits are breached and built-in automatic control systems for each essential process to bring it back inside the acceptable limits. A useful example of the type of change needed within the animal industries comes from the world steel industry. In the 1950's, all tasks were undertaken by humans compared with today when the whole process is controlled electronically, almost all manual work tasks are automated and monitored centrally. This is a vision for Precision Livestock Farming, where animal welfare, environmental sustainability, productivity and profitability are all at an optimum using electronic measurement, interpretation and control.

Integration of traceability with PLF

Traceability within livestock management has largely been limited to movement and disease control applications such as the European passport system for cattle, the PigPass for pigs in Australia and the movement permit across state/provincial borders in Malaysia and Vietnam. Virtually no attempts have been made to unlock the economic benefit that traceability can have for livestock enterprises. There are a number of objective reasons why the integration of traceability and PLF has not progressed further, which include (1) availability of easy to implement and affordable automated identification systems, (2) overemphasised privacy concerns related to data captured on-farm, (3) inconsistent offering of traceability products to farmers and (4) too much focus on particular numbering technologies (simple numbering, barcode, RFID).

The most interesting example of the integration of traceability with PLF in our opinion is the exchange of information along the feed – animal – food chain. This information exchange (Figure 1) has a number of benefits.

- Feed and feed input providers can greatly improve the composition of their products if they have access to slaughterhouse statistics resulting from the feeding profiles applied on the farm.
- Farms can use such a system for the selection of the right feed (or right feed provider). They can also

optimise their feed use/intake from the statistics of other farms on the network
- Abattoirs can use the system as a basis for cooperation with farms to produce and source more animals on weight and conformation specification.
- Industry statistics are a very important tool for both governments and the industry itself to steer the sector. Reliable statistics can be used for political decision making, benchmarking, lobbying and business decision making.

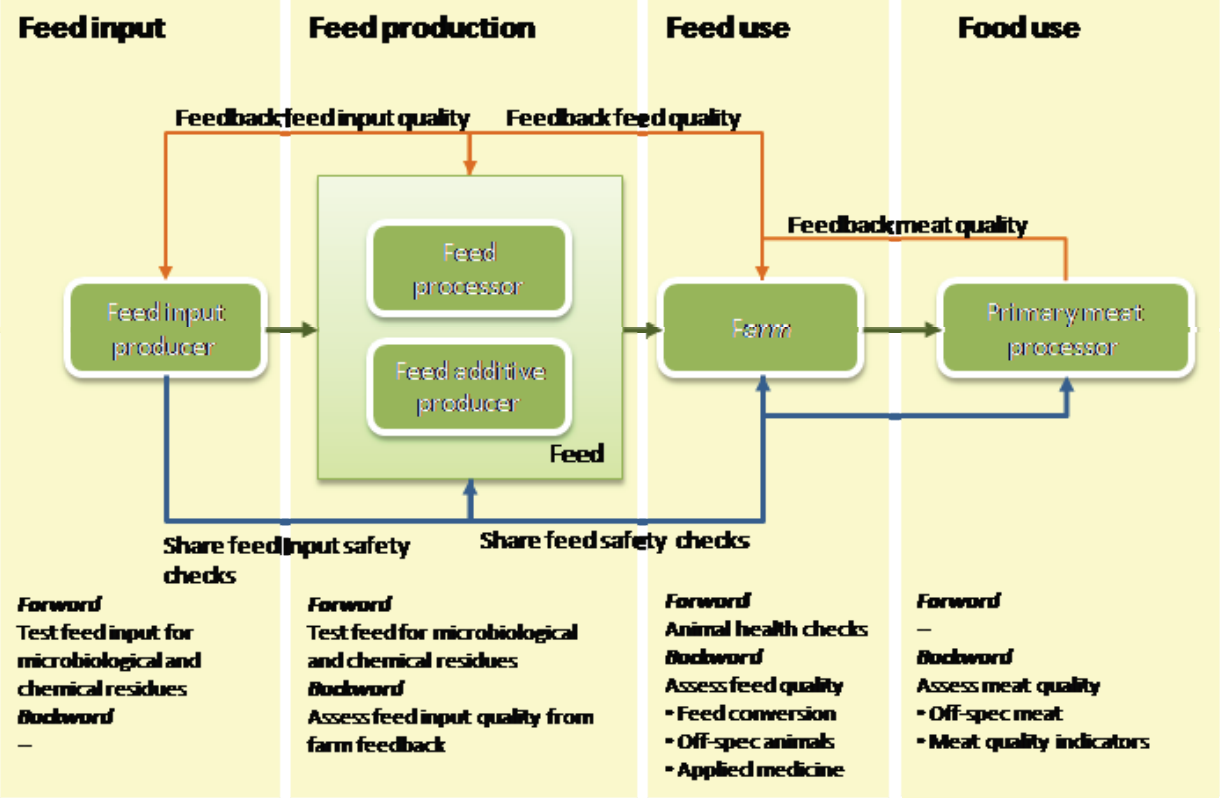

Figure 1: traceability systems and linkages with PLF (Lehr, 2011a)

Scientific and technological developments

Many of the early PLF developments were predominantly instigated in Europe/UK. Early pioneers of the PLF concept were researchers at the Silsoe Research Institute, UK and Leuven University, Belgium. Additional developments took place in other EU countries, such as Germany, Denmark, the Netherlands, Finland and the Volcani Research Centre, Israel (Devir *et al.*, 1997; Halachmi *et al.*, 1998). In 2002 Australian PLF developments started with assistance provided by scientists based at the UK and Belgium (Banhazi *et al.*, 2003). Most pig industry related PLF developments were lead by scientists in South Australia (Banhazi *et al.*, 2007; Banhazi and Black, 2009) while researchers at USQ developed PLF applications for the beef industry. CSIRO researchers extensively investigated virtual fencing technologies (Bishop-Hurley *et al.*, 2007; Umstatter, 2011). In the table 1 a number of publications and resultant technologies are presented as an example of PLF tools developed over the years without aiming to accurately review of all developments over the years.

Table 1: examples of PLF technologies developed over the years

Reference	Technology/tools
(Exadaktylos *et al.*, 2011)	Improved egg incubators via synchronisation of hatching
(Gates *et al.*, 2001)	Intelligent ventilation control in livestock buildings
(Schofield, 1990; Brandl and Jorgensen, 1996; Wang *et al.*, 2008; Banhazi *et al.*, 2009b)	Weight estimation of pigs via machine vision tools
(Maltz *et al.*, 2003)	Dairy management to maximise profit
(Niemi *et al.*, 2010; Banhazi *et al.*, 2009a)	Improving profitability via precision feeding for pigs
(Frost *et al.*, 2000)	Sensor placement robot for pigs
(Mottram, 1997; Stewart *et al.*, 2007)	Cattle monitoring system
(Bull *et al.*, 1996)	Udder health and hygiene monitoring in dairy cattle
(Chao *et al.*, 2000; Park *et al.*, 1998; Park *et al.*, 2007)	Poultry carcass inspection
(Cronin *et al.*, 2008)	Automated egg counting and identification
(Doeschl-Wilson *et al.*, 2005)	Carcass composition prediction for pigs
(Hsieh *et al.*, 2011; Ruff *et al.*, 1995; Zion *et al.*, 2007)	Automated fish sizing and sorting
(Shao and Xin, 2008; Wouters *et al.*, 1990)	Improved thermal control for pigs via machine vision
(Guarino *et al.*, 2008; Chedad *et al.*, 2001; Moshou *et al.*, 2001)	Cough recognition in pigs

Recent developments in communication technology through mobile phone technology, telecoms and the internet offer a huge potential benefit to the design, application and value of PLF. Whilst independent applications on individual farms may be desirable to some customers, the advantages of centralised data collection, processing, management and reporting are significant. Data collected by sensors on the farm can be sent, by FTP for example, to a central site for processing, storage and reporting. The farm manager is saved this task and his time and expertise is instead available for farm and animal husbandry tasks. The centralised processing should supply him with only the data pertinent to his daily needs, with more detailed reports available as required, including through the centralised database the comparative performance of his unit, for example. In short, the benefits offered by a good PLF system should be obvious to the user and ideally should reduce his management workload, not increase it (Lehr, 2011b).

COMMERCIAL ISSUES

Examples and principles of commercialising PLF technologies

In livestock production there are already a few examples of commercialisation of PLF techniques. Good examples of commercial adoption of PLF techniques include the use of robotics in dairying, measurement of water usage, egg counting, bird weighing, better control of environment in poultry houses, computerised feed systems, climate control, automated disease detection, (Guarino *et al.*, 2008) growth measurement and real-time production site data capture in piggeries. The recent EU sponsored project BrightAnimal project (Lehr, 2011a) has looked for evidence of PLF technologies in laying hens, pigs, diary and aquaculture fish used in a commercial environment in a number of countries, including Estonia, Denmark, Norway, United Kingdom, Australia, Malaysia, Vietnam and South Africa. In general, there was limited evidence of commercial PLF products used on farms. As expected, farmers in techno-friendly countries, like Estonia, are more inclined to use technology to reduce their dependency on hard-to-get (and expensive) workers and make their life a little more comfortable. However, even there the amount of technology deployed is very limited and key aspects of animal welfare or productivity are not monitored in an automated fashion routinely.

The commercialisation principles of PLF technologies need to include (1) a verification of the benefits of the PLF technique being proposed, (2) a clear communication of those verified benefits to customers, (3) identification of principle beneficiaries (i.e. operator vs. owner of the business), (4) provision of appropriate training and technical support, (5) correct specification, installation, commissioning and monitoring of the installed system. However, PLF developments have been largely spear-headed by academic organisations so far. In general, there is an inadequate engagement of commercial companies in the PLF technology development process. In order to increase the interest of suitable companies in providing services to farms, a collaboration between smaller specialist firms and larger generalist firms such as DeLaval, Fancom, Petersime etc is desirable. Transferring PLF technologies to companies that will supply and manage the systems is a significant step towards

developing commercial PLF tools/products that customers want and that can be sold with confidence.

Limitation factors of commercialisation

The greatest problem of commercialisation is the lack of a consistent service offering for farmers. Farmers are biologist by nature and only technologists ocasionally. There is a need for a service sector that will be able to (1) take care of technology components, (2) interpret data captured by sensors, (3) formulate and send simple, relevant advice to farmers on a regular basis and (4) involve users in technology developments. This service sector would need to use suitable business models that avoid high initial investment costs for farmers. Affordable monthly or annual fees might well be compatible with farmers cash flow, especially is they are linked to performance improvements or animal sales. Although farmers usually invest part of their gains in technology, it is typically machinery that they would look forward to buying (as opposed to software or sensors).

The food industry in general is a very conservative industry and with good reason. Although it is one of the largest industries world-wide, its margins are very small and its products are usually very delicate. Agriculture is in addition a fragile industry, because it depends directly or indirectly on climatic factors and seasonal demand/supply circles. In addition, even for the more adventurous farmer it is very difficult to judge the applicability of a particular technology and 'guesstimate' its benefits. In other words, an important missing element is the absence of clear cost benefit data on PLF that takes into consideration the complexity of farmers' purchase decisions. Demonstrating and verifying the economical, welfare and environmental benefits of these technologies is an essential part of the commercialisation process.

The other key limiting factor of adoption rate of PLF technologies on farms is the lack of co-ordination between researchers, developers and technology suppliers. Achieving better co-ordination between the developers and suppliers of PLF tools is very difficult, but would result in the development of better integrated systems. That in turn would results in greater commercialisation of PLF systems as integrated systems would serve the farmers better. In addition, many of the PLF "products" have actually never been 'productised' (developed into a proper 'product'); but they went directly from the lab to the farm. Only some larger firms with enough development funds have taken up PLF as their guiding principle, such as Fancom, DeLaval, Petersime and a few others.

PLF as a facilitator of progress: likely benefits and motivators of implementation

In the next 10 years it is very unlikely that PLF will revolutionise the livestock industries. However, in the next 5-10 years sensors will be deployed routinely around animals that might allow farmers to monitor effectively a range of useful parameters for all livestock species. This will enable a range of new services to be developed and implemented on farms, such as individual feeding, heat detection, health monitoring and animal localisation. Mobile robots will emerge for milking and other tasks both in the shed as well as in the open. Virtual fencing will contribute to better herd and meadow management and improved financial returns for grazing enterprises. Most farms in Europe will be computerised in 10 years time and will use software tools for their management.

PLF can greatly contribute to an objective discussion on animal welfare by providing real data to the otherwise very subjective discussion process. While PLF will not be able to necessarily resolve all welfare related questions, it will allow interested parties to detect and act upon time periods when animals were kept under sub-optimal conditions.

Green house gas emissions are going to be very important in the future and PLF can contribute to the reduction of such emissions by measuring emission and by potentially adjusting feeding, temperature and other parameters that influence the emission of gases. Farm enterprises in the supply chain are making a concentrated effort to keep animals under optimal conditions, to keep emissions down and to provide the best livestock product at the lowest possible price. PLF can assist in transporting this information to other parties within the supply chain, and ultimately to the consumer. It can facilitate more informed choices by consumers and can be the base for other business models, such as selling meat by protein contents, emitted GHG gases, food miles, or other concepts. The exchange of information on the feed-animal-food chain has a great potential for optimising livestock production. Feed producers could reap very important information from carcass composition data. Farmers could improve their feeding regime and chose the feed provider with the "best" feed for their animals. Traceability and PLF are the basis for such an information exchange. If there is a continued decline in the profitability of farms in Europe, perhaps retailers will start buying farms and require data exchange along the supply chain. Environmental control will be much improved within this time period and most farmers ten years from now will know how much GHG they emit. Driven by consumers and retailers they will be striving to reduce their emissions by capturing gases, adapting their feed and dealing better with waste. PLF will have its role in feeding strategies, perhaps linked to gas and waste production.

PLF can also contribute to the avoidance of illegal trading of livestock and livestock products. Smuggling animals is a major problem (health and financial) in countries like Malaysia. Illegal and unregistered (IUU) fishing is a billion dollar enterprise and cuts deeply into our fish banks. Misusing the available fish stock could be significantly reduced if the information chain was quicker to react.

The way forward: conclusions and recommendations

1. The principles of PLF are well established and the routine utilisation of PLF technologies could be certainly contributing to improved livestock management on farms.

2. Integrating traceability with PLF would be a positive step forward and would improve the usefulness of PLF systems.

3. A number of interesting PLF developments have occurred over the past years that have great potential to revolutionise livestock management. PLF/smart farming technologies, (if properly implemented) could (1) improve or at least objectively document the level of animal welfare on farms (2) reduce GMG emission and improve environmental performance of farms (3) reduce illegal and facilitate product segmentation/better marketing of livestock products (4) improve rural economy and stabilise rural populations.

4. However, when it comes to commercialising these technologies (1) there are only a few good examples of successful PLF technology commercialisation exist and (2) only a small number of commercial companies are involved actively in the PLF commercialisation process.

5. Thus to facilitate the proper development and implementation of PLF products on farms (1) a new service industry needs to be established to be responsible for maintenance of hardware tools and management of collected data (2) benefits provided by PLF technologies need to be independently verified under commercial farm conditions (3) development and marketing efforts of different industrial and academic partners need to be better coordinated and (4) the involvement of commercial sector in the process of professional product development needs to be facilitated.

In addition, a "Federation of PLF focused companies" might be created with the aim of developing a "road map" document highlighting the critical steps that need to be taken to stimulate the commercial uptake of PLF/Smart Farming technologies. Such document should be based on the outcomes of a recently completed international PLF project and might be developed as part of a commercially focused PLF conference/meeting. PLF participants need to also engage their respective governments in order to secure public funds required for verification studies that would be unlikely to be financed by private companies.

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RELATIONSHIP BETWEEN ENVIRONMENTAL MICROBIAL POLLUTANTS AND MASTITIS IN EGYPTIAN BUFFALOES

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SUMMARY

The work was done on 145 Egyptian lactating buffaloes 4-5 years old. 91 milk samples were aseptically collected from mastitic animals. Also, 494 apparently normal milk samples were used for C.M.T. 28 air samples and 40 soil samples were collected from environmental of animal enclosure. All samples as well as those positive for C.M.T. were bacteriologically examined. Acutely mastitic animals showed anorexia, fever and signs of udder inflammation. Cases with chronic mastitis were recorded. The most prevalent bacterial isolates were S. agalactiae(76.5 and 92.9%) and S. aureus (52.9 and 71.4%). There is a great correlation between bacterial milk isolates and those of air and soil. Gentamycin and Kanamycin were seen to be drugs of choice for control of mastitis in buffaloes.

INTRODUCTION

Buffaloes are regarded the most important dairy animals in Egypt due to their high milk production with high fat content. The disease causes great economic losses in animal wealth [4] and zoonotic diseases to human. Air dust particles, soil and bedding materials are environmental sources of pathogenic bacteria to animals [1and8]. The objective of our work is to investigate the correlation between occurrence of mastitis in buffaloes and environmental microbial pollutants. Vitro sensitivity tests for the isolated bacteria will done.

MATERIAL AND METHODS

The work was conducted on 145 Egyptian native breed dairy buffaloes aged 4-8 years old, and belonged to some governmental and private farms in Assiut governorate. 91 milk samples were aseptically collected from mastitic animals of both farms (GF and PF). 494 milk samples were aseptically collected from normal udder quarters of both animals groups and used for CMT [3]. The milk samples positive to CMT were 52 G.F. and 43 PF.28 air samples and 40 soil samples were collected from the environment of animals enclosures. All clinical mastitis, subclinical mastitis, air and soil samples were subjected to bacteriological examination [9]. The test of antibiogram was applied on most isolated strains using discs diffusion technique of antibiotics [7].

RESULTS

The data obtained in this work were recorded in tables 1, 2, 3 and 4.

Table 1: Prevalence of bacterial mastitis among buffaloes

Animals	No. of exam. animals	No. of exam. quarters	Total no. of +ve quarters	%	Types of mastitis				Negative quarters	
					clinical		Subclinical			
					Total		Total			
					No.	%	No.	%	No.	%
Governmental Farms	80	320	95	29.7	43	13.4	52	16.3	225	70.3
Private farms	65	260	91	35	48	18.5	43	16.9	169	65

Table 2: prevalence of isolated bacteria among dairy buffaloes with mastitis

Isolated bacteria	Clinical mastitis								Subclinical mastitis			
	G.F.				P.F.				G.F. (52)		P.F. (43)	
	Acute(33)		Chronic (10)		Acute (34)		Chronic (14)					
	Frequency				Frequency				Frequency			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
S. Agalactiae	20	60.6	5	50	26	76.5	13	92.9	13	25	25	58.1
S.Dysagalactiae	0	0	1	10	3	8.8	0	0	0	0	8	18.6
S.Epidermidis	8	24.2	6	60	0	0	2	14.3	6	11.5	11	25.6
S. Aureus	12	36.4	7	70	18	52.9	10	71.4	7	13.5	10	23.3
E.coli	5	15.2	5	50	5	14.7	3	21.4	5	9.6	13	30.2
K.Pneumoniae	8	24.2	4	40	2	5.9	0	0	0	0	3	7.0
P.Aeruginosa	3	9.1	3	30	1	2.9	3	21.4	0	0	4	9.3
E.aerugenes	4	12.1	5	50	11	32.4	5	35.7	1	1.9	5	11.6
Coryn. spp.	9	27.3	6	60	8	23.5	4	28.6	3	5.8	12	27.9

G. = governmental , P. = private , F. = farms

Table 3: Occurrence of bacteria in air and soil samples in houses of dairy buffaloes

Isolated bacteria	Governmental farms				Private farms			
	Air (no.14)		Soil (no.20)		Air (no.14)		Soil (no.20)	
	Frequency		Frequency		Frequency		Frequency	
	No.	%	No.	%	No.	%	No.	%
S.Aureus	3	21.4	5	25	4	28.6	6	30
S.Epidermidis	1	7.1	3	15	1	7.1	3	15
S.Faecalis	0	0	2	10	1	7.1	3	15
S.bovis	1	7.1	2	10	2	14.3	2	10
S.Faecium	1	7.1	3	15	2	14.3	4	20
S.durans	0	0	3	15	1	7.1	2	10
Coryn.spp.	3	21.4	5	25	5	35.7	7	35
E.coli	2	14.3	13	65	3	21.4	16	80
K.pneumoniae	0	0	1	5	1	7.1	3	15
P.aeroginosa	1	7.1	2	10	1	7.1	4	20
Proteus spp.	0	0	3	15	1	7.1	2	10

Table 4: Antibiogram pattern of most bacterial isolates from mastitic buffaloes

Pathogens(No.) → / Chemotherapeutic antibiotic discs ↓	S.Agalactiae(102)	S.Dysagalactiae(12)	S.Epidermidis(33)	S. Aureus(64)	E.coli(36)	k.pneumoniae(17)	P.aeruginosa(14)	E.Aerogene(31)	Coryn. Spp.(42)
Ampicillin	36	0	12	13	0	0	0	0	4
(25 ug)	35.2	0	36.4	29.7	0	0	0	0	9.5
Cefacetrile (30ug)	48	1	17	10	3	9	0	2	8
	47.1	8.3	51.5	15.6	8.3	52.9	0	6.5	19
Choramphenicol (30ug)	62	0	24	41	0	2	0	0	11
Erythromycin (5ug)	60.8	0	72.7	64.1	0	11.8	0	0	26.2
	10	3	5	7	2	3	0	12	15
Gentamycin (10ug)	9.8	25	15.2	10.9	5.6	17.6	0	38.8	35.7
	100	10	29	56	21	51	11	8	28
Kanamycin (30ug)	98	83.3	87.9	87.5	58.3	88.2	78.6	25.8	66.7
	98	10	28	58	29	16	14	26	21
Oxolinic acid(2ug)	96	83.30	84.8	90.6	80.6	94.1	100	83.9	50
	0	0	0	0	0	0	0	0	0
Penicillin (10ug)	0	5	0	0	0	0	0	0	0
	78	41.6	18	15	8	8	0	0	3
Streptomycin (10ug)	76.5	7	54.5	23.4	22.2	47.1	0	0	7.1
	76	58.3	12	45	36	7	9	7	0
	74.5		36.4	70.3	100	41.1	64.3	22.6	0

DISCUSSION

Prevalence of clinical mastitis and subclinical mastitis varied among animals of both farms (table 1). The reported changes may attributed to the variations in hygienic management. The isolation of different types of bacteria from mastitic buffaloes showed various frequencies (table 2). Marked variations in the incidence of different bacterial isolates were reported between mastitic cases of both animal groups (GF and PF). Lower data of bacterial incidence in German dairy cows with clinical mastitis and subclinical mastitis were previously reported [5]. Incidences of isolated bacteria from other mastitic Egyptian buffaloes [2]. Were varied with our data. The different microbial incidences may be explained on basis of environmental conditions, endemic state of mastitis pathogens, animal management, resistance of animals, technique of milk sampling and handling of collected samples. Table3, showed various bacterial isolated with various frequencies in air and soil samples in enclosures of both farm animals. Our results were nearly similar to some investigators [6]. Soil might act as a reservoir of several pathogens as causative agents of diseases. Table 4, indicted that Gentamycin and Kanamycin were found to be the antimicrobial agents of choice to control mastitis in buffaloes.

CONCLUSION

The animal environment constitutes a dangerous vehicle for certain pathogens of veterinary and human importance. It has been proved that the air and soil of animal enclosures are the most important sources of many pathogenic and potentially pathogenic microorganisms. There was a great prominent correlation between mastitis and the environmental pathogens. Air inside animal enclosures should be improved. Soil of animal enclosure should be always kept clean with hygienic disposal of secretions and excretions as well as animal wastes. Teat disinfect must be carried out either immediately prior to milking or more commonly after milking. Gentamycin and kanamycin were proved to be highly effective antibiotics that can be used for treatment of mastitis in buffaloes. Culling all buffaloes with recurrent or chronic mastitis was among the most important preventive measures of control.

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UPDATE ON KOI-HERPESVIRUS – A GLOBALLY CHALLENGING AQUATIC DISEASE (Abstract)

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SUMMARY

During the last decade infection with the koi herpesvirus (KHV or cyprinid herpesvirus 3, CyHV-3) has become an emerging disease among common carp and ornamental koi carp. First described in 1997 in Germany [1], the virus has spread across the European continent, to many Asian countries, the USA, Canada and South Africa. The virus affects common carp and koi carp causing great economic losses due to high morbidity and mortality rates. Clinical overt disease has been described only for this species up today. However, the virus was detected in other fish of the family *Cyprinidae* and even in non-cyprinid fish like sturgeon and mussels [2, 3, 4, 5]. The extent of the host range is quite unclear but seems to involve more species than thought. Like mammalian herpesviruses KHV becomes latent in fish once infected. It is supposed that the viral genome is maintained in white blood cells [6]. Reactivation and shedding of the virus is possible [6] and thus new infection of native organisms.

Reliable diagnosis of KHV can only be performed on internal organs post mortem, preferentially by PCR-testing of kidney, gill, liver or spleen. However, the viral load in tissues may be very low in carriers und thus beyond limits of detection [6]. Serological testing is another diagnostic method, but no routine tests are available and methods greatly differ in sensitivity and reliability. Specific anti-KHV antibodies are supposed to last up to about one year. However, both PCR and serological testing, may fail in detecting KHV latency in fish where the viral genome is maintained for an extended period in the absence of productive infection. Thus, the detection of latent carrier fish causes great difficulties and fighting the disease remains as a challenge.

Actually, the virus has spread globally in natural water systems as well as in aquaculture farms and ornamental fisheries causing serious economic losses. The international fish trade and the common cohabitation of different fish species benefit to the on-going spread of KHV. Particularly in natural aquacultures preventing the introduction of KHV and eradication of the pathogen are of major difficulty.

To reduce the economic threat the application of a vaccine would be easily conceivable. To date, an attenuated live vaccine (KV3, KoVax Ltd,) is available in Israel but has not been approved by several countries due to its lack in efficacy and safety. The import of vaccinated koi into the EU has to be judged quite critically. Recombinant and DNA vaccines are generally considered to be the vaccines of the future to protect carp from overt clinical disease. However, vaccine development is still in progress.

Strategies and efforts in controlling and preventing the disease in aquaculture and ornamental fisheries are complex. Improved biosecurity, including quarantine to keep new introductions separate from existing stock and responsive management of aquaculture farms are pivotal measures. Further, surveillance monitoring and testing should be carried out on a regular basis at sites holding susceptible species with serology as monitoring tool in focus. Control of fish movements and suitable health attestation from suppliers especially from areas where KHV infection is widespread would be other aims to be mentioned. In Addition, more knowledge on epidemiology is needed, identifying the routes of virus transmission and the range of carrier species. Detailed knowledge on KHV provides the basis for the implementation of efficient control strategies.

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ALARIA ALATA – NEW APPROACHES FOR IDENTIFICATION AND DIFFERENTIATION OF A RE-EMERGING PARASITE

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SUMMARY

Reports on human larval alariosis in North America and repeated findings of *A. alata* mesocercariae in meat of wild boars in Europe necessitate an assessment of the parasites potential threat to public health. In order to obtain the data and information required in this respect, the development of a reliable detection method is mandatory. It is needed to determine (a) the actual prevalence of *A. alata* mesocercariae in sylvatic populations of animals with respect to their introduction into the food chain, (b) the distribution of the mesocercariae within their paratenic hosts, and (c) the level of infection, i.e. number of mesocercariae per infected wild boar.

Until now 128 wild boars from different German regions have been analyzed by application of the official method for the detection of *Trichinella* spp. in muscle tissue according to Annex I, Chapter I of the regulation (EC) No. 2075/2005 (TIM) and the newly developed *Alaria alata* mesocercariae migration technique (AMT).

Our results demonstrated already at an early stage that TIM is unsuitable for the detection of *A. alata* mesocercariae in meat, and a so far unknown but substantial underestimation of cases of alariosis within the German wild boar population has to be assumed. Only the comprehensive application of AMT will allow the collection of full and accurate data about the parasites prevalence in sylvatic populations of animals, and their tenacity against different chemical and physical influences. Finally, this will facilitate an assessment of the human exposition risk to *A. alata* mesocercariae in meat of wild boars and the introduction of effective measures for consumer protection, if needed.

INTRODUCTION

Alaria alata is a trematode, the adult of which is found in mammalian carnivores throughout Europe and Asia. From its definitive host the life cycle of the *alariae* takes them into two intermediate hosts - first a snail, then a frog or a tadpole. The life cycle is completed when a second intermediate host is ingested by a definitive host. The infective mesocercarial stage of the trematode (*Distomum musculorum suis*, DMS) can also infect paratenic, or nonessential hosts, such as many species of mammalians, birds, reptiles, amphibians, and even humans. In those paratenic hosts, mesocercariae are *larva migrans*, without defined target tissue and life cycle evolution [1, 2]. The pathological consequences of human alariosis, relevant symptoms, and therapeutic strategies have been described for *Alaria americana* [3-9] but no sound data are available regarding human infection with *A. alata*. However, the wide variety of possible symptoms makes this parasitic disease difficult in diagnosis and requires specific detection methods for exclusion of other diseases.

Since 2002 an increasing number of accidental findings of *A. alata* mesocercariae in meat of wild boars in western EU (Germany, France) and other European countries (Balkans: Croatia) during official meat inspection for *Trichinella spp.* initiated an assessment of the potential foodborne human health risk posed by this parasite. In view of the insufficient data regarding *Alaria spp.* and more specially *A. alata*, a final risk assessment could not be performed, which lead the responsible authorities to preliminary recommendations on how to proceed with carcasses of wild boars found to be infested with DMS. German Federal Institute for Risk Assessment (BfR) pointed out, that a human health risk could not be precluded which lead to a preliminary recommendation to declare carcasses of wild boars, found to be infested by *A. alata* mesocercariae, unfit for human consumption [10]. Furthermore, the BfR concluded, that there is a great need for the development of suitable detection methods for *Alaria alata* mesocercariae in meat.

Against this backdrop, our own studies concentrate on the most relevant questions concerning (a) the development, optimization and validation of methods for reliable DMS detection, (b) the distribution of the mesocercariae within their paratenic hosts, i.e. identification of potential predilection sites, particularly in wild boars, and (c) their prevalence in sylvatic populations of animals with respect to their introduction into the human food chain.

MATERIAL AND METHODS

We examined the carcasses of 108 wild boars from different regions in Brandenburg, Saxony-Anhalt, Lower Saxony, Saxony, and Schleswig-Holstein. In all animals, samples were taken from anatomically defined sites: pillar of the diaphragm, tongue, masticatory muscles (Mm. *masseter, temporalis, pterygoidei*), "cheek" (i.e. various tissues in the caudoventral region of the head containing among others muscle, connective, adipose, glandular, and lymphatic tissue), neck, distal foreleg, shoulder, intercostal muscle, loin, back, abdominal muscles (Mm. *rectus abdominis, obliquus externus and internus abdominis, transversus abdominis*), haunch, distal hind leg, and subcutaneous adipose tissue. In addition, we were able to sample additive material in some cases where the carcasses were not already disembowelled as follows: larynx with annexed connective tissue, oesophagus, peritoneum with retroperitoneal adipose tissue, *omentum majus*, heart, liver, spleen, kidney, and lungs. Sample sizes ranged from 11.6 to 125 g, resulting from the differing availability of the respective tissues. All carcasses and sample materials were stored at +2°C until dissection, e.g. preparation and examination, which was performed within 24 hours. In our studies on DMS in wild boars, we originally tested the reference method for *Trichinella* detection in meat samples (in the following, abbreviated as Trichinella inspection method, or "TIM") as stipulated in Annex I, Chapter I of the regulation (EC) No. 2075/2005 [11]. In addition to the protocol for TIM we tested various modifications of this method in order to optimize its efficiency to detect DMS. In the course of these investigations we were able to develop an alternative method based on a larvae migration technique.

For a direct comparison of the newly developed Alaria alata mesocercariae migration technique (AMT) and TIM, 100 positive meat samples containing pillar of the diaphragm, cheek, masticatory muscles and abdominal muscles with annexing adipose tissue from another 20 wild boars were chopped, thoroughly mixed, and sectioned into appropriate aliquots, 60 g for TIM and 2 × 30 g for AMT.

RESULTS

Using TIM, Pankreatin©/bile acid digestion, and the newly developed AMT the distribution patterns of DMS in 108 wild boars were analyzed. The main results as pertaining to the detection techniques were as follows: (a) DMS distributes heterogeneously within the paratenic host, (b) DMS shows a differing distribution pattern in comparison with *Trichinella*, and (c) DMS also prefers localizations with considerable amounts of intermuscular/intramuscular connective and adipose tissue as well as lymphatic tissue, glandular tissue and cartilage. The body lymph nodes showed the highest average level of infection (80.9 larvae/100g). Larynx with annexed connective tissue (50.2 larvae/100g), peritoneum (40.9 larvae/100g), pillar of the diaphragm (28.3 larvae/100g), and tongue (20.2 larvae/100g) followed.

During our studies, we soon realised that the repeatability and reproducibility of TIM were very unsatisfactory, when this method was used for the detection of *Alaria alata* mesocercariae. So, from the beginning the main focus of our investigations was on the development of a reliable and secure detection method for DMS. We developed a method in which Pankreatin© and bile acid in a magnetic stirrer apparatus were used for the digestion of adipose tissue with and without additional HCl/pepsin supplements. Even though a maximum of only 10% to 15% of the sample material was actually digested, the number of DMS we retrieved from samples was increased in some cases as compared with TIM, which was performed in parallel with a representative aliquot of the respective samples. Visualization by stereomicroscope demonstrated that these parasites moved actively from the sample material into surrounding liquid and that the isolated DMS were very motile, whereas DMS retrieved in parallel by TIM showed a distinctly decreased vitality, or were dead. Based on the observations that the parasite (a) shows a high affinity to liquids and (b) moves actively out of the tissues the new approach of a larvae migration was tracked and AMT was developed in this course. An operating procedure for the *Alaria alata* mesocercariae migration technique (AMT) was prepared and is displayed in Table 1.

From the beginning the AMT demonstrated a high larvae recovery rate but not until direct method comparison with TIM, the superiority of the AMT could be proved: 96 out of 100 positive samples from 20 wild boars could be clearly identified with AMT, whereas only 40 samples were demonstrated DME-positive with TIM. The number of larvae in 100g tissue examined with TIM ranged from 0.6 (minimum) to 224 (maximum) DMS/100 g (median 9.38 DMS/100g). The arithmetic mean was 30.17 and the standard deviation was 44.77 DMS/100 g. AMT detected on average 30.14 larvae/100g, with DME contents ranging from 0.2 (minimum) to 373.3 (maximum) larvae/100 g (median 9.38; SD 53.3 DMS/100 g).

To confirm our hypothesis that the unsatisfactory larvae counts with TIM could be associated with an impairment of DMS during HCl/pepsin digestion, isolated DMS as recovered from AMT were put into contact with HCl/Pepsin concentrations as used in TIM (see regulation (EC) No. 2075/2005, Annex I, Chapter I). The larvae lost their characteristic motility and their morphologic structures soon after exposition and started to degenerate after only 15 minutes. After 90 minutes, DMS began to dissolve. Conditions as used in this experiment did not include warming and stirring of the digestion fluid. Thus, one must expect that when using TIM, the DMS loss is even faster and more pronounced.

Table 1: *Alaria* spp. mesocercariae migration technique (AMT) - Operating procedure for the detection of *Alaria* spp. mesocercariae in different body matrices of wild boars

1 Equipment	1.1	Knife or scissors and tweezers
	1.2	Cutting board
	1.3	Stands, rings and clamps or multiple funnel stand
	1.4	Glass funnel, Ø 10 cm
	1.5	Plastic sieves, Ø 9 cm, mesh size 0.8 mm
	1.6	Rubber hose, Ø 10 mm, 10 cm long
	1.7	Hose clamp, 60 mm
	1.8	50 ml glass measuring cylinders
	1.9	A trichinoscope with a horizontal table or a stereomicroscope
	1.10	Petri dishes, Ø 9 cm, marked on their undersides into 10 × 10 mm squares
	1.11	Larval counting basin according to Regulation EC No. 2075/2005, Annex I, Chapter I, No. 1 (m)
	1.12	Tap water heated to 46 to 48 °C cooling down to room temperature
	1.13	Balance accurate to at least 0.1 g
2 Samples / Sampling	2.1	For wild boar carcases, a sample weighing at least 30 g containing muscle tissue, adipose tissue, connective tissue, and glandular tissue is to be taken.
	2.2	It is recommended to take aliquot tissue samples (approx. 5 g each) from preferably different sampling sites (n=6) added together for a 30 g specimen.
	2.3	Suitable tissues: "cheek" (i. e. various tissues in the caudoventral region of the head containing among others muscle, connective, adipose, glandular, and lymphatic tissue), peritoneum with retroperitoneal adipose tissue, pillar of the diaphragm, larynx with annexed connective tissue, tongue, and masticatory muscles (*Mm. masseter, temporalis, pterygoidei*)
3 Proceeding	3.1	The glass funnels are supported on the funnel stands.
	3.2	The rubber hose is fitted to the funnels stem and closed with a clamp.
	3.3	The sieve is placed in the funnel.
	3.4	An aliquot of 30 g of the sample material is roughly chopped (0.5 cm edge length).
	3.5	The chopped meat is transferred to the sieve and 150 ml of lukewarm tap water is filled in the funnel. The sample material has to be totally emerged in the water.
	3.6	The sample is allowed to stand for 90 minutes at room temperature.
	3.7	After 90 minutes, a 20 ml sample of fluid is quickly run off into the measuring cylinder and transferred to larval counting basin/petri dish.
	3.8	The cylinder or centrifuge tube is rinsed with not more than 10 ml of tap water, which has to be added to the sample. The sample is examined by trichinoscope or stereo-microscope at a 15 to 20 times magnification.
	3.9	In all cases of suspect areas or parasite-like shapes, higher magnifications of 60 to 100 times must be used.

DISCUSSION

A steadily increasing number of incidental findings of *A. alata* mesocercariae in meat of wild boars during official *Trichinella* inspection underline the demand for a reliable and feasible method for the detection of DMS in meat. The magnetic stirrer method for pooled sample digestion has been specially adapted and optimized for the detection of *Trichinella* spp. and is obviously not transferrable one-to-one to the detection of a parasite with a completely differing biology. Objections in principle relate to both sampling sites and methodology. Application of the newly developed *A. alata* mesocercariae migration technique proved that the number of DMS retrieved from samples was increased in comparison with pure HCl/pepsin digestion, irrespective of the type of tissue (muscular, adipose, lymphatic, and/or connective tissue) we used. A direct comparison between AMT and TIM demonstrated that the sensitivity of AMT to detect DMS in tissues of wild boars is nearly 60% higher than that of TIM. The low sensitivity of the official reference method for *Trichinella* mainly arises from a higher susceptibility of DMS toward the digestion fluid. This was clearly demonstrated in challenge tests with HCl/pepsin concentrations and digestion times as used in TIM, where the larvae die off or get damaged during digestion. Moreover, a substantial number of mesocercariae might loose their characteristic motility, which is a major diagnostic feature.

The AMT works without chemicals. This guarantees a distinctly higher survival rate and motility of the mesocercariae and facilitates diagnosis. Furthermore, the chemical-free implementation minimizes the working and environmental risks and reduces costs. Analytical time requirement is in the range of the TIM or even lower. In addition, the further development of the molecularbiological approaches (12) touches on a field of great importance for both, diagnostics and epidemiological studies.

CONCLUSIONS

Our results demonstrated already at an early stage that TIM is unsuitable for a reliable detection of *A. alata* mesocercariae in meat and a so far unknown but substantial underestimation of cases of alariosis within the German wild boar population has to be assumed. The AMT proved to be by far superior to TIM, particularly as pertaining to sensitivity. AMT is a simple, robust, highly applicable, low-cost, and fast method, which we can already recommend—on the basis of a cautiously formulated standard operating procedure—for routine application in official laboratories.

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ALGORITHMS OF BIOMARKERS FOR MONITORING INFECTION/INFLAMMATION PROCESSES IN PIGS

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SUMMARY

In this study, time series of cytokine responses in pigs were analysed after infection by *Actinobacillus pleuropneumoniae*. First, autoregressive (AR) models were used for the quantification of the dynamics of the cytokine responses to infection. Second, model-based criteria were developed in order to classify disease outcome of the pigs (survival vs. non-survival). The results suggest that IL-6 responses of non-surviving pigs react more clearly to infection indicated by differences in dynamics and higher fluctuations of the IL-6 responses. These findings might be the first steps towards the development of an objective individualised method for sensor-based quantification of sepsis and inflammation processes and early prediction of disease outcome in pigs.

INTRODUCTION

Nowadays, there is a lack of automated objective methods for quantification of sepsis and inflammation in pigs. Implementing such methods in a monitoring system would allow optimising treatment efficacy in piggeries. In this way treatment costs could be reduced substantially.

Several complex biological processes are involved in sepsis and inflammation, making it a challenging task to quantify infection and inflammation processes in real-time. Based on earlier work, it might be expected that the dynamics of biomarkers are related with disease outcome [1, 2].
This study had two main objectives:
i) To apply data-based autoregressive (AR) modelling methods quantifying the dynamics of three cytokines after infection by *Actinobacillus pleuropneumoniae* in pigs.
ii) ii) To investigate whether the dynamics of the cytokine responses during a short period (16 hours) after infection can be used to classify disease outcome (survival vs. non-survival).

MATERIAL AND METHODS

Infection protocol

Prior to the disease challenge, there was a 14-day period of socialisation and acclimatisation. The pigs were catheterized three days before infection. At challenge, all pigs (n=22) were endobronchially inoculated with 1 x 10^7 CFU *A. pleuropneumoniae* under anesthesia. For all experiments, blood samples were taken with a sample frequency of 1 sample/day during three days before infection and a sample frequency of 1 sample/2 hours starting from two hours before the moment of infection until maximally 76 hours after infection for the surviving pigs. The cytokines, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10) were analysed resulting in three blood cytokine time series for every pig. In addition, all pigs were clinically scored by a veterinarian. The experiments were approved by the ethical commission of Ghent University.

Dynamic data-based modelling methods

For each pig, the dynamic characteristics of the cytokines responses were calculated using dynamic data-based autoregressive (AR) models. All calculations were performed in Matlab using the Captain Toolbox [3].

AR models are time series models and can be described by the following general equation:

$$A(z^{-1}).y_t = e_t \quad (1)$$

y_t is the output (cytokine level in the blood at time t); e_t is the noise term. $A(z^{-1})$ is a polynomial which can be written as:

$$A(z^{-1}) = 1 + a_1 z^{-1} + \cdots + a_n z^{-na} \quad (2)$$

The polynomial is a function of z^{-1}, which is a *backward shift* operator that is defined as:

$$z^{-1}y_t = y_{t-1}. a_1, a_2, \cdots, a_n$$

are the model parameters. The number of a-parameters represents the order of the system.
For the used AR models, n_a ranged from 1 to 2 resulting in two possible AR models per biomarker for every pig. Since no significant results were found for second order AR models, all AR models described in this report are simple first order models which can be written in the time series form as:

$$y_{(t)} = -a_1 \cdot y_{(t-1)} + e_{(t)} \quad (3)$$

For each pig, the a_1-parameter was calculated representing a measure for the dynamics of the cytokine responses. Afterwards, the a_1-parameters of surviving and non-surviving pigs were compared in order to develop model-based criteria to classify surviving and non-surviving pigs. In order to maximise the predictive value of the models, only the data of a short period after infection (first 16 hours) were used for the development of the AR-models and the model-based criteria for disease outcome.
Finally, ROC curves were calculated in order to determine the sensitivity and specificity of the model-based criteria developed for the cytokine responses [4].

RESULTS

In this paper, we focused on the results of IL-6, since the other analysed cytokines (TNF-α, IL-10) showed no significant results. Figure 1 shows the IL-6 responses for all pigs. As can be seen in figure 1 (upper left), all pigs that survived for 76 hours after inoculation (or longer) are defined as survivors. Pigs that survived for less than 76 are defined as non-survivors. The IL-6 responses to infection were clearly different between the individual pigs for the survivors as well as for the non-survivors. Even before infection, significant differences in the IL-6 values were present.
The a_1-parameters of the first order AR models are graphically represented in figure 1 (upper right). Since 4 of the 22 infected pigs died within the first 16 hours after infection, only 18 pigs were analysed. Interestingly, there was a significant difference between survivors (S, n=7) and non-survivors (NS, n=11) for the a_1-parameters of the AR models of IL-6 ($mean_S$ = -0.1272, $mean_{NS}$ = -0.5751, p = 0.0085). Figure 1 (down left) represents the ROC curve for the a_1-parameters of all pigs. The ROC analysis showed that the optimal a_1-parameter to distinguish between survivors and non- survivors was -0.229 with a sensitivity and specificity of 1 and 0.71 respectively. Therefore, the classification based on the dynamics of the IL-6 responses to infection could be defined as follows:

Pigs with an a_1-parameter ≤ -0.229 will not survive
Pigs with an a_1-parameter > -0.229 will survive

Using this a_1-parameter as criterion, only two pigs were misclassified. The Area Under Curve (AUC) of the ROC curve was 0.84 with a standard error of 0.11 (cfr [5]).
In addition, there was also a significant difference between the variances of the IL-6 responses for surviving and non-surviving pigs ($mean_S$ = 0.1678, $mean_{NS}$ = 1.2945,
p = 0.04). Again, these variance were calculated only for the first 16 hours of the responses after infection. Figure 1 (down right) shows a scatter plot of the a_1-parameters, as a measure for the dynamics, with corresponding variances (σ^2) of the IL-6 responses for each pig.

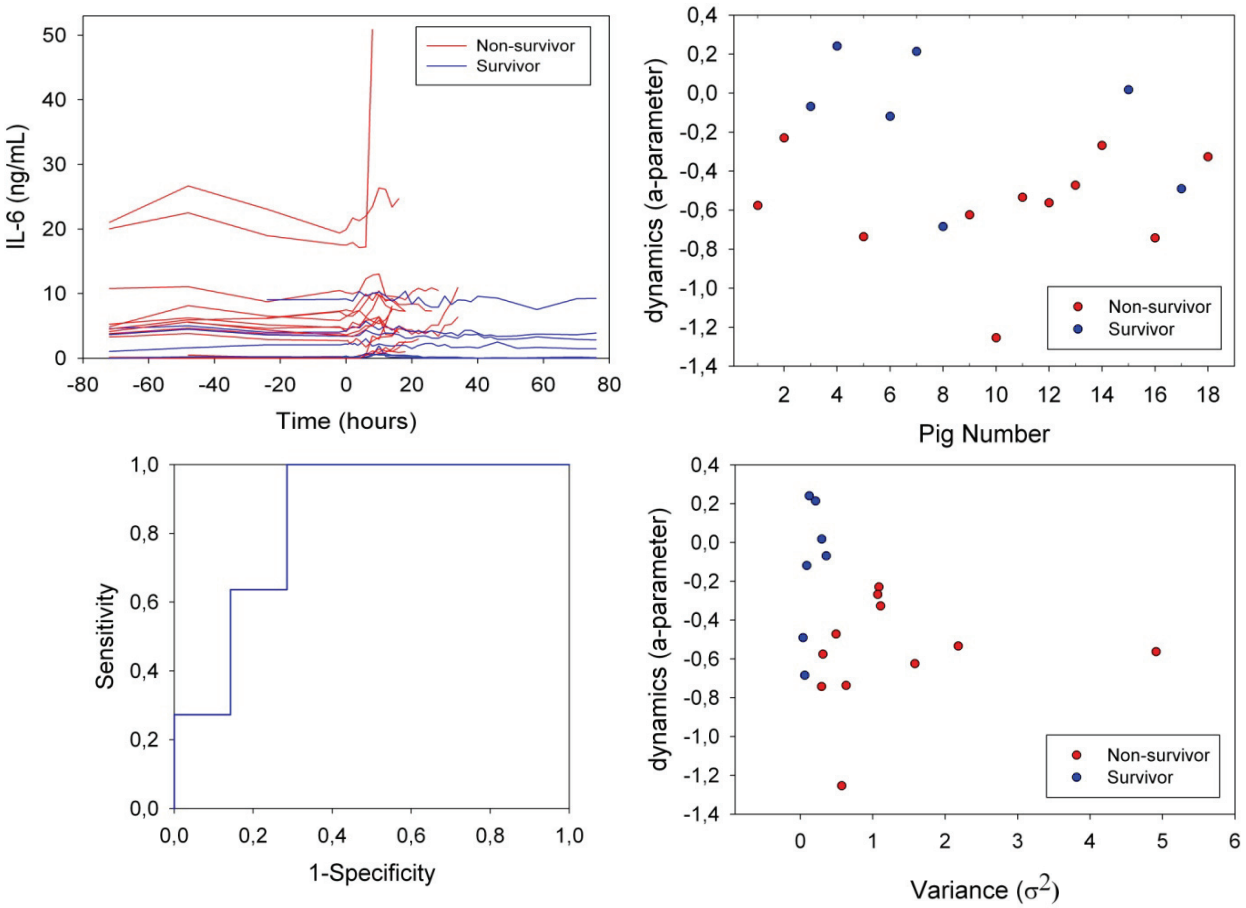

Figure 1. Upper left: Overview of IL-6 responses for all pigs. Time point 0 corresponds with the moment of infection. Upper right: Overview of a-parameters of AR-models for all pigs. Down left: ROC curve for determination of model-based criterion for early detection of survival/non-survival based on a-parameters. Down right: Plot of a-parameters of AR-models against variance of IL-6-responses for first 16 hours after infection.

DISCUSSION

IL-6 was selected as being most promising for monitoring and distinguishing between survivors vs. non-survivors. Before as well as after infection, the IL-6 responses to infection differed considerably between the individual pigs. The dynamics (a_1-parameters of the AR models) of the survivors and non-survivors differed significantly ($p=0.0085$). For the surviving pigs the a_1-parameters were lying close to zero, which suggests that there is no relation between succeeding IL-6 values after infection for these pigs. Thus, for surviving pigs the response of IL-6 on the infection approximates the *noise term* in eq. 3 (with $a_1=0$). The non-surviving pigs had models with more negative a_1-values suggesting a trend in the succeeding IL-6 values for these pigs. Using the AR models and the corresponding ROC curve, a criterion could be defined based on the dynamics of the IL-6 responses to classify surviving and non-surviving pigs. Two surviving pigs were misclassified as non-survivors using this rule. It has to be noticed that such misclassifications (false positives: pigs that were predicted to die, but survive) are less problematic, then misclassifications in the other direction (false negatives: pigs that were predicted to survive, but die) [4]. However, several limitations of the criterion have to be pointed: small number of test animals, no validation group and low sampling frequency.

Interestingly, when the a_1-parameters were plotted against the variances of the early IL-6 responses (first 16 hours) a more clear distinction could be made between survivors and non-survivors (figure 1, down left). These findings indicate that IL-6 responses to infection of non-surviving pigs contain larger fluctuations compared to surviving pigs.

CONCLUSIONS

Based on the analyses of the IL-6 responses of pigs to infection there were two main conclusions:

i) The results indicate differences in the dynamics of the IL-6 responses between surviving and non-surviving pigs.

ii) IL-6 responses of non-surviving pigs contain fluctuations with larger amplitudes compared with the IL-6 responses of surviving pigs.

Thus, both findings indicate that non-surviving pigs react more pronounced to an infection then surviving pigs regarding to the early IL-6 responses (first 16 hours).

Models as developed in our study could be the first steps towards the development of an objective individualised method for online sensor-based quantification of sepsis

and inflammation processes in pigs and for early prediction of disease outcome.

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BIOBUSINESS RESEARCH PROJECT: TRAINING AND DEVELOPMENT OF INNOVATIVE SOLUTIONS FOR ANIMAL HEALTH AND WELFARE PROBLEMS BY MEANS OF PRECISION LIVESTOCK FARMING (PLF)

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SUMMARY

In order to satisfy an increasing global hunger for meat, dairy, and eggs, livestock production is increasingly becoming industrialized worldwide. This agricultural transformation, however, is revealing animal health and welfare problems. A solution to this dilemma may lie in implementing new technology complimentary to industrial production that is founded in animal biology and capable of identifying and correcting health and welfare problems. However people educated in biology are not aware of the possibilities of modern technology and people developing new technology are not familiar with the world of biology. The main objective of the 4 year BioBusiness project is to train biologically educated people (veterinarians and biologists) to collaborate with technology driven people (bio-engineers and computer engineers). The project is being conducted by a strong Precision Livestock Farming (PLF) related consortium formed by 10 partners of research institutions, universities, and companies. A total of 11 early stage researchers were recruited as Marie-Curie Fellows to work as a team for the development of high-tech products in the following areas: 1) optimal embryonic development in chickens; 2) lameness in dairy cows; 3) aggressive behaviour in pigs. Each fellow has a supervisor responsible for scientific and research education. The fellows affiliated with academic partners are seeking a PhD degree in this project. All fellows are trained in animal applied research, technology, product development, and marketing. The progress of the fellows is monitored by a group of expert researchers under 3 specific conditions: a) individual academic/scientific progress; b) industrial progress in product development activities and; c) individual development by the network and synergy on group activities. The research outcome of the project will be 11 well-trained people who understand the innovative potential of PLF from an animal health and welfare position. Moreover they will develop innovative product concepts and the corresponding business models. Together, farmers will have a tool to improve their business and animal health and welfare through PLF technology.

INTRODUCTION

There is increasing demand for more food production, mainly in developing countries [9]. Population growth is also now coupled with the rise of the middle class and their increasing desire to consume meat and animal products. Livestock production has become larger in scale and more mechanized to satisfy this demand. This major agricultural transformation has revealed industrial livestock related health and welfare problems [3, 4, 5, 6, 7, 8, 10, 12]. These problems have drawn consumer concern; and simultaneously are being addressed through legislation in many countries. Modern livestock production systems have already been improved by the increasing adoption of new technologies. There is interesting potential for Precision Livestock Farming (PLF) to automatically monitor and help manage large numbers of animals as well as complex biological production processes [1, 2, 11]. In this regard, the combination of new technology with biology offers great opportunities for the EU in the implementing of directives and economic terms.

The obstacle is that people educated in biology are not always aware of the possibilities of modern technology. At the same time those developing technology are not participating in the world of animal welfare. Animal scientists know the most important variables to be measured from animals, while engineers can provide the best technology solutions to measure those requested variables.

The main objective of the 4 year EU BioBusiness project (from December 2009 to November 2013) is to train biologically educated people (veterinarians, biologists and animal scientists) to collaborate with technology driven people (bio-engineers and computer engineers) and make them familiar with modern technology by means of PLF. They will be trained in research, product definition and development, marketing and sales for BioBusiness in the EU. The 11 early stage researchers will understand the evolutionary origins of measuring, modelling, and controlling in relation to bioprocesses in order to foster the development of high-tech products or services for livestock within industry.

MATERIAL AND METHODS

The project is conducted by a strong PLF-related consortium formed by 10 partners. There are two research institutions: Agence Nationale de Sécurité Sanitaire de 'alimentation, de l'environnement et du travail (ANSES – France); The Agricultural Research Organization of Israel – The Volcani Centre (ARO – Israel). There are five universities in the consortium: The Royal Veterinary College of University of London (RVC – United Kingdom); Katholieke Universiteit Leuven (KUL – Belgium) as a coordinator of the project; Stiftung-Tieraerztliche Hochschule Hannover (TIHO – Germany); Animal Science Group of the Wageningen University (ASG – The Netherlands); Universita degli Studi di Milano (UNIMI – Italy). There are 3 representatives of companies: DeLaval International AB (DeLaval – Sweden); Fancom B.V. (Fancom – The Netherlands); Petersime N.V. (Petersime – Belgium).

A total of 11 early stage researchers were recruited as Marie-Curie Fellows to work in a team for the development of high-tech products and services in the field of PLF, focusing on the complex biological organism, and practical implementation of their science.

The consortium network focuses on research that will enhance animal welfare and health management. The main areas of interest are behaviour monitoring, disease detection and monitoring, animal growth monitoring and management. In particular there are three collaborative projects for fellows to work on, all of which the partners bring their particular expertise to, as following:

1) Chicken embryo development: the aim is to improve the conditions for incubating the eggs of broiler chickens by focusing on the relationship between the environment and embryonic development and by identifying optimal incubation conditions and their post-hatch effects.
2) Lameness in dairy cows: the focus is on the development of an automatic algorithm for the detection of lameness in dairy cows using video images.
3) Pig aggression behaviour: the work is concentrated on the automatic monitoring of pigs to detect aggression in order to reduce poor welfare and economic consequences.

The fellows will be trained in distinct work packages, which cover the above described topics in relation to animal and welfare indicators, experimentation, labelling, algorithm development, mathematical modelling, and field testing. There are also work packages for training in the dissemination and exploitation of the scientific results, as well as on conference and workshop organization.

In addition, the 11 fellows will be introduced to the BioBusiness methodology from research to sales by means of complementary trainings on animal applied research, technology, product development and market introduction.

Each fellow has a supervisor responsible for the scientific education. Fellows affiliated with academic partners are seeking a PhD degree linked to the project and following specific doctoral training programme at one of the partner universities. Furthermore, fellows who are affiliated with one of the industrial partners are being trained in product development activities.

The progress of the fellows is constantly monitored by a group of selected expert researches, which composes the Advisory Board of the project, under the three following specific conditions: a) individual academic and scientific progress; b) industrial progress considering the product development activities and; c) individual development by the network and synergy on group activities. Regular meetings occur between the fellows and the Advisory Board members as part of the evaluation system, as well as meetings between fellows and the EU-commissioners linked to the project.

RESULTS

The project still has a significant amount of time before the current funding period ends in November 2013. BioBusiness will allow the selected fellows to take part in all the stages that are required for an innovative idea reach to the market. Also, coming closer to the industrial environment (by direct employment or secondment by the industrial partners), they will be introduced to product-oriented research in economics and marketability. Therefore, a main result of the project will be 11 well-trained people who understand the innovative potential of PLF. Moreover they will show their innovative product concepts and the corresponding business models. They will provide tools to improve the farmer’s business and animal welfare and health.

The early stage researchers that successfully complete the training will be able to conduct high quality research which is required by the academic environment while, at the same time, they will be able to conduct research supporting product development. They will understand the scientific, time, and financial constraints that this imposes and they will be able to work under such conditions either in SMEs, high-tech companies, and research institutes. Fellows will have the appropriate skills in bringing PLF-related solutions to the market, benefiting the industry as well as consumers.

CONCLUSIONS

Final results of the BioBusiness will benefit the livestock production system in general at the EU and global level. Farmers will be able to use a guaranteed continuous automatic monitoring system to manage their animals regarding health and welfare. There is an advantage to consumers of animal products, who know that continuous monitoring and follow up of animal health, well-being, and performances is operational. For innovative companies and high tech SMEs, this technology can provide opportunities to enter a worldwide market with guaranteed safe, humane, and specialty products.

In the future a close link between research, industrial partners, and innovative SMEs on PLF applications is expected in order to change livestock production into a new and modern economic activity.

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REVIEW OF THE KEY RESULTS OF A LARGE INTEGRATED AIR QUALITY PROJECT IN AUSTRALIA

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SUMMARY

It was established that improvement in air quality in livestock buildings could produce significant benefits for animals, workers and the environment. In order to achieve a sustained reduction in the concentration of airborne pollutants in livestock building; the different management, environmental and housing factors, which could influence the concentrations within and emissions of airborne pollutants from livestock buildings had to be statistically evaluated. Thus a broad study of air quality in piggery buildings was designed (1) to determine the key piggery design and management factors that affect the internal concentrations and emissions of airborne pollutants and then to (2) model and therefore able to predict the concentrations and emission rates of the main pollutants. This article considers the implications of the main results of this significant research project.

INTRODUCTION

The significant amounts of airborne pollutants, which can be found in the airspace of some piggery buildings could potentially affect the external environment, production efficiency of pigs, human as well as animal health and welfare (Banhazi *et al.*, 2009). In order to achieve the maximum safe concentrations recommended in Australia a broad study of air quality in piggery buildings was designed (1) to determine the key piggery design and management factors that affect the internal concentrations and emissions of airborne pollutants and then to (2) model the concentrations and emission rates of the main pollutants. This article is a summary of the main results of this national research project.

MATERIAL AND METHODS: BRIEF DESCRIPTION OF STUDY DESIGN

To enable this study to be conducted, the sampling methods and instrumentation kit used during the survey was standardised and an "environmental monitoring kit" (EMK) was developed. In the later stage of the study the original EMK was further simplified, so it could be used as an extension tool after the completion of the survey component of the study (Banhazi, 2009). A field survey of airborne pollutant concentrations within and emissions from 160 piggery buildings in four states of Australia was then executed. The measurement techniques chosen proved to be practical, reliable and cost effective (Banhazi *et al.*, 2008b; Banhazi *et al.*, 2008c). Using the collected data, comprehensive statistical modelling was undertaken to explain the variation in the measured concentrations and emission rates. An output from this component of the study was equations to reliably predict concentrations and emission rates of key airborne pollutants (Banhazi *et al.*, 2008b; Banhazi *et al.*, 2008c; Banhazi *et al.*, 2008d; Banhazi *et al.*, 2008a). Later on the models developed were fine-tuned and validated using an innovative statistical approach (Banhazi *et al.*, 2010).

DISCUSSION OF KEY STUDY RESULTS

General comments on concentrations and emissions measured

This study delivered a number important outcome. First of all, the validated model developed can be used as a practical management tool to predict the concentrations and emissions of major pollutants without undertaking costly measurements. Routine use of the combined predictive model is expected to make pig producers aware of potential problems associated with air quality on their farms. In turn, this will facilitate the inclusion of pollutant abatement techniques into routine management procedures on farm, improving the health and welfare of pigs and piggery workers as well as the environmental sustainability of piggery operations. Furthermore, designing piggery buildings to minimize airborne pollution now is a theoretical possibility. Using the predictive equations, mathematical optimization of building and engineering parameters will be possible in order to minimize the concentrations and emissions of different airborne pollutants. The optimization process could calculate the best combination of building features to achieve minimum pollutant loading internally and externally. Such calculation would be very useful for building companies as well as for individual producers contemplating building renovations. These "low-pollution" buildings could improve piggery environment for the

benefits of both pig and piggery staff and could reduce the environmental impact of piggery operations. In addition, if reliable economical data on the effects of airborne pollutants becomes available, the potential financial advantage of one building design over another could be predicted. The range of pollutant concentrations measured in the study buildings are presented and compared to European concentration values in Table 1.

Table 1: Mean concentrations of key airborne pollutants measured in Australian piggery buildings (based on building averages) (Banhazi *et al.*, 2008c)

Pollutant	*Concentrations suggested in Australia*	*Australian study*
Ammonia (ppm)	10	3.7
Inhalable particles (mg/m^3)	2.4	1.74
Respirable particles (mg/m^3)	0.23	0.26
Respirable endotoxins (EU/m^3)	50	33
Total airborne bacteria (10^5 cfu/m^3)	1.0	1.17

Recommendations for airborne pollutant concentration targets in livestock buildings in Australia are available and the study demonstrated that the average airborne pollutant concentrations in piggery buildings in Australia are generally below or near the recommended limits. Australian piggery buildings generally have lower or comparable airborne pollutant concentrations compared to published results from Europe (Seedorf *et al.*, 1998; Takai *et al.*, 1998; Groot Koerkamp *et al.*, 1998). Atmospheric NH_3 concentration on average is not a major concern in Australian buildings, as ventilation rates are much higher compared to buildings in colder regions of the world, such as parts of Europe or North America. Only 1% of the buildings surveyed had concentrations measured above recommended levels for CO_2 and approximately 8% of buildings were above the recommended 10 ppm concentrations. The concentrations of airborne particles were high in deep-bedded shelters (DBS); the mean concentration of endotoxin, total bacteria, inhalable and respirable particle concentrations exceeded the recommended limits frequently (Banhazi *et al.*, 2008c). Pigs housed in DBS and workers undertaking manual tasks in those buildings were potentially exposed to high concentrations of these airborne pollutants. In the absence of more specific information, the concentrations measured in DBS do provide a ground for concern.

Factors affecting concentrations and emissions

A number of individual models were developed during the study to explain the variation observed in the concentrations and emission of the airborne pollutants, as well as in environmental variables. Table 2 summarizes all significant main effects identified for the concentrations and emissions of the five major airborne pollutants measured during the study.

Table 2. Significant effects associated with the concentrations and emission rates of the five major airborne pollutants measured.

Concentration	Ammonia	Airborne Bacteria	Respirable Endotoxin	Respirable Particles	Inhalable Particles
		Building type	Building type	Building type	Building type
	Cleanliness	Cleanliness		Cleanliness	
	Management			Management	Management
	Seasons			Seasons	Seasons
	Shed size				Shed size
				Ventilation	Ventilation
				Temperature	Temperature
			Humidity	Humidity	
				Sow number	Sow number
Emission		Ventilation type	Ventilation type	Ventilation type	Ventilation type
		Inlet height	Inlet height	Inlet height	Inlet height
		Building height		Building height	Building height
	Building type			Building type	Building type
	Temperature			Temperature	
	Humidity				Humidity
	Management			Management	
	Seasons				Seasons
					Building width
		Cleanliness			
	Sows number				

The concentrations of four airborne pollutants were affected by the classification of the buildings. Three pollutant concentrations were affected by cleanliness, management and seasons, while the concentrations of two pollutants were affected by temperature, humidity, ventilation, sow numbers and shed size (Table 2). The emission rates of four airborne pollutants were affected by the classification of the ventilation system and the height of the air inlets. Three pollutant emission rates were affected by the type and height of the buildings. The emission rates of two airborne pollutants were affected by temperature, humidity, management and seasons. The

emission rate of one pollutant was affected by building width, pen hygiene and sow numbers (indication of farm size) (Table 2). In general, the statistical analysis accurately identified the important factors affecting the concentrations and emissions of major airborne pollutants and therefore improved the understanding of the behaviour of those pollutants. In the section below, the primary building and management effects identified during the study are discussed.

Type of buildings

The type of building (dry sow, farrowing, weaner, grower/finisher buildings and DBS) had a highly significant effect on total bacteria, respirable endotoxin, inhalable and respirable particle concentrations and on the emissions of NH_3, inhalable and respirable particles (Banhazi *et al.*, 2008c; Banhazi *et al.*, 2008a). Overall, DBS recorded the highest concentrations for all four pollutants. Inhalable particle emission was the highest from weaner buildings and from DBS (Banhazi *et al.*, 2008a). It was hypothesised that the presence of bedding material in DBS is a risk factor for the high particle concentrations and high inhalable particle emissions under Australian climatic conditions. Similar findings were reported by European researchers for cattle buildings (Takai *et al.*, 1998). DBS were also implicated in generating very high endotoxin, airborne bacteria, inhalable and respirable particles emission rates (Banhazi *et al.*, 2008a). Other types of buildings had relatively small emissions when compared to DBS. The high emissions from DBS are a concern in terms of environmental sustainability and appropriate reduction methods should be investigated. It has been demonstrated that increased humidity appeared to decrease particle concentrations in and emissions from DBS (Banhazi *et al.*, 2008c; Banhazi *et al.*, 2008a). The increased humidity levels would make the bedding material more adhesive, trapping smaller particles within the larger fibres of the bedding material, reducing both concentrations and therefore emissions of respirable particles from these type of structures. However increasing humidity would not be advised as a management tool, as it can compromise thermal comfort of animals in both summer and winter. Therefore, implementation of treatments, which will not increase humidity but result in increasing the adhesion of bedding material used, should be considered in DBS.

Pen hygiene and pig flow management

The effect of pen floor hygiene (essentially pen cleanliness) on airborne bacteria, NH_3 and respirable particle concentrations was an important finding of the study and partially confirmed the results of previous studies on air quality (Aarnink *et al.*, 1997; Ni *et al.*, 1999). Dunging patterns need to be controlled in order to improve pen hygiene. It is interesting to note that while hygiene was an important factor in concentrations of airborne pollutants, it only influenced emission of bacteria. All-in/all-out management proved to be beneficial for reducing the concentrations of NH_3. Management interacted with seasons for NH_3, indicating that summer in continuous flow (CF) buildings is a risk factor for high NH_3 concentrations. These findings confirmed the results of Dutch researchers, reporting on the strong influence of temperature on incorrect dunging behaviour in pigs.

Season

The effects of season on the concentration of various airborne pollutants were complex and varied for different airborne contaminants. In piggery buildings, an increase in the concentrations of inhalable particles has been demonstrated in this study for the winter period. However, for smaller particles, the turbulence associated with higher air velocities associated with summer conditions, could increase respirable particle concentrations under certain conditions. Higher concentrations of NH_3 and significantly higher emission rates were recorded in summer than in winter in CF management system but not in buildings managed on an AIAO basis. Therefore, it can be concluded that while winter is a risk factor for inhalable particles, in summer greater emphasis needs to be placed on reducing potentially high NH_3 and respirable particle concentrations.

Ventilation related factors

Factors related to the operation of ventilation systems have been demonstrated to have a very significant influence on emission rates (Banhazi *et al.*, 2008d; Banhazi *et al.*, 2008a). The emission rates of all pollutants (with the exemption of NH_3 emission) were influenced by the size of ventilation inlet opening. Airborne bacteria, respirable endotoxin, inhalable and respirable particles emission rates are all increased with increasing size of air inlets. The classification of ventilation systems also had a very significant influence on emission rates. Airborne bacteria, respirable endotoxin, inhalable and respirable particles emission rates were the highest from tunnel-ventilated buildings, which is a typical feature of DBS. The high emission rates observed for these pollutants were partially related to high internal concentrations measured typically in DBS (Banhazi *et al.*, 2008c; Banhazi *et al.*, 2008d; Banhazi *et al.*, 2008a).

Temperature and humidity

Generally, temperature had a positive correlation with both inhalable and respirable particles concentrations and emission. As temperature increases, piggery buildings tend to become a drier environment, creating greater opportunities for particle generation (Pedersen *et al.*, 2001). Because of increased temperature, respirable particle concentrations increased significantly in AIAO buildings. Inhalable particle concentrations were also significantly affected by temperatures, but the relationship was more complex due to interaction with the classification of the buildings. The effect of humidity interacted with building type for respirable particles and there was a pronounced reduction effect of increased humidity in DBS. Increased humidity also sharply reduced respirable particle emissions from DBS and NH_3 emission generally. However, for other building types the effect of humidity was not simple and in interaction with management type, demonstrated a positive correlation with respirable particle concentrations. This study also found that humidity affected endotoxin concentrations (Banhazi *et al.*, 2008c). This finding could have implications for dust reduction methods, such as spraying of oil/water mixture.

Farm size

The size of farm (as described by the number of sows) had a significant effect on both inhalable and respirable particle concentrations. Inhalable particle concentrations were strongly and positively associated with sow numbers. However, the effect of sow number on respirable dust was more complex. It has been hypothesized that on larger farms, due to work pressures, less time is available for cleaning and general maintenance of the environment of the pigs. Therefore, the reduced hygiene and increased intervals between cleaning episodes creates an ideal environment for higher dust concentrations in buildings on large corporate farms (Banhazi *et al.*, 2008c). (Banhazi *et al.*, 2008c)

CONCLUSIONS

This study demonstrated that compromised pen hygiene is an important risk factor for elevated concentrations of NH_3, viable and non-viable particles. The effect of housing type was greatest on a number of pollutants, however applying improved management of these buildings is more readily applicable. Therefore, this source of airborne pollution could be eliminated to a large extent by controlling dunging patterns and improving the hygienic conditions of pens. The current practice of managing buildings using all-in/all-out strategy with thorough cleaning of the facilities between batches of pigs is advisable. Treatment of bedding materials in DBS is highly advisable to reduce the opportunities for particle generation. The knowledge generated by this study will also enable piggery managers to focus on reducing the concentrations of specific pollutants under different seasonal conditions. Ventilation, humidity and temperature can be theoretically adjusted to minimize airborne pollution emission and concentration in piggery buildings. In terms of emission rates, it appears that ventilation related factors have the most influence on the amount of airborne pollutants emitted from piggery buildings. Although, some of the management methods suggested might be successfully used to reduce the concentration and potentially the emission rates of airborne pollutants, in reality there are limitations associated with managing emission rates via concentration reduction. Therefore, the immediate focus has to be on developing new techniques and evaluating existing ones (such as air scraping and bio-filters), which have the capacity of capturing emitted pollutant plumes from livestock buildings.

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PREVALENCE AND ANTIBIOTIC RESISTANCE OF THERMOTOLERANT *Campylobacter* spp. IN RETAIL CHICKEN MEAT - TRENDS IN SLOVENIA AND EU

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SUMMARY

The increasing antimicrobial resistance is a well-known and world-wide problem, especially microbial multidrug resistance phenotypes, being dispersed also among food-related bacteria. In this study 242 food products from retail market in 2009 were tested for thermotolerant campylobacters by ISO 10272-1:2006 and real-time PCR detection. All *C. jejuni and C. coli* isolates (N=74) from 48 out of 67 (72 %) and 12 out of 25 (48 %) positive samples of fresh chicken carcasses and spiced chicken meat samples, respectively, were tested for antibiotic resistance against seven antibiotics by broth microdilution method (Sensititre® (Treck Diagnostic System) and CellTiter-Blue® reagent and automated fluorescence signal detection. The antibiotic resistance was highly prevalent, especially against fluoroquinolone ciprofloxacin (91% and 82%), tetracycline (56 % and 52 %) and also erythromycin (9 % and 10 %) for *C. coli* and *C. jejuni*, respectively. Only 11 % of the isolates were sensitive to all antibiotics, but 82% were resistant to at least two out of four non-related antibiotics. These rates are much higher than previously studied (2001-2003), higher than from the other sources (e.g. human isolates) and also among the highest comparing to other EU countries. An inevitable side effect of the use of antimicrobials is the emergence and dissemination of resistant bacteria, so more attention should be paid for possible solutions and alternatives.

INTRODUCTION

In the last decade campylobacteriosis has become the leading bacterial food-borne illness and most frequently reported zoonosis in humans. In the period 2005-2009, it was the most commonly reported zoonosis transmitted to humans in the EU, with 190,566 confirmed cases reported in 2008 (EFSA, 2010). In Slovenia, in 2009, the number of reported cases of campylobacteriosis for the first time exceeded reported salmonelloses (1). Most infections with *Campylobacter* are transmitted via the food chain. In foodstuffs, *Campylobacter* was by far the most commonly detected in fresh broiler meat, although other food animals were also quite frequently contaminated at the farm level (EFSA reports 2005-2010, Fig. 1).

In addition, *Campylobacter* antibiotic resistance is prevalent and still increasing, but the epidemiology of resistant strains and the importance of strain origin is not yet fully understood. Thus, the aim of this work was to determine resistotypes and multidrug resistance profiles of thermotolerant camyplobacters from retail chicken meat and meat products in Slovenia in 2009 against 6 non-related antibiotics and comparative analysis of this with the results a) from the previous years (2001-2003) b) of campylobacters from different sources and c) from different EU countries (EFSA reports, 2005-2010).

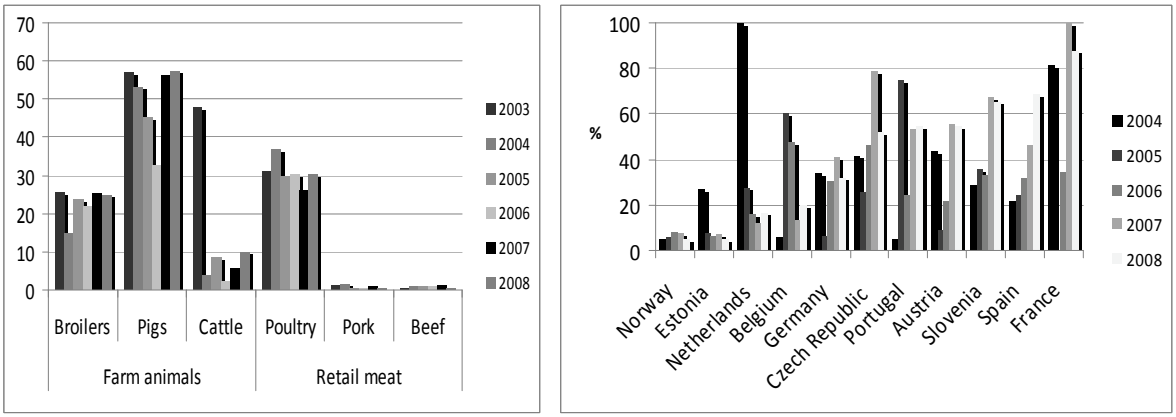

Fig. 1: Reported prevalence of *Campylobacter* (%) in animals on the farm and in retail meat in EU countries in the period 2003-2008 (left) and in retail poultry meat in some European countries (right) (data were available for slaughter and/or processing plants or retail meat) in the period 2004-2008 (Source: EFSA reports and EFSA specific country reports, 2005-2010).

MATERIAL AND METHODS

Meat samples, isolation and identification of *C. jejuni* and *C. coli* isolates

In total, 242 food products from retail market in Slovenia in 2009 were tested for thermotolerant campylobacters by ISO 10272-1:2006 and real-time PCR detection. The presumptive isolates, which were isolated from fresh chicken carcasses and spiced chicken meat samples were identified phenotypically and by mPCR identification (7).

Antibiotic resistance

It was determined for all *C. jejuni and C. coli* identified isolates against the following antibiotics (ciprofloxacin, gentamycin, streptomycin, tetracycline, nalidixix acid, chloramphenicol, erythromycin) by broth microdilution method (Sensititre® (Treck Diagnostic System) following the instructions of the supplier and CellTiter-Blue® reagent and automated fluorescence signal detection (5). For comparative analysis we included also human *C. jejuni* and *C. coli* isolates (N=200), where resistotypes were determined for ampicillin, gentamycin, ciprofloxacin, tetracycline and erythromycin with disc-diffusion test, but we confirmed the same results by microdilution method in all examined cases.

RESULTS

Prevalence of thermotolerant *Camyplobacter* in retail chicken meat

In total, 74 *C. jejuni* (N=37) and *C. coli* (N=37) isolates from 48 out of 67 (72 %) and 12 out of 25 (48 %) positive samples of fresh chicken carcasses and spiced chicken meat samples, respectively, were isolated, identified and included in further antibiotic resistance testing. The samples of fresh leafy vegetables and salads with chicken meat ingreadients were all confirmed as negative *for Campylobacter* presence.

Antibiotic resistance of *C. jejuni* and *C. coli* chicken meat isolates

The results of testing meat isolates against different antibiotics are presented in Table 1. There was a very high prevalence of resistance against nalidixic acid (86.5 %) and ciprofloxacin (85.1 %) among food strains. We also noticed a high prevalence of resistance against tetracycline and streptomycin and erythromycin, 54.1 %, 39.2 % and 9.5 %, respectively (Tab 1.).

Multiple resistance of *C. jejuni* and *C. coli* chicken meat isolates and comparison with the isolates from different sources

There were noticeable differences between the resistance of strains isolated from food and from human stool. The food strains had considerably higher resistance rate and multiple drug resistance was also more frequent in food isolates, as there was only 16 % of food isolates completely sensitive to four non-related antibiotics (Tab.1, Fig. 2). A high part of human isolates was sensitive to all antibiotics tested (41 %). There were also no human strains, resistant to 3 antibiotics, whereas 6 % of food isolates have been resistant against three antibiotics and 50 % against two antibiotics (Fig.2).

Table 1: The prevalence of antibiotic resistant isolates of *C. jejuni* and *C. coli* (%) from retail chicken meat in Slovenia in 2009 tested against seven antibiotics (left) (GEN: gentamycin, STR: streptomycin, NAL:nalidixic acid, CIP:ciprofloxacin, TET:tetracycline, Ery:erythromycin, CHL:chloramphenicol. Data were calculated according to EFSA recommendations and in the concentration range as stated in the table (right).

	C. coli	*C. jejuni*	*Campylobacter* spp.	*C. jejuni* Cut-off value (µg/ml)	*C. coli* Cut-off value (µg/ml)	Used Conc. range (µg/ml)	Conc. range recommended by EFSA (µg/ml)
GEN	0.0	2.0	1.4	>16	>16	2-32	0.125-16
STR	52.2	34.0	39.2	>1	>1	0.06-4	0.5-32
NAL	89.0	87.5	86.5	>1	>2	0.12-8	ND
CIP	91.3	82.0	85.1	>16	>32	2-64	0.06-8
TET	56.5	52.0	54.1	>2	>4	1-16	0.125-16
ERY	8.7	10.0	9.5	>4	>16	0.5-32	0.5-64
CHL	2.7	0.0	1.4	>2	>2	0.25-16	ND

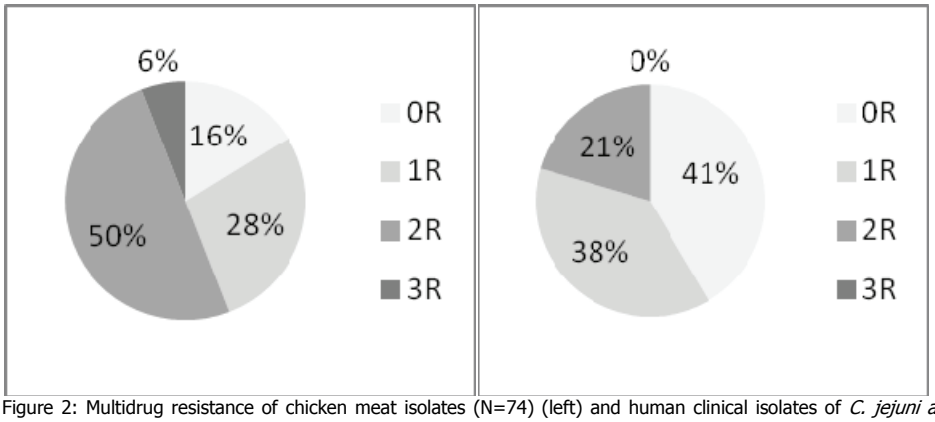

Figure 2: Multidrug resistance of chicken meat isolates (N=74) (left) and human clinical isolates of *C. jejuni and C. coli (*N=200) (right) against four antibiotics (gentamycin, ciprofloxacin, tetracycline, erythromycin) in Slovenia in 2009 (0R means sensitive to all tested antibiotics).

DISCUSSION

The prevalence and antibiotic resistance rates were compared also with the resistance of chicken meat isolates tested in Slovenia 7-8 years ago. Much lower resistance rates were noticed in this period for most of tested antibiotics (4). Similar as in the other European countries the resistance has increased a lot recently. According to EFSA national report for 2008 (3) and this study, which was experimentally performed in the same way, only from the year 2008 to 2009 the prevalence of ciprofloxacin, nalidixic acid and erythromycin resistance for *Camyplobacter* food isolates has increased for 24 %, 29,3 % and 6,5 %, respectively. Although EFSA national reports are not available yet for all EU countries with the most recent monitoring data, it is evident from some other publications (6), that there was an evident increase in prevalence and multiple resistance of *Campylobacter* in food production environments in central and southern EU-member states. Despite the differences in results and some insufficiency in the methodological harmonization among reports from different countries, this is an evident and increasing problem.

CONCLUSIONS

From our results we can conclude that the prevalence of antibiotic resistance in *Campylobacter* strains isolated from food is still increasing compared to the previous years. There is a particularly large part of tested strains resistant to ciprofloxacin, which is an important antibiotic used in human medical treatment, similar is true also for the erythromycin. Much more attention should be paid for possible solutions and alternatives in different fields, including animal breeding, veterinary and human medical practice as well as in food and feed processing and general public health concerns.

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LAYING HENS AS A SOURCE OF *CAMPYLOBACTER JEJUNI*

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SUMMARY

Campylobacter spp. are frequently found not only in broiler meat but also in laying hens. Little is known about the variety of genotypes present in laying hens and in the litter of different farms. A total of 150 cloacal swabs and 5 litter samples were taken from 5 laying hen farms and investigated for the presence of *Campylobacter jejuni*. Isolation was carried out by microaerophilic incubation on selective media. Identification was performed by morphology, biochemical characterization and *mapA* PCR. Typing of isolates was carried out by using restriction fragment length polymorphism (*flaA* restriction by *DdeI*). Typing showed a high diversity among the isolates; 25 different genotypes were detected out of 68 *C. jejuni* isolates. Genotypes differed between farms and seemes to be farm specific. Up to 8 different types were isolated from one farm. The results display that there is a great genetic diversity of *C. jejuni* within and between flocks and that laying hens and litter a considerable sources of *Campylobacter* for both animal and humans.

INTRODUCTION

Campylobacteriosis is the most commonly reported gastrointestinal bacterial pathogen in human world wide. In 2008 the total number of confirmed cases was 190,566 in Europe and 64,731 in Germany (2). *Campylobacter* are widely present in nature. It colonizes the alimentary tracts of most warm blooded animals and humans. However, the most preferential environment appears to be the intestine of poultry. Poultry meat especially fresh broiler meat is considered to be the major vehicle of *Campylobacter* causing disease in humans (8). In contrast to broilers, occurrence of *C. jejuni* in laying hens had not been widely studied in the past (4). However, recent research reveals the presence of *Campylobacter spp.* in internal organs, faeces and eggs of commercial laying hens (1 , 5). That means, from the hygienic point of view, that laying hens can contaminate their own farm environment, other farms during the laying period and later other carcasses at slaughter. To our knowledge, no studies have been carried out on the role of the litter in laying hen houses in *Campylobacter* transmission. Therefore, investigations were carried out in order to estimate the colonization level and genetic diversity of *C. jejuni* in laying hens and litter from five different farms.

MATERIAL AND METHODS

Sample collection

Samples were taken from 5 laying hen flocks kept in floor keeping systems from 5 different farms. Flock sizes ranged from 1300 to 13,000 birds per flock. The birds were about 18 to 42 weeks old. In each flock, samples were taken from 30 randomly selected birds from right, middle and left of the hen house. Cotton swab samples were taken from cloacae of each of the birds and streaked directly on modified Charcoal Cefoperazone Desoxycholate Agar (mCCDA) and *Brilliance* CampyCount Agar (BCCA, both Oxoid, Germany). Thereafter, each swab was put in a vial with 9 ml Bolton Broth (Oxoid, Germany). A pool litter sample per flock was collected from top litter levels at randomly selected places in the barn. Samples were cooled to about 4°C and transported to the laboratory for same day analysis.

Isolation and Identification of isolated *C.jejuni*

The samples were examined for *C. jejuni* according to ISO 10272-1:2006. The litter was enriched in Bolton Broth (1:9) and homogenized for 1 min at 120 rpm in a stomacher (Seward, UK). After homogenization, the broth was ten fold diluted in 0.9% NaCl, and 100 µL aliquot of each dilution was direct streaked onto mCCDA and BCCA in duplicate. Streaked plates and bags were then incubated at 42ºC under microaerobic conditions for 48 h. For swabs: plates and broth were incubated in a microaerobic atmosphere as described above.

From each plate, 1 to 3 suspected *Campylobacter spp.* colonies were confirmed by microscopic observation of characteristic spiral shape and corkscrew-like motility using wet mount. Pure cultures were then tested for Gram Staining, hippurate hydrolysis, catalase test, oxidase test, microaerobic growth at 25°C and aerobic growth at 42°C.

Initially positive isolates were further identified biochemically using API Campy test (BioMerieux, Germany). All *C. jejuni* positive isolates were stored in FBP medium at -80°C until confirmation by PCR.

Polymerase Chain Reaction of *C. jejuni* isolates

As a positive control, two reference *Campylobacter* strains, *C. jejuni* DSMZ 4688 and *C. coli* DSMZ 4689 were obtained from German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). The DNA from the *C. jejuni* isolates as well as the reference strains were extracted from 48 h cell cultures on Columbia Agar using peqGOLD bacterial DNA Kit (PeQlab Biotechnologie, Germany) following the manufacturer instructions. The *mapA* gene was used for the identification of *C. jejuni* using primers which were designed by Invitrogen (Germany). Amplification reactions were carried out using Mastercycler (Eppendorf, Germany) using FastStart Taq DNA Polymerase kit (Roche, Germany). The PCR product was separated by electrophoresis using 1.5 % agarose gel (Application AG, Germany) with ethidium bromide. A DNA Molecular weight marker XIV 100 bp ladder (Roche, Germany) was used as the standard molecular size marker. The gel was visualized with an ultraviolet transilluminator (Biometra, Germany) and photographed.

Restriction fragment length polymorphism of PCR products

The amplification of the *fla A* gene of *C. jejuni* was performed using peqGOLd Taq-DNA-polymerase (PeQlab Biotechnologie, Germany). The PCR products were purified using MinElute PCR Purification kit (QIAGEN, Germany) following the manufacture's instruction. The purified PCR products were restricted using *DdeI* (Roche, Germany). The digested DNA was loaded in 2.5 % agarose gel with ethidium bromide and proceeds as described above.

RESULTS

Positive *C. jejuni* cloacal swabs were obtained from all five farms. The numbers of positive birds in the 5 farms were 8, 9, 14, 16 and 18 corresponding to 27, 30, 47, 53 and 60 %, respectively at the tested birds. *C. jejuni* was isolated from litter of three farms (flocks 2, 3 and 5). The molecular typing resulted in 25 restriction profiles which were farm specific (Table 1). The litter genotype in farms 2 and 3 was also detected in the birds. In flock 5 the litter showed different pattern than those isolated from birds.

Table 1: Number of positive samples (hens and litter) per farm and genotypes detected by RFLP.

Flock number	n	RFLP genotypes detected (no. of isolates)
1	8	A_1 (8)
2	10	B_1 (3), B_2 (3), B_3 (2), B_4 (2)
3	15	C_1 (3), C_2 (3), C_3 (1), C_4 (2), C_5 (2), C_6 (1), C_7 (3)
4	16	D_1 (2), D_2 (6), D_3 (4), D_4 (1), D_5 (3)
5	19	E_1 (3), E_2 (1), E_3 (1), E_4 (3), E_5 (3), E_6 (5), E_7 (1), E_8 (2)

Bold indicate positive litter samples

DISCUSSION

Many studies on the prevalence and epidemiology of Campylobacter in broilers and poultry meat products have been realised as the fresh poultry meat is considered being a common source for Campylobacteriosis in humans (2). However, laying hens have not been seen as a major vehicle of food born *Campylobacter* due to low incidence of *Campylobacter* isolation from eggs (3). This study shows that all 5 investigated farms were tested positive for *C. jejuni*. The isolation rate of *C. jejuni* from laying hens faeces was ranging from 27-60%. The positive samples from cloacal swabs and caecum was 26% (13/50) and 54% (19/35), respectively (6). Whereas, *Campylobacter* isolation rate from ceaca was reaching up to 73% (1). This wide variation of colonisation was usually explained by age and immunity of the bird or different biosecurity levels on the farms.

Genotyping of the *C. jejuni* isolates show large diversity *flaA* pattern which confirms earlier results by (5). Diffrent tapes ocuurs in different farms. This may be due to the wide distance between each farm or might be due to multiple exposure of the birds or the different origin of the hens (7). No *Campylobacter* was isolated from broiler litter (9) but in this study *C. jejuni* was isolated from litter of farm 2, 3 and 5. In a previous study by the *flaA* pattern of litter in farm 2 and 3 was similar to those isolated from birds. In contrast, the *flaA* pattern of litter in farm 5 was not detected in the birds. It is not clear that the origin of this isolate is undetected strain from hens or environmental contamination. The study shows that there is a need for more and careful investigations on the occurrence and persistence of *Campylobacter* spp. in laying hens and their environment in order to increase our understanding about the reservoirs and transmission pathways of *Campylobacter*.

CONCLUSIONS

The results show that there seems to be a great genetic diversity of *C. jejuni* within and between flocks and that laying hens and litter have to be regarded as considerable sources of *Campylobacter* for both animal and man. It seems useful to continue investigations on the presence of *Campylobacter* in herds and in their environmental compartments to improve our understanding about the transmission pathways of *Campylobacter*.

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RELATIONSHIP BETWEEN USE OF FLUOROQUINOLONES IN BROILER AND HUMAN CAMPYLOBACTERIOSIS

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SUMMARY

Therapeutic use of Fluoroquinolone (FQ) in poultry production is controversial, in connection with a more frequent resistance to FQ in *Campylobacter (C.* spp.), carried by chicken and transmitted by contamination from the carcass to humans. In the framework of the multidisciplinary project "Evalu FQ Vol", the socio-economical consequences of a ban of FQ in poultry production were investigated. In a first survey, FQ was shown to be used only during the 10 first days of life to treat broilers batches (n=286) against Colibacillosis. A cost-benefit analysis showed that it was on average beneficial to treat with FQ compared to other antimicrobials (n=96 batches). Nevertheless, sensitivity analyses provided more contrasted results. Another survey showed that the contamination of the carcasses by *C.* spp. in colonized batches of broilers (n=108) was not related to previous drug administration against colibacillosis. Among other health indicators, only heterogeneity of carcass weight in the batch was found to be a risk factor of carcass contamination. Continuous increase of FQ resistance in *C.* spp. in broiler carcasses in France is questionable as FQ appeared to be administered in a period where *C.* spp. is most probably absent of the chicken gut. The hypothesis is presented that *C.* spp. could acquire resistance by selection in the litter at the contact of persistent FQ residues in faeces from early treatment in the same batch and then followed by an oro-fecal recycling of FQ resistant *C.* spp.

INTRODUCTION

Therapeutic use of Fluoroquinolone (FQ) in poultry production is responsible of a more frequent resistance to FQ in *Campylobacter (C.* spp.) carried by chicken. Most often, FQ resistant *C.* spp. are transmitted to broiler meat at slaughter, exposing, by the way, humans to more frequent FQ resistant Campylobacteriosis. In the framework of a multidisciplinary research project (EvaluFQVol), granted by the French National Agency for Research (ANR), our task was to investigate useful parameters for modeling health and economic consequences of a ban of FQ in poultry production for both public health and poultry production. This communication aimed to highlight some of the key results of this project.

MATERIAL AND METHODS

In a first study, a cost-benefit analysis about the choice between FQ and other antibiotics in the treatment was conducted: health, technical and economical data were retrospectively collected from 286 batches of standard broilers from 2 production organizations where a treatment against colibacillosis was identified. Cost of treatment was estimated for each treatment based on age and length of treatment together with a common price list of drugs and related growth curve. Benefit was estimated by the "chick-feed margin" for 1000 chickens which is the prize of the broilers paid to the farmer (based on the number and average weight of broilers in the batch) minus the prize of feed (based on conversion rate of the batch) and the prize of day-old chicks with common prizes for broilers, feed and day-old chicks. The decision problem was formalized using a simplified decision tree framework, to characterize representative therapeutic paths and in order to compare only a limited number of therapeutic alternatives. Therapeutic paths to consider were then decomposed, distinguishing three kinds of nodes: decision nodes, chance nodes and terminal nodes. Observed proportion of each path was used as the probability for each chance node. The 'optimal' strategy was the strategy maximizing this expected net benefit. To test the robustness of the model's prediction and take into account the uncertainty in the value of the parameters, a sensitivity analysis was performed. This sensitivity analysis allowed us to evaluate the impact of modifications of the model's parameters on the model's predictions (results not displayed). Finally, a probabilistic sensitivity analysis was performed to evaluate the simultaneous effects of variations in the parameters on the results by using Monte Carlo simulations (one million iterations).

A second study investigated the relationship between technical and health related data of 108 *C.* spp. colonized batches and the contamination of their carcasses by *C.* spp. in 3 slaughterhouses. It was based on *C.* spp. numeration in pools of 10 ceca and 10 neck-skins per batch. Presence or absence of *C.* spp. in pools (detection limit: 2 $\log_{10}$ cfu) was submitted to a risk factor analysis by the mean of a logistic regression. Explanatory variables were presence or absence of treatment (against colibacillosis, bacterial enteritis and coccidiosis), age,

carcass weight, standard deviation of carcass weight, mortality rate, condemnation rate, slaughter rate *C.* ssp counts in ceca, with the slaughterhouse as a random variable.

RESULTS

In the first study, FQ (i.e. enrofloxacin) was only used before 10 d. of age in chicken in the sample. Therefore, no economical comparison between FQ and other antibiotics was possible in case of colibacillosis after 10 d. Moreover, no treatment other than FQ was implemented before 10d in one of the two organizations: comparison before 10 d. was only possible in one organization (96 batches).

In our model (Fig. 1), the average cost and benefit associated with FQ treatment were respectively €11.35 and €355.33, leading to a net benefit estimate for the producer of €343.98. This net benefit was slightly higher than the expected net benefit arising from the use of another antimicrobial (€341.71), because the cost and benefit associated to these treatments were respectively calculated as €5.84 and €347.55.

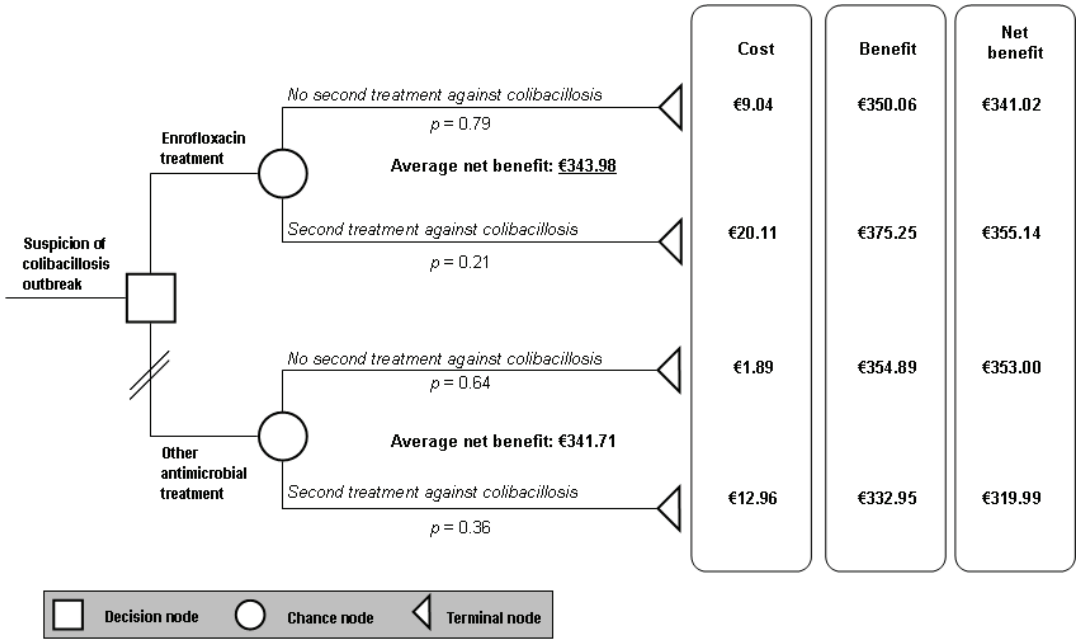

Figure 1: The decision tree model

Monte Carlo simulations support the fact that enrofloxacin treatments are more profitable but enrofloxacin strategy was the 'optimal' strategy in only 50.9% of cases.

In the second study, the counts of *Campylobacter* in the cecal contents of the 108 colonized batches varied between 4.8 and 10.2 $\log_{10}$ cfu (mean = 7.97 $\log_{10}$ cfu, s.d. = 0.95 $\log_{10}$ cfu). Seventeen of the positive batches (15.7%) were below the detection limit of 100 cfu/g while in the remaining 91 batches, the count of *Campylobacter* on the neck-skin varied between 2.0 and 5.2 $\log_{10}$ cfu (mean = 3.48 $\log_{10}$ cfu, s.d. = 0.62 $\log_{10}$ cfu, n= 91). A comparison of variables according to skin contamination status showed significant differences between the two skin status groups: on average, the broilers in the batches that were below the detection limit grew more rapidly, were slaughtered at a lower rate and at a younger age, had a smaller standard deviation of carcass weight, a lower condemnation rate and a lower level of cecal contamination than the poultry in the batches with skin counts above the detection limit. Various treatments were administrated for colibacillosis, coccidiosis, and enteritis. No treatment had an identifiable effect on cecal counts. Finally, only heterogeneity of carcass weight had a significant effect in the multivariable analysis (Table 1.): batches with higher standard deviation of carcass weight (>165g) were found to be 5.7 to 9.2 fold more at risk of carcass contamination.

Table 1. Final multivariable logistic regression model for risk-factors for *Campylobacter* neck skin contamination in batches of broilers carrying *Campylobacter* in the ceca (n=108).

	Odds ratio		
Variable	EstimateGlobal	P value	95 % CI
Standard deviation of carcass weight at slaughter (g)			
< 165			
165 – 200	9.2	0.01	1.8, 47.7
≥ 200	5.7		1.2, 28.1

DISCUSSION

Campylobacter spp. can be transmitted to human by the poultry products colonized by the bacteria. Issuing our latter study, carcass-weight homogeneity might contribute to limit this contamination. At the farmer level and in case of disease, a sound use of an efficient antibiotic may, occasionally, contribute to preserve this homogeneity by improving health. Among antibiotics used to treat the very common avian colibacillosis, as described by the building of the decision tree, use of FQs depends on the age of the broiler. The exhaustive examination of treatments in two production companies showed that every FQ treatment was achieved before 10 days of age. The national system for continuous monitoring of antimicrobial use in poultry production [2] stated that, for the year 2008, 96 % of FQ treatments were initiated before the 10^{th} day of age in standard broiler production (n=961). This early administration of FQ in broiler appeared therefore to be largely dominant in France, mainly based on antibiotic prize considerations. Predominant use of FQ for colibacillosis at this stage may be related to the lowest resistance rate of *E. coli* strains to FQ compared to the other licensed antimicrobials for broiler as documented in France [7], together with a lower occurrence of new cases after a first treatment [3]. In our decision tree, this treatment seemed to be beneficial on average, but at batch level, individual situations may be more contrasted. Similar studies in other companies/other years are obviously necessary to discuss the issue that FQ ban would be economically detrimental to broiler production.

Colonization of gut by *C.* spp. is considered to occur usually between the second and the fourth week of life, with possible early infection at 7 days as reviewed in [1]. Therefore the use of FQ during the first week of age, as used in France, has been thought to be a possible "strategic way" to preserve Campylobacter susceptibility to FQ [5]. The present situation does not match this suggestion as FQ resistance in *C.* spp. in broiler is still arising in France [6]. As *C.* spp. acquires FQ resistance by chromosomal mutation and not by plasmid transmission, we propose that this resistance could arise from the presence in the manure of previously administered FQ, excreted in the feces or spoiled in the litter with the drinking water. Recycling of selected *C.* spp. from the litter by oro-fecal route might be thereby a significant source of resistant FQ for further colonization of the digestive tract. Thus, FQs exhibit large chemical stability and enrofloxacin may be recovered from chicken manure at relatively high concentration 7 d. after the end of a treatment (11mg/kg in [4]). Another hypothesis could be early contamination of a few chicks during the first few days after treatment when enrofloxacin residues are still present in the digestive tract of previously treated birds. These hypotheses are only based, at this stage, on epidemiological considerations and deserve further investigations.

CONCLUSIONS

Use of FQ against colibacillosis before 10 d. of age in broiler might somewhat economically benefit to the farmer compared to other antibiotics. If it may contribute to higher homogeneity of carcass weight in case of disease, it could by the way contribute to lower the risk of carcass contamination. Nevertheless this use could, through persistence of FQ residues in the manure or in the digestive tract, impair the susceptibility of *C.* spp to this antibiotic while colonizing later the broiler.

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ADAPTATION OF A PROBABILISTIC MODEL ON CAMPYLOBACTER AT THE STAGE OF SLAUGHTER

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SUMMARY

Campylobacteriosis is the most frequent reported bacterial, gastrointestinal infection in Austria [8] and other developed countries. Broiler meat and the chicken reservoir as a whole are considered to be the major source amongst others. As *Campylobacter* is an important zoonotic pathogen several quantitative microbiological risk assessments (QMRA) have been conducted "from farm to fork". Especially for gaining insights of mechanisms during the slaughter process and the evaluation of interventions at this stage of food production the technique of probabilistic modelling is commonly used.

An existing, previously published model of broiler slaughtering served as a basis for the adaption process. The input parameters of this model have been evaluated and modified if feasible. The presented probabilistic model with Austrian data is ready for the evaluation of specific interventions in terms of the original work and might be adapted further for particular questions.

INTRODUCTION

Campylobacter is a major cause of bacterial infectious enteritis in the developed world. In the EU ~ 198.300 human cases have been reported in 2010 [3], within the EU27 the estimated number of infections is about 9 million people [2]. Contaminated chicken is the most important source/vehicle of human Campylobacter infection and is estimated to be involved in up to 80% cases [2]. The cost of human *Campylobacter* infection to Western economies is high, e. g. in Belgium in 2004 the estimate was ~€27 million [4].

The framework of QMRA has been used to estimate risk and risk mitigation strategies associated with *Campylobacter*, some containing economic calculations. As part of these efforts several industrial processing models on *Campylobacter* in poultry meat have been developed in the last ten years ([5], [11], [7], [9]). They have the use of probabilistic modeling in common, but they vary for instance in their included details, end products, and they consider national differences. For more details the reader is referred to the elaborate comparison of quantitative risk assessments on *Campylobacter*, concerning more than just the processing stage [10].

The objective of this work is the adaption of a published broiler-processing model to the Austrian situation for evaluation of conceivable interventions at broiler slaughter plants.

MATERIAL AND METHODS

The published poultry processing model has been developed for of the CARMA-Project ("campylobacter risk management and assessment" in the Netherlands) and is used as the base for the adaption to the Austrian situation [9]. The simulation model is implemented in @risk 5.5 (Palisade, Newfield, NJ; an add-on to Microsoft Excel), all changes and simulations were implemented in the same software package. The functionality of the model has been studied elaborately and the need of appropriate data has been ascertained instantly. In a first step the influence of truncated distributions is analyzed. Next the input parameters have been compared to local data, and changed, if appropriate. For lacking data (e. g. exterior contamination) hypothetical distributions (binomial distributions, normal distributions with different mean and standard deviation values) are used as new input parameters. The impact of these modifications on the outputs of the model is measured. Subsequently modifications are validated with quantitative data at the stage of carcass chilling [1].

RESULTS

Three different probabilistic models have been at choice, and the Dutch model was identified to suit best. The Danish model [11] primarily is based on data (e.g. chronology of slaughtered flocks in combination with Campylobacter results), which are not available in Austria at the moment. Closing all the data gaps again would have been expensive. As the UK model [6] holds a lot of simplifying assumptions, the Dutch model [9] is used for adaption.

Some input parameters are replaced, such as the variability of bacterial loads in poultry faeces entering the

process. The difference in the mean is 100 fold and the difference in the standard deviation is 25. Results of faecal samples which have been analyzed in the course of previous validation studies imply these changes.

Another input parameter is the animal prevalence within a herd (inner herd prevalence). The dynamics of Campylobacter infection within a flock are assumed to be the same in the Netherlands and in Austria, as the social behavior of chicken does not vary due to nationality. Furthermore literature ([1], [12],) with Austrian results indicates that the inner herd prevalence is comparable to the input table of the Dutch model. This data underpins the above mentioned assumption.

The impact of the exterior contamination is examined with sensitivity analysis. Hypothetical distributions (various normal distributions with different mean values, standard deviations and bimodal distributions) have been examined. The overall effect of these changes was negligible, indicating a minor influence of Campylobacter on the exterior of the birds (skin, feathers).

As a major result of the described work, the original model is adjusted to the Austrian situation and ready for use.

DISCUSSION

The development of a mechanistic, probabilistic processing model for broilers is time consuming and expensive. For this reason the idea of this work was to adapt an existing model to the Austrian situation, making use of current available knowledge.

As a basic prerequisite the assumptions and aims of the utilized model have to be appropriate for the local purposes and questions. In this case the Dutch model meets the regional requirements, as the general questions regarding intervention strategies are comparable in Austria and the Netherlands. The adaptations of the model to fit the Austrian situation are only minor, but, nevertheless, some expertise on modelling is necessary.

A European risk assessment model has been developed recently [13] which has not been available at the time of this investigations. Nevertheless, the scopes of this QMRA differ to our aims, as we intended to investigate specific stages within the slaughter plant exclusively. Each step of processing – like scalding (at 50-52°C), defeathering, evisceration, washing, chilling and cutting (breast cap, filet) - is considered at the Dutch model. As the whole process is described quite mechanistically, at each step changes in cross contamination, transfer rates, temperature, etc. may be implemented. This is an important difference to the recently published report, where the cross contamination is represented as the change of variance in the whole slaughter process, not accounting for the chronology in reduction of independent steps. This approach is an advantage for the goal of the European risk assessment, but does not support the aims of this work. Finally the European risk assessment does not use Austrian data therefore at least this modification would have to be done with this model anyway.

Further work would be necessary to inspect expert judgements considered in the original work in detail. Additionally specific interventions, like the realistic reduction of faecal leakage at defeathering, as one example, could be assessed theoretically. Finally it seems reasonable to use this specific tool for further investigations on intervention strategies located at Austrian processing plants.

CONCLUSIONS

It is attainable and reasonable to adapt an established probabilistic model provided it is fit for the particular purpose. The adapted model will be used for further investigations, e. g. on interventions within the slaughterhouse processes in Austrian processing plants. Further modification of the original model might be necessary to cover specific questions. Summing up the adapted model can serve as a valuable tool to support the combat against Campylobacteriosis in Austria.

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EVALUATION OF RISK FACTORS ASSOCIATED WITH *CAMPYLOBACTER SPP*. IN BROILER FLOCKS

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SUMMARY

To evaluate risk factors associated with *Camplybacter* spp. in broiler flocks in the period from May to November 2010, the caecal contents from broilers of 53 farms from 2-3 fattening trials including all batches delivered to the slaughter house where testes for *Campylobacter jejuni/coli* to make a rating from 1 (all batches positive) to 5 (all batches negative). All this farms were visited and graded from 1 to 5 (latest best) on the basis of 18 criteria, including for example farm structure, environment, hygiene criteria, pest control, hygiene barrier or logistics.

The relation of the hygiene status of the farm on bases of a risk priority number and the faeces category is significant with a correlation coefficient of r_s = 0.67. Supplementary details show that the slaughter age of *Campylobacter* colonized herds is significant higher than in non-colonized. Including the influence of thinning on *Campylobacter* free herds the odds ratio is calculated as 1.7. No significant value could be determined by taking into account flock size.

INTRODUCTION

In the last years, Campylobacterioses was the most frequently reported zoonosis in Austria and the European Union. The infection is usually due to *Campylobacter jejuni*, which accounts more than 90 % of the cases. This serovar is dominant in cattle und poultry. Due to its processing poultry meat is therefore one of the most important risk factor for human *Campylobacter* infections.

The aim of this project was to collect basic data for an evaluation of risk factors associated with *Campylobacter* spp. in the broiler flocks in Austria.

MATERIAL AND METHODS

In the period from May to November 2010 pooled faecal samples of 5 broilers per batch from a total of 53 broiler farms including 2-3 fattening trials were tested for the presence of *Campylobacter jejuni/coli*. Detection was made by direct plating on modified Charcoal-Cefoperazone-Deoxycholate Agar (mCCDA). Concerning the consistency of the faecal results the farms were first categorized into always positive (1), always negative (5) and in between (2-4). Additional all farms were visited from October 2010 to January 2011 by the official veterinarian or the vet care. Thereby a total of 18 criteria were documented. These criteria were graded from 1 to 5 (5 is the best) and subsequently weighted with a risk factor (1, 10 and 100), as illustrated in Table 1.

Consequently every farm receives its own RPN (risk priority number), comprising the sum of the weighted hygiene criteria. The assessment and weighting of the selected criteria was based on literature research and close cooperation with specialized veterinarians, slaughter plant and the Austrian Poultry Health Service.

The statistical analysis was performed with SPSS (IBM, SPSS Statistics, v19).

Table 1: Description of the hygiene criteria classification and their weighting

hygiene criteria	classification (1-5)	risk factor (1, 10, 100)
number of stables	5 = only 1 stable/unit at the farm;	10
	1 = 2 or more stables at the farm, 2 or more units in the stable	
water supply	5= mains water	10
	1= ground water, contaminated with *E. coli*/Enterococci	
dung storage	5 = distance more 10 km, fortified	10
	1 = directly beside the stable	
other animals or pets on/nearby the farm	5 = no other animals (poultry) and pets on and nearby the farm	
	1 = other animals in the same stable, more pets	
stable environment	5 = completely fortified	10
	1 = marginally fortified, plant growth, storage of materials and waste	
structural/technical stable condition	5 = new stable, good condition	10
	1 = old stable, bad condition	
ventilation system	5 = side wall – ceiling ventilation	10
	1 = side wall in/out ventilation,	
pest security	5 = stable (incl. hygiene slice and anteroom) protected, fly control	100
	1 = stable (incl. hygiene slice and anteroom) in bad condition	
chicken watering systems	5 = nipple drinker with tray, bedding material dry	10
	1 = round drinker, bedding material wet, litter flour plates	
feeding	5 = closed supply system, protected area	10
	1 = open food container, food self-transport	
stable cloth	5 = own clothes and food wear for farmer and visitor, correct change	10
	1 = no stable clothes and food wear, no change	
hygiene sluice, barrier, changing room	5 = hygiene sluice or barrier, changing room ok. and clean, hygiene barrier outside of farm	100
	1 = no hygiene slice an vestibule, no disinfectant mat	
c & d equipment for personal	5 = shower, sink ok & clean, detergents and disinfectants ok	1
	1 = no shower, sink, no detergents and disinfectants	
litter materials	5 = dry corn cob, soft cells or straw pellets	100
	1 = straw or food shavings moist and crusted	
litter storage	5 = closed, protected, clean	10
	1 = largely unprotected area, other equipment materials and waste closely	
c & d of the stable	5 = complete manure removal, dry cleaning, hot water cleaning, spray disinfection in no wet area, pat dry	100
	1 = no correct litter removal and dry cleaning, cold water cleaning, disinfection in wet area, no disinfection	
collection system	5 = own and clean chicken harvester from farm; harvester crew without contact to other poultry farms	100
	1 = harvester crew two or more plants per day, no cloth change and correct cleaning and disinfection of the crew	
thinning frequency	5 = no partial slaughter	100
	1 = collection on 2 or more days over a period of 5-8 days	

RESULTS

Descriptive statistics of the RPN (risk priority number) and the faeces category were performed and reveal an important overview on frequentness and distribution. Additionally their relation was investigated and the correlation coefficient was calculated.

The hypothetical minimum value of the RPN is 801, the maximum is 4005 points. Within this survey the observed values are within [1772; 3114]. The mean is 2434 with a standard deviation of 341 points, and the distribution is positive skewed (0.219). Skewness measures asymmetry, in this case the majority of the farms have fewer points than the mean.

Every hygiene category was inspected individually and criteria concerning the structural and technical standard, the hygiene and the collection system at depopulation showed a moderate standard (detailed results will be presented).

The relation of the hygiene status used in this investigation and the *Campylobacter* results from caecal samples is visualized in Figure 1. Farms with high RPN demonstrate better *Campylobacter* results than farms with a low RPN. The calculated Spearman rank correlation coefficient between RPN and faeces category is significant ($\alpha<0.01$) with a value of 0.67, indicating a strong positive correlation between good hygiene status and *Campylobacter* negative results. Additionally the correlation of each hygiene criterion was calculated, the highest value was examined for stable environment (r_s = 0.69), for other significant correlations we refer to [2].

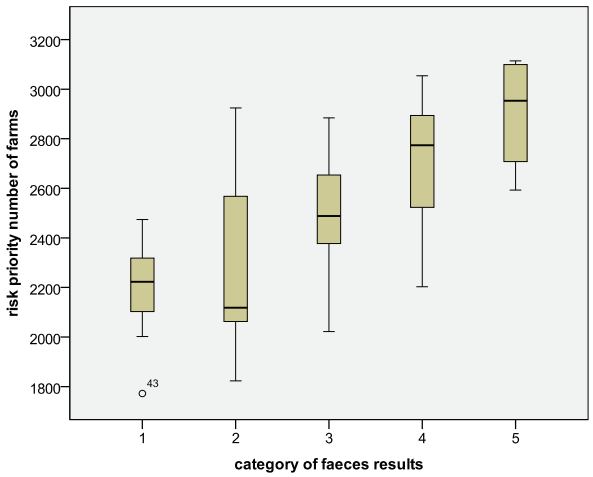

Figure 1: Correlation of the risk priority number and faeces category

Supplementary to the evaluation of the above mentioned hygiene criteria frequently discussed parameters in relation to *Campylobacter* status of flocks were investigated in detail: slaughter age, herd size and thinning.

The average age and the median age of *Campylobacter* colonized herds (average=36 days, mean=34 days) are higher than in non-colonized (average=34.4 days, mean=33 days). The difference in the average of this two distinct groups according to statistical testing is significant ($\alpha<001$).

The mean herd size within this survey is 17 000 animals per flock. As former investigations indicated an influence of the flock size on the *Campylobacter* status, the same statistical technique was performed (odds ratio). No significant value could be determined, neither for the size of 15 000 nor 17 000 animals per flock.

The influence of thinning was determined with the same technique, as two distinct binary categories are established: the *Campylobacter* status (negative/positive) and partial depopulation (thinning/no thinning). The odds ratio for *Campylobacter* free herds regarding to "no thinning" was calculated as 1.7, confidence interval [1.218-2.474]. In other words, the probability of having a negative *Campylobacter* result at the first harvest is about 2 times higher than a negative *Campylobacter* result in the following rounds.

DISCUSSION

It is beyond question that hygiene is essential to prevent *Campylobacter* colonization in broilers. Despite this common knowledge single factors contribute to the overall risk of *Campylobacter* positivity on different scales. The developed model includes 18 hygiene parameters and the weighted, summed risk priority numbers (RPN) reveal a strong and significant correlation with the faecal results of the investigated farms, which indicates the model validity.

The RPN of the farms reveals a condensed impression of the management behaviour and hygiene status at specific Austrian broiler farms. Most of the farms have a RPN less than the mean which indicates possible improvement in general.

The connection of slaughter age and *Campylobacter* positive results has been shown previously [1], [4] which

is in conformity to our results. Apart from this our results do not indicate a significant contribution of herd size to the risk of *Campylobacter* detection. This is in contrast to previous work [3], [5]. The mentioned, Austrian investigation was carried out about 10 years ago (sampling in 2001-2003). Since then agriculture structure (average herd size), management and legislation (animal welfare) have changed, to mention some of possible reasons for the differing result. Additionally another Austrian survey [4] could not identify the herd size as a risk factor.

The effect of thinning described with the odds ratio of 1.7 looks quite small. The influence of positive flocks even before the first depopulation has to be considered as this proportion biases the effect powerfully.

CONCLUSIONS

For the future a better implementation of the legal standards in coordination with an economic and trade quality system must be in the interest of the official veterinarian, the animal health service and of the slaughterhouses, respectively.

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MICROBIOLOGICAL IMPLICATIONS ON THE REMOVAL OF BRUISES ON OSTRICH CARCASSES POST-EVISCERATION OR POST-CHILLING

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SUMMARY

Bruising on ostrich carcasses reduces meat yield as these are usually removed as part of the primary meat inspection, performed directly after evisceration. Three separate studies were conducted to determine the advantages and disadvantages of removing the bruises at primary meat inspection or after overnight cooling of the ostrich carcasses (0-4°C). It was established that trimming bruises on warm carcasses caused higher total aerobic viable counts on the trimmed surfaces than cold trimming. This was most probably caused by microbial contamination from the environment.

A further evaluation of the occurrence of bruising on ca. 2280 birds indicated that neck bruises represented 52.58% of all bruises; the high side railings of the transport vehicles being the most probable cause. Large and multiple bruising noted were probably from the trampling of birds lying down. Cold trimming together with better management of trimming practices also led to a decrease in meat yield losses.

INTRODUCTION

Within South Africa, ostriches are transported to the abattoir uses stringent guidelines [5]. Even so, bruising occurs either from the herding, loading, transport, offloading, lairage and the slaughter process itself or from inter-bird interactions. Bruising on ostrich carcasses reduces meat yield.

Bruises or contusions are described as superficial discoloration due to haemorrhage into the tissue from ruptured blood vessels beneath the skin surface, without the skin being broken. In the contusions the blood accumulates in surrounding tissues, producing pain, swelling and tenderness [2]. These bruises are usually removed as part of the primary meat inspection, performed directly after evisceration (within 1 hour *post mortem*) according to the appropriate Veterinary Procedural Notice [1]. This action of warm trimming of bruises has in the past been known to contribute to significant losses in meat yield per carcass (on average 300 g per bird) in the abattoir where the studies were conducted.

The effect of warm or cold trimming of the carcasses was examined together with possible causes of contamination along the processing line. The prevalent microbial growth on carcasses before and after overnight cooling in an ostrich abattoir and de-boning plant was thus investigated. An attempt was made to link the prevalent microorganisms that were identified from carcasses to those from specific external contamination sources [3].

MATERIAL AND METHODS

Three separate studies were conducted to determine the advantages and disadvantages of removing the bruises at primary meat inspection or after overnight cooling of the ostrich carcasses (0-4°C)[3]. On each of the three occasions ca. 760 ostriches were slaughtered, bringing the total ostriches considered to ca. 2280 birds. Once inside the abattoir, the ostriches were slaughtered, bled, plucked, de-skinned and eviscerated according to the prescribed standard operating procedures. Ostriches with bruises on their thighs or back muscles were selected. Bruised areas between 2.5 cm and 10 cm in diameter that did not show green discoloration (i.e. an old injury) or any sign of infection or abscess were selected. The size of the bruises was selected on the basis of experience on the slaughter floor that suggested that smaller bruises (< 2.5 cm in diameter) are mostly negligible and larger bruises (> 10 cm in diameter) are usually so severe that they have to be removed by the inspectors.

For each of the three studies, 10 carcasses were identified with visible bruises: five bruises (one bruise per carcass from five carcasses) were removed (warm trimmed) during the primary meat inspection (on day one, 30-45 minutes post-mortem) and five bruises (one identified per carcass) were left on the carcass. The first set of microbiological samples was taken directly/immediately after primary meat inspection. A set of control samples were also taken adjacent to the bruised areas that were sampled.

The five bruises left on the carcass were cold trimmed the next morning (on day two, ca. 24 h post-mortem, temperature = 0-4°C) and the second set of

microbiological samples was taken (meat temperature ≤4ºC). The sampling sites on the carcasses were identified on day 1 with sterilized steel pins that were wedged in the physical sample area to ensure that a similar sample area was tested on day two.

Microbial samples of the bruised carcass muscle surfaces were taken in a destructive manner, i.e. pieces of meat were removed from the surface muscles, using aseptic techniques. Groups of five samples per sampling point were pooled to give one value for the five carcasses.

At the first sampling point (day one) the following samples were taken: For the five bruised carcasses that were warm trimmed; A1: One sample per carcass cut from the newly exposed trimmed area where the bruises were removed, pooled into one sterile bag. A2: One sample per carcass from the undamaged area adjacent to the trimmed area, pooled into one sterile bag (control sample). For the five bruised carcasses that were not trimmed on day one; B1: One sample per carcass cut from the bruised area, pooled into one sterile bag. B2: One sample per carcass from the undamaged area adjacent to the trimmed area, pooled into one sterile bag (control sample).

Day two, at the second sampling point (day two) the following samples were taken: For the five bruised carcasses that were warm trimmed on day one; A3: One sample per carcass cut from the exposed trimmed area where the bruises were removed, pooled into one sterile bag.

A4: One sample per carcass from the undamaged area adjacent to the trimmed area, pooled into one sterile bag (control sample). For the five bruised carcasses that were cold trimmed on day two; B3: One sample per carcass cut from the newly exposed trimmed area after the bruises were removed, pooled into one sterile bag. B4: One sample per carcass from the undamaged area adjacent to the trimmed area, pooled into one sterile bag (control sample).

The sterile sampling bags were analyzed according to standard ISO and SANS[3].

In another trial, at the primary meat inspection point of the slaughter-line all the carcasses slaughtered over eight slaughter days (3153 ostriches) were visually inspected for the presence of bruises. These bruises were identified to one of four areas on the carcasses: the neck; the back, the front of the thighs; and the back of the thighs. The number of bruises were recorded and commented on in terms of size. An A, B and C classification were awarded to the bruises on the basis of size, where A = <2 cm in diameter, B = 2-5 cm in diameter and C = >5 cm in diameter.

RESULTS

The increase in aerobic plate counts (APC) on the bruised and non-bruised (control) areas over the 24 hour cooling period is depicted in figure 1. Results from an ANOVA analysis did not show evidence that there was any reason to select one of the four treatments over the other *(p =* 0.2241 for the APC values).

On 3153 birds evaluated, 789 bruises were found (Table 1). The necks (52.58%) were clearly the most pronounced area for bruising folowed by the front of the thighs which represented 36.98% of the bruising [3]. On 168 carcasses, more than one bruise was found.Theses bruises tended to be larger in size (from 2 cm to > 5 cm). From the 789 bruises evaluated, 47.06% of the bruises were indicated as >5 cm in diameter, thus, representing fairly large areas of meat and causing significant losses [3].

On 168 carcasses (5.33% of the ostriches evaluated), more than one bruise was found (up to 4 large bruises on a single carcass).The bruises evaluated from the carcasses with multiple bruising also tended to be larger in size (from 2 cm to > 5 cm). From the 789 bruises evaluated, 47.06% of the bruises were indicated as >5 cm in diameter, thus, representing fairly large areas of meat and causing significant losses [3].

DISCUSSION

The final microbial load on the primal meat cuts in the de-boning area was lower when bruises were cold trimmed rather than warm trimmed and this should lead to an increase in shelf-life. The reason for this increase in microbial growth on the trimmed areas could be the fact that the muscle area is exposed during trimming; the meat is thus more susceptible to aerobic bacterial contamination and growth. Sources of bacterial contamination in any abattoir are present all along the slaughter-line, in the cooling rooms and the de-boning facilities. The sources identified (unpublished results) in this abattoir include the air, the hygiene of workers and surfaces and most importantly, the water pooling on platforms and drainage areas. Microbiological results indicated pooled water in the abattoir as the most hazardous vector for carcass contamination and that contaminants from this source are mostly Gram-negative pathogens. *Pseudomonas* and *Shigella* were frequently isolated from surface and air samples and indicated that the control of total plant hygiene is a requirement for producing ostrich meat that is safe to consume and has an acceptable shelf-life [4].

The high side railings of the transport vehicles were the most probable cause of neck bruises. The relatively high frequency of bruising on the thighs was most likely caused during loading and off-loading practices, when the ostriches jump on or off the trucks and often into either the sides of the trucks or the walkways. To a lesser degree these bruises might be caused by injuries in the

pens and during herding between pens. Protecting the sides of the vehicles and the ramps with a softer barrier might aid in minimizing this area of bruising. Large and multiple bruising were probably from the trampling of birds lying down [3].

CONCLUSIONS

Cold trimming together with better management of trimming practices led to a decrease in meat yield losses. The average meat loss due to the trimming of bruised meat decreased from an average of > 250 g per bird for warm trimmed carcasses to an average of just over 130 g per bird for cold trimmed carcasses. It is further argued that a bruise is not a health risk but rather an aesthetic and quality issue [3].

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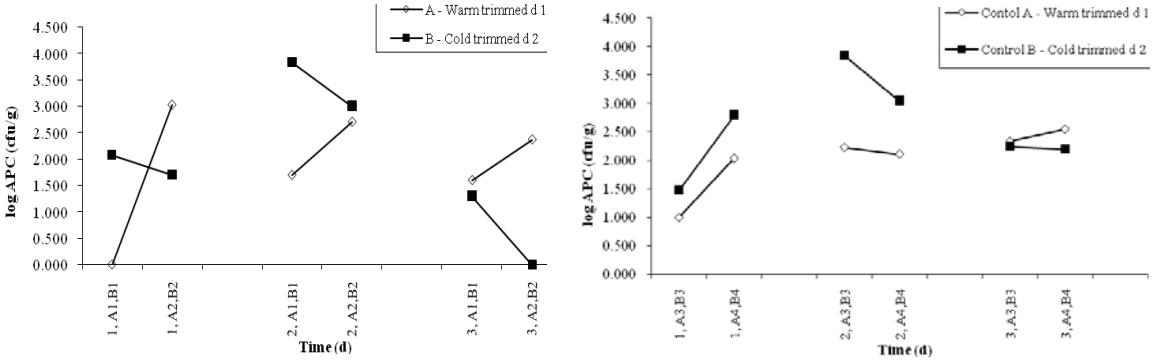

Figure 1: The change from slaughter to 24 h post slaughter (a one day period) in aerobic plate counts on bruised areas (left figure) and the undamaged control (right area) for the three trials for both cold and warm trimmed carcasses (pooled from n = 5 x 3 carcasses) [3].

Table 1: Total distribution of bruises on ostrich carcasses monitored over an eight day slaughter period [3].

Number of			Number of bruises on				Percentage (%) of bruises on				
	Birds	Bruise	Neck	Back	Thigh front	Thigh back	Total	Neck	Back	Thigh front	Thigh back
Total	3153	789	418	9	294	74	25.21	52.58	1.13	36.98	9.31

PRELIMINARY REPORT ON THE ZOONOTIC SIGNIFICANCE OF TUBERCULOSIS IN CATTLE IN THE HIGHLANDS OF CAMEROON

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SUMMARY

Bovine tuberculosis (TB) which is an important zoonosis is neglected in Cameroon, where the incidence of human TB and TB-HIV/AIDS co-infection are among the highest in the world and increasing annually. Also, many communities depend on their livestock for livelihood. Comparative tuberculin skin tests of cattle in the highlands of Cameroon revealed that bovine TB is prevalent in live cattle (3.09%) and widely distributed in the regions, though at a low prevalence in some areas. Over 1.47% of cattle slaughtered in the Bamenda city abattoir showed suspect tuberculous lesions. Severe interpretations of the comparative tuberculin skin tests could be more useful for maximum diagnosis of the disease in the local environment. Genomic deletion typing for regions of difference (RD) 9 and 4 of killed isolates showed evidence of *M. bovis* and *M. tuberculosis* in both cattle and humans suggesting possible animal / human cyclic transmissions. Comprehensive investigations of the molecular epidemiology of bovine TB in cattle, the zoonotic risks and public health importance of bovine TB in Cameroon cannot be overemphasised.

INTRODUCTION

Bovine tuberculosis (TB) caused by *Mycobacterium bovis* is a debilitating disease of cattle but can also cause severe hazards to human health [15] from ingesting of contaminated milk and raw beef or inhaling cough spray from infected cattle [4]. The detection of tuberculous lesions at post mortem examination of carcasses including meat inspection of slaughtered animals is usually the basis for indicating the occurrence of bovine TB in Cameroon [3,7]. However, the disease is poorly investigated or neglected in Cameroon which contributes over 60% of the cattle population in the entire Central African sub-region [13]. There are scanty reports of the prevalence of bovine TB in live animals in some restricted areas of the country [8,10].

The incidence of human TB in Cameroon is among the highest in the world and is increasing annually and extraordinary rates of TB-HIV/AIDS co-infection also exist [2,11,18]; but the contribution of *M. bovis* to human TB based on the risks of exposure, transmission and the actual infection is not clear. Furthermore, many communities depend on their livestock for livelihood and there are plenty of close human / animal interactions.

This study was therefore carried out to determine the prevalence of bovine TB based on tuberculin skin tests of live cattle and the detection of tuberculous lesion at meat inspection of slaughtered cattle in the highlands of Cameroon. Preliminary genomic deletion typing findings of tubercle bacilli isolates from cultured samples of suspected tuberculous cattle lesions and sputa from human TB cases in the regions are also described.

MATERIAL AND METHODS

Live cattle in the highlands of Cameroon were subjected to comparative tuberculin skin tests (between March and August 2010) through the intradermal injections of bovine tuberculin and avian tuberculin at separate sites (~ 12cm apart) in the skin of the neck. The differences in skin responses at 72 hours post injection of the tuberculins were interpreted using the recommended cut-off points of the increase in skin thickness for a comparative tuberculin test to be positive [12,1].

Also, during January 2009 to May 2010 a thorough investigation of the detection and collection of tuberculous lesions in slaughtered cattle in the Bamenda municipal abattoir of the highlands of Cameroon were carried out. The suspected cattle lesions and sputa from willing human TB cases in local TB clinics showing acid-fast bacilli at Ziehl-Neelsen staining / microscopy were cultured on Lowenstein Jessen media enriched with pyruvate or glycerol [17].

All isolates were heat killed and the multiplex-PCR based deletion typing of various genomic regions of difference (RD) for differentiating *Mycobacterium tuberculosis* complex [16] were done to check the presence or absence

of RD9 and RD4. Flanking primers indicated the absence of the RD while the associated Internal primers indicated the presence of the RD being analysed. The RD-PCR reactions were performed in 96-well plates and contained per reaction 8 µl DNA template, 1 µl each of 10 pmol / µl Flanking and Internal primer (Eurofins MWG Operon, Ebersberg, Germany) and 10 µl HotStarTaq DNA polymerase (Qiagen, Hilden, Germany) to give a total volume of 20 µl. Amplification was initiated by incubation at 95°C for 15 minutes, followed by 45 cycles at 94°C for 1 minute, 61°C for 1 minute and 72°C for 2.5 minutes. After the last cycle, the samples were incubated at 72°C for 10 minutes. The products were electrophoresed using 1.5% agarose gel in 1xTAE running buffer (pH 8.03). SYBR Safe dye at a ratio of 1:10000, 100 bp DNA ladder and orange 6 x loading dye were used in the gel electrophoresis.

RESULTS

Of 1,166 tuberculin skin tested cattle from 29 herds and using the OIE-recommended ≥ 4-mm cut-off point, positive (3.09%) and inconclusive (9.18%) reactors were observed to be widely distributed in the study regions. Also, ~45% of the tested herds presented ≥1 positive reactor.

Over 1.47% of 12,775 inspected slaughtered cattle showed suspected tuberculous lesions and the monthly detection rate ranged from 0.51% to 3.35% (Figure 1); 1.33% for 2009 and 1.93% for January – May 2010. Peak occurrences were observed during March and May but season did not seem to affect the detection rates of the lesions.

Table 1 shows RD deletion analysis of mycobacterium isolates in cattle and humans in the study. Overall, a total of 174 isolates (108 cattle and 66 humans) showed results at RD9 and or RD4 deletion typing. There was also evidence of inconclusive result analysis for some isolates. However, culture of 169 suspected tuberculous lesions (125 cattle) and genomic deletion typing of the isolates yielded acid-fast bacilli in ~83% of cases, with *M. bovis* accounting for ~ 45% (~65% of cases) and *M. tuberculosis* for >10% (~18% of cases) of the acid-fast cultures. Furthermore, >26% of a total of 64 human sputa cultures was linked to *M. bovis* or associated to another organism other than *M. tuberculosis*.

DISCUSSION

Bovine TB which has intense zoonotic potential and public health significance is widespread in cattle in the highland regions of Cameroon based on post mortem findings of tuberculosis lesions in carcasses and comparative tuberculin skin test analysis. Also, RD deletion analysis confirmed *M. bovis* in cattle and showed evidence of *M. bovis* in humans and *M. tuberculosis* in cattle isolates which suggest animal / human cyclic transmissions. However, the reason for the inconclusive deletion results observed in this study was not clear.

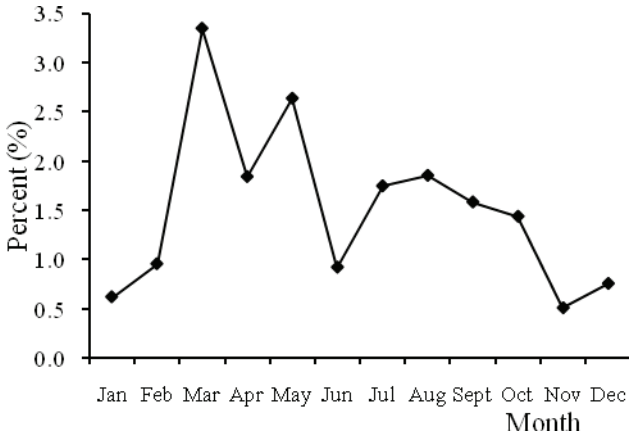

Figure 1: Distribution of suspected TB lesions in slaughtered cattle in Bamenda abattoir, Cameroon

Table 1: RD deletion typing results for Mycobacterium isolates of cattle and humans in the highlands of Cameroon

RD	Cattle		Humans	
	Present	Absent	Present	Absent
RD9 (n_c=169; n_h=78)	13 (3)	113 (103)	55 (41)	14 (3)
RD4 (n_c=162; n_h=78)	22 (4)	81 (54)	45 (21)	19 (1)

RD: genomic Region of different
n_c and n_h: number of cattle and human isolates
( ) : value represent strict assessment for the category

All active bovine TB cases do not show tuberculous lesions at post mortem examination and the presence of lesions usually indicate advance stages of the disease [5,14]. Comprehensive molecular studies would be needed to provide more precise epidemiology data on issues of the mycobacterial strains causing TB in Cameroon, transmission of TB in animals and humans, the reservoir (and maintenance) hosts and their role as risk factors. Furthermore, an all-inclusive investigation of these risks and the public health implications of zoonotic bovine TB in cattle from a Cameroon context can not be overemphasized.

The performance of tuberculin skin tests are affected by many factors such as environmental factors, prevalence of TB, host factors (status of immunity, genetics, etc.) and nature of the tuberculin used; and a perfect cut-off point in a specific geographic area may not be so useful in

another environment [1,6,9]. Also, the ability of a tuberculin test to accurately predict the true positive disease status is not constant and depends on its sensitivity and specificity, as well as the prevalence of the disease in the population tested [6]. The OIE recommended cut-off point of > 4mm after 72 hours of increase in skin thickness for a comparative tuberculin test to be considered positive [12] was established mainly in the developed countries and for *Bos taurus* cattle. However, different reference cut-offs are applied according to a particular country's disease status and the objective of its disease control program [1,9]. Therefore, it would be important to re-evaluate tuberculin skin test cut-off values in the Cameroon's environmental conditions.

CONCLUSIONS

Bovine TB is widespread in the highlands of Cameroon, though at a low prevalence in some areas. Severe interpretations of the comparative tuberculin skin test responses at a cut-off value of less than 4-mm could be more useful for maximum diagnosis of the disease in the local environment. The identification of *M. bovis* from human and *M. tuberculosis* from cattle cases suggest possible animal / human cyclic transmissions. The findings of this study provide overwhelming evidence for further investigation of the incidence of human TB due to *M. bovis* and useful hints on high zoonotic risks and importance of bovine TB in Cameroon.

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PREVALENCE OF TUBERCULOSIS IN RED DEER (CERVUS ELAPHUS HIPPELAPHUS) IN TYROL - PRESENTATION OF A PILOT STUDY

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SUMMARY

This paper describes the preliminary results of an ongoing cross sectional survey on the prevalence of Tuberculosis (TB) in free living red deer in the province of Tyrol.

INTRODUCTION

TB in wildlife is a chronic infectious disease caused by bacteria of the *Mycobacterium tuberculosis* complex (MTC). Several members of the MTC can be distinguished, *Mycobacterium caprae (M. caprae)* being one of them. TB is a zoonosis, hence wild animals may act as a source of infection for domestic animals and humans. *M. caprae,* the aetiological agent of TB in wild ungulates found in our pilot study, can infect a wide range of wild-, domestic animals and humans. Since 1999, we observed single outbreaks of TB affecting red deer in defined areas of the Tyrol, Western Austria [1]. A serious outbreak of TB in cattle which shared the same ecosystem as red deer during the summer grazing period was observed in 2008 having a great impact on cattle production in concerned region. Due to further investigations, singular cases of TB in red deer were continually observed. Together with the cooperation of all relevant stakeholders a systematic survey was conducted in the red deer population. Sampling started in the hunting season 2008/2009.

MATERIAL AND METHODS

During the hunting season 2008/2009 a network of local hunters cooperating with official veterinary authorities was established throughout the observation area. Red deer of specific age and sex where included in the survey. During the pilot phase 143 carcasses of hunted harvested red deer where submitted for further examination for the presence of tuberculosis infection to the AGES Institute of Veterinary Disease Control in Innsbruck. Retropharyngeal, tracheobronchial, mediastinal, mesenterial as well as ileocaecal lymph nodes were examined for gross lesions and histology performed. Tissue material from lymph nodes and organs were submitted to the National Reference Laboratory, AGES, at Mödling for microbiological cultivation and molecular identification. *M. caprae* isolates were further analysed by genotyping in two subsequent steps: first by spoligotyping and secondly by MIRU-typing (Mycobacterial interspersed repetitive units typing) at the Medical University of Innsbruck.

RESULTS

Out of the 143 examined red deer, 8 showed gross lesions and 9 yielded mycobacteria after cultivation. All isolates were identified as *M. caprae* by the GenoType MTBC Assay. The results of the genotyping showed homogeneity in all 9 isolates with marginal variation, each in one of 25 MIRU loci, in two isolates. Culture positive animals showed gross and microscopic lesions typical for mycobacterial infection except one animal. In this animal no pathological changes were visible.

DISCUSSION

Since 1999, we observed single outbreaks of TB affecting red deer within the Province of the Tyrol. A serious spill over to singular cattle holdings which shared the same ecosystem was observed since the beginning of 2008. TB in wildlife is a common problem in several European countries threatening the eradication in livestock. In cattle, TB can be addressed as an emerging disease of major economic and public health importance. In the geographical region under observation the indirect as well as direct contact of domestic animals and red deer on alpine pastures is likely. Isolates from wildlife and cattle were indentified as *M. caprae,* all isolates were genetically homogenous belonging to one cluster. The role of *M. caprae* in human tuberculosis contracted from animal sources presumably long before disease manifestation was described by Prodinger et al.[2]. Until now, no *M. caprae* isolate was isolated from TB cases among residents of the geographical area concerned.

CONCLUSIONS

We conclude that red deer is being recognized as sources of TB infection in cattle. Red deer can serve as a reservoir and constitutes a significant risk to cattle husbandry within this defined geographical area. Transmission to humans appears to be rare, as no matching human TB case has been identified in the affected region within 12 years.

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DEVELOPMENT AND EVALUATION OF A NEW AND ORIGINAL EXTRACTION PROTOCOL TO DETECT *MYCOBACTERIUM AVIUM* SUBSP *PARATUBERCULOSIS* IN BOVINE FAECES BY REAL TIME PCR

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SUMMARY

Mycobacterium avium ssp *paratuberculosis* (Map) is the etiological agent of Johne's disease, granulomatous enteritis that can affect cattle, sheep and goats and other non ruminant wildlife species.

We have developed an original protocol to detect Map from faecal sample. This protocol allows concentrating the Map from 10g of faecal sample. Our study assessed the performance of this new Map extraction protocol and of the Adiavet®PARATB real time PCR detection kit. The new MAP extraction protocol using 10 g of faeces has been compared to a classical extraction procedure using only 1 g of faeces.

329 faecal samples were examined with the two extraction protocols. 24% were identified as positive for Map with the classical extraction protocol compared to 64% with the new protocol. This new protocol for Map detection could be a useful sensitive procedure for the clinical diagnostic but also, as it is more sensitive, to subclinical Johne's disease.

INTRODUCTION

Mycobacterium avium ssp *paratuberculosis* (Map) is the etiological agent of Johne's disease, a granulomatous enteritis that can affect cattle, sheep and goats and other non ruminant wildlife species (1). Although its role as a zoonotic agent has not been determined yet, the potential association of Map with human Crohn's disease has been the subject of a number of reviews (2). Numerous countries have set up programmes to control the disease. It requires the development of high-throughput sensitive diagnostic methods for the direct detection of infected animals. The performance of the PCR methods was often hampered by low sample input and inhibition. To increase the sensitivity of diagnostic test we developed an original protocol to concentrate Map from 10g of faecal samples and compared the real time PCR results obtained after a classical DNA extraction from 1g.

MATERIAL AND METHODS

Limit of detection of the PCR method

A negative faeces, collected from a herd free of *paratuberculosis*, was spiked with serial dilutions of a MAP titrated suspension to 4670, 2333, 1167, 233 and 117 bacteria per gram. The new protocol of extraction was four time repeat for each infected faeces. 5µL of each DNA extract was analysed with the ADIAVET® PARATB real time PCR kit (Adiagene)

Faecal samples exposed to Map

329 faecal samples were collected from different veterinary diagnostic laboratories. The common point among these samples is they were sent to the laboratory for MAP analysis; they came from different areas and from different herds with or without history of *paratuberculosis*.
All samples were examined with both extraction protocols.
The first extraction (Standard Protocol : SP method) was performed using Adiapure® DNA extraction protocol (Adiagene) and with 1 gram of faecal sample as recommended by the manufacturer. In the same time, the new extraction protocol (New Protocol : NP method) was achieved on the same faeces. 6g +/- 0.2 g of faeces were diluted with 40 mL of sterile distilled water, mixed 15 seconds and allowed to settle during 10 to 20 minutes. Then 10 ml of the obtained supernatant were transferred on an ADIAFILTER and centrifuged 5 minutes at 3000g. The obtained pellet was diluted with 500 µL of sterile distilled water. The solution was then disrupted 10 minutes at 30 Hz with 300 mg of glass beads on a mixer mill and centrifuged 5 minutes at 15 000 g.
Then DNA was purified from 200 µL of the supernatant with a standard extraction protocol from Qiamp DNA mini kit (Qiagen) or NucleoSpin tissue (Macherey-Nagel).
5 µl of each DNA obtained from both extraction methods were analysed with ADIAVET® PARATB Real Time PCR (Adiagene)

RESULTS

The lowest reproducible level of detection in quadruplet was 1167 bacteria / gram of faeces (average Ct 38.48 +/- 1.06)

79/329 (24%) faecal samples were identified as positive for Map with the SP method and 210/329 (64%) with the NP method. All the 79 positive samples found positive with the SP method were also found positive with the NP one. The average Ct value of these 79 faecal positive samples was 26.2 +/- 6 with the NP method and 31.9 +/-6.2 with the SP one. The 131 additional positive samples presented an average Ct value of 36.8 +/- 2.7 indicating that all these additional positive samples were lower contaminated with MAP.

DISCUSSION

Several Polymerase Chain Reaction (PCR) tests have been developed to detect *Mycobacterium paratuberculosis* DNA (3, 4, 5, 6). However, it has been difficult to obtain a good sensitivity of the test because of the presence of inhibitors and the biology of the bacteria. MAP are present in a complex matrix: the faeces. They formed clumps, they are not homogenous spread in faeces, they are hydrophobous, and are difficult to disrupt because of a strong bacteria wall. For these reasons, it appears crucial to improve sample preparation and DNA extraction steps for a better sensitivity and reproducibility.

So we have developed a new method of extraction to increase the sensitivity of the direct PCR detection. Foremost, it was important to increase the amount of faecal sample treated. Most of the work done for MAP detection with methods like culture or PCR were limited to treat 1g or less (3,4,5,6). Even new technologies combining magnetic beads separation and PCR use a small amount of samples (7). The main originality of our protocol is to be able to treat up to 10g of faeces in order to concentrate MAP present in the sample. In the standard protocol, only 1g of faecal sample is treated; after homogenization and DNA purification only 0.15mg of "equivalent faeces" is used for PCR analysis. With the new protocol 10g of faeces are treated and extracted, leading to 15mg of "equivalent faeces" used for PCR analysis. The limit of detection of the NP method was calculated to be 1167 bacteria/g and if we assume that 1CFU of MAP contains 100 bacteria, then the limit of detection of the NP method can be estimated to 10 CFU/g. We are able to concentrate 100 times the sample and so we can increase the sensitivity of the whole PCR method. Prior studies have suggested that high shedders yield more than 50CFU/tube and low shedders less than 10CFU/tube (8). So with the NP protocol we would be able to detect MAP even in the low shedders.

CONCLUSIONS

We know that during subclinical phase of infection, small amounts of MAP are shed in faeces. However, over time shedding can led to significant contamination of the environment and spread of the infection throughout the herd. Even if they are low shedders, subclinical infected animals are an important source of infection and therefore a correct diagnosis of these animals is essential for the control of bovine *paratuberculosis* in a herd. In this way, the New Adiavet protocol for Map detection is a very useful sensitive tool for MAP diagnostic.

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A PRACTICE ORIENTED THREE-STEP BASIC PROGRAM AGAINST PARATUBERCULOSIS IN CATTLE

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SUMMARY

Paratuberculosis or Johne's disease (JD) is caused by *Mycobacterium avium* subsp. *paratuberculosis* (*MAP*). The disease is untreatable and can cause considerable losses in affected herds. Suggested control programs for JD in cattle are often neglected due to high costs and intensive work load. By the introduction of a basic program against *MAP,* as an alternative to intensive control programs, it should be discussed how JD can be reduced in the cattle population by the use of effective, simple and practice oriented measures. This suggested "minimal-program" consists of diagnostic evaluation of diarrhea and culling of animals with clinical JD, the implementation of basic management measures and regularly evaluation of the *MAP*-herd status. Implementation of such a program can be performed with reasonable efforts on most farms. It helps to reduce clinical JD, to avoid new infections and to protect free herds. Furthermore it can help to reduce the introduction of *MAP* into the food chain, to increases hygiene and efficiency as well as animal welfare.

INTRODUCTION

Paratuberculosis or Johne's disease (JD) is caused by *Mycobacterium avium* subsp. *paratuberculosis* (*MAP*) and is considered one of the most important diseases in ruminants today [9, 10]. JD is found world wide and can cause considerable economic losses in affected herds. Furthermore paratuberculosis is gaining special interest because of its long and controversial discussed possible connection to Crohn's disease in humans [2].

Calves are usually infected soon after birth by oral ingestion of the organism, originating from feces, colostrum or milk containing *MAP*, but adult cattle can get infected too [4, 11]. JD in ruminants is characterized by untreatable, chronic enteritis, leading to emaciation and death. Subclinically infected animals do not show visible signs of JD, but may shed *MAP* and thereby serve as source for infections within the herd. JD is untreatable and ends with the death of the affected animal [4].

Animals suffering from clinical JD are the "tip of the iceberg" of *MAP*-infections. For every animal with advanced clinical JD 1 to 2 animals with clinical disease, 6 to 8 inapparent carriers and 12-25 individuals with silent *MAP*-infection have to be calculated within a cattle herd [4].

Many different laboratory tests are available for the detection of *MAP* or specific antibodies. Diagnostic tools for detection of JD or *MAP* have made great progress and current research activities are high, but diagnosis is still difficult in young animals and animals in an early stage of the infection [3].

As JD is difficult to diagnose, untreatable and may cause severe economic losses, control and reduction of *MAP*-infections in positive herds and prevention of spreading of the disease to negative herds is considered to be most important. Hygienic precautionary measures have to be taken to prevent further spreading of JD in *MAP*-positive herds. The mayor aim of these measurements is to prevent infection of calves and young stock and to purchase *MAP*- free animals only.

MATERIAL AND METHODS

Despite an ongoing scientific interest and high research activities concerning paratuberculosis in cattle, most farmers and veterinarians still share the opinion that there is nothing they can do against this disease. At the same time several effective measures that can be settled to fight paratuberculosis on herd level there do exist but suggested programs for control and eradication of JD in cattle are expensive, require significant changes in the farm management and are time consuming. These discouraging facts often lead to the refusal of such an intensive program by farmers and veterinarians. By the introduction of a basic program against *MAP* as an alternative to intensive control programs it should be discussed how JD can be reduced in the cattle population by the use of effective, simple and practice oriented measures.

RESULTS

The suggested 3-step basic program for the abatement of paratuberculosis in cattle includes:
1. Diagnostic evaluation of diarrhea, culling of animals with clinical JD.
2. Implementation of individual basic management measures.
3. Regularly evaluation of the *MAP*-herd status.

As first, and probably most important step, every case of diarrhea in adult cattle has to be clarified by appropriate diagnostic measures with special emphasize on JD. Animals with confirmed clinical JD have to be culled immediately under all circumstances.

The second step is the implementation of basic management measures to prevent new infections within the herd. Many different and extensive suggestions covering al aspects of cattle farming have been published [1, 5, 12]. These hygienic measures have to be adapted and implemented according to the possibilities (economy, time…) on the individual farm.

The last step involves regularly evaluation of the *MAP*-herd status to detect *MAP*-shedding animals as soon as possible. Environmental fecal samples can serve as a cheap and reliable tool to assess the paratuberculosis status in cattle [8]. Studies have shown that this sampling scheme can also be successfully performed in small cattle herds. Most positive samples were found in manure channels, manure storage sites and around water troughs [6, 7]. To increase the reliability of environmental samples multiple samples should be gained repeatedly (for example every 6 months) and tested for the presence of *MAP* by culture and PCR.

DISCUSSION

Implementation of a 3-step basic program as suggested above can be performed with reasonable costs and work load in most cattle herds. Furthermore, the program can serve as an entry into the control of JD and may be intensified and extended at any point if desired. Of course such a "minimal program" can't replace an intensive "true" control program and might not be able to eradicate *MAP* within a farm. Therefore it is crucial to discuss and define the goals of the program with all people involved before its start. The aim of a basic program against JD can be to reduce the number of cows with clinical JD, decrease new infections within the herd and to protect herds which are free of the disease.

Furthermore this program can help to reduce the introduction of *MAP* into the food chain.

Additional positive side effects of *MAP*-control programs are a general increase of hygiene and that they also help to reduce other diseases and increase production efficiency and animal welfare.

CONCLUSIONS

The introduction of a basic program against JD in cattle can be performed with reasonable efforts on most farms. The aim of such a basic program is to reduce the amount of cows with clinical paratuberculosis shedding high numbers of *MAP* into the environment, decrease new infections within the herd and to protect herds which are free of the disease. Furthermore these programs can help to reduce the introduction of *MAP* into the food chain and to increase general hygiene and efficiency as well as animal welfare.

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PARATUBERCULOSIS CONTROL IN AUSTRIA

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INTRODUCTION

Paratuberculosis is a chronic and untreatable infectious disease in ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). A compulsory control program based on government regulation affects cattle, sheep, goat and farmed deer. Animals showing clinical signs of paratuberculosis have to be notified to the district veterinarian and separated. Blood and faeces respectively lymphnodes and intestines in case of fallen stock or slaughtered animals have to be sent to the national reference laboratory (NRL) for diagnosis. Positive tested animals must be destroyed in combination with implementation of hygienic and management measures on the affected farms under control of the district veterinarian.

MATERIALS AND METHODS

Blood samples are tested by ELISA for MAP specific antibodies, whereas faeces or lymphnodes are tested by realtime PCR for MAP specific DNA.

In 2010 samples from 81 cattle (60 farms), 6 goats (1 farm) and 3 farmed deers (3 farms) were sent to the NRL for laboratory confirmation of clinically suspicious animals.

RESULTS

34 cattle from 25 farms, 6 goats and 1 farmed deer were tested positive for MAP in 2010.

CONCLUSIONS

Beside Sweden, Austria is the second European country to declare clinical paratuberculosis a notifiable disease.The Austrian control program is an important step in the fight against the disease, although, due to the deficiency in diagnosing subclinically infected animals, only ruminants showing clincal paratuberculosis are affected by the law. It has been reported that animals showing clinical signs of paratuberculosis signify only the tip of the iceberg and that there is always a significantly higher number of subclinically infected animals. But on the other hand, a severly affected animal can shed high amounts of MAP to the environment and can excrete even more bacteria with faeces than approximately 20.000 subclinically infected animals. Therefore the detection and elimination of these clinically diseased „super-shedders" in lifestock will cause a significant decrease of MAP in farms and environment.

ON THE OCCURRENCE OF PARATUBERCULOSIS IN CATTLE AND WILD ANIMALS IN AUSTRIA/STYRIA

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SUMMARY

Due to an increased prevalence of *paratuberculosis* in Austrian cattle herds, recent years have also shown a rise in infections with *M. paratuberculosis* in wild red and roe deer, chamois and mouflon. Mesenteric lymph nodes were taken from a total of 851 wild animals hunted or found dead and from 338 deceased cattle. Samples were analysed using PCR and cultivation methods. In the case of pathomorphological changes or anamnestic indications, investigations also included an analysis of organ samples (e.g. liver, lung) or foetuses.

The major symptoms in wild animals affected were weight loss and significantly enlarged mesenteric lymph nodes. Evidence of diarrhoea was only observed in about 15% of positive cases, the majority of which, however, were emaciated. The study for the first time provided evidence of intrauterine transmission of *M. paratuberculosis* in red deer (3 cases) and chamois (1 case) and succeded in the isolation of the pathogen from the liver, lung and subcutaneous granulomas of wild animals. Of the total of 338 mesenteric lymph nodes of cattle from 303 herds, 80 samples of 77 herds tested positive for *paratuberculosis*.

31 wild animals and 27 cattle isolates have so far been molecularly typed using IS900-RFLP and RAPD analyses [Pillai et al., 2001] in order to prove epidemiological relationships between occurrences in cattle and wild animals.

INTRODUCTION

Infections with *Mycobacterium avium* ssp. *paratuberculosis (M. paratuberculosis, Map)* are increasingly recognised worldwide. The reservoir of *Map* is generally accepted to be environmental, in particular, water and soil are sources of the organism. From 2002, a soaring number of cases of *paratuberculosis* could be observed in wild animals in Styria. Thus a Paratuberculosis-project has been implemented in Styria in 2002, followed by a nationalwide Regulation in 2006, when *paratuberculosis* has become a notifiable disease in Austria in cattle, sheep, goat and farmed game.

MATERIAL AND METHODS

Isolates of cattle and wild animals were compared for their genetic relation and origin. Mesenterial-, ileocaecal- and portal lymph nodes were collected for sampling. In case of pathomorphological changes or anamnestic irregularities, further organ samples were taken, e.g. liver, lung, hypodermic granulomas. Both blood samples and fecal samples were taken in clinical suspect cases from cattle in accordance with the national regulation BGBL II 2006/48 (paratuberculosis regulation). A total number of 338 dead cattle were taken to rendering facilities and dissected in order to exclude possible epizootics. PCR, microscopy and cell cultur methods have been performed. For cultivation, samples were decontaminated and subsequently inoculated onto Harold's egg yolk and Middlebrook agar and incubated at 37°C for up to 20 weeks. Molecular detection of *M. paratuberculosis* was accomplished by PCR amplification of IS*900* [Bauerfeind et al., 1996, Pavlik et al., 1999] and IS*Mav2*. Up to now, only *M. paratuberculosis* of the cattle type has been detected in the wild animal samples examined by IS*1311* PCR restriction enzyme analysis.

RESULTS

187 out of 851 samples taken from wild animals were tested positive for *M. paratuberculosis*. *M. paratuberculosis* could be detected in red-, roe- and fallowdeer, chamois, ibexes and moufflons, as well as in foxes, mountain hares, yellow-necked mouses, marmots and wood grouses. From cattle a total of 338 mesenterial lymph nodes were taken. 80 samples of 77 farms were tested positive for *M. paratuberculosis*. Affected wild animals suffered from extreme weight loss and weakness and had enlarged mesenteric lymph nodes. Evidence of diarrhoea was only observed in individual cases. During 2006 and 2010 a total of 371 samples in cattle and total of 6 samples in Reddeer have been taken in accordance with the national regulation BGLB II 2006/48 (Tab. 1).

Table 1: Taken samples in cattle and redder during 2006 and 2010 in accordance with the national regulation BGBL II 2006/48

Year	Cattle	MAP positiv	MAP negativ	Reddeer	MAP positiv	MAP negativ
2010	26	11	15	3	1	2
2009	53	20	33	1	0	1
2008	96	30	66	2	0	2
2007	118	44	74	0	0	0
2006	78	17	61	0	0	0
Total	371	122	249	6	1	5

However, RAPD analysis revealed five different DNA fingerprint patterns. Identical RAPD profiles were obtained in cattle and wild animal isolates recovered from animals from a geographically limited area. This leads to the assumption that infections of this wild population originated from a few genetically closely related strains (Fig 2). Distinct genomic polymorphisms were determined using RAPD analysis.

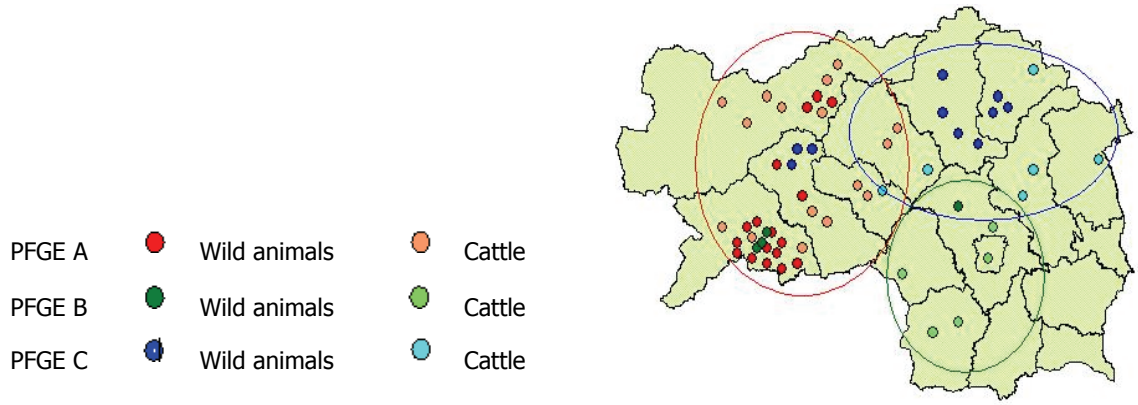

Figure 1: Demonstration of the geographical accumulation („cluster") in different regions in Styria

DISCUSSION

A sharp increase of clinical cases of *paratuberculosis* in wild ruminants has been observed in southern Austria since 2002. The increase of *paratuberculosis* in wild animal species is assumed to have been caused by the purchase of animals, a strong increase in suckler cow farming (cow-calf herds) with a concentration of pathogens in the environment and by inadequate feed hygiene for wild animals. However, environmental and management-associated factors can hardly separated giving the fact that lack of biosecurity may cause an increase of *MAP* in the farm environment.

CONCLUSIONS

Due to a geographical accumulation („cluster") in the isolated 3 distinct RAPD-profiles in cattle and wild animals a transmission of the agent between cattle and wild animals can be assumed. However, the faecal-oral route has been identified as primary cause for MAP infection, meaning that *MAP* contaminated environment plays a major role as a potential source of infection for animals living in this area. The results indicate a transmission from wild animals to cattle and vice versa caused by a lack of feeding hygiene, import of infected animals (e.g. Limousin, Holstein) and due to the fact that wild and domestic animals often share the same environment in certain regions in Styria.

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MEAT PRODUCTION, CLIMATE CHANGE AND ETHICS

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SUMMARY

Humans contribute substantially to the emissions of carbon dioxide causing global warming, and meat producing livestock contributes to this. Estimates of the global greenhouse gas emissions from the livestock sector vary from 5% to 50%. Nevertheless, the emissions are in absolute terms substantial, and the issue needs to be mitigated. The present paper analyses and discusses various solutions for how to mitigate climate change to the extent it is caused by animal production. The ethical problem of mitigation of climate change, to the extent it is caused by animal production, is an extremely complex and difficult issue. There are different views on the nature and scale of impact and different mitigation approaches, as well as different ethical aspects of the mitigation approaches.

INTRODUCTION

During the last few years it has been argued that humans contribute substantially to the climate changes (6; 8). The main causes of climate change are the emissions of greenhouse gases (GHG), mainly due to fossil fuel use (6), but FAO showed that the contribution of the livestock sector to the global warming is larger than previously estimated (10). In particular sustainability of meat producing livestock has been questioned, but several critical voices have been heard defending meat production in this context.

Animal production contributes to climate change, but there are different views on how much. According to the report Livestock's Long Shadow from the UN Food and Agriculture Organization, the global emissions from the livestock sector constitute 18% (10). The FAO report has since 2006 been at the centre of the debate and it has been criticized for providing a too high estimate as well as a too low. Estimates of the global GHG emissions from the livestock sector vary substantially, from 10% to 51% (5). Pitesky and co-workers (9) argue that the FAO estimation cannot be trusted applied regionally, as the US livestock sector contributes only 3% and the US transport sector 26%, whereas countries like Paraguay, livestock production may contribute more than 50% because of Paraguay's much smaller transport and energy sectors. The emissions in the UK have been estimated to be 7-8% (3) and in Sweden to be 5-6% (11). A much higher estimate is made by the World Watch Institute, 51% (4), as it argues that e.g. livestock respiration should be included. However, even if the rather low estimates of the relative emissions in developed countries would be correct, the emissions are in absolute terms substantial, and the meat consumption per capita in developed countries is much higher than in developing countries. The US consumption of meat is on average 127 kg per person and year, while the average consumption in the world is 41 kg. As a comparison Bangladesh has the lowest consumption with 3 kg (2; figures from 2005).

To the extent climate change is caused by animal production (and consumption), what should we do to mitigate it? This question has technological and policy aspects, but it is also an ethical question. It is a question about how we should act when facing the fact that the global warming in part is caused by production of meat, eggs and dairy products. A research project in ethics was initiated regarding this issue, and it was funded by the Swedish Research Council. The general aim of the project was to analyse and discuss various solutions for how to mitigate climate change to the extent it is caused by animal production.

MATERIAL AND METHODS

Theoretical analyses of various solutions for how to mitigate climate change due to meat production and consumption were performed. On the basis of these analyses, a preliminary investigation of ethical aspects of the mitigation approaches was carried out.

The material from another study within the project regarding stakeholder interviews in Sweden on animal agriculture and climate change will be presented by Lerner et al (2011) at this ISAH congress. The present presentation is mainly based on theoretical results reported by Nordgren (7).

RESULTS AND DISCUSSION

The answers to this ethical question depend partly on how we conceive of the nature and scale of the impact of animal agriculture on climate change. There are at least three different views in this regard, below called "models" and that may function as starting-points for different approaches (technological, political and ethical) to climate change mitigation. Three different models of the impact of animal production on climate change were found in the literature, namely; I) the life cycle model, II) the complex impact model, and III) the additional emissions model.

I) The life cycle model provides a picture of the nature and scale of the impact of animal agriculture on climate change that is based on results of life cycle analysis (LCA). The results of LCA show that food products may differ substantially in GHG intensity or "carbon footprint" (commonly measured in terms of carbon dioxide equivalents per kilogram (CO2e/kg) commodity). There are several studies applying this approach on different types of agricultural production (e.g. 12). Regardless of the exact figures, there is a similar pattern in all LCA reports: the emissions from beef production are much higher than those from pork production, which are higher than those from production of chicken and eggs. In addition, the emissions from animal products are generally much higher than those from non-animal products.
II) The complex impact model accepts LCA but stresses its limitations and that LCA should be considered within a broader framework that encompasses also indirect impacts and opportunity costs (3). Garnett (3) argues that cattle and sheep can actually help to prevent emissions of carbon dioxide by grazing on non-arable land.
III) The additional emissions model has recently been proposed by Goodland and Anhang (4). They argue that an adequate estimate is rather 51%, as the GHG emissions of livestock breathing is a neglected source. This should be included, since livestock like cars are human inventions and the carbon dioxide exhaled by livestock therefore is no less unnatural than the carbon dioxide emitted from car, and that livestock respiration is responsible for 21% (1). Furthermore, land use is an overlooked source, and forest regeneration, growing crops for human consumption and biofuels would have the potential of mitigating half or even more of all human-caused GHG emissions (4).

How can we mitigate climate change to the extent it is caused by animal agriculture? The technological methods reducing the animal production the GHG emissions can be split into three approaches: (I) improving productivity, (II) changing management, and (III) managing outputs.

(I) Improving productivity is aiming for to making the animals grow faster and thereby emit less greenhouse gases over their lifetime. This could be done by changing the feed, changing the genetic make up of the animals, increase the fertility or longevity of the animals, or shorten the fattening period.
(II) Changing management includes e.g. to manage the soil inputs, by improving soil drainage or optimising nitrogen fertiliser applications. Other options have been much debated in terms of the pros and cons of organic versus conventional production, and the pros and cons of intensive versus extensive production. Many studies have tried to compare the impacts of conventional and organic animal production, including their GHG emissions. The results vary substantially.
(III) "Managing outputs" is type of mitigation approach that focuses on manure storage and handling. For dairy cattle enteric fermentation is responsible for 80% of methane and manure for 20%, but for pigs it is the other way around, with manure responsible for 70%. However, the total methane emissions from cattle are much higher than from pigs. The manure can also be used as a nitrogen fertiliser for plants. A potential problem is, however, that these options might lead to keeping the animals indoors for longer periods of time in order to collect more manure, and this may affect animal welfare negatively.

Nevertheless, as technological solutions may be considered to be too slow or insufficient, a more radical mitigation approach is to reduce livestock numbers over and beyond the outcome of increased productivity. Within the general approach of reduction of livestock numbers there are at least three different theoretical options that need to be explored further; (I) a "less beef" approach based on the life cycle model, (II) a "less meat" approach based on the complex impact model, and (III) a "no meat" approach based on the additional emissions model previously defined.

(I) A "less beef" approach based on the life cycle model, which means shifting consumption from animal products with high associated GHG emissions (beef and sheep meat) to products with lower emissions (poultry, vegetable protein) can reduce total global GHG emissions (2).
(II) A "less meat" approach based on the complex impact model downplays the differences in GHG intensity between different types of animal products, but it can be based on any of the three impact models. The meat production/consumption should be reduced and humans should eat more non-animal food. There is a positive role for livestock in climate change mitigation, provided that their numbers are much lower than they presently are.
(III) A "no meat" approach based on the additional emissions model is based on the additional emissions model, i.e., the view that the total emissions from the livestock sector are much higher than the FAO states, and it takes this as a reason to adopt a policy of eliminating the production of animal products altogether. Goodland and Anhang (4) stress that the problem is extremely urgent and that this requires extreme measures: no meat should be produced.

Five ethical principles may be applicable for handling the climate change mitigation:
(I) There should be a just distribution of goods and burdens between the present generation and future generations.
(II) There should be a just distribution of goods and burdens among countries and individuals within each generation.
(II) Unnecessarily compromising animal welfare should be avoided in animal production.
(IV) A mitigation approach with a sufficient potential to mitigate climate change should be chosen, rather than an approach with an insufficient potential to do so.
(V) A mitigation approach that is more feasible should be chosen, rather than one that is less feasible.

Finally, there are several political problems. Would politicians within a particular developed country be prepared to argue in favour of reduced meat production/consumption? Can a sufficient number of states agree on such a policy on global level? There is also a delicate problem of what kind of political steering is feasible, e.g. climate labelling or meat taxes that are difficult to implement.

CONCLUSIONS

The ethical problem of mitigation of climate change, to the extent it is caused by animal production, is an extremely complex and difficult issue. There are different views on the nature and scale of impact and different mitigation approaches, as well as different ethical aspects of the mitigation approaches.

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LIVESTOCK'S "SHORT SHADOW"? BALANCING MITIGATION OF CLIMATE CHANGE AGAINST OTHER VALUES

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SUMMARY

Livestock has been said to cast a long shadow on climate change. However, environmental protection has also other aspects. This paper analyses one possible conflict between environmental goals in Sweden: to decrease the number of ruminants in order to minimise the impact on climate change and to maintain the number of ruminants in order to preserve biological diversity. Some Swedish stakeholders seem to argue that preserving biological diversity carries as much weight as mitigating climate change. This can be interpreted as if ruminants are considered to be good for the environment despite their impact on climate change, and thus shortening the long shadow. We point out, however, that only a fraction of ruminants are grazing pastures with high biodiversity. For most Swedish meat production, this argument is of minor importance for environmental sustainability.

INTRODUCTION

During the last few years, the sustainability of livestock production has been questioned due to its impact on climate change (depicted as "livestock's long shadow") [1]. On the basis of life cycle analysis, several studies have shown that there are substantial differences in greenhouse gas intensity among different species, with ruminants being clearly the most intensive [2]. Despite these reports meat producers defend livestock production [3]. They claim that other values need to be taken into account. We will discuss such a defence as presented in an interview study on Swedish stakeholders. The argument is mainly that preserving biological diversity is as important as mitigating climate change. We will investigate this argument and put it into the broader picture of sustainability.

MATERIAL AND METHODS

During 2010 we interviewed 12 representatives of Swedish stakeholders within the livestock sector. We used a structured qualitative interview method. The stakeholder organizations were chosen through purposive sampling. Each organization chose its own representative. Their answers on questions regarding the impact of livestock on climate change, mitigation approaches and international responsibilities were analyzed regarding content.

The empirical material from this study will be presented elsewhere and in this paper we analyze theoretically a conflict evident in this empirical material.

RESULTS AND DISCUSSION

The analysis of our interviews showed that according to some stakeholders mitigation of the impact of livestock on climate change need to be balanced against other values for a more holistic and realistic approach. A common theme was that the climate aspect was only one environmental aspect among many. Therefore, only considering the impact on climate change for decisions regarding livestock production is too narrow an approach. Other important values to take into account are biological diversity, food safety and transport distances of products.

The informants seemed to accept the reports that state that ruminants contribute more to climate change than pigs and chickens. Still there was a strong defence for ruminants. This means that even though ruminants contribute to a higher extent to climate change than other farm animals one should still keep ruminants. The main line of defence for ruminants was to claim their value as maintainers of biological diversity. How can this claim be understood more precisely?

Ruminants have been regarded as contributing more to climate change than other species, mainly due to their digestion process, which produces methane (with 23 times the global warming potential of CO_2) [1]. In Sweden, ruminants are the key species to graze pastures and natural grasslands.

The biological diversity in natural pastures in Sweden is mainly dependent on grazing ruminants, creating the proper landscape for several endangered species. If consumers chose meat from pigs and chickens rather than products from cattle the foundation for keeping natural pastures open would disappear. The natural pastures would then slowly turn into forests and vital diversity values would be lost. Several plant and insect species are

highly dependent on grazed natural pastures as their living habitat [4]. For some species of birds living in farmlands, a high variety in the landscape is needed for the success of breeding. If the variety is preserved, these populations of birds would not continue their decreasing trend [5].

Unfortunately, keeping ruminants on natural grasslands may be less effective from the perspective of climate change mitigation than keeping them on a more optimized diet. Natural grasslands are often in areas that are less productive than other farmland. Animals eating less nutritious food develop slower and contribute for a longer time to climate change until they reach their slaughter weight.

The Swedish Government has decided that all sectors in society have environmental goals to fulfil [6]. These goals concern different environmental aspects. Among the goals there are goals for decreasing the contribution of greenhouse gases to climate change but also to preserve biological diversity. There is therefore a possible conflict for the different agencies in Sweden that are to fulfil these goals.

There is a geographical difference concerning these two goals that might be crucial when one has to choose between them. Climate change is not so closely tied to a specific local setting as is biological diversity. Humans contribute to climate change through all their actions. In all sectors, including agriculture, greenhouse gases are emitted. The gases travel large distances and contribute at the global level. Therefore reduction of the emissions in one country may not directly influence this country itself. Changes in the preconditions for biological diversity in a country, on the other hand, will directly influence the biological diversity in this particular country. Biological diversity is very much dependent on local settings, the soil, surrounding species, the specific management of the farm, etc. Moreover, biological diversity may also be seen as diversity on the genetic level, and among species with a low ability to move there are often local genetic adaptations [7]. Therefore preservation needs to be carried out in the places where the biological diversity exists today. It is site-specific. One way of preserving natural diversity is for example to preserve *in situ* traditional agricultural methods to make sure that no biological diversity–as well as knowledge about the methods–will be lost [8].

How should this argument proposed by some stakeholders be assessed? Should we keep ruminants on natural grasslands for reasons of preserving biological diversity despite the fact–according to a number of reports–that they contribute more to climate change than do other options? It seems that this line of defence has only limited value. Most ruminants–as well as chickens and pigs–do not contribute to biological diversity at all. Only a limited amount of ruminants within the total of Swedish ruminants are grazing such pastures. The consumption of meat from ruminants is by far higher in Sweden than the number of ruminants contributing to biological diversity. And chickens and pigs do not contribute to biological diversity whatsoever. Moreover, imported meat may contribute to deforestation, for example in the Amazonas, and thus reduce biological diversity in those areas [9].

Although biological diversity is site-specific and could be useful as an argument for keeping some meat production, the argument does not hold for other kinds of meat production. Even if the stakeholders may have found an argument for defending some of the national meat production, this is still a minor defence and holds only for a fraction of the total production (and consumption) of meat.

CONCLUSIONS

In the present-day debate, there is a risk that the climate change aspect of livestock production is the only aspect considered. Mitigation of climate change needs to be balanced against other values such as biological diversity. However, in a country like Sweden only a minor part of all ruminants are important for maintenance of biological diversity. For other meat production this defence will not hold. The appeal to biological diversity shortens livestock's long shadow only to a limited extent.

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HOUSING EMISSIONS OF NH_3, N_2O AND CH_4 AND OUTDOOR EMISSIONS OF CH_4 AND N_2O FROM ORGANIC BROILERS

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SUMMARY

This study presents an assessment of gaseous emissions from an organic broiler production system composed of a broiler house and an outdoor run. In the house, concentrations of NH_3, N_2O, CH_4, CO_2 and H_2O were monitored during 56 days over 3 discontinuous periods. To assess gaseous emissions, both indoor and outdoor gas concentrations were monitored using a photo-acoustic infrared analyser (INNOVA 1312). Air flow rates were estimated using SF_6 tracer gas. Mass balances were also performed with zootechnical data to validate the measurements and interpolate data between measurement periods. Outdoor N_2O and CH_4 emissions were measured using manual static chambers placed on the outdoor run. In the house, emissions were 130 mg NH_3 day^{-1} $broiler^{-1}$, 46 mg N_2O day^{-1} $broiler^{-1}$ and 13 mg CH_4 day^{-1} $broiler^{-1}$. Indoor NH_3 emissions were lower than for broilers raised in confinement, which can be explained by the access to the outdoor run. On the outdoor run, emissions of N_2O and CH_4 were low and close to background fluxes (< 40 ng s^{-1} m^{-2}). Emission peaks were occasionally detected, especially in chambers close to the house where the density of droppings was higher. With good assessment of dropping distribution on the outdoor run, emissions of CH_4 and N_2O could be extrapolated over the whole rearing period at the outdoor run scale

INTRODUCTION

In organic broiler production, organic farming principles require that broilers have access to open air areas (outdoor runs) as specified for instance in European Regulations. Yet, the environmental impact of organic broiler production has not been well studied, especially gaseous emissions from the broiler house and from the outdoor run. Poultry production was indentified as a producer of ammonia (NH_3), and to a lesser extent, of nitrous oxide (N_2O) and methane (CH_4). As NH_3 is responsible for eutrophication and soil acidification and CH_4 and N_2O are two major greenhouse gases involved in climate change, gaseous emissions of organic broiler production should be quantified in order to assess and minimize its environmental impact. This paper proposes therefore an estimation of gaseous emissions from an experimental organic broiler production facility in the framework of the French project AlterAviBio.

MATERIAL AND METHODS

Study site

The study took place on an experimental facility of the French National Institute for Agricultural Research (INRA) at "Le Magneraud" (in the Charente-Maritime department). The climate is temperate oceanic, with an average annual air temperature about 13 °C and an annual rainfall about 500 mm. The broiler housing has an area of 75 m² with two pop-holes for the access to the outdoor-run (grassland of 2500 m² with grass/clover mixture). Indoor and outdoor air temperature and relative humidity were measured and soil temperature and water filled pore space (WFPS) were also recorded during the study to provide environmental conditions for the estimation of gaseous emissions.

Livestock and litter management

A total of 758 Hubbard I657 broilers arrived in the housing on 8 December 2009. From day 36 until day 91 (slaughtering), broilers had unlimited access to the outdoor-run. Broilers were fed with three organic feed types. In the housing, organic wheat straw was used as litter for a total weight of 6.56 kg m^{-2}, with half of it added during the rearing.

Dropping repartition between the house and the outdoor run

Total excretion of N, P and K was estimated as the difference between ingestion and body retention. Ingestion was estimated from feed consumption and N, P and K feed contents. Body retention was calculated from CORPEN values (32.8 g $N.kg^{-1}$ BW, 4.8 g $P.kg^{-1}$ BW and 2 g $K.kg^{-1}$ BW) [1]. Litter in the house was weighted, sampled and analyzed. Indoor excretion was calculated for P and K as the difference between amounts in final litter and in bedding straw. Outdoor excretion was estimated as the difference between total and indoor excretion. As nitrogen is lost through gaseous emissions in the house, we proposed an outdoor nitrogen excretion range based on P and K outdoor excretions (in % of total excretion).

Measurement of NH_3, N_2O and CH_4 fluxes in the broiler house

Within the housing, monitoring of gaseous emissions was performed continuously over 3 periods of 20, 15 and 21 days respectively during the rearing of broilers and until litter removal. To estimate air flow rates, the tracing method was used with SF_6 as tracer gas [3]. NH_3, N_2O, CO_2, CH_4, SF_6 and water vapour concentrations were measured using infrared photoacoustic spectrometry, with a gas analyser (INNOVA 1312) coupled with a sampler-doser (INNOVA 1303). Two sampling channels were placed outside the housing to measure outdoor gas concentrations, and four were placed within the building. Missing datapoints were estimated by linear interpolation.

Measurement of CH_4 and N_2O fluxes on the outdoor run

CH_4 and N_2O fluxes on the outdoor run were measured using static chambers [4]. Sixteen chambers were placed on the outdoor run. Eight were placed within the first 15 m in front of the housing because previous behavioural observations showed that broilers prefer this zone. Moreover, 3 chambers were used as control chambers outside the outdoor run. At the start of each measurement period, the chambers were covered with a removable lid equipped with a septum. Four air samples were taken (at 0, 10, 20 and 30 min) and injected into evacuated vials closed with leak-free septa before being analysed using gas chromatography (Agilent 6890N). Gas fluxes for each chamber were calculated from the slope of the linear regression of concentration over time.

RESULTS

Zootechnical performances

At 91 days, average live weight was 2.21 kg, average daily gain was about 24 g per day and food conversion ratio was 3.61 kg of feed per kg of live weight.

Dropping repartition between the house and the outdoor run

Table 1. Ingested, retained and excreted amounts of N, P, K by the broilers.

	N	P	K
Total ingested (kg)	152	35	44
Total retained (kg)	53	8	3
Excreted (kg)			
Total	99	27.0	41.0
Indoor	88 - 96	26.2	36.5
Outdoor	3 - 11	0.8	4.5
Dropping repartition (% of total excretion)			
Indoor	89 - 97	97	89
Outdoor	3 - 11	3	11

Total amount of solid manure was 3400 kg with a dry matter content of 57%. N, P and K litter contents were respectively 17, 8 and 12 $g.kg^{-1}$. Indoor excretion represented 89-97% of total excretion. Mass balances for N, P and K are presented in Table 1.

Indoor NH_3, N_2O and CH_4 emissions

Indoor NH_3, N_2O and CH_4 emissions for each measurement period and for the whole rearing period are given in Table 2.

Table 2. Indoor NH_3, N_2O and CH_4 emissions (g broiler^{-1} day^{-1}) for each measurement period and for the whole rearing period.

	P1 16 Dec 2009 - 5 Jan 2010	P2 18 Jan - 2 Feb 2010	P3 16 Feb - 9 Mar 2010	Rearing period
NH_3	0.010	0.140	0.220	0.130
N_2O	0.086	0.010	0.006	0.046
CH_4	0.0006	0.023	0.016	0.013

Total NH_3 and N_2O emissions were respectively 7.8 kg and 2.8 kg and represent 7% and 2% of total N excretion respectively. Total N losses represented about 30% of total N excretion. Total CH_4 emissions were low (0.8 kg).

Outdoor CH_4 and N_2O fluxes

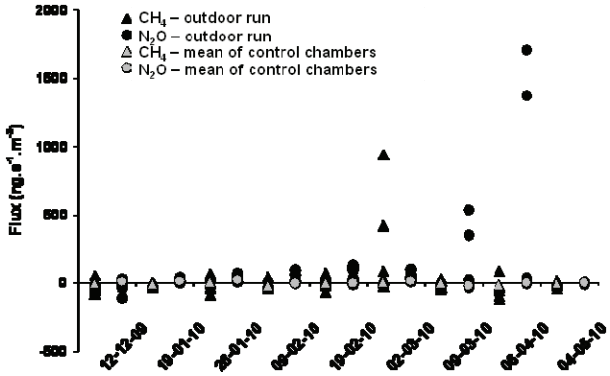

Figure 1. CH_4 and N_2O fluxes measured on the outdoor run and in the control chambers.

On the outdoor run, CH_4 and N_2O fluxes were extremely variable in time and space. Measured CH_4 and N_2O ranged from -112 to -944 ng m^{-2} s^{-1} and from -105 to 1710 ng m^{-2} s^{-1}, respectively (Figure 1). CH_4 and N_2O median fluxes were -7 and 1 ng m^{-2} s^{-1}, respectively. Mean fluxes on control chambers (outside the outdoor run) were low for both gases. WFPS was nearly 75%. Peaks of CH_4 and N_2O emissions were observed during the study. These peaks were always measured on the chambers placed just in front of the two pop-holes of the broiler housing.

DISCUSSION

Dropping repartition between the house and the outdoor run

Outdoor excretion was between 3 and 11% of total excretion, which is lower than the value given by the CORPEN [1] for this type of production system (25%). This can be explained by the fact that the study was carried out in winter conditions with low temperatures and snow which could have affected broiler exploratory behaviour.

Indoor NH_3, N_2O and CH_4 emissions

NH_3 emissions were lower than values for claustration systems found in the literature [2]. This could be explained by the low temperatures in the house during the experiment (<11°C in periods 2 and 3).

N_2O emissions were rather high for a poultry-production system on litter [2], mainly due to the high emissions during the first measurement period. However, we suspect that during this period, emissions were overestimated due to interference between two absorption spectra when heating was on. Further analysis should confirm this hypothesis.

The low CH_4 emissions could be explained by the low temperatures in the house during the experiment, amount of bedding straw or litter porosity. These values are lower than values for claustration systems [2].

Outdoor CH_4 and N_2O fluxes

Peaks of CH_4 and N_2O emissions were observed only from the chambers placed just in front of the two pop-holes of the broiler housing. This confirms our hypothesis that emissions should be higher when close to the housing, due to a higher concentration of broiler droppings in the first few metres of the outdoor run.

Moreover, the frequency of broiler droppings seems to have a greater impact on fluxes than WFPS. Measured fluxes were indeed low at the beginning of the study, even though WFPS was high. The low winter temperature could also have limited gaseous emissions.

CONCLUSIONS

This study provides the first data concerning gaseous emissions from a French organic broiler production system. Outdoor gaseous emissions have yet to be interpolated for the whole rearing period, and outdoor NH_3 emissions still must be estimated. Finally, new references concerning other types of organic production or outdoor runs (e.g. under tree canopies) are still necessary.

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CONCENTRATIONS OF AIRBORNE PARTICULATE MATTER, AMMONIA AND CARBON DIOXIDE IN LARGE SCALE UNINSULATED LOOSE HOUSING COWSHEDS IN ESTONIA

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SUMMARY

The concentration of airborne particulate matter in large scale uninsulated loose housing cowsheds as a potential risk factor to the human and animal health was investigated in this study. In addition, correlations between indoor particulate matter, noxious gases' concentrations and other microclimate parameters were investigated. The concentration of particulate matter (all fractions) inside the uninsulated loose housing cowsheds was low compared to pig and poultry housing systems. There was a clear seasonal variation between measurements in summer and winter.

INTRODUCTION

There is little experimental data about indoor inhalable airborne particle concentrations, particularly respirable airborne particles (<2.5 μm), in uninsulated loose housing large scale dairy farms in Estonia. The fine respirable airborne dust is considered to be one of the potential risk factors for animal and human health as dust may penetrate into the alveoli through the respiratory tract. In livestock production, intensive poultry and pig houses are the main sources of particulate matter emissions, contributing about 50% (poultry) and 30% (pigs) of total particulate matter emissions from agriculture in Europe [1]. Dust in livestock buildings is comprised of feed and other plant-derived fractions, epithelial cells detached from the animal body, urine, faeces, micro-organisms and other particles. In pigs, the bulk of particulate matter (PM) comes from feed [2, 3]. Faecal PM is also an important contributor to PM in pigs. Faecal PM is found to a greater extent in the respirable fraction, indicating a potential high risk to the alveoli in the lungs [2]. In broiler houses, down feathers, mineral crystals from urine, and litter are the main PM sources [3]. In layer houses, skin, feathers, faeces, urine, feed and litter, are amongst the most important sources of PM [4]. Other livestock production systems may contribute other relevant sources of PM different from these. For instance, bedding material can considerably contribute to PM [5]. Type of litter and moisture content may also affect PM concentrations [6]. The gaseous compounds such as ammonia and odours in livestock housing can be adsorbed onto dust particles. Particles can carry NH_3 molecules for a long time and can adsorb large amounts of NH_3 [7]. The formation of PM, its concentrations and emissions from livestock houses, depend on many physical and biological factors [8]. The concentration of PM in livestock houses depends mainly on the animal type, as well as on the housing system, season and within-day sampling period [9]. Important are also animal activity, animal stocking density and moisture condition. A relative humidity of 70% and higher may contribute to low PM concentrations due to a high equilibrium moisture content. Ventilation rate, also related to temperature and humidity, is an important factor, because it determines to a great extent particle formation, concentrations and emissions, and especially its distribution in the airspace of livestock houses [10]. The microclimate of uninsulated large scale dairy farms is strictly related to environmental factors [11], thus it can be assumed that these also affect the concentration of airborne dust particles.

MATERIAL AND METHODS

Microclimate parameters (temperature, °C; relative humidity, %; CO_2 and NH_3, ppm) as well as inhalable (PM total, PM10) and respirable (PM2.5, PM1.0) particulate matter concentrations (μg m^{-3}) were measured in nine large uninsulated loose housing cowsheds once per month on five dairy production units in Estonia over the period from September 2008 to August 2009. The number of animals in the buildings varied between 300 and 600. The concentrations of inhalable and respirable particles, carbon dioxide and ammonia were measured at one meter height from the floor, at eight to 13 locations, depending on the size of the building, for 10 minutes per measuring site. The concentrations of inhalable particles and their fine fractions were recorded at the same time. Grimm 1.108 portable aerosol spectrometer was used for the measurements of the concentrations of airborne dust particles, and Dräger X-am 7000 multigas detector for recording gas emissions. In all buildings the temperature and relative humidity were constantly determined at intervals of 15 min. The measurements were performed with a Rotronic HygroLog data logger. Statistical variation between the air quality data was analyzed by using the procedure of SAS.

RESULTS

The mean particulate matter concentration (Fig. 1a) in loose housing dairy farms during the measuring period was 205μg m^{-3}, but this had a wide monthly range from 130 to 313 μg m^{-3}. The mean inhalable airborne particle (PM10) concentration (Fig.1b) was 65 μg m^{-3}, with a monthly range between 27 and 123 μg m^{-3}. The mean respirable airborne particle (PM2.5) concentration (Fig. 1c) was 18 μg m^{-3}, with a monthly range between 7 to 32 μg m^{-3} and the respirable airborne particle fraction PM1.0 concentration (Fig. 1d) was 10 μg m^{-3}, with a monthly range between 3 to 20 μg m^{-3}. The mean carbon dioxide concentration of the indoor air (Fig. 1e) was 553 ppm, with a monthly range from 313 to 822 ppm. The mean ammonia concentration (Fig. 1f) of the indoor air was 1.2 ppm, with a monthly range between 0.24 and 2.38 ppm. The mean temperature was 9.6 °C, with a monthly range from 2.0 to 20.4 °C. The mean relative humidity (Fig. 1h) was 83%, with a monthly range between 60 and 96%.

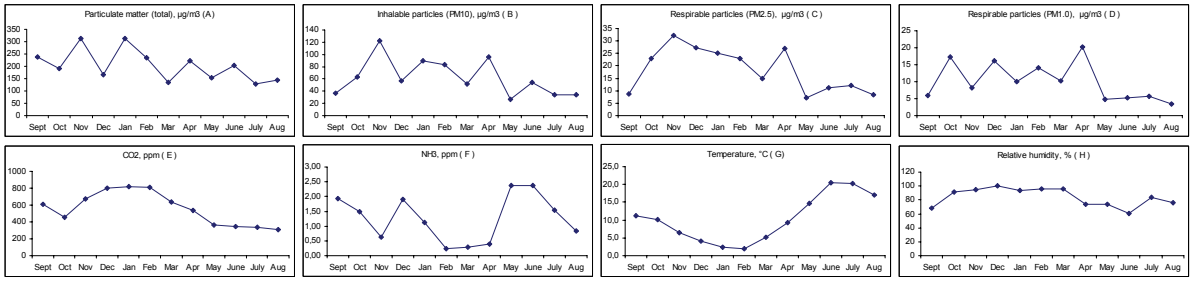

Figure 1. Mean concentration of PM (A), inhalable (B) and respirable PM (C, D), CO_2 (E), NH_3 (F), air temperature (G) and relative humidity (H) in cattle buildings

DISCUSSION

The uninsulated loose housing large scale farms showed lower inhalable and respirable airborne particles concentrations compared with insulated farms with tied housing, which is in accordance with other findings [12]. This might presumably be associated with the amounts of litter used in the farms. The carbon dioxide concentration in the indoor air of dairy buildings was directly affected by the outdoor environmental conditions. The more the curtain walls and other ventilation outlets were opened, the greater was the volume of the air that passed through the building and the lower was the concentration of carbon dioxide. The concentration of ammonia in indoor air was affected primarily by the indoor temperature and ventilation rate. The indoor air temperature of an uninsulated cattle building is directly determined by the external temperature [13]. There was a clear seasonal variation between summer and winter. In the summer (from May to September) the concentration of different PM fractions, carbon dioxide and relative humidity inside the uninsulated loose housing cattle buildings was lower. However, the temperature and the ammonia concentration were higher. Also, the fluctuation of parameters between these months within the summer period was smaller. Statistical analysis of the results showed that the concentration of PM1.0, PM2.5 and PM10 were positively correlated with the concentration of total particulate matter concentration. There was a strong positive correlation between the concentration of total PM and the concentration of PM1.0, PM2.5, PM10 particles in the air inside the cowshed (r=0.174***; r=0.379*** and r=0.796***). There was also a strong positive correlation between the concentrations of all particulate matter fractions and carbon dioxide concentration – the lower the ventilation rate (higher carbon dioxide concentration), the higher the concentration of PM particles of different fractions (r=0.395***; r=0.377***; r=0.403*** and r=0.463***). Ammonia concentration in the air inside the cowsheds was most strongly correlated with the concentration of the fine fractions of PM [7]. The stronger correlation was observed between the ammonia concentration and fine fraction of PM (r=0.045**; r=0.205***; r=0.155*** and r=0.086***). No statistically significant relationships were found between the concentration of PM total and either the indoor temperature or the relative humidity. However, when the temperature and relative humidity increased, the concentration of total particulate matter concentration decreased, weak negative correlation between the parameters could have been followed. A rise in indoor temperature significantly reduced the concentrations of PM1.0; PM2.5 and PM10 (r=-0.263**; r=-0.346** and r=-0.261**). A correlation between the indoor temperature and carbon dioxide concentration was also strongly negative (r=-0.691***). This is logical as, with an increase in temperature, the wall curtains (openings) will be opened and a higher ventilation rate will be achieved. The temperature and concentration of ammonia inside the cattle buildings were positively correlated (r=0.355**). As the ammonia is a result of microbial processes, then the higher than optimal temperature stimulates ammonia formation. The correlation between the particulate matter concentrations and the relative humidity inside the cattle buildings is of interest. While the total PM concentration was weakly negatively correlated with relative humidity (r=-0.135), the PM10 and especially the PM2.5 and PM1.0 were strongly positively correlated with the relative humidity (r=0.191; r=0.560*** and r=0.544***). A possible explanation is that the coarse fractions (commonly solid) clump together in a moister environment, into bigger particles and precipitates out of the air and fall to the ground. No correlation between the concentration of moisture and ammonia was detected.

CONCLUSIONS

The concentration of particulate matter (all fractions) inside the uninsulated loose housing cowsheds was low compared with pig and poultry housing systems. The mean total PM concentration was 0.21 mg m^{-3}; that of PM10 - 0.07 mg m^{-3}; of PM 2.5 - 0.02 mg m^{-3} and of PM1.0 - 0.01 mg m^{-3}, respectively. The concentrations of PM1.0, PM2.5 and PM10 depended directly on the total particulate matter concentration. There was also a strong positive correlation between the concentration of all particulate matter fractions and the carbon dioxide concentration. Ammonia concentration was associated most with the concentration of fine fractions of PM. No statistically significant relationship was found between the concentration of total PM and the indoor temperature and relative humidity. Increasing indoor temperature significantly reduced the concentrations of PM1.0; PM2.5 and PM10. The correlation between the indoor temperature and carbon dioxide concentration was also strongly negative. The indoor temperature and the ammonia concentration were positively correlated. While the total PM concentration had a weak negative correlation with relative humidity, the PM10, but especially PM2.5 and PM1.0, had strong positive correlations with relative humidity. There was no correlation between the relative humidity and ammonia concentration.

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THE EFFECT OF RAMBUTAN PEEL (*Nephelium lappaceum*) AS REDUCING AGENT ON IN VITRO METHAN PRODUCTION WITHIN CREATING ENVIRONMENT FRIENDLY FARMING

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SUMMARY

Syringes were filled with 300 mg mixture of forage and concentrate (60:40) and 30 ml medium consisted buffered rumen fluids derived from the gas production technique. Syringes were divided into four group of treatments with rambutan peel as saponin source at the level of 0% (P1), 0.2% (P2), 0.4% (P3), and 0.6% (P4) of the respective medium and incubated at 39ºC for 72 hours. Variables being measured were methan production, protozoal number, pH value, ammonia, and microbial protein. Additional rambutan peel of 0.2%, 0.4%, 0.6% decreased methan production as much as 6.63%, 14.90%, 25.65% ($P<0,01$) and decreased protozoal number as much as 17.50%, 63.28%, 72.02% ($P<0,01$). However, the treatments did not have any effect on together variables. It could be concluded that addition of saponin from rambutan peel as low as 0.2% of the medium decreased methan production and protozoal.

Key words: Rambutan peels, Saponin, Rumen fermentation, Methan production

INTRODUCTION

According to the Food Agriculture Organization (FAO) of the United Nations declared that the international meat industry is responsible for 18% of green house gas emissions and according to Guardian Unlimited in the UK, methane gas produced by cows partly responsible for 4 percent of greenhouse gas emissions.1.3 billion cows produce 100 million tonnes of methane. Cow manure also resulted in emissions of N_2O and CH_4 release dung decay. Methane gave a negative effect on the environment since estimated to contribute approximately 14 to 20% of total gas that cause the greenhouse effect. (Asanuma *et al.*, 1999).

Methane formation by Jouany (1991) that protozoa symbiotic with bacteria by producing H_2 methanogenic which will be utilized by the bacteria, and then converted into CH_4. Jouany (1991) states that defaunation is a process of elimination or reduction of protozoa in the rumen, although protozoan ciliates was removed digestive participation in the rumen, ruminant digestive function was still normal in its fermentatif. Further asserted that the defaunation process will increase the total bacteria in the rumen, because the protozoa are active against microbial pagosit especially rumen microbial amylolitic. Reducing the impact of protozoa would provide greater protection against protein degradation and increase the flow of feed protein into the duodenum. With descriptions of protozoa can be removed with defaunation agent, the agent of defaunation can be used as methane inhibitors or lower because if the protozoan is reduced, the symbiosis between protozoa and bacteria also reduced so that the H_2 methanogenic utilized methanogenic bacteria are few and gas methane (CH_4) produced also decreases.

Saponin is a compound that can be used as agents defaunation. Saponin will react with the cholesterol contained in the membrane of eukaryotic cells including protozoan causing damage cell membranes and lyse cells of protozoa (Bangham and Home, 1962 cit. Klita et al., 1996). Defaunation action of saponins appear with the formation of an irreversible complex with cholesterol in the protozoal cell membrane causing cell lysis (Cheeke, 2005). Saponin found in several plants, one of which is the rambutan. Rambutan is a fruit of tropical fruit and inventory is abundant in Indonesia so that rambutan fruit peel waste was also abundant. Rambutan fruit peel contain saponin (Setiawan, 2003) so that the peel of rambutan fruit has potential for use as a feed additive that can reduce the formation of methane production in ruminants.

MATERIALS AND METHODS

Materials were used in this research is the rambutan peel as a source of saponins, rumen fluid from cows fistula for rumen microbial inoculum source, reagents for testing of microbial protein and ammonia, as well as for the analysis of the number of protozoa and pH. Among other equipment used syringes as a fermenter, a microscope and haemocytometer to count the number of protozoa, waterbath, flasks, centrifuge, micropipet, pHmeter, spectrophotometer, CO_2 gas cylinder, electric scales, and the oven.

Determination value saponin from rambutan peel

2 g samples and standards as much as 516.6 mg/50 ml hydrolyzed by adding 5 ml 1 N H_2SO_4, then direflux for 30 minutes. After the cold and then extracted with $CHCl_3$ and added 5 ml of homogenized with a vortex of about 2 minutes. After that, separated using sentrifuge and will get the acid phase and organic phase. Obtained organic phase evaporated, dissolved with methanol, samples and standards were put on silica gel GF 254 plates, followed by elution. After that, the determined sample area and the area standard to determine levels of saponins in the sample (Wagner et al., 1984).

Determination of the dose levels of saponins rambutan fruit peel (on the basis of fermentation medium)

Rambutan peel saponin content 7.86% or 7.86 mg/100 mg. To make a 0.1 mg/100 ml of saponin, the requires rambutan peel = (0.1 / 7.86) x 100 mg = 1.27 mg. In a 30 ml fermentation medium skin requires rambutan = (30/100) x 1.27 mg = 0.38 mg. Additional level of rambutan peel: 0% (0 mg/100 ml), 0.2% (0.2 mg/100 ml), 0.4% (0.4 mg/100 ml), 0.6% (0, 6 mg/100 ml). And the addition of rambutan peel: 0 mg/30 ml. 0.76 mg/30 ml. 1.52 mg/30 ml. 2.28 mg/30 ml.

In vitro fermentation

The medium is the medium used for the measurement of gas production in vitro, by mixing the fluid with a solution of carbonate buffer. Rumen fluid taken from Ongole fistule cow. Intake of rumen fluid conducted in the morning before the cattle were given feed. Rumen fluid is inserted into the flask which was previously filled with water with a temperature of 39º C, which aims to adjust the temperature in the rumen, then removed just before it entered the rumen fluid. Flask filled to the brim and rumen fluid sealed to keep anaerobic conditions, then taken to the laboratory for use as a source of microbes. Fermentation medium prepared by mixing 474 ml H_2O, 0.12 ml micro mineral solution, 237 ml of buffer solution, 237 ml mineral solution macro, resazurin 1.22 ml, and 49.5 ml of reducing solution. These materials were homogenized with a CO_2 gas flowed, then rumen fluid was added in buffer solution after the solution is colorless which indicates conditions are anaerobic. Mixing ratio of rumen fluid: medium was 1:2 (v / v). The syringes were filled with 300 mg of a mixture of grass king with rice bran with counterweight 60; 40. The syringes that already contain the substrate and then put into an incubator at a temperature of 39 º C for overnight. The syringes were then divided into four treatment levels with the rambutan fruit peel as a source of saponins with saponin levels 0%, 0.2%, 0.4% and 0.6%. Each treatment was done with three replications. Thirty milliliters of a mixture of rumen fluid fermentation medium and inserted into the syringe. Anaerobic process is carried out by the CO_2 gas flowing into the syringe. Then the syringe sealed with clamps and incubated in an incubator at a temperature of 39º C for 72 hours. Results of fermentation and then filtered using a given cruss goch glass wool to separate the rest of the feed. Filtrate obtained partly used for the analysis to calculate the number of protozoa and pH test. Filtrate was sentrifuged with speed 3000 g for 15 minutes to separate feed particles, and then be repeated with speed 13 000 g for 15 minutes, the sediment was obtained for the test of microbial protein content, whereas the supernatant obtained was used as a sample for testing ammonia.

Observed variables

Methane analysis. Total gas production measured after 72 h incubation with a view on syringes scale based on the increase in gas pressure caused the piston to the top (Getachew et al., 1998). To measure the levels of methane gas, samples gas were analyzed using gas chromatography. Total methane production is known to convert the methane gas levels in a sample of the total gas production.

The number of protozoa. Preparation calculation of protozoa by Diaz et al., (1993).

The degree of acidity or pH. pH is measured using a pH meter which was calibrated with buffers pH 4 and pH 7. pH measurements made at the end of fermentation.

Microbial protein. Measurement of microbial protein by Lowry protein analysis method (Plummer, 1987).

Ammonia levels. Determination of ammonia using the method of Weatherburn (1976).

Data analysis. Data results were analyzed with one way analysis. The average difference was tested by Duncan's multiple range test test (DMRT) (Astuti, 1981).

Results

Table 1: Protozoa number, gas Production, methane production and characteristics of rumen fermentation at several level of rambutan peel addition as the saponin source.

Parameter Observation	Level saponin of peel rambutan (%)			
	0	0,2	0,4	0,6
Protozoa number (10^3/ml)	6,89[a]	4,35[b]	2,53[c]	1,78[d]
CH_4 production (ml)	17,64[a]	16,47[b]	15,01[c]	13,13[d]
pH value[ns]	7,12	7,10	7,10	7,11
Microbial protein[ns]	0,21	0,17	0,22	0,21
Ammonia concentration[ns]	17,86	17,05	16,87	16,99

[abcd] The values in the same row with different superscripts show the different effects of treatment (P<0.01)
[ns] not significant

Discussion

Methane gas production with the addition of saponin levels rambutan fruit peel at 0%, 0.2%, 0.4% and 0.6% respectively was 17.64 ml, 16.47 ml, 15.01 ml, and 13.13 ml. The results showed that methane production decreased (P <0.01) at 6.63%, 14.90%, 25.65%. These results indicate that the higher levels of saponins in the diet of cattle given the lower production of methane in the rumen. The decrease in methane gas production is due defaunation causing protozoan symbiosis between methanogens and protozoa in the rumen decreased so that the methane gas production also declined. Cheeke (2005) states that the saponins has the ability to reduce the number of protozoa. In addition, Miller (1995) stated that bacteria and protozoa that no one can directly ferment carbohydrates into CH_4 (methane). Species of methanogens and then transform the H_2 and CH_4. Format and H_2 is the primary substrate to produce methane. By the action of the protozoa defaunation, methane production is reduced because the supply of hydrogen is lower, thus reducing bacterial activity in the form of methane methanogenik (Finlay et al., 1994).

Number of protozoa with the addition of rambutan fruit peel with saponin levels of 0%, 0.2%, 0.4% and 0.6% respectively was 6.89 x10^3/ml, x10^3/ml 4.35 x10^3/ ml, 2.53 x10^3 / ml, and 1.78 x10^3/ml. The results showed that the number of protozoa decreased (P <0.01) by 17.5%, 63.28% and 74.61% compared to controls. These results indicate that the higher the lower the saponin content of protozoa in the rumen. These results are in accordance with the revelation Cheeke (2005), that the saponin had the effect of fermentation in the rumen and inhibit the growth of cilliata protozoa in the rumen . Decrease the number of protozoa occurs because cholesterol in the membrane of eukaryotic cells including protozoan react with saponin so will damage the cell membranes and lyse cells. The population of protozoa in the rumen is influenced by pH, type of substrate, and the competition between both the protozoa eat bacteria and protozoan predation among themselves (Church, 1988). In addition, the presence of protozoa is influenced by the additive compound (Hart et al., 2007). One of the additive compounds that affect the number of protozoa is a saponin. Saponin causes damage cell membranes and lyse cells of protozoa (Bangham and Home, 1962 cit. Klita et al., 1996).

pH value with the addition of rambutan peel with saponin levels of 0%, 0.2%, 0.4% and 0.6% respectively were 7.12, 7.10, 7.10, and 7.11 . The results showed that the pH for four different levels of saponins are not real. According to Deacon (2004) in rumen pH optimum ranging from 6.3 to 7.5. This is in accordance with the statement Jouany (1991), that although protozoa digestive removed from participation in the rumen, ruminant digestive function was still normal in fermentation.

Microbial protein value with the addition of rambutan peel with saponin levels of 0%, 0.2%, 0.4% and 0.6% respectively was 0.21 mg / ml, 0.17 mg / ml, 0,22 mg / ml, and 0.21 mg / ml. The results showed that the addition of rambutan peel did not affect microbial protein. Addition of rambutan fruit peel as a source of saponins caused a significant decrease in the number of protozoa, however, microbial protein synthesis results showed diffrrence no significant. According Jouany (1991) reduction will increase the total number of protozoa rumen bacteria. Increased protein synthesis by rumen bacteria with the increase in total rumen bacteria. Thus, microbial protein fixed or not there is a decrease or increase. The main factors affecting microbial protein synthesis is the availability of precursors in sufficient concentration in rumen fluid. Widyobroto (1992) states that the precursor for microbial protein synthesis is the availability of sufficient carbon skeleton, NH_3, energy and minerals. Besides, that takes place under ideal conditions microbial protein synthesis would be achieved if the source of carbohydrate fermentation, are available simultaneously with the source of protein.

Based on table 5. ammonia content with the addition of rambutan skin with saponin levels of 0%, 0.2%, 0.4% and 0.6% respectively was 17.86 mg/100 ml, 17.05 mg/100

ml, 16.87 mg/100 ml, and 16.99 mg/100 ml. The results showed that the ammonia content for the fourth level was not significantly. Although not significantly but ammonia levels were within normal range. It was in accordance with that stated by Leng (1985), maximum growth and microbial activity necessary concentration of ammonia in the rumen fluid for 5 to 23.5 mg/100 ml. According to Arora (1989) level of 5.8 mg/100 ml of rumen fluid was able for the formation of microbial protein from rumen. Ammonia content was influenced by the level of rumen wall absorption speed. Ammonia released quickly if it will increase the absorption of ammonia by the rumen wall (Noor, 1990) and the less ammonia is available that can be used by bacteria (Orskov, 1992).

Conclusion

It could be concluded that addition of saponin from rambutan peel as low as 0.2% of the medium decreased methan production, protozoal and did not have any effect on rumen fermentation.

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Block 3

SEROPREVALENCE OF CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN TIGRAY AND AFAR, NORTHERN ETHIOPIA

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SUMMARY

Goats play a significant role in the socio -economy of Ethiopia because of their better adaptation to unfavorable arid environment and their suitability for resource poor farmers. Diseases affecting goats such as *peste des petitis ruminants (PPR), contagious caprine pluropneumonia (CCPP), pasteurellosis*, and *pox* cause substantial losses through high morbidity and mortality. *Contagious caprine pleuropnemonia* (CCPP) which is caused by *Mycoplasma capricolum subsp. capripneumoniae (Mccp)* is a rampant and highly contagious animal disease with potential of rapid spread irrespective of national borders. The exact picture, dynamics and distribution of CCPP in areas bordering Tigray and Afar regions is not well documented. Hence, a cross sectional study was conducted to determine the seroprevalence of the disease in goats in three districts namely Kafta Humera and Alamata (Tigray) and Aba-'ala (Afar) and to check the potential of sheep in maintaining the disease. Proportions and chi-square test statistics were used to analyze the data. The tested serum samples were collected from 863 goats and 137 sheep; 282 (32.68%) and 25 (18.25%) were positive for antibodies of *Mycoplasma capricolum subsp. capripneumoniae (Mccp)* respectively using Complement Fixation Test (CFT). The sero-prevalence was higher in Alamata 123 (43.93%) followed by Aba-'alla 136 (38.64%) and Humera 23 (9.96%) and the variation was statistically significant (X 2=76.00, P<0.0001). How ever; there was no statistically significant difference in the seroprevalence of CCPP in goats in both sexes (X 2= 3.619, P= 0.0571) and age (X 2=0.990, P=0.6095) groups. The finding of high seroprevalence of CCPP in sheep (18.25%) could indicate that sheep are potential carriers of *Mccp*, hence sheep could be considered as one potential reservoirs of *Mccp* infection warranting that control and prevention strategies such as vaccination in goats should include sheep as well.

INTRODUCTION

Goats being an important component of livestock sub-sector play a significant role in the socio- economy of the developing countries because of their better adaptation to the unfavorable arid environment and suitability for resource poor farmers [3]. *Peste des petitis ruminants (PPR), contagious caprine pluropneumonia (CCPP), pasteurellosis, sheep and goat pox* diseases cause substantial losses through high morbidity and mortality [21]. CCPP is defined as an infectious disease which clinically affects only goats [17] and it is one of those rampant and highly contagious animal diseases with potential of rapid spread irrespective of national borders [4]. *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) is the causal agent of CCPP. *Mccp* originally known as the F38 biotype was first isolated in the Sudan, Tunisia, Oman, Turkey, Chad, Uganda, Ethiopia, Niger, Tanzania and the United Arab Emirates. CCPP was first reported in the mainland of Europe in 2004, when out breaks were confirmed in Thrace, Turkey, with losses of up to 25% of kids and adults in some herds [14]. Several tests may be used for serological diagnosis such as complement fixation test (CFT), passive haemagglutination test [13], latex agglutination (Robust test) [18] and indirect ELISA [24]. CFT remains the most widely used serological test for CCPP and it has been found to be more specific though less sensitive than the indirect haemagglutination test. Moreover; it is the official test recommended for international trade [14]. The exact picture, dynamics and distribution of CCPP in areas bordering Tigray and Afar regions is not well documented. Hence, the objectives of this study were to assess seroprevalence of CCPP in Alamata, Humera (Tigray) and Ab-'Ala (Afar) and to evaluate the presence of *Mccp* antibodies in sheep.

MATERIALS AND METHODS

Randomly selected goats (n=863) and sheep (n=137) with no history of vaccination for CCPP were used as a source of serum samples regardless of age, sex or status of health [23] and were grouped in to three age categories using dental formula [7]. 5-7 ml of blood was collected directly from jugular vein of each animal using sterile plain vacutainer tubes and needles [9] and was allowed to stand in slant position for 2-6 hours at room temperature until sufficient amount of clot is formed [2]. Then the samples were put at +4oc in the refrigerator till serum was extracted. Serum was then separated in to cryovials. All the samples were labeled (date, age, sex)

and stored temporarily at -20oc. Transportation to the referral laboratory National Veterinary Institute (NVI) was made using an ice box. The serum samples were examined for the presence of specific antibodies against *M. capricolum subsp. capripneumoniae* by using complement fixation test (CFT) in NVI. The test was under taken according to the standard operating procedures of [15]. The sera were tested at dilutions rates of 1/10, 1/20, 1/40 and 1/80. Data entry and analysis was made through JMP 5 statistical software. Proportions were used to calculate the prevalence of CCPP while X2 test was used to asses the status of the disease with age, sex and area. Results were reported as statistically significant if *P-value* was less than 0.05.

RESULTS

A total of 863 goats sera collected from the three study sites were tested and 282 (32.68 %) was found to be positive for the presence of antibodies of *Mycoplasma capricolum* subsp*. capripneumoniae* considering at 1:20 dilutions and above as positive. The seroprevalence was higher in Alamata 123 (43.93%) followed by Aba-'alla 136 (38.64%) and Humera 23 (9.96%) with significant statistical difference (X 2=76.00, P<0.0001) (Table 1). However, there was no significant statistical difference (X 2= 1.806, P= 0.1790) in seroprevalence of CCPP between the two neighboring districts; Alamata 123 (43.93%) and Aba-'alla 136 (38.64%). 74 (30.96%),105 (32.01%) and 103 (34.8%) of the goats with age between 6 months to 1 year ,1 to two years and greater than two years respectively were positive and there was no significant statistical variation (X 2=0.990, P=0.6095) among the three age groups (Table 2). Contingency analysis of the sero prevalence of CCPP with respect to sex in goats also showed no significant statistical difference (X 2= 3.619, P= 0.0571) between male 37.84 % (84/222) and female 30.89% (198/641) (Table 3). 137 sheep's sera collected from the three woredas were tested for *Mccp* antibodies and 25 (18.25%) became positive and there was significant statistical variation (X 2= 8.188, P=0.0042) in comparison to the prevalence in goats. The statistical analysis of the seroprevalence in sheep among the three districts indicated that there was a significant variation (X 2=32.585, P< 0.0001) except between Alamata and Aba-'alla (X 2= 0.227, P=0.6337).

Table 1: Sero-prevalence of CCPP by study sites and species

	Goats		Sheep	
	No. Positive (%)	No. Negative (%)	No. Positive (%)	No. Negative (%)
Alamata	123 (43.93)	157 (56.07)	11 (40.74)	16 (59.26)
Aba-'alla	136 (38.64)	216 (61.36)	10 (47.62)	11 (52.38)
Humera	23 (9.96)	208 (90.04)	4 (4.49)	85 (95.51)

1 X2 = 76.000, DF = 2, P<0.0001 (significant for goat among the three sites)
1 X2 = 32.585, DF = 2, P<0.0001 (significant for sheep among the three sites)
* X2 = 8.188, DF = 2, P=0.0042 (significant between sheep and goat)

Table 3: Prevalence of CCPP in goats with respect to sex

Sex	No. of Positive (%)	No. of Negative (%)
Female	198 (30.89)	443 (69.11)
Male	84 (37.84)	138 (62.16)

X 2 = 3.619 DF = 1, P= 0.0571 (not significant)

Table 2: Prevalence of CCPP in different age groups of goats

Age group	No. of Positive (%)	No. of Negative (%)
6 month – 1 year	74 (30.96)	165 (69.04)
1-2 years	105 (32.01)	223 (67.99)
Above 2 years	103 (34.80)	193 (65.20)

X2 = 0.990, DF = 2, P = 0.6095 (not significant)

DISCUSSION

Based on the antibody titre 1:20 taken as positive threshold, the 32.68% (282/863) seroposetivity of goats for CCPP antibodies was comparable with the reports made by [5] 29.08% in selected districts of Afar, [19] 29% in Wolloo and North Shoa using CFT. Similarly 31% prevalence had been reported by [2] in an export abattoir from goats that had been collected from Afar, Borena, Bale and Jinka using CFT. Higher proportions of sero-prevalence were observed in other studies underwent in different times and areas in the country; a prevalence of 51.5% using CFT [6], 50 % using CFT [11], 53 % using B-ELISA [16] in east Shoa, Melkasedi (Hararge) and Gewane (Afar) respectively. This variation may be due to the situation of the disease during the time of sampling in the study areas. A relatively lower sero-prevalence of 16.5% using CFT [20], 1.3% using C-ELISA [1] and 0.56% using B-ELISA [25] in south Omo and Gamogofa, Diredawa and eastern Ethiopia respectively had been reported. The difference may be as a result of the temporal and spatial factors associated with sampling, the situation of the disease during the time of sampling and the variation in the specificity and sensitivity of the different serological tests employed. The prevalence of the disease has been evaluated between the three different study sites, Alamata (43.93%), Humera (9.96%) and Ab-'Ala (38.64%), by using contingency analysis (chi-square) and the results

revealed (P<0.0001) a highly significant statistical difference (Table 1) which is in agreement with [5, 20, 1] that reported the significant difference in the distribution of the disease among the different agro ecological zones. The prevalence of the disease in goats between Alamata (43.93%) and Ab-'Ala (38.64%) was not statically significant (X 2= 1.806, P= 0.1790). This may be associated with the non restricted animal movement between the two neighboring districts of Afar and Tigray region and the highly contagious nature of *Mccp* infection. The occurrence of the disease across age and sex factors showed that there was no significant statistical difference among the three age categories and between male and female goats (Table 2 and 3). This result was compatible with the similar observations made by [5, 20] in studies conducted in different parts of Ethiopia. It has also been reported that CCPP is highly contagious and fatal to susceptible goats irrespective of age and sex by [15]. These might also indicate that the humeral immunity to *Mccp* infection is not age and sex dependent. Sheep being reared together with goats in the study areas, they were included in the investigation to weigh up their role in the dissemination of CCPP to goats, which has been described by [15] that goats are the only species to be clinically affected by *Mccp* infection. A prevalence rate of 18.25% was observed in sheep in the present study. This is a relatively higher result obtained as compared to sero-prevalence report of 5% and 7% using B-ELISA by [12,22] respectively. The higher value might be associated with the higher sensitivity of CFT in contrast to B-ELISA [19]. The result clearly illustrates that sheep can act as reservoirs of *Mccp* organisms and can play a tremendous role in the dissemination of CCPP to the highly susceptible goats which has also been explained by [10, 8] by isolating *Mccp* organisms from apparently healthy sheep.

CONCLUSION

Although there were no official reports of outbreak and clinical cases of CCPP during the study period in the study areas, the CFT sero-surveillance had shown that CCPP exists at least in sub clinical level warranting the need for regular vaccination programs. More over; though the exact role of sheep in the epidemiology CCPP was not established, it can be concluded that sheep can have the infection with detectable antibody response. Thus, an over all epidemiological study of CCPP in goats and the role of sheep in maintaining and dissemination of CCPP need to be further studied to design best control strategies. Lastly, rapid, inexpensive, easily applicable diagnostic tests for primary screening of CCPP in field (for example, latex agglutination or capsular polysaccharide antigen antibody) should be developed adequately and be easily accessible as part of surveillance and control program in regional laboratories.

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EPIDEMIOLOGICAL STUDY OF CANINE VISCERAL LEISHMANIASIS IN SYRIA

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SUMMARY

Visceral leishmaniasis (VL), also known as kalaazar, accounts for an estimated 75,000 deaths annually. Dogs are the main domestic reservoir of VL caused by Leishmania infantum in the Mediterranean area and in Central and South America. Early diagnosis of the disease in dogs is essential for the effective surveillance and control national programs. Syria is one of the areas where cutaneous and visceral leishmaniasis highly endemic. Using the fast strep test (rK-39) 217 dogs were tested randomly in different provinces of Syria (Lattakia, Hama, Homs, Edleb, Aleppo, Tartous, Sweida and Daraa), distributed in 20 areas of these provinces (Esawe`eh, Slayeb, Bet Qidar, Hama, Hurbnefseh, Mesiaf, Kufrtanour, Sheikhbahar, Maara, Krurymeh, Safita, Menan, Jenderis, Al-Frerieh, Hadedah, Houleh, AL-Qareteen, Era, Laja and Zabayer), to study the distribution of canine visceral Lieshmaniais (CVL).

Serological analysis showed a positive visceral leishmaniasis result in 55 dogs (25.3%) of total cases, the highest prevalence was in Edleb 40%, followed by Aleppo 32%, Daraa 31% and Hama 27.8%. The highest infected areas were Al-Maara 60%, followed by Menan 44%, Jenderis 43%, Safita 40% and other areas (Hama, Laja, Kufrtanour) 33%. Results reveals a significant variation between dogs age and leishmania infection, the positive prevalence was 8%, 25%, 32% in the different groups of age <1, 1-3, and >3 respectively. Females were more infected (48%) than males (32%). The study showed that 62% of positive dogs were asymptomatic, while 38% showed very visible coetaneous lesions, among them 9% had weakness and only 4% movement disturbances.

The high prevalence of VL in dogs in Syria (25%) considered to be the highest percentage in the middle east region. The infections were located mostly in the humid and sub-humid western belt of Syria, starting from the coastal zone of Mediterranean to the nearby mountain ranges in the second climatic zone of Syria. Another foci of infection was found in Daraa province in the south of Syria.

INTRODUCTION

Canine visceral Leishmaniasis is a zoonotic disease caused by *Leishmania donovani* complex (*L. donovani, L. infantum, L. chagas*). The main resrvoire are dogs, and the disease occurred traditionally in the Mediterranean area, some new references indicate the occurrence of the disease in Italy, Canada and the United States [1,2].

The serological prevelance of the disease in the middle east variated between 10-37% [3]. In the Arab world the epidemiological serology show 19% positive cases in Yemen and Saudi Arabia and infection foci in Iraq, 4% positive serological prevelance in north Palastine [4] and 10% in dogs and 5% in foxes and Jackals near Jerusalem [5], and 8,6% in dogs in the West Bank [6].

MATERIAL AND METHODS

Using the fast strep test (rK-39) 217 dogs were tested randomly in different provinces of Syria (Lattakia, Hama, Homs, Edleb, Aleppo, Tartous, Sweida and Daraa), distributed in 20 areas of these provinces (Esawe`eh, Slayeb, Bet Qidar, Hama, Hurbnefseh, Mesiaf, Kufrtanour, Sheikhbahar, Maara, Krurymeh, Safita, Menan, Jenderis, Al-Frerieh, Hadedah, Houleh, AL-Qareteen, Era, Laja and Zabayer), to study the distribution of canine visceral Lieshmaniais (CVL).

RESULTS

Serological analysis showed positive visceral leishmaniasis results in 55 dogs (25.3%) of total 217 cases, the highest prevalence was in Edleb 40%, followed by Aleppo 32%, Daraa 31% and Hama 27.8%. The highest infected areas were Al-Maara 60%, followed by Menan 44%, Jenderis 43%, Safita 40% and other areas (Hama, Laja, Kufr Tanour) 33%. Results reveals a significant variation between dogs age and leishmania infection, the positive prevalence was 8%, 25%, 32% in the different groups of age <1, 1-3, and >3 respectively. Females were more infected (48%) than males (32%). The study showed that 62% of positive dogs were asymptomatic, while 38% showed very visible cutaneous lesions, among them 9% had weakness and only 4% movement disturbances. Five dogs died during the first stage of the study (6 monthes).

DISCUSSION

As visceral leishmaniaisis exist in the Mediterranean area its very normal to have 55 positive cases (25,3%) in Syria. This results are similar to the prevelance of 10 to 37% in the middle east [3,7,8].

The highest dog prevelance was in Idleb province 40% , where the highest incidence of human cutaneuos and visceral leishmaniasis has been recorded [9]. Another study show that the highest number of people visited hospitals for leishmaniasis were in Idleb and then Daraa[10,11], and we found in our study that the Laja area of Daraa with 31% dog cases is the same traditional area of leishmania distribution. The same was registered in Lattakia, Tartus and Hama.

CONCLUSIONS

Visceral canine leishmaniasis occure in the most Syrian provinces in different prevalences, the areas of high prevelances have the highest human cutaneous and visceral cases.

There are very rare studies about the disease in dogs and other farm animals, these studies can improve the national strategies for more effective control programs of the disease.

More studies should concentrate on the prevelance of the disease in zoo's, wildlife to find other reservoirs of the causative agent.

More studies on dogs should be carried out to examine their role as reservoire for the cutaneous leishmaniasis (L.troipca)in the areas with no other reservoir found.

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ISOLATION AND PREVALENCE OF PATHOGENIC *LEPTOSPIRA INTERROGANS* IN SLAUGHTERED CATTLE IN TWO ABATTOIRS IN SOUTHWESTERN NIGERIA

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SUMMARY

Leptospirosis is a waterborne bacterial disease, transmitted to humans through contaminated water, usually by urine of rodents that are chronically infected by the pathogenic strains.The prevalence and incidence of leptospirosis in slaughtered cattle in relation to the risk of exposure of abattoir workers and the public consumers was determined in this study. The objective of this study was to evaluate the prevalence of bovine leptospirosis in slaughtered cattle in southwestern Nigeria. Kidney samples from 108 cattle were examined. Samples were collected from Bodija abattoir in Ibadan, capital city of Oyo State, and Lafenwa abattoir in Abeokuta, capital city of Ogun State. Leptospira was isolated in Ellinghausen-McCullough-Johnson-Harris broth medium (EMJH). Pathogenecity test was carried out in Guinea pigs. Gross and histopathological lesions were observed in their kidneys. Leptospira species were isolated from 89 (82.4%) out of 108 kidneys from the slaughtered cattle. Twenty (95.2%) out of 21 kidneys and 69 (79.3%) out of 87 kidneys collected from Ibadan and Abeokuta respectively were positive**.** Only 31 (28.7%) (9 kidneys from Ibadan and 22 from Abeokuta) kidneys showed visible macroscopic changes, while histomorphological changes such as interstitial nephritis, tubular nephrosis and tubular protein cast were observed. Death of guinea pigs that were inoculated occurred within 24 hours to 168 hrs. The isolation of *leptospira interrogans* and the pathology associated with kidneys obtained in this study indicates that cattle slaughter in public abattoir in South-western Nigeria may be sources of exposure and infection to abattoir workers and the public to leptospirosis.

INTRODUCTION

Leptospirosis is a zoonosis of ubiquitous distribution, caused by infection with pathogenic *Leptospira* species. The spectrum of disease caused by leptospires is extremely wide, ranging from subclinical infection to a severe syndrome of multiorgan infection with high mortality. The syndrome, icteric leptospirosis with renal failure, was first reported over 100 years ago by Adolf Weil in Heidelberg (Levett, 2001)

Documented information on the role of cattle in the epidemiology of leptospirosis in Nigeria is scanty. At present, there is no specific control strategy against leptospirosis in Nigeria as little is known about the epidemiology of the infection. Cattle, sheep and goats are known in Nigeria to be kept in smallholder units in close proximity with their owners, thus infection with *leptospira* may pose human health hazard. Hence, the present work was designed to determine the prevalence of leptospirosis in cattle, by leptospire isolation in EMJH medium and histopathological changes associated with infected cattle in south-western Nigeria.

MATERIALS AND METHODS

This project was carried out in Ibadan and Abeokuta the capital cities of Oyo and Ogun States respectively, in the south western Nigeria. The animals were slaughtered in the central metropolitan abattoirs in Ibadan and Abeokuta where more than 500 and 200 heads of cattle respectively, are slaughtered daily. One-hundred and eight kidney samples from 108 different cattle with unknown leptospirosis history, slaughtered at the abattoirs were selected for the study. Approximately10-15g of kidney sections each was taken for bacteriological and pathological evaluated5.

The isolation of *Leptospira* was made from direct inoculation of two drops of blood in 5 mL of Ellinghausen-McCullough-Johnson-Harris broth medium (EMJH) (Difco®-USA) with the addition of 10 % of Rabbit serum and 5-fluorouracil (400 mg/L; Sigma®-USA and chloranphenicol (5 mg/L; Sigma®-USA), nalidixic acid (50 mg/L; Inlab®-BR), neomycin (10 mg/L; Sigma®-USA) and vancomycin (10 mg/L; Acros®-USA).Each sample was inoculated into EMJH medium tubes, incubated at room temperature (28-30ºC) in the dark and examined under dark field illumination at intervals of 10 days to check for the growth of leptospires for at least three months.The bacteria load was manually counted with a Petroff Hausser counting chamber for experimental infection. Ten guinea pigs of either sex each weighing 150 to 200grams were inoculated intraperitoneally with 1 ml of randomly selected isolates of the culture leptospiral (1×10^6). Two normal

guinea pigs were inoculated with EMJH medium as the negative controls.

RESULTS

Culturally, leptospires were isolated from 89 (82.4%) out of 108 kidneys from the two states. This consist of 20 (95.2%) out of 21 kidneys and 69 (79.3%) out of 87 kidneys collected from Ibadan and Abeokuta respectively.

Out of the one hundred and eight (n = 108) kidney samples randomly collected from the two abattoirs, only 31 (28.7%) (9 kidneys from Ibadan and 22 from Abeokuta) kidneys showed visible macroscopic changes. The lesions include multifocal necrotic areas, multifocal petechiat ions,icterus and diffuse nephrosis

The specific histopathology lesions observed in samples that tested either positive or negative for leptospires in EMJH expressed in percentages are shown in table 1.

Most of the guinea pigs that were inoculated with the isolates died between 24 and 168hrs (seven guinea pigs) and the remaining three showed signs of infection associated with leptospirosis.

Table 1: Prevalence of specific histopathological lesion in kidney tissue samples collected from Abeokuta and Ibadan that were cultured in EMJH medium.

Histological lesions		Abeokuta abattoir (n=87)		Ibadan abattoir (n=21)	
		Positive (n=69)	Negative (n=18)	Positive (n=20)	Negative (n=1)
Interstitial Oedema		8 (11.6%)	-	1 (5%)	-
Tubular Nephrosis		52 (75.4%)	2 (11.1%)	16(80%)	1 (100%)
Tubular Epithelail\ .uolation		47 (68.1%)	4 (22.2)	6 (30%)	-
Interstitial Fibrosis		31 (44.9%)	1 (5.5%)	8 (40%)	-
Interstitial Mononuclear Cells Infiltration		58 (84%)	1 (5.5%)	13(65%)	1 (100%)
Perivascular Mononuclear Cell Infiltration		38 (55.1%)	-	7 (35%)	1 (100%)
Periglomerular Mononuclear Cell Infiltration		32 (46.4%)	-	3 (15%)	-
Glomerular Atrophy		25 (36.2%)	-	4 (20%)	-
Cast		40 (58.0%)	13(72.2%)	11(55%)	1 (100%)
Tubular Dilation		30 (43.5%)	5 (27.8%)	4 (20%)	1 (100%)
Glomerulonephritis	Membranous	10 (14.5%)	-	1 (5%)	-
	Membranoproliferative	19 (25.5%)	-	6 (30%)	-
Crystals		31 (44.9%)	4 (22.2%)	6 (30%)	1 (100%)

DISCUSSION

Leptospires were isolated from 89 out of 108 kidney samples collected from the two abattoirs in the Southwestern Nigeria. The diagnosis was based on either or both isolation of the leptospira species with EMJH medium at 27°C-30°C and pathogenicity test with guinea pigs. In Nigeria, there is no record of the isolation of

leptospires from animals, as the majority of the data are based on serology(Agunloye *et al* 1997).

The gross renal lesions, such as cortical haemorrhage, multifocal necrosis, diffuse palor and icterus, reported in this study are typical of renal bovine leptospirosis and are consistent with those in previous reported cases in cattle and other animals (Faine *et al.*, 1999). In this study, there is no relationship between gross lesions and isolation of leptospira organism in the kidney samples, since most of the kidney samples without gross lesions were culturally positive. Histological changes observed in this investigation were in correlation with the reports of other workers (Marinho *et al* 2009). However, Skilbeck *et al* (1988) did not observe significant histopathological lesions in kidneys from which leptospires were isolated. In this study the lesions range from locally extensive cellular infiltrates to diffuse lesions, characterized by tubular nephrosis, glomerular atrophy and renal haemorrhage. Most of the kidneys samples studied presented changes suggestive of leptospirosis in the histopathological lesions of the kidneys in accordance with Faine *et al.*, (1999).

CONCLUSION

The isolation of *leptospira interrogans* and the pathology associated with the kidneys obtained in this study indicate that cattle slaughtered in the public abattoir in the South-western Nigeria may be sources of the infectious agent to human population. It is recommended to improve on the sanitation and personal hygiene of abattoir workers and implement a hazard analysis critical control system in the abattoir.

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MOLDAVI: A MODEL TO PREDICT NUTRIENT AND ENERGY FLUXES FROM MEAT-POULTRY PRODUCTION SYSTEMS

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SUMMARY

MOLDAVI is a dynamic model developed to predict nutrient (water, C, N, P, K, Cu, and Zn) and energy fluxes from meat-poultry production systems at an hourly time-step. It can simulate a wide range of poultry production systems with or without an outdoor run. MOLDAVI predictions are based first on animal growth, simulated by a Gompertz function. Heat, water and carbon dioxide produced by animals and nutrient excretion are then calculated. Animal feeding is included in the model, with the ability to simulate different feed compositions. Feed intake, body retention and excretion are also predicted by the model. The indoor climatic conditions are calculated, and ventilation and heating rates are regulated in order to meet indoor condition targets. The amount and chemical composition of manure also are predicted. Emissions of NH_3, CH_4 and N_2O from manure are calculated with emission factors from literature and modulated according to manure management practices. This paper presents the model and simulations that illustrate the effect of different farming practices on manure characteristics and gaseous emissions.

INTRODUCTION

In the recent decades, livestock-farming practices have evolved considerably. The intensification of production systems has proven to be economically effective but is noted for its negative impact on the environment (climate change, eutrophication, soil acidification, etc.). Precise knowledge of the dynamics of nutrient and energy flows of livestock systems is required to improve both their economic and environmental performances. Modelling appears as a relevant tool to study the environmental impacts of livestock systems, but such models are still missing for poultry production. In this context, the aim of this paper is to present a model predicting the effects of farming practices and climate on nutrient and energy flows from meat-poultry production systems.

MATERIAL AND METHODS

General description

MOLDAVI is composed of two interacting sub-systems (Fig. 1): (i) a decision system which takes into account farmer's strategy and practices and objectives daily and (ii) a biotechnical system which simulates nutrient and energy flows on an hourly basis. The information relative to climate (temperature and relative humidity) interacts with these two sub-systems.

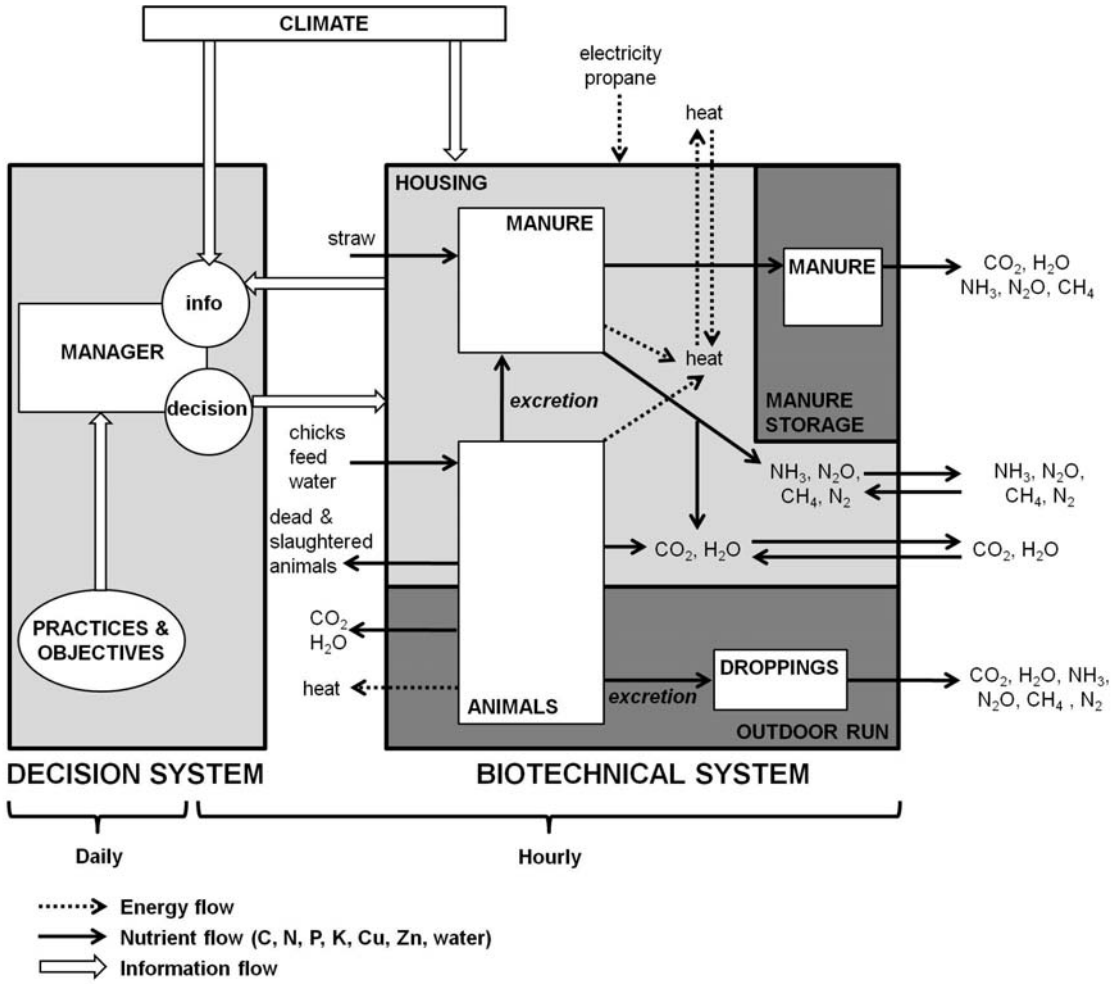

Figure 1. General organization of MOLDAVI components.

Animals

Body weight (BW) of animals is calculated using a Gompertz function and is modulated according to nutritional and environmental factors [6]. Water and CO_2 production related with heat production is calculated using CIGR equations [1].

Feed intake is calculated using a mean feed conversion ratio (FCR) applied to BW. Water, dry matter (DM), N, P, K, Cu and Zn excretions are calculated as the difference between intake and retention. Intake is calculated from feed intake and nutrient content. Retention is calculated as a function of BW using CORPEN [2] values. Drinking water consumption is calculated from the water/feed ratio. When animals have access to an outdoor run, all nutrient and energy flows (heat, excretion, etc.) are shared out with a percentage of time spent on the outdoor run.

Litter

N losses from manure are mainly NH_3, N_2O and N_2 emissions, and several factors can influence them. Therefore, emissions are estimated in the model with emission factors (EFs) applied to nitrogen excreted and then modulated with variation factors (VFs) using the methodology developed by Rigolot *et al.* [7]. EFs are defined for total N, NH_3 and N_2O emissions. N_2 emissions are calculated as the difference between total N losses and NH_3 and N_2O emissions. EFs and VFs were identified from a literature survey [5] and expert's judgment. CH_4 emissions are estimated using IPCC Tier-2 methodology [4]. After manure removal, manure is stored outside the house and emissions of NH_3, N_2O, N_2 and CH_4 are calculated using specific EFs.

Contents of P, K, Cu and Zn in manure are calculated as the sum of excreted amounts and amounts in bedding material (straw). N, DM and water contents in manure are calculated as the difference between inputs (straw, excretion) and outputs (gaseous emissions).

Outdoor run

Nitrogen losses on the outdoor run are calculated with EFs adapted for outdoor conditions. Outdoor CH_4 emissions are estimated using IPCC Tier-2 methodology [4] applied to outdoor excretion. The amounts of nutrients that accumulate in soil are calculated as the difference between excretion and gaseous emissions on the outdoor run

Indoor climatic conditions and regulation

Indoor climatic conditions are calculated as a function of outdoor climatic conditions and housing characteristics (ventilation rate, thermal insulation, etc.). The algorithm for regulation of ventilation rate, heating power and evaporative cooling is based on the model CalorSta [3].

RESULTS

Table 1 presents model predictions for a broiler house (1000 m², 20 animals/m², 4% mortality) in western France in spring 2011. The objectives of performance were to reach a BW of 2 kg at 42 days with a FCR of 1.8 (kg feed/kg BW). The influence on gaseous emissions and solid manure characteristics for 8 different practices were tested. These practices resulted from the factorial combination of:

- 2 dietary crude protein (CP) contents: recommendations *vs.* -10 g of CP/kg of feed,
- 2 types of waterer: nipples *vs.* bells,
- 2 amounts of straw: 4 kg/m² *vs.* 6 kg/m².

Table 1. Predicted influence of farming practices on mass, dry matter (DM) and nitrogen contents of solid manure and on gaseous emissions (NH_3, N_2O, N_2) from the broiler house.

Practices			Litter characteristics			NH_3	N_2O	N_2	Total N losses
CP content	Waterer type	Straw (kg/m²)	Mass (t)	DM (%)	N (kg/t)	kg (% excreted N)			(% excreted N)
Ross PM3 recommendations	nipples	6	27.5	52%	32.9	371 (25%)	47 (2%)	36 (3%)	30%
		4	23.6	53%	37.8	370 (25%)	47 (2%)	37 (3%)	30%
	bells	6	28.7	50%	27.4	555 (37%)	51 (3%)	1 (<1%)	40%
		4	24.7	51%	31.3	553 (37%)	55 (3%)	1 (<1%)	40%
-10 g of CP/kg of feed	nipples	6	28.5	52%	29.7	345 (25%)	44 (2%)	34 (3%)	30%
		4	24.6	52%	33.9	344 (25%)	44 (2%)	35 (3%)	30%
	bells	6	29.7	49%	24.7	516 (37%)	48 (3%)	1 (<1%)	40%
		4	25.7	50%	28.1	514 (37%)	52 (3%)	1 (<1%)	40%

DISCUSSION

Lowering dietary CP content by 10 g/kg feed reduced the amount of nitrogen excreted by animals and total emissions of NH_3, N_2O and N_2 by about 10%, whereas emissions rate, as % of N excreted, remained unchanged. This decrease in excretion and emissions has been observed in previous studies [5].

The type of waterer type had a significant influence on gaseous emissions. When nipples are replaced by bells, N losses increased. This is mainly due to NH_3 and N_2O emissions and can be explained by the fact that more water is wasted with bells than with nipples, increasing manure water content.

The increase by 50% in the amount of straw used per m^2 resulted in an increase by only 16% of the amount of manure produced. The influence was even more limited on the final characteristics of manure and on gaseous emissions. This suggests that these amounts of straw are within the optimal range to provide a good thermal insulation for animals and limit litter moisture and N emissions.

Total CH_4 emission remains unchanged in all simulations (62 kg). This can be explained by the fact that CH_4 EF is not modulated by VFs because knowledge is still lacking.

CONCLUSIONS

MOLDAVI is an original model to predict nutrient and energy flows in poultry-production systems. It can be used for the environmental assessment of different farming practices and innovative systems. Furthermore, the model complements experimental approaches or environmental assessment methods such as Life Cycle Assessment. In the future, it be extended to laying hens or include new knowledge on nutrient and energy flows of poultry systems.

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MODELLING AND CONTROL OF BROILER ACTIVITY

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SUMMARY

Lameness in broilers is a major welfare concern. Increased activity could reduce the incidence of lameness, thus greatly improving broiler welfare. Furthermore, active control of illuminance increases activity of broilers, especially walking. In this work, a differential recurrent neural network (DRNN) was identified from experimental data to predict broiler activity using a recently developed nonlinear system identification algorithm. The DRNN model was then used as a simulation and design tool to synthesize various control inputs, such as illuminance, feeding frequency and duration and to predict overall broiler activity. A discrete control algorithm was developed using these data, so that the overall broiler activity could be maintained at a desired level. Fresh experimental results demonstrated that the developed DRNN model captured the underlying dynamics of the broiler activity well, whilst the DRNN based control algorithm was able to produce accurate input changes to maintain broiler activity at the desired level.

INTRODUCTION

Broiler lameness has been one of the main welfare concerns over recent decades [10]. Various solutions have been proposed to improve broiler leg health and thus alleviate lameness. Changes in feeding regime [7,9] and environmental conditions (illuminance) [5,6] increase activity. Controlling these interconnected processes, as well as growth, is a task normally conducted by experienced stockmen. The precision livestock farming approach, using integrated closed-loop, model based control systems, could be implemented here since novel sensor technology has become available [10].

MATERIAL AND METHODS

To generate data for training and validating the model, broiler chickens were grown from day-old to 51 days and exposed to dynamic changes in the inputs, namely feed amount, light illuminance and absolute humidity. To ensure a measurable response in output, the change in the input was set unrealistically large compared with normal practice. Feed amount was set at either 90% or 110% of normal dosage rates. Light intensity was set at either 10 or 200 lux and relative humidity at 56 or 70%. The frequency of change was set according to the time required to reach a new steady state in the output, i.e. 2-4 hours for the light intensity and 3-7 days for feed amount and absolute humidity.

A three factor (growth, activity and ammonia emission), two-level (change or no change), (2^3) factorial design, repeated three times, requiring 8 identical rooms was used. This design potentially allowed interactions between the processes (growth, activity and ammonia emission) to be identified.

Following a 30 minute dawn period, light intensity was changed either up to 200 lux down to 10 lux every 3 hours and 24 minutes during the 17 hours of daylight, followed by a 30 minute dusk period to darkness. On "odd" days the light intensity was increased directly after dawn, thus allowing for 3 increases to 200 lux a day, whereas on "even" days the light intensity was increased 3 hrs 24min after dawn, allowing just 2 increases to 200 lux a day. Odd and even days were alternated daily.

Each room housed 262 broiler chickens (Ross 308) on a bed of wood shavings. Stocking density was 33 $kg.m^{-2}$. Bird weight was measured continuously using a weighing platform suspended from a load cell (Fancom nv). Specially formulated broiler feeds were weighed and dosed automatically to each room four times a day (Fancom nv). Feed quantity dosed, time of dosage and bird weights in each room were recorded four times per day from day 3 to day 51. Other environmental variables such as temperature, RH and water consumption were monitored at least four times per day. Light intensity was measured using a calibrated light sensitive diode. Activity was measured by scanning consecutive video frames for changes in pixel colour within specified sections of the image. The number of pixel changes was a measure of activity [6]. Light intensity and activity were recorded every minute.

RESULTS

The main challenge in developing a model for broiler activity was that the model had to capture both long-term changes in activity due to bird growth and short-term behaviours of the birds due to illuminance changes and feeding time. The variability of activity increased as the bird weight increased over the whole batch. On the other hand, the activity response to illuminance and feeding time, was very quick and similar throughout, mainly because the pattern for the main drivers, illuminance and feeding was constant during the batch (Figure 1).

A nonlinear dynamic model was devised to control the chicken's activity patterns. To cope with variable sampling rates, the differential recurrent neural network (DRNN) developed by Al-Seyab and Cao [1,2] and the associated automatic differentiation-based training algorithm [3] were employed. A 1st order DRNN model with two inputs (illuminance and feeding incidence), one output (activity), two states and three hidden nodes was designed to represent activity. Broiler growth was incorporated as a scaling factor. The time-step of the model was 1 minute. The measured activity was scaled with the previous day's mean and standard deviation of measured activity.

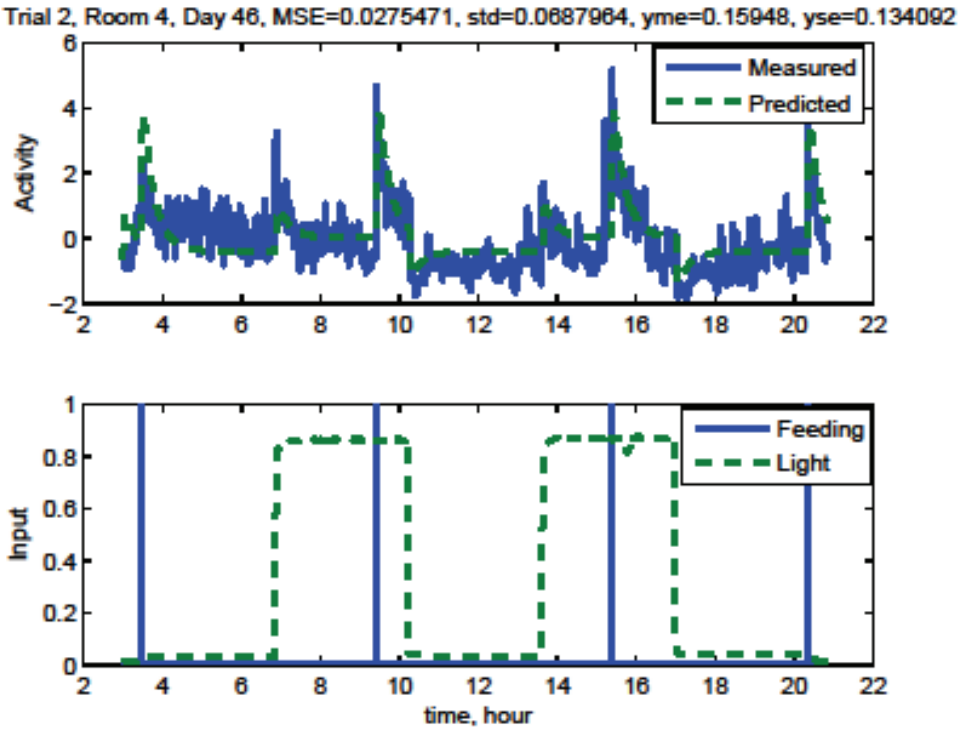

Figure 1 Actual and model prediction of scaled activity of broilers resulting from 4 illuminance changes and 4 feeds a day.

The 16 model parameters of the DRNN model were determined using 9 days of data and validated using a further 54 days of the extensive data set, which comprised 864 days. The trained model was successfully applied to data for all the remaining days with satisfactory accuracy. A typical result is given in Figure 1.

The validated DRNN model was subsequently used to develop a control system for maintaining broiler activity at a desired level. The inputs, frequency of illuminance change and feeding frequency, are discrete variables, so continuous control of the average daily activity was not possible, and a discrete controller managing the daily average activity of the broilers was designed. A simulation model incorporating the trained DRNN model was used to obtain the theoretical activity levels for combinations of illuminance change and feed incidence. The combination most closely matching the desired activity level was selected by the controller.

Fresh experiments were conducted with new combinations of feed incidence and illuminance changes to validate the controller. The simulation model was capable of predicting the instantaneous broiler activity during the growth period. Typically the mean squared error for the scaled activity was 0.03 and the standard error 0.11. Mean daily activity differed significantly for the range of combinations tested over the growth cycle of the broilers (Figure 2). The differences were particularly evident after two and four weeks for feed incidences and illuminance change respectively. The mean activity values depicted for the feed incidence are for the range of illuminance changes used and *vice versa*.

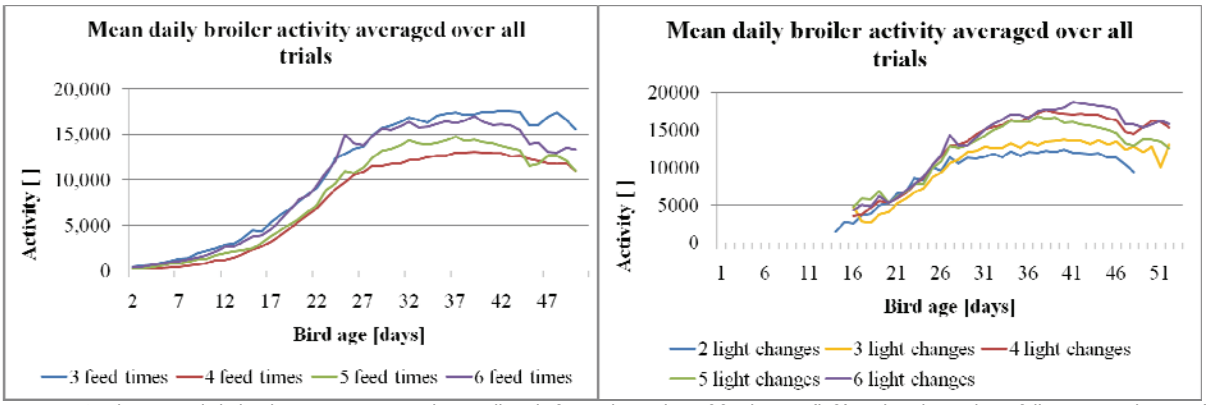

Figure 2: The mean daily broiler activity averaged over all trials for each number of feed times (left) and each number of illuminance changes (right).

DISCUSSION

The daily pattern of broiler activity was dominated by feed incidence and to a lesser extent by illuminance changes. The increased bird activity during feeding was also reported by Nielsen *et.al.* [7], whilst Su *et.al.* [9] found increased activity as a result of feed restriction. Feed was restricted throughout these trials, but Su's result could not be confirmed due to the absence of a control group fed *ad libitum*. The rapid increase in measured activity of the broilers and subsequent slow decrease in activity was also reported by Kristensen *et.al* [5]. Kristensen *et.al.* [6] reported that the effect of illuminance on bird activity was negligible. This was confirmed here, as there was no significant difference in activity for the high and low illuminance batches without light changes.

As with the recursive linear model approach used by Kristensen *et.al.* [6], the DRNN model developed in this work proved very suited to predicting the instantaneous activity of the birds. In addition, the DRNN model was also capable to predict the effect of the birds' growth on the measured activity throughout the growth cycle. The discrete inputs, frequency of illuminance change and feeding incidence, were used for the development of the controller.

In contrast to our previous findings [8], the daily average activity in response to changes in feeding frequency and changes in illuminance was significantly different (Figure 2). The main reason for this difference was the inclusion of activity due to feeding in this work, whereas it was omitted from our previous work.

The controller based on the DRRN model proved very capable of predicting instantaneous daily activity patterns as well as average daily activity for a new range of input values, and also revealed significant, but relatively small, differences in mean daily activity. The latter implies that use of the controller in future to increase broiler activity significantly by varying the frequency of feeding and illuminance change is debatable.

CONCLUSIONS

This project has shown that a data-based approach is a viable means to model and control a biological process. This offers the livestock industry a valuable tool in the management of livestock. The model of broiler activity in response to changes in illuminance and frequency of feeding is robust and accurate. The controller, incorporating a simulation model based on the differential recurrent neural network, was proved to be sound. Although the mean daily bird activity could be controlled to a desired level, the increase obtained was small.

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CALORSTA: A TOOL FOR DESIGN AND EVALUATION OFEVAPORATIVE COOLING SYSTEMS IN POULTRY HOUSES

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SUMMARY

Evaporative cooling of livestock buildings, associated with ventilation, is one way to reduce poultry mortality and improve animal performance in intensive livestock herds during hot periods or in countries with a hot climate. We present the theoretical bases of the tool CalorSta developed to improve the design and management of evaporative-cooling systems and to evaluate existing systems. This tool uses simple choices as inputs, which the user can adapt when necessary. For instance, the user can configure the building characteristics (ventilation type, dimensions, insulation), the type of poultry production (age, species, density) and the outdoor climate conditions. Animal heat production is modelled using CIGR recommendations. Three types of needs were treated: (i) the design of cooling systems and their adaptation to the climate, the animals, and the building; (ii) the simulation of a management scenario during a hot week; and (iii) the evaluation of a cooling system using measurements of temperature and humidity inside and outside the building. Simulations predict that the optimal ventilation and flux of cooling water differ depending on whether the priority is to minimise inside temperature or to minimise inside air humidity. Evaluating a functioning cooling system can show if better performance can be achieved by increasing the flux of cooling water or by reducing warm air entries through leaks. CalorSta was developed for professional uses, and simulations showed that to limit economic damage due to heat stress, it is necessary to design the cooling system accurately and then to ensure that it functions correctly.

INTRODUCTION

Breeding poultry in enclosed conditions requires controlling temperature, moisture and air velocity in the breeding houses. In hot weather conditions, ventilation is not sufficient to reduce animal heat stress, poultry mortality and poor performance. Evaporative cooling of livestock buildings, associated with ventilation, is one of the means used in intensive livestock herds during hot periods when outdoor relative humidity is not too high. These systems are based on conversion of sensible heat into latent heat of evaporated water. They allow a decrease of the indoor temperature with an increase of the indoor moisture [4]. The main evaporative cooling systems are sprinkling, pad-and-fan and fog based [3].

For a better understanding of the effect of evaporative cooling systems on indoor climate conditions, we summarized useful knowledge and organized it into a computer tool named CalorSta. This paper presents the sub-models included in the tool, its outputs and some implications of simulation results.

MATERIAL AND METHODS

CalorSta is intended to be used by scientists but also by breeders and designers of ventilation and evaporative cooling systems. Thus, we wanted the tool to remain as simple as possible while being able to represent a large range of configurations. The tool was developed in Microsoft Excel® using simple choices as inputs (Fig.1). The user can chose the characteristics of the poultry houses (ventilation type, dimension, insulation), the type of poultry production (age, species, density) and the outdoor climate conditions. Crucially, the tool can be used either for mechanically or naturally ventilated buildings. The design of the air inlets for naturally ventilated buildings is based on equations of Souloumiac and Itier [7] which include effects of both temperature and air humidity on air density.

Calculations are based on a steady-state heat balance, using the following equations:

Indoor moisture

Indoor moisture is predicted from the moisture balance:

$$q_i = q_e + \frac{\left(\frac{\Phi_{lat,a} + \Phi_{lat,lit}}{m \times Lat}\right) + cool}{Q_{dim} \times \rho_i} \times 3600$$

with q_e and q_i, outdoor and indoor specific humidities (kg water/kg dry air),
Lat, latent heat of vaporisation of water (2.45 x 10^6 J/kg water),
m, live weight (LW) per animal (kg),
cool, water input through evaporative cooling per animal (kg water/s/kg LW),
ρ_i, density of inside air (kg dry air/m^3 moist air),
Q_{dim}, air flow rate (m^3 moist air/h/kg LW),
$\Phi_{lat,a}$ and $\Phi_{lat,lit}$, animal and litter latent-heat production (W/animal), respectively.

$\Phi_{lat,a}$ is given by CIGR [1]:

$\Phi_{lat,a} = \Phi_{tot,a} \times \mathrm{Min}\left(0.2 + 1.85 \times 10^{-7} \times (t_i + 13)^4; 0.95\right)$, with t_i, indoor temperature (°C).

We assumed that the minimal sensible heat production of the animal is 5% of total animal heat production because body temperature is always higher than air temperature.

Based on results of De Oliveira [5], we considered that latent heat production of the litter represented 70% of total litter heat production.

Indoor temperature

Indoor temperature is predicted as follows:

$$t_i = \frac{\frac{h_i}{4184} - q_i \times 595}{0.24 + q_i \times 0.47},$$

with h_i, indoor air enthalpy (J/kg dry air), calculated from the total heat balance:

$$h_i = h_e + \frac{\Phi_{tot,a} + \Phi_{tot,lit} + \Phi_{ind} - \Phi_{build}}{\frac{Q_{dim}}{3600} \times \rho_i \times m},$$

with h_e, outdoor enthalpy (J/kg dry air),

Φ_{build}, heat losses from the poultry house (W/animal; [6]),
Φ_{ind}, heat inputs of human (heating power) and natural (solar) origins (W/animal),
$\Phi_{tot,a}$, total animal heat production (W/animal), proportional to animal metabolic mass
$(10 \times m^{0,62})$,
$\Phi_{tot,lit}$, total heat production of the litter (W/animal), representing 10% of the total heat production per animal for litter that is neither too wet nor too thick [5].

RESULTS

Three types of needs were treated:

(i) design of cooling systems and their adaptation to the climate, the animals, and the building. Ventilation rate (or opening size in the case of natural ventilation) and water-flow rate from the evaporative cooling system were estimated for selected poultry houses, outdoor climate conditions and animals at the end of the breeding period.

(ii) simulation of a management scenario during a hot week. Two contrasting outdoor climate scenarios in France were used to simulate the indoor climate conditions of a given poultry house over the entire breeding period.

(iii) evaluation of a cooling system using measurements of temperature and humidity inside and outside the building. Observed measurements (indoor temperature and moisture or ventilation and water-flow rates) were compared with predicted values based on information about the breeding conditions and the outdoor climate conditions.

DISCUSSION

Simulations made to address design needs show that the optimal ventilation and flux of cooling water differ depending on whether the priority is to minimise inside temperature to avoid animal mortality at the end of a breeding period or to minimise inside air humidity speeds to keep the litter dry. The development of technical solutions which allow simultaneous control of air- and water-flow rates, in which the farmer can choose to lower either indoor relative humidity or temperature, seems to be required.

To address evaluation needs, the tool can use information about the poultry house, breeding conditions and indoor climate measurements to interpret temperatures which are higher than expected. It also can detect possible dysfunctions of the evaporative cooling and ventilation systems, such as water-flow or ventilation rates which are too low.

Performing simulations with a variety of parameter values is a rapid and inexpensive way to check if better performances can be achieved by increasing the flux of cooling water or by reducing warm air entries through leakages or ventilation.

CONCLUSIONS

CalorSta was developed for professional uses. Simulations show that to limit the economic damage due to heat stress, it is necessary to design the system correctly and to ensure that it functions correctly. This model is also a research tool to increase knowledge about temperature control in animal houses during hot periods.

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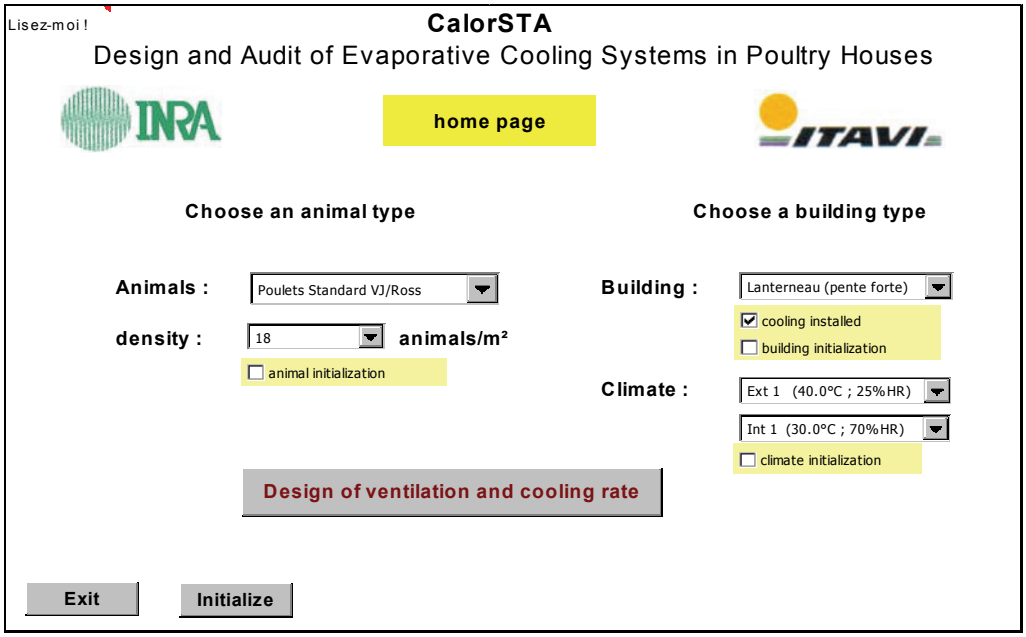

Figure 1: The home page of the CalorSta tool, allowing the user to choose the breeding conditions

IMPACTS OF FURNISHED CAGE DESIGN ON CAGE FLOOR HYGIENE AND EGG QUALITY

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SUMMARY

This experiment examined the effect of group size on floor hygiene in furnished cages for layers. Three treatments of 36 furnished cages (768 cm²/hen including nest and litter areas) were compared: group size S20 (20 hens per cage), S40 (40) and S60 (60). The nest was lined with artificial turf mat (Astroturf Poultry Pads®) and the pecking and scratching area (PSA) was placed opposite the nest and consisted of an Astroturf mat. At 73 weeks when birds were removed from cages, hygiene scoring of nest, PSA and cage floors was conducted in all cages. The scoring comprised 0 to 3 points, in which a higher score indicated a cleaner condition. Overall cages, areas lined with a mat (nest, PSA) were more heavily soiled than wired areas because droppings were stuck in the mats. Droppings also accumulated on wired floor under perches because hens were perched during the night and droppings under perches were not trampled through the floor by bird walking. Nests were cleaner in S40 (average score: 1.9 ± 0.4) and in S60 cages (1.9 ± 0.7) than in S20 cages (1.5 ± 0.6, $P<0.01$) but the frequency of dirty eggs laid in the nest and the mesophilic aerobic bacteria counts on the eggshell were similar in all group sizes. Cage floor under perches was not as clean in S20 cages (1.5 ± 0.6) as in S40 (1.9 ± 0.4) and S60 cages (1.9 ± 0.7, $P=0.07$) because droppings accumulated under the crossed perches in S20 cages.

INTRODUCTION

To improve the welfare of laying hens, the European Union Council Directive 1999/74/EC is banning the use of conventional cages from 2012 onwards. From that date, egg production will only be allowed in non-cage systems or in cages furnished with a nest, perches and a Pecking and Scratching Area (PSA). In France, 81 % of laying hens are kept in cages, essentially in conventional cages [5]. However some of these cages are designed to make it possible to add furnishment after 2012. The cages in which furnishings could be added are generally larger than the conventional cages and are known as large furnished cages (more than 12 hens per cage) [3]. Increasing group size in furnished cages has sometimes been associated with higher mortality rates and degradation of performance [2, 4] but the effect of large group size on egg quality has been rarely studied in cage with more than 12 hens. Cage floor cleanliness and hygiene of hens'feet and plumage are generally inferior in furnished cages than in conventional cages [8]; this may have an impact on eggshell bacterial contamination [9]. In order to contribute to improve cage design, an experiment was therefore set up to test various group sizes at a constant density in large furnished cages.

MATERIAL AND METHODS

4320 beak-trimmed ISA BROWN hens were housed in 3 sizes of furnished cages, built by the same manufacturer (model MEC®, Zucami Poultry Products) with a capacity of 20 hens per cage for group size S20, 40 hens for group size S40 and 60 hens for group size S60. Hens in all 3 group sizes had 768 cm² floor space per hen including 67 cm² for the nest and 67 cm² for the PSA, with 12 cm trough space per hen and a claw-shortening system (abrasive strips, one for 10 hens). Perches were disposed longitudinaly to the feed trough except for S20 cages where perches perpendicular to the feed trough were added (crossed perches) to provide 15 cm of perches per hen in the 3 group sizes. The nest was lined with an artificial turf pad (Astroturf Poultry Pads®) and enclosed by plastic yellow curtains. The PSA was placed opposite the nest and consisted of an artificial turf mat.

When the hens were 71 weeks of age, the eggs laid on 2 consecutive days were recorded per cage for 18 cages per treatment. The eggs from each cage were visually examined to record the number of dirty eggs. These data were classified according to where the eggs were laid, i.e. in the nest, in the PSA or in the rest of the cage. The percentages of dirty eggs per laying location in a cage were calculated. From 66 to 70 weeks of age, 4 samples of 15 sorted eggs laid in the nest (eliminating dirty and broken eggs) and 4 samples of 15 sorted eggs laid in the rest of the cage were taken per treatment on 4 non-consecutive days. The eggs were analysed for the count of the mesophilic bacteria on the eggshell according to the method developed by Protais et al. [7]. The count was expressed as $\log_{10}$ CFU/egg (Colony Forming Unit) for statistical analysis. At 73 weeks when birds were removed from cages, hygiene scoring of nest, PSA and cage floors was conducted in all cages. The scoring comprised 0 to 3 points, in which a higher score indicated a cleaner condition. Each cage was divided into 6 parts: mat in the

nest, wire in the nest, mat in the PSA, wire in the PSA, wire under perches and rest of the cage (middle part of the cage). A score was attributed to each part of the cage.

RESULTS AND DISCUSSION

After a 53-weeks laying period, the areas of the nest and of the PSA covered by a mat had lower average hygiene scores than the wired-areas of the cage: 0.8 ± 0.3 for the nest mat and 0.8 ± 0.4 for the PSA mat vs. 2.3 ± 0.5 for the middle part of the cage ($p<0.01$). Droppings were stuck in the mats while they fell through the wire floor. Despite the lack of hygiene associated with mats, these facilities are needed to improve the welfare of laying hens housed in cages: it has been demonstrated that lining the nest with a mat enhanced nest box use for laying [9, 10]. Similarly artificial turf pad appeared to be more attractive for the hens to perform dust baths than wired floor in the PSA [6]. However mats have to be removed and cleaned separately at the end of the laying period to ensure a good standard of cleaning. Nest hygiene was similar in S40 cages and S60 cages (table) but was better in the two largest group sizes than in S20 cages: the mats were cleaner in S40 and S60 cages than in S20 cages. However neither the cleanliness of eggs laid in the nest nor the bacterial load on the eggshell were affected by group size.

In the PSA, no difference of cleanliness was observed for the mat but the wire floor of S60 cages was significantly cleaner than in the other cages. Surprisingly eggshell of eggs laid in the PSA of the largest cages tended to be more contaminated than in S40 and S20 cages. In the middle part of the cage, wire under perches had an inferior hygiene (1.8 ± 0.6) than the rest of the wired-area (2.4 ± 0.6, $P<0.001$). Indeed droppings accumulated on wire floor under perches because hens were perched during the night and droppings under perches were not trampled through the floor by bird walking [1]. Hygiene score of wire floor under perches was lower in S20 cages (1.5 ± 0.6) than in S40 cages (1.9 ± 0.4, $P=0.05$) and in S60 (1.9 ± 0.7, $P=0.07$). In S40 and S60 perches were disposed parallel to the food trough whereas in S20 a transverse perch was added to the perch parallel to the feed trough. The crossed perches may limit bird movements and therefore degrade cage floor hygiene as demonstrated by Wall and Tauson [8].

CONCLUSION

The smallest group size tested seemed to be associated with a lower hygiene of the nest and of the cage floor but no impact was observed on the cleanliness and on the bacteriological quality of eggs laid in the nest. Crossed perches have a negative impact on cage floor cleanliness.

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Table Effect of group size on hygiene, proportion of dirty eggs and count of aerobic flora on shells of eggs. Results are shown as mean ± standard deviation.

Trait	S20	S40	S60	P[1]
Hygiene of nest mat	0.5 ± 0.3	0.9 ± 0.4	0.7 ± 0.3	0.01
Hygiene of wire floor in the nest	2.4 ± 0.5	2.6 ± 0.5	2.7 ± 0.5	0.17
Hygiene of PSA mat	0.8 ± 0.3	0.8 ± 0.4	0.8 ± 0.3	0.84
Hygiene of wire floor in the PSA	2.0 ± 0.6	2.2 ± 0.5	2.5 ± 0.5	0.01
Hygiene of wire floor under perches	1.5 ± 0.6	1.9 ± 0.4	1.9 ± 0.6	0.05
Hygiene of wire floor in the middle part	2.2 ± 0.5	2.4 ± 0.7	2.4 ± 0.6	0.35
Dirty eggs in the nest, %	1.2 ± 2.6	1.1 ± 1.4	0.5 ± 0.7	0.48
Dirty eggs in the PSA, %	1.3 ± 5.2	0.0	5.8 ± 14.7	0.17
Aerobic flora, eggs in the nest, $\log_{10}$ UFC/eggshell	4.7 ± 0.4	4.7 ± 0.4	4.9 ± 0.5	0.24
Aerobic flora, eggs in the PSA, $\log_{10}$ UFC/eggshell	4.8 ± 0.3	4.9 ± 0.4	5.2 ± 0.6	0.05

[1] Probability of the Kruskal-Wallis rank-test

EFFECT OF FREE EXERCISE IN GROUPS ON THE BEHAVIOUR OF COMPETITION HORSES HOUSED IN SINGLE STALLS

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SUMMARY

Three management practices were investigated with regard to their effect on the behaviour of four competition horses housed in single stalls: daily training without free exercise (NT), turnout before training (TBT) and turnout after training (TAT). The behaviour in the stable was observed via video recordings, the distance covered during turnout was measured by GPS devices and the behaviour during training was evaluated by the riders with the aid of a questionnaire. The behaviour within the stall was more restless in the treatment NT compared to the treatments with turnout, which became apparent in significantly more frequent changes between behaviours. The results of GPS measurement during turnout showed a significantly shorter distance covered in the treatment TAT compared to TBT. The horses' willingness to perform was not significantly different between the three treatments.

INTRODUCTION

In Germany most competition horses are housed in single stalls and free exercise is not permitted in many cases [6]. The reason for not allowing free exercise is mostly the risk of injury [3]. Additionally, opinions exist that the horses' demand for exercise is fulfilled by training or that the horses' willingness to perform is negatively influenced by free exercise. The aim of the study was to determine the effect of common management practices on the behaviour of competition horses housed in single stalls.

MATERIAL AND METHODS

The research was undertaken in a stable in Settmarshausen (county of Goettingen, Lower Saxony, Germany) in the period from 11th May until 19th June, 2009. The stable contained 24 single stalls (3.00 x 3.80 m) and four experimental horses were located in stalls next to each other. The horses were between 4 and 10 years old and were presented at competitions (dressage/show jumping) before, during and after the experiment. To evaluate the effect of three management practices on their behaviour, they were divided into two groups (Group 1 = Horses 1 and 2; Group 2 = Horses 3 and 4). The groups had to pass three treatments, each lasting for two weeks: daily training without free exercise (NT: weeks 1 and 2), two-hour turnout (for free exercise) before training (TBT: weeks 3 and 4 for Group 1, weeks 5 and 6 for Group 2) and two-hour turnout after training (TAT: weeks 3 and 4 for Group 2, weeks 5 and 6 for Group 1). Turnout was carried out on a grassless paddock of 10 x 35 m and the horses were trained by two experienced riders who also presented the horses at competitions. The horses' behaviour in the stable was observed continuously via video recordings (12.30 pm to 7.30 am) on three days at the end of each treatment. The frequency and total duration of the behaviour patterns eating, standing alert, occupation with equipment, occupation with bedding, dozing, sternal and lateral recumbency, aggression and locomotion were recorded. The distance covered during turnout was measured by GPS devices. The horses´ behaviour during training was evaluated by the riders with the aid of a questionnaire. The riders documented the horses' behaviour during the working phase of training (answer possibilities: particularly quiet, rather quiet, normal, rather agitated, particularly agitated), its concentration (answer possibilities: particularly good, rather good, normal, rather bad, particularly bad) and contumacy (answer possibilities: particularly little, rather little, normal, rather intense, particularly intense). The riders' answers about these parameters were summarized into the feature "willingness to perform".

The statistical evaluation of the data was carried out with the software program SAS 9.1 (SAS Inst. Inc., Cary NC, USA). If possible, data sets were transformed in Gaussian distributions and analysis of variance was carried out using the procedure GLM. Significant differences were identified using *t*-test. For data sets that could not be transformed into Gaussian distributions differences between the treatments were calculated with the procedure NPAR1WAY and WILCOXON-TWO-SAMPLE-TEST.

RESULTS

The analysis of the behaviour of the horses in the stable revealed no significant differences between treatments in the time budgets. The frequency of most of the behaviour patterns was highest in the treatment NT. Except of

dozing; frequency of the behaviour patterns did not differ significantly between the treatments with turnout (Figure 1).

The distance covered by the horses during turnout was significantly shorter in TAT compared to TBT. The individual horses did not show the same reaction regarding the particular turnout time. Horses 1 and 2 covered a significantly shorter distance in TAT. The distances of the horses 3 and 4 were not significantly different, but horse 4 covered a slightly longer distance in TAT (Figure 2).

The horses showed "good" willingness to perform most frequently in the treatment TBT and "bad" willingness to perform most frequently in the treatment NT. In TAT the horses' behaviour hardly varied from "normal" performance (Figure 3: right). The duration of training was significantly shorter in the treatments with turnout compared to the treatment NT (Figure 3: left).

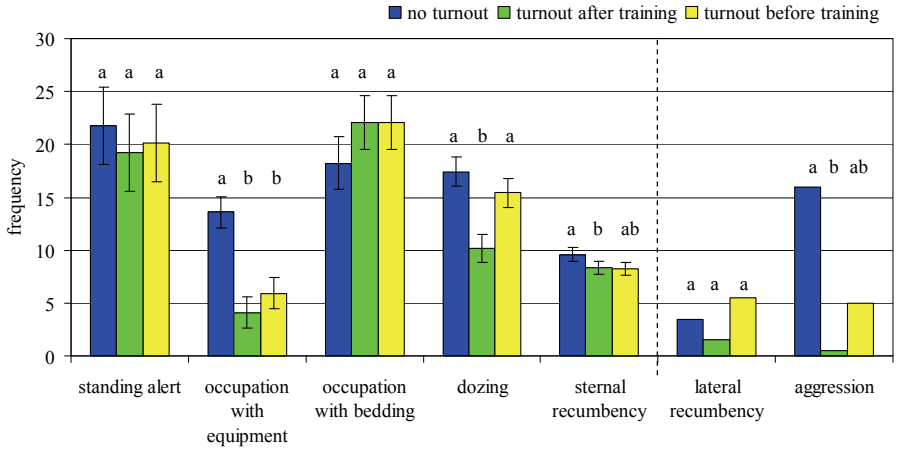

Fig. 1. LSM and SE of the frequency of standing alert, occupation with equipment, occupation with bedding, dozing and sternal recumbency; median of the frequency of lateral recumbency and aggression, subdivided according to treatment [n = 36; a, b = LSM/median within a behaviour with different letters are significantly different ($P < 0.05$)].

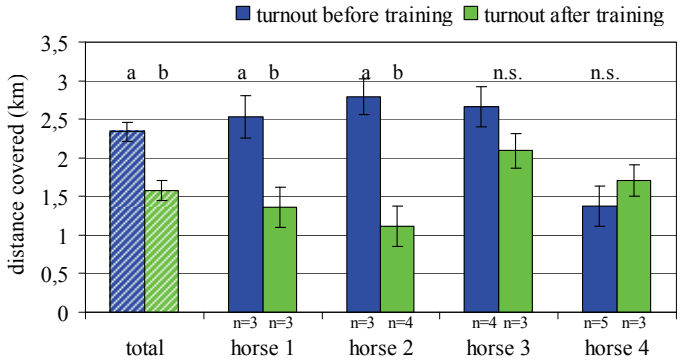

Fig. 2. LSM and SE of the distance covered during turnout subdivided according to treatment. [n = 28; n.s. = not significant; a,b = LSM within a horse with different letters are significantly different ($P < 0.05$)].

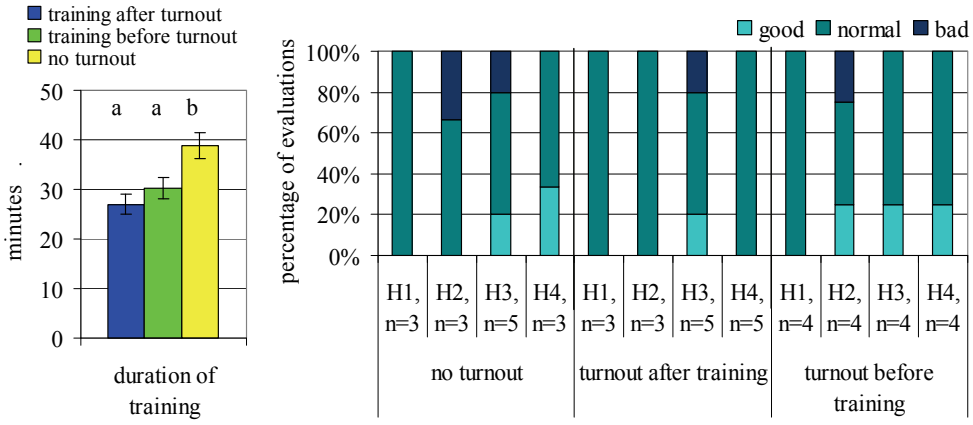

Fig. 3. Left: LSM and SE of the duration of training subdivided according to treatment [n = 44; a,b = LSM with different letters are significantly different ($P < 0.05$)]. Right: Evaluations of the willingness to perform (good, normal, bad) in percent subdivided according to treatment and horse (H1-H4).

DISCUSSION

The horses in the present study showed the highest frequency of appearance of the behaviours in the treatment NT, though the total duration was not different. That means the horses changed their behaviour more frequently in this treatment, which indicates a greater degree of nervousness and restlessness [7]. The horses appeared unsettled as well. Earlier studies also found that the prevention of exercise leads to an accumulation of unspent energy [2], as does a long-term stay in stimulus-poor environments [4]. The decreased aggressive behaviour against their neighbours in the treatments with turnout supports the observation of a more relaxed behaviour in the horses.

The horses showed less activity during turnout in TAT and so covered a shorter distance. This result indicates that training does fulfil the demand for exercise at least to some extent. Jørgensen and Bøe [5] also found more time standing and less time walking in horses on exercise days than on no exercise days. The fact that the horses still showed exercise in TAT indicates that the demand for exercise was not fulfilled entirely by training. Chaya et al. [1] concluded that riding is not a sufficient substitute for turnout. In horses 1 and 2 the difference between the treatments was probably more pronounced than in horses 3 and 4, because the horses 1 and 2 passed TBT after NT and the horses 3 and 4 passed TAT after NT. Presumably, the horses performed a compensatory increase in locomotion as a consequence of their confinement. This reaction has also been observed previously in horses [4, 7].

The horses' willingness to perform was not significantly different between the three treatments. Anyway, the evaluation of the riders was best in TBT and worst in NT. Rivera et al. [8] found that young horses kept on pasture acclimatise easier to a training environment and equipment than horses housed in a stable. The significantly longest duration of training occurred in NT. Presumably, in this treatment the horses needed more time to acclimatize and concentrate on training due to accumulated energy caused by the long time spent in the stimulus poor environment of the stable [2].

CONCLUSIONS

The study shows that allowing or not allowing free exercise and the particular time of turnout effects horses' behaviour in the stable as well in training and as during turnout. The behaviour of the horses in the stable was more relaxed when turnout was allowed in addition to training and the willingness to perform was not negatively affected by turnout. Furthermore, the study indicates that training does not fulfil the horses' exercise requirements. Regarding the risk of injury caused by free exercise, it is advised to allow turnout after training, because locomotion activity is decreased with this order of events compared to turnout before training.

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EXPRESSION OF THE CORTISOL RECEPTOR AND (11β-HYDROXYSTEROID DEHYDROGENASE TYPE 1 AND 2) IN EQUINE TESTICULAR AND EPIDIDYMAL TISSUE

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SUMMARY

Little is known about the effects of stress on fertility in the equine species. However, influences of glucocorticoids on testicular function have been suggested. In many tissues, intracellular glucocorticoid levels are controlled by either or both of the two known isoforms of 11 β-hydroxysteroid dehydrogenase (11β HSD) enzyme. Testicular and epididymal samples from 11 stallions were collected at castration during both the breeding and non-breeding season for analysis of expression of receptors for glucocorticoids (GC-R) and 11β HSD-1 and 11β HSD-2. In addition, expression of receptors for luteinizing hormone (LH-R), follicle stimulating hormone (FSH-R), growth hormone (GH-R), melanocortin 2 (MC2-R) as well as aromatase P-450 was studied. Differences and correlations between the relative gene expression were determined. The results support the hypothesis of an interaction between glucocorticoids and testicular functions in the stallion. The expression of genes studies was not affected by age, but by testicular pathology.

INTRODUCTION

In the stallion, influences of glucocorticoids on testicular function have been suggested [1]. Little is known about the effects of stress on fertility in the equine species. The action of stress on tissue functions is mediated via adrenocorticotrophic hormone (ACTH) and glucocorticoids (GCs). In males stress may interfere with reproductive functions by direct action of glucocorticoids mainly caused by decline in testosterone levels. Studies in boars [2] have shown that cortisol suppresses Leydig cell steroidogenesis by inhibiting the expression of proteins involved in testosterone biosynthesis including steroidogenic acute regulatory protein and steroidogenic enzymes. However, in many tissues, these effects can be modulated by the two known isoforms of 11 β-hydroxysteroid dehydrogenase (11β HSD) enzymes type 1 and 2 which convert active cortisol into inactive cortisone and vice-versa [3]. The aim of the present study was to investigate potential interactions between the pituitary-reproductive and the pituitary-adrenal axis in the stallion. Therefore, the expression of genes possibly involved in the regulation of testicular and epididymal function on the one hand and genes involved in stress responses on the other hand was determined.

MATERIAL AND METHODS

In the present study, testicular and epididymal samples from 11 stallions were collected at the time of routine castration during the breeding season for analysis of expression of receptors for glucocorticoids (GR) and 11β HSD-1 and 11β HSD-2. In addition, expression of receptors for luteinizing hormone (LHR), follicle stimulating hormone (FSHR), growth hormone (GHR), melanocortin 2 (MC-2R) as well as aromatase P-450 was studied. The stallions were divided in four groups: young (1.5 to 3 years, n=3), mature (4-8 years, n=3), elderly (> 9 years, n=2) and with seminoma (n=3). The samples were stored at -80°C until analysed by qualitative RT-PCR. Total RNA from all tissues was extracted. Removal of template DNA was performed using DNaseI, RNase-free. For qualitative PCR, primer sequences of the different genes: 11β HSD-1, 11β HSD-2, eLH-R, eFSH-R, eGH-R, eGC-R, eMC-R and aromatase P-450 were designed based on sequences obtained from PubMed Genbank (http://www.ncbi.nlm.nih.gov/pubmed/). The reaction was performed using the One-Step RT-PCR kit according to the manufacturer's instructions. After the reverse transcriptase-PCR reaction, the products were analyzed by gel-electrophoresis in a 1.5% agarose gel and visualized under UV illumination. PCR products were isolated and subjected to DNA sequencing to confirm specificity. Quantitative real time-PCR was performed using SuperScript III Platinum One-Step Quantitative RT-PCR System (Invitrogen, Carlsbad, CA, USA). Fluorescence was detected on a Realplex Mastercycler epgradient S (Eppendorf, Hamburg, Germany). Each sample was run in triplicate, in parallel with template controls. Normalization of the expression data for genes of interest was realized with β-actin.

RESULTS

Qualitative PCR demonstrated a consistent expression of the receptors for eGC and glucocorticoid metabolizing enzymes (β-HSD-1 and -2) as well as for aromatase P-450 and the receptors for FSH, LH and GH in equine testicular tissue. In contrast, their expression bands occurred only inconsistently in epididymal tissue.

Similar relative gene expression of GC-R in testicular and epididymal tissue (testis: 1.1±0.6, caput: 0.7±0.3, cauda: 0.8±0.6, n.s.) was observed. Expression of β-HSD-1 and -2 was significantly higher in caput than in cauda epididymidis ($p<0.05$). While expression of β-HSD-1 did not differ between caput epididymidis and testis, expression of β-HSD-2 was significantly higher in testicular tissue (2.2±0.8) when compared to caput epididymidis (0.3±0.2, $p<0.05$). For the MC-R, similar differences were seen. Furthermore, significant differences in the expression of aromatase P-450 and the receptors for LH, FSH and GH with regard to the different tissues existed. The relative gene expression of β-HSD-2, but not β-HSD-1 was positively correlated to the gene expression of the receptors for LH ($r=0.606$, $p<0.001$), FSH ($r=0.540$, $p<0.01$), glucocorticoids (GC; $r=0.691$, $p<0.001$) and aromatase P-450 ($r=0.691$, $p<0.001$). In two stallions with seminomas, a very pronounced gene expression was found in all tissues investigated.

DISCUSSION

Glucocorticoids suppress Leydig cell steroidogenesis by inhibing the expression of proteins involved in testosterone biosynthesis and induction of cellular apoptosis. It has been demonstrated that enzymes of glucocorticoid metabolism (11β HSD-1 and 11β HSD-2) modulate the concentration of cortisol and corticosterone providing a physiological balance [4]. The present study demonstrates the expression of 11β-HSD-1 and 11β-HSD-2 in equine testicular and epididymal tissue and their differential tissue expression suggests a fine cellular control in the glucocorticoid levels. The predominant expression of 11β HSD-2 in the testes is in accord with the data showed for pigs [5], suggesting a major role of this enzyme in the glucocorticoids mechanism control.

Positive correlations between 11βHSD-2 and aromatase as well as receptors for LH and FSH suggest interactions between cortisol release and regulation of testicular functions.

CONCLUSIONS

The results support the hypothesis of an interaction between glucocorticoids and testicular functions in the stallion. The glucocorticoid-metabolizing enzymes β-HSD-1 and -2 may have protective effects via regulation of a physiological balance between cortisol and cortisone.

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PARTICLE SEPARATION FROM ROUGHAGES AND BEDDING MATERIALS FOR HORSES WITH A NEW TECHNOLOGY

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SUMMARY

Four bedding materials (wheat straw, wood shavings, hemp, flax) and two roughages (hay, haylage) were treated using an air-driven particle separation technology. The airborne particle generation and the mould spore content of both treated and untreated samples were then analyzed under standardized laboratory conditions. The particle separation resulted in a reduction in the airborne particle (PM_{20}) generation in all materials: hay 49.16 to 22.79 mg/m³ (53.6%), haylage 28.57 to 25.04 mg/m³ (12.3%), wood shavings 141.68 to 15.04 mg/m³ (89.4%), wheat straw 143.08 to 22.97 mg/m³ (83.9%), flax 135.11 to 53.31 mg/m³ (60.5%) and hemp 63.67 to 17.64 mg/m³ (72.3%). The separation treatment reduced the mould spore content by 92.4% in the wood shavings, 88.0% in the wheat straw and by 85.8% in the hay.

INTRODUCTION

One of the main causes of respiratory disease in horses is high concentrations of airborne particles (dust) in the stable air [6]. Due to the fact that the large numbers of horses are housed in stables, there is a direct correlation between the stable climate (including permanent mechanical irritation of particles) and the occurrence of respiratory diseases. Holcombe et al. [5] established that horses housed in a conventional stable environment showed evidence of inflammatory airway disease (IAD). In addition to the total concentration of the airborne particles, the individual particle factions are important in respiratory disease [2]. Particularly the thoracic (PM_{10} - aerodynamic diameter ≤ 10 µm) and alveolar (PM_4 - aerodynamic diameter < 4 µm) fraction are known to penetrate deep into the lower parts of the airways and can directly cause severe damage to the tissues [3]. The primary sources of airborne particles in stable air are the bedding material and roughage [9]. For example, by comparing various bedding materials under laboratory conditions, Fleming et al. [4] showed that a significantly lower concentration of airborne particles was generated by wood shavings (140.9 ± 141.9 mg/m^3) than by wheat straw (227.5 ± 280.8 mg/m^3) (PM_{10}). However, not only dust but also mould spores are known to play a central role in the development of respiratory disease. Tanner et al. [7] found the following concentrations of fungal spores in stable air when comparing different types of bedding materials: phone book paper (3782 ± 2953 CFU/m^3) and straw (5845 ± 5113 CFU/m^3). Accordingly, the aim of this study was to analyze the influence of the treatment of diverse bedding materials and roughages under standardized laboratory conditions with a machine using a dry-air particle separation technology on the subsequent generation of different airborne particle fractions and the mould spores content.

MATERIAL AND METHODS

Following four types of bedding material and two types of roughage were investigated: a) wheat straw, b) wood shavings, c) hemp shives, d) flax shives, e) hay and f) haylage. All of the untreated materials were subjected to the same treatment with a machine using a form of air-driven particle separation technology (Fig. 1).

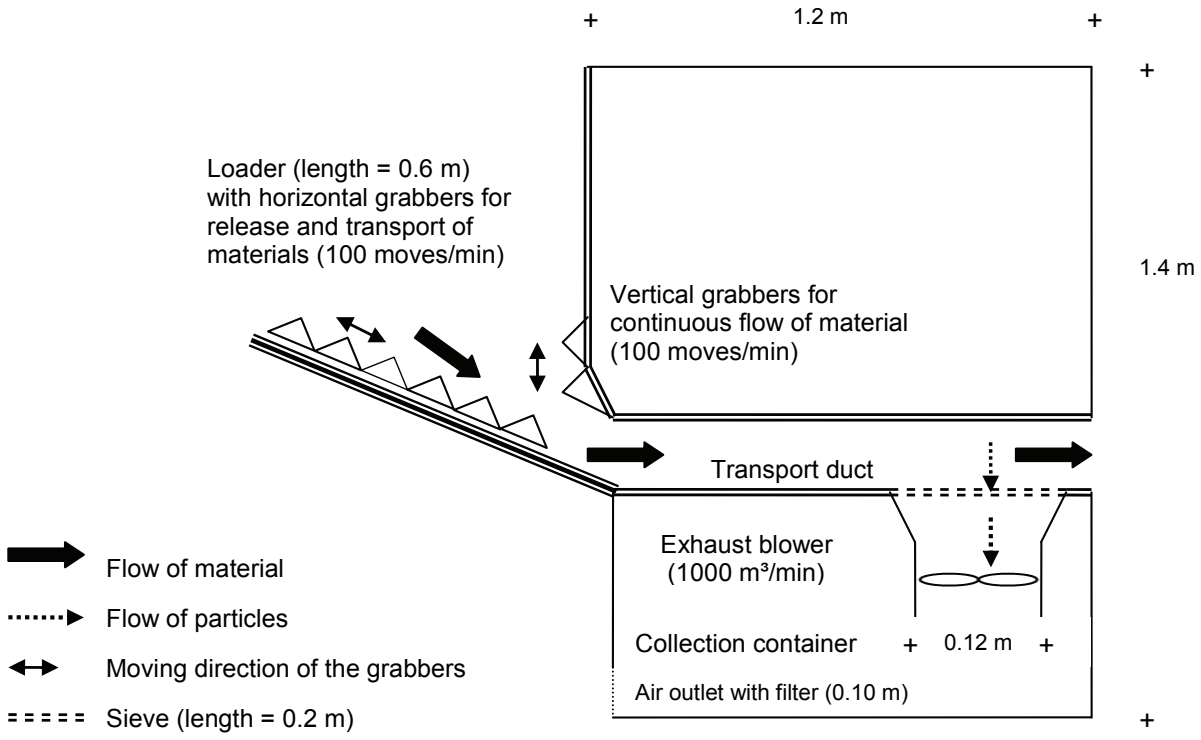

Figure 1. Particle separation technology with collection container.

The machine (Fig. 1) consists of a loading and feeding plate with numerous horizontal grabbers, which are in continual motion (100 moves/min). The input material is loosened, distributed evenly over the plate's surface and moved in the direction of the transportation canal. Vertical grabbers, which move upwards with sudden jolting movements (100 moves/min), are situated in front of the transportation canal, thereby ensuring a continual flow of material into the machine (ca. 2.5 kg/min.). The actual separation system is situated in the transportation canal. It separates particles out of the material using a constant air flow (1000 m³/h). The particles are then captured in a collecting sump. The treated material is pushed into a container due to the pressure from the constant supply of material flowing into the machine.

For the analysis, two special chambers were developed. A pan (diameter 1.0 m) with three rotating paddles (1.0x0.45 m) were integrated in the chamber A (1.0x1.4x1.3 m). In a second separate chamber (B; 1.0x0.8x0.8 m), the gravimetrically measuring particle analyzer TEOM 1400a (Rupprecht & Patashnick Co., Franklin, MA USA) was placed. For the differentiation of the airborne particle concentration, the TEOM 1400a detected the following four different particle fractions using four different sampling inlets: a) $PM_{1.0}$ b) $PM_{2.5}$ (aerodynamic diameter [ad] <1 µm respectively <2.5 µm, alveolar passable), c) PM_{10} (ad <10 µm, thorax passable), d) PM_{20} (ad <20 µm). The two chambers were connected by a tube. Another tube leading out of Chamber B contained a ventilator that transported the air out of Chamber A into Chamber B (air flow 2.31 m^3/min) and from there to a separate room. The measurements started and ended with the starting and cessation of the paddle motor (1.5-kg of each material) over a 90-minute period (n = 3). For determining the presence of mould spores, two manually operated drawers were installed in chamber B, so that Petri dishes with a special agar could be placed inside for two short intervals (10 and 20 seconds) (n = 3).

The statistical evaluation was carried out with the software program SAS 9.1 (SAS Inst. Inc., Cary NC, USA). The average maximum airborne particle concentration (C_{max}) of all particle size fractions and materials were evaluated. In addition, the mean mould spore content (10 sec.; 20 sec.) was analyzed. The analysis of variance (ANOVA) was computed using the GLM procedure, which estimates the influence of the material and processing, and the interaction between both on the airborne particle and mould generation. The data are reported as least square means (LSM) ± standard error (SE). The significance level was $P \leq 0.05$ (t-test).

RESULTS

The highest reduction in airborne particles in the roughages was found in the $PM_{1.0}$ fraction (treated hay 67.0%; treated haylage 87.4%) (Fig. 2). The treated haylage PM_{20} fraction with an airborne particle reduction of 12.3% showed no significant difference to the untreated haylage (P = 0.0524). In comparison, the treated hay did have a significantly lower airborne particle concentration than the untreated hay (P = 0.0008). The effect of treatment on the PM_{10} (Fig. 2) and $PM_{2.5}$ fractions was obviously lower in the hay than in the haylage. In terms of bedding materials, the largest effect of the particle separation treatment could be found in the

wood shavings in the PM_{20} fraction. The treated wood shavings generated an 89.4% lower airborne particle concentration as the untreated wood shavings (reduction in treated wheat straw -83.9%, treated flax -60.5%, treated hemp -72.3%). The treatment of the wood shavings also led to a significant reduction in the $PM_{2.5}$ fraction of 90.1% and in the $PM_{1.0}$ fraction of 61.5% (Fig. 2). No significant reduction as a result of the separation treatment was found only in the $PM_{1.0}$ fraction in the treated wheat straw (P = 0.8456) and treated hemp (P = 0.2268). A significant reduction of more than 50% could be found in the PM_{10} and $PM_{2.5}$ fractions for all the bedding materials as a response to the separation treatment. Figure 2 shows the results of PM_{10} and $PM_{1.0}$ fraction.

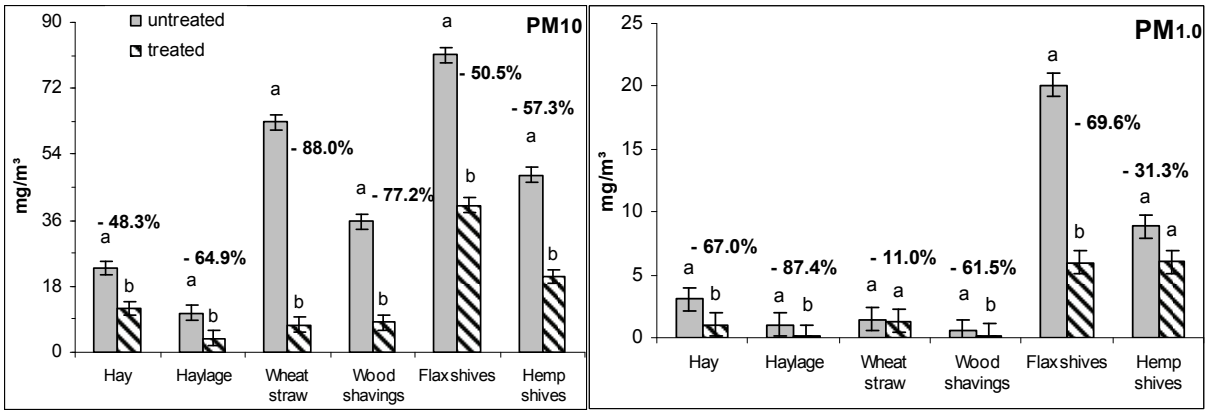

Figure 2. Least squares means and standard error of airborne PM_{10} and $PM_{1.0}$ particle concentration of all untreated and treated materials; a,b = different letters within a material means the values differ significantly (P <0.05). (-) = reduction [%] in the generation of airborne particles due to the treatment (effect of treated vs. untreated materials)

The generation of mould spores (10-sec. sample) was only significantly reduced by subjecting the materials to particle separation in the wood shavings (from 586.7 to 44.3 CFU/m³), wheat straw (from 383.3 to 46.0 CFU/m³) and hay (from 61.0 to 8.7 CFU/m³). In the 20-sec. samples – as with the 10-sec. samples - only the treated wood shavings, wheat straw and hay generated significantly lower mould spore content than the untreated materials, whereby the greatest effect was in the wood shavings (-87.2%) and straw (-81.3%).

DISCUSSION

The separation of the airborne particles in this study occurred without the application of fluid; dry air was used. In a number of previous studies, it could be shown that the wetting of roughage with water only led to a short-term reduction in the generation of dust and in addition provided a good medium for the growth of moulds [1]. In this study, the reduction in particle generation caused by the separation treatment was definitely much lower for hay, haylage, flax and hemp compared to wheat straw and wood shavings (PM_{20}, PM_{10}). The reason for the obviously lower reducing effect of the treatment in haylage could have been due to its high moisture content (25.0%); the moisture content of the other materials was under 15%. Such moisture present in the haylage especially binds large dust particles (≤20 µm), which are only minimally removed by dry separation treatment [8]. The separation treatment had a lower reducing effect in both flax and hemp in comparison to wheat straw and wood shavings. A possible reason for this lower mitigation effect in flax and hemp could be that these materials produced new particles as a result of breakages and abrasion during the treatment. No significant reduction in the airborne particle generation could be found in the treated wheat straw and treated hemp in the $PM_{1.0}$ fraction. This means that the separation of particles <1 µm in these materials is much less efficient. The obviously lower initial concentrations found in the untreated materials of this fraction in comparison to the initial concentrations of the PM_{20} and PM_{10} fractions could be the reason for the reduced reduction effect found in these smaller-sized fractions. No significant reduction in the mould spore content could be found as a result of the separation treatment in either the hemp or flax. Untreated flax and hemp generated clearly lower mould contents than wheat straw and wood shavings, leading to the assumption that there could only be a much lower reduction effect. Due to the much higher moisture content found in the haylage, it could be assumed that the mould spores adhered to the material and so could not be removed by dry separation alone. Through the wetting, it is known that particles such as dust or fungal spores adhere more effectively to the material.

CONCLUSIONS

The results of this study show that the particle separation technology implemented here can be used for roughages and bedding material to cause a manifold reduction in the generation of airborne particles, not only in the coarse particles but also in those that penetrate into the thorax and alveoli. The release of mould spores could also be significantly reduced by the separation treatment,

although there was a high degree of material-specific variation.

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THE EFFECTS OF LIQUID ADDITIVES MIXED WITH OATS FOR HORSES ON THE GENERATION OF AIRBORNE PARTICLES

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SUMMARY

Three different liquid additives (tap water, rapeseed oil, sugarbeet molasses) in three different concentrations (1%, 2%, 3%) were mixed with whole or rolled oats to analyze the reduction potential in terms of the generation of airborne particle generation, which were then analyzed under standardized laboratory conditions. The mixing of oats with just 1% of each of the liquid additives led to a significant reduction in airborne particle generation in all particle fractions with respect to the samples without additives (P <.0001). By the addition of 1% oil, a reduction in the PM_{20} fraction of 90.6% could be achieved. The same dosage of water or molasses only resulted in a reduction of 60.4% or 69.1%, respectively. Overall, the highest significant reduction in the PM_{20} fraction was achieved using 3% rapeseed oil: 96.5% compared to 75.6% for 3% water and 81.9% for 3% molasses.

INTRODUCTION

The quality of concentrated feeds has a decisive influence on the performance and health of horses. Especially during the feeding of concentrated feed, airborne particles can generate in the horse's direct breathing zone. The equine respiratory tract is very sensitive to airborne dust [8] and although horses are usually offered a small amount of oats per day which they consume within a few minutes a not unimportant concentration of airborne particles can also occur from oats. Hessel et al. [4] showed that cleaned whole oats generated a mean PM_{20} fraction concentration of 2.5 g/m³. Just the dry cleaning of oats (sieving and suction cleaning of particles) led a reduction in particle generation of up to 80% (PM_{20}, PM_{10}). The rolling of oats led to an increase in airborne particle generation of ca. 20% [4]. In addition to the total concentration of airborne particles, the individual particle factions are important in respiratory disease [1]. Particularly the thoracic (PM_{10} - aerodynamic diameter ≤ 10 µm) and alveolar (PM_4 - aerodynamic diameter < 4 µm) fraction are known to penetrate deep into the lower parts of the airways and can directly cause severe damage to the tissues. One option for the reduction of airborne particle generation, which has been primarily investigated in pig husbandry, is the mixing of concentrated feedstuffs with animal fat or plant oil, whereby a reduction in the feed dust of 35-70% could be achieved [3]. Welford et al. [10] showed that a 31% reduction in airborne particle generation (PM_{20}) could be attained by adding 2% canola oil to pig feed. Using liquid additives mixing with horse concentrated feeds could be one possibility to reduce airborne particle. The aim of this study was to analyze the effect of three different liquid additives in three different concentrations mixed with cleaned whole or rolled oats on the generation of airborne particles under standardized laboratory conditions.

MATERIAL AND METHODS

A total of 800 kg of commercial cleaned whole oats was used for the analyses. All of the oats came from a single batch and were automatically cleaned using the Aspirateur OPTIMA 2002 NA (Company ZUTHER GmbH, Karwitz, Germany). Half (400 kg) of the cleaned oats were rolled with the roller mill "Universal" (Company Sommer Maschinenbau, Osnabrueck, Germany). Three different liquid additives were chosen for this investigation (tap water, rapeseed oil and sugarbeet molasses). To calculate the most effective amount of liquid additive required to reduce the airborne particle concentrations, each of the liquid additives was added to 2 kg oats in three different concentrations: 1% (20 g), 2% (40 g), 3% (60 g). For each of the airborne particle measurements, 2 kg oats (whole or rolled) were weighed out and placed in the bowl of the mixer (table cutter ST11, Albert Schumann GmbH, Germany). After starting the mixer, the liquid additive was injected through a slit in the mixer's lid into the oats. The mixing process lasted 5 minutes. Once completely mixed, the oats/additive mixture was reweighed and immediately placed in the funnel tube on the outside of a special dust chamber (1x1x1.5 m) including a bowl, which simulated the trough (Fig. 1).

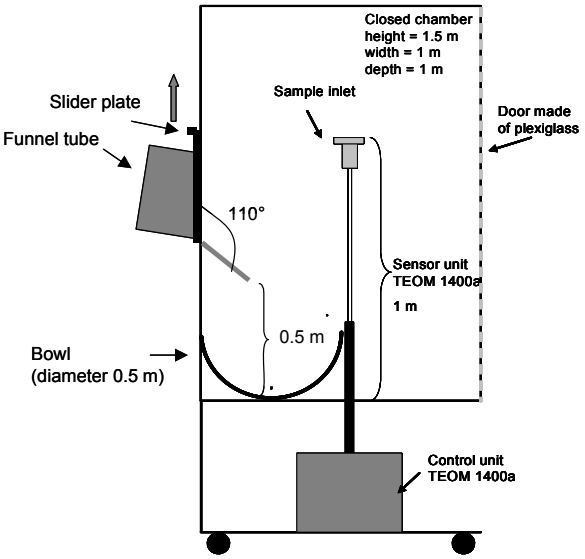

Figure 1. Outline of the closed chamber and particle analyzer TEOM 1400a (side view, cross section)

The gravimetrically measuring particle analyzer TEOM 1400a (Rupprecht & Patashnick Co., Franklin, MA USA) was placed in this chamber (Fig. 1). For the differentiation of the airborne particle concentration, the TEOM 1400a detected the following three different particle fractions using three different sampling inlets: a) $PM_{2.5}$ (aerodynamic diameter [ad] <2.5 µm, alveolar passable), b) PM_{10} (ad <10 µm, thorax passable), c) PM_{20} (ad <20 µm). In order to simulate normal horse feeding, the feed samples were let into the chamber via a funnel tube and slider plate. The measuring of the particle fraction concentrations started with the opening of the slider plate over a 60-minute period (n = 3) under standardized laboratory conditions (temperature 20°C, relative humidity 45%).

The statistical evaluation of the data was carried out with the software program SAS 9.1 (SAS Inst. Inc., Cary NC, USA). The mean airborne particle concentration (C_{mean}) over the 60-minute measurement periods of all particle size fractions was used. The analysis of variance (ANOVA) was computed using the GLM procedure, which estimated the influence of "additive" and "concentration" and the interaction between both on the airborne particle generation. The data are reported as least square means (LSM) ± standard error (SE). The significance level was $P \leq 0.05$ (t-test).

RESULTS

Within the three liquid additives, the addition of rapeseed oil had the highest effect in all of the particle fractions. The oats-oil mixture generated the lowest mean PM_{20} fraction (372.2 µg/m³) compared to the oats-water mixture (643.4 µg/m³; P <.0001) and the oats-molasses mixture (565.4 µg/m³; P = 0.0008). The fixed factor "concentration" had a highly significant effect on the airborne particle generation in all particle fractions (P <.0001). As a consequence of the addition of 1% of any of the additives, there was a significant reduction in the airborne particle generation compared to the controls (P <.0001). There was also a significant reduction in airborne particle generation in all fractions when the additive was increased from 1% to 2%. There was only a significant reduction in the PM_{10} fraction by increasing the additive concentration further from 2% to 3% (P = 0.0025).

The interaction between the factors "additive" and "concentration" had only a significant effect on the airborne particle generation in the PM_{10} fraction (P = 0.0016). The mixture of oats and 1% rapeseed oil generated the lowest airborne particle concentration of 81.2 µg/m³ (-89.4%) in this fraction (P <.0001) compared to the addition of water (300.3 µg/m³) or molasses (236.2 µg/m³) at the same dose (Fig. 2). Again, at an addition of 2%, the oat-oil mixture generated the lowest airborne particle concentration (PM_{10}) compared to water (P <.0001) or molasses (P = 0.0024). Through the addition of 3% rapeseed oil to the oats, a 95% reduction in airborne particle generation was achieved. The concentrations of airborne particles in the mixtures containing either 3% water or 3% molasses were significantly higher than the addition of 3% rapeseed oil (P <.0001 and P = 0.0349, respectively). However, they caused a 73.7% (water) and 90.6% (molasses) reduction in airborne particle generation. Figure 2 shows the mean airborne particle concentrations (C_{mean}) in relationship to the factors "additive" and "concentration".

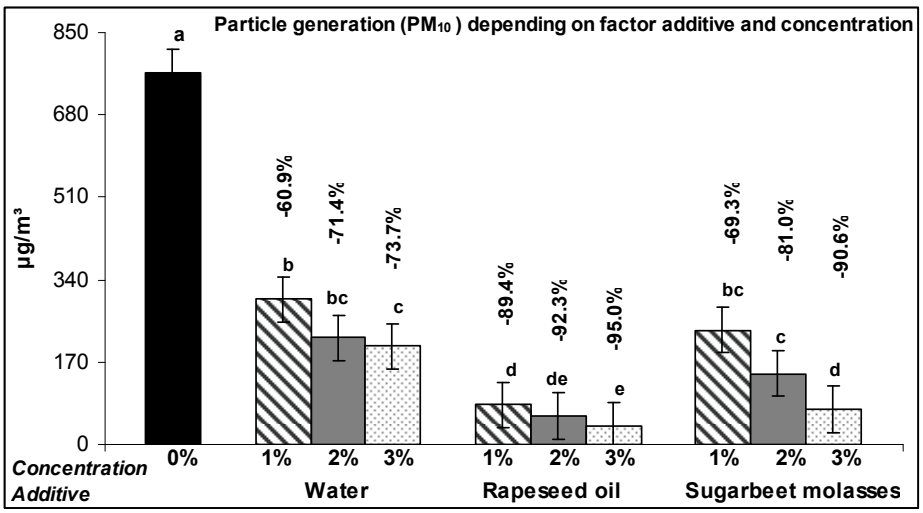

Figure 2. Least squares means (LSM) and standard errors (SE) of C_{mean} [µg/m³] (PM_{10}) for oats mixed with three additives in three concentrations (interaction between factors "additive" and "concentration") and the reduction in airborne particle generation (% - with respect to the 0-concentration). a,b,c,d,e = LSM with different letters are significantly different (P <0.05).

DISCUSSION

The importance of this study arises from the fact that feeds such as oats generate dust directly in the breathing zone of the horse when filling in the trough [6]. The inhalation of particles takes place directly during feeding and is known to decrease airway function [6,2]. Whether, the addition of these additives prevent respiratory diseases, can not substantiate in this study, but for horses which have already respiratory diseases, all processes to reduce airborne particles are helpful. By the addition of small amount of the additives, a main source of dust contamination was eliminated almost completely. This is an important finding with respect to the prevention of respiratory disease in horses. The analyses show that in all three particle fractions the addition of rapeseed oil causes the highest reduction in airborne particle generation in comparison to water or molasses. Takai et al. [9] described the effect of dust reduction caused by the spraying of rapeseed oil in pig stalls through the adherence of the dust particles to the surfaces covered by the oil. Salyer et al. [7] described that the higher adhesion of rapeseed oil with respect to water as being the cause of the formation of the oil film on the surface of oats. The oil film on the surface of the grain enables the dust particle to be completely engulfed, thereby preventing a renewed dispersal of the dust particles. Another reason for the higher reduction in airborne particle generation of oil could be justified by the higher amount of oil compared to molasses and water. Because 3% oil equals 69 ml compared to 54 ml molasses and 60 ml water.

In all of the particle fractions, the reduction in airborne particle generation was increased by upping the concentration of the liquid additives from 1% to 3% (Fig. 2). This result is in agreement with a number of other investigations. Mankell et al. [5] investigated the reduction in airborne particle generation (ad > 5µm) in pig food through the addition of 1% or 3% soyabean oil under laboratory conditions. Through the addition of 1% soyabean oil, a reduction in airborne particle generation of 88% could be achieved in comparison to a sample without additive. The addition of 3% soyabean oil caused a ca. 97% reduction in airborne particle generation.

CONCLUSIONS

Considering all the results together, it can be said that the addition of liquid additives leads to an obvious reduction (50% - 97%) in the generation of all fractions of airborne particles from whole or rolled oats. It can be concluded that from the point of reducing airborne particle generation, rapeseed oil is clearly a better additive than either molasses or water. In this study, the mixing of the oats with the liquid additives was done immediately before they were used (i.e. feeding in practice) so that predictions over possible, time-related storage influences (mould formation, rancidness) on the mixture could not be made.

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ASSESSMENT OF ANTI-*SALMONELLA* ACTIVITY OF BOOT DIP SAMPLES

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SUMMARY

The introduction of pathogens from outside poultry houses by the boots of farm workers and visitors is a significant risk on poultry farms. The use of boot dips containing disinfectant to prevent this from happening is common practice. The effectiveness of boot dips as a preventive measure can vary in relation to various factors. The aim of this study was to assess the anti-*Salmonella* activity of boot dips that are being used on farms.

Boot dip samples were collected from commercial laying hen farms in the United Kingdom and tested within 24 hours of receipt in the laboratory to assess their anti-*Salmonella* activity. All boot dip samples were examined in three test models: (1) pure culture, (2) paper disc surface matrix and (3) yeast suspension model. Of 52 boot dip samples tested 73.1% were effective against *Salmonella* in model 1, 28.8% in model 2 and 34.6% in model 3.

Certain factors may influence the efficacy of the disinfectants. Disinfectants used in the dips may not always be ideal for surface or organic matter contamination, be accurately measured out or made up to a concentration other than that specified or recommended. Dips may not be changed frequently enough or have been exposed to rain and other environmental elements. This study showed that boot dips that are in use on poultry farms are frequently ineffective.

INTRODUCTION

Salmonella is the second most frequent cause of bacterial foodborne disease and is primarily found in the intestinal tracts of animals. It is excreted in the faeces and could contaminate the environment, water and food derived from infected animals [7,12].

In an attempt to restrict the spread of bacterial and viral pathogens on farms, it is common practice to use boot dips containing disinfectant. Organisations, such as the Food Standards Agency, recommend the use of boot dips [4]. Studies have shown that the use of boot dips can be helpful in controlling the spread of bacteria like *Salmonella* and *Campylobacter* by reducing the introduction of pathogens from outside the poultry houses into the animal housing [10,11]. However, a study by Refregier-Petton *et al* showed that the use of boot dips may not have any effect on flock infection with organisms such as *Campylobacter* [1,9].

Pathogens may be introduced on footwear by staff working on the farm, or by visitors. The risk is significant and the rationale is that using boot dips may help in preventing or minimizing the possibility of these pathogens from entering the poultry houses [4]. Clearly the boot dips must contain effective products in order to exert the intended control of microorganisms.

In this study, boot dip samples were collected from commercial laying farms in the United Kingdom and tested to assess their anti-*Salmonella* activity.

MATERIALS AND METHODS

Samples of boot dips were collected on the farms into sterile glass universals by our sampling team and were tested within 24 hours of arrival in the laboratory. All samples were examined using three test models: (1) pure culture, (2) paper disc surface matrix and (3) yeast suspension model and were tested against a field strain of *S.* Enteritidis (SE) from a commercial laying farm that had been used in previous studies [8].

The pure culture method involved adding 1ml of 2 x 10^9 cfu *Salmonella* to 9ml of boot dip with no added organic matter and holding it at 4°C for 30 minutes. After the holding period 0.1ml of the mixture was added to 10ml of Nutrient Broth with 5% horse serum in order to neutralise the disinfectant. A serial dilution was then made in Buffered Peptone Water (BPW) before incubation in order to semi-quantify any surviving *Salmonella*.

The paper disc model provided a porous contaminated surface to be placed in the dip. The disc used was a 6mm Whatman AA filter disc and was calculated to contain roughly 6 x 10^7 cfu of SE per disc at the point of immersion into the dip. The disc was immersed in 5ml of the dip and held at 4°C for 30 minutes with no added organic matter after which it was immersed in 225ml BPW and incubated without further elution or dilution.

The yeast suspension method was based on the British Standards method BS6734:1986 and 2004 [2]. A volume of 0.4ml of the SE challenge strain was added to 9.6ml of

a 5% killed yeast suspension. A volume of 2.5 ml of the mix was then added to 2.5ml of the boot dip sample and held for 30 minutes at 4°C. As in model 1, 0.1ml was neutralised in 10ml Nutrient Broth with 5% horse serum. A volume of 1ml of the mixture was then added to 5 replicate resuscitation broths of 9ml BPWs and incubated.

All the samples were incubated in BPW for 18 hours ± 2 hours at 37°C ± 1°C and were further cultured with a sensitive isolation procedure employing Modified Semi-solid Rappaport Vassiliadis (MSRV) and Rambach media. A volume of 0.1ml was inoculated onto MSRV plates which contained 0.01% Novobiocin and incubated for 24 ± 3 hours at 41.5°C ± 1°C and subsequently plated onto Rambach agar and incubated for 24 ± 3 hours at 37°C ± 1°C. The Rambach plates were examined for *Salmonella* and suspect colonies were confirmed by slide agglutination test.

RESULTS

If the challenge strain of SE was isolated from any of the dilutions in model 1, it was classed as a fail. Any isolation of the strain in model 2 was regarded as a fail. In model 3, a result was considered a pass if the strain was not isolated from two or more of the replicates [2].

In the study, 73.1% of 52 boot dip samples tested were effective against *Salmonella* in model 1, 28.8% were effective in model 2 and 34.6% in model 3.

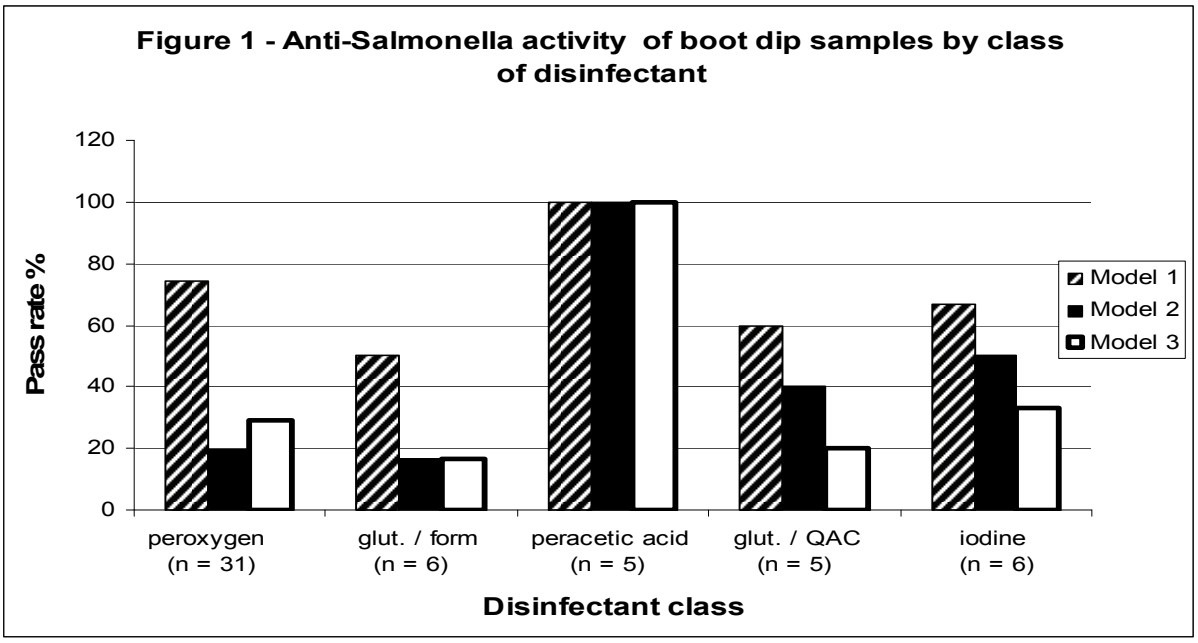

Figure 1: summary of the results

A total of 15 different disinfectants were tested. Most of the boot dip samples contained disinfectant with peroxygen (n=31) as the main active ingredient. Other classes represented in the study include gluteraldehyde/formaldehyde (glut./form.)(n=6), peracetic acid (n=5), gluteraldehyde/quaternary ammonium compound (glut./QAC)(n=5) and iodine (n=6) based disinfectants.

DISCUSSION

Numerous factors may have an influence on the effectiveness of the boot dips, such as the type of disinfectant, concentration, organic soil levels and the frequency with which the dips are changed. Environmental factors and conditions may also have had an influence on the efficacy of the dips.

Disinfectants that are proven to be effective in the presence of organic matter and used at a concentration that is recommended for *Salmonella* (such as the Department for Environment, Food and Rural Affairs (Defra) General Orders Rate) should be selected. This is the concentration specified on the Defra Approved list for use where an order is made under the Animal Health Act 1981 prescribing the cleansing and disinfection of places used for the holding of markets, fairs, exhibitions or sales of animals or lairage, yards, sheds, stables, vessels, aircraft, vehicles, pens etc. [3].

If disinfectants are not measured accurately according to specifications, they may be too dilute and may not have the necessary activity to control or kill *Salmonella* [4].

Dips need to be replenished frequently and should be monitored and refreshed before the antibacterial activity deteriorates. It is advisable to change the dips at least twice a week, more frequently if usage is heavy [4].

Environmental factors such as rain and wind may also influence the efficacy of the dips by either diluting it or possibly contaminating it via dust. Freezing conditions also prevent the effective use of foot dips, but the effect of prior freezing on their chemical activity is unknown. Providing the dips with a cover may prevent environmental factors from compromising the dips [1,6]. However, all these factors are irrelevant if the dips are not correctly used by the workers and visitors to the farm.

The majority of disinfectants collected in this study were from dips that were not (by admittance) measured accurately when made up. The gluteraldehyde/formaldehyde disinfectants, which we have previously observed to be very effective for surface disinfection, did not perform as well as expected in the current study when compared to peracetic acid or peroxygen disinfectants that are known to be more easily inactivated by organic matter [5,8]. Visual inspection of the boot dip samples that were peracetic acid based indicated that there was little particulate organic matter present. This was in contrast to samples of other boot dips which looked more soiled.

CONCLUSIONS

Disinfectants used in the dips may not always be suitable for surface or organic matter contamination, accurately measured by farmers or may be made up to a concentration other than what is specified or recommended. Some of the samples were obviously not changed frequently enough, judging by their appearance. The presence of organic matter is known to adversely affect the efficacy of disinfectant and we found that generally where organic matter was visible in our boot dip samples, the pass rate was lower. Dips may also have been exposed to rain and other environmental elements. The findings may also result from evaporisation of the active ingredients in aldehyde based disinfectants or may merely be a feature of operator error in preparation and maintenance of dips.

This study showed that boot dips that are in use on poultry farms are frequently ineffective.

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MICROBIAL EXPOSURE OF SERVICE PERSONAL IN BIOLOGICAL AIR CLEANING INSTALLATIONS

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SUMMARY

Biological air cleaning constructions such as bio-scrubbers and bio-filters are increasingly required by the authorities when new large pig housing facilities are built in order to protect the environment and the respiratory health of residents living in close vicinity. While these installations are able to reduce odour and dust emissions by about 90% as well as ammonia by 70 to 90%, little is known about the exposure of the cleaning personnel which has to work inside the scrubber and filter systems during the regular maintenance intervals. In order to provide data to the occupational health services regarding the microbiological burden for maintenance personnel, the content of airborne microorganisms inside a combined scrubber/bio-filter system was measured. During cleaning of the filter of exhaust air treatment systems the total bacteria count ranged between 10^4 to 10^5, staphylococcae 10^5, MRSA 10^4, streptococcae 10^4 and enterococcae 10^2 CFU m^{-3}. Therefore it seems useful that workers are provided with filter masks which are still effective in these very humid conditions.

INTRODUCTION

Intensive livestock areas with animal farming units for cattle, pigs and poultry lead to a general increase in emissions and immissions in their surroundings. Pig facilities particularly emit high amounts of ammonia (NH_3), dust particles and odorous substances during fattening periods [7]. To reduce these emissions of gaseous substances, particles and aerosols of animal housing, multi-staged exhaust air treatment systems (EATS) are used, which can consist of acidic-scrubbers, bio-scrubbers or trickle-bed filters [5]. Filter of EATS are the primary contact surface for a variety of microorganisms from the stable and provide an ideal surface for microbial growth. During the manual cleaning of filter by personnel, health impairment can occur through the negative effects of dust particles and bioaerosols on the respiratory tract. In addition such substances have a potential to cause infection, allergy, toxicity or pharmacological effects [6, 11]. Bioaerosols are defined as "airborne particles of biological origin" [3] and consist of dust, biologically active organisms (viruses, bacteria, fungi, protozoa, mites) and particles of biological origin (endo- and mycotoxins, biogenic amines, allergens). Indicator microorganisms for bioaerosols from animal housing systems primarily include: (i) Gram-negative bacteria such as enterococci, pseudomonads and their endotoxins; (ii) Gram-positive bacteria such as staphylococci, particularly the multi-resistant *Staphylococcus aureus* (MRSA) and streptococci; (iii) actinomycetes; (iv) molds (spores, mycotoxins and β-(1-3)-glucanes); (v) plants (aeroallergens) [11]. It has been shown that in pig housing facilities the livestock-associated (LA) MRSA is the predominant strain of MRSA [13]. Little information is available about the exposure of the cleaning personnel to bioaerosols inside EATS during cleaning processes. In this preliminary study for potential employment protection, certain indicator organisms in bioaerosols were detected.

MATERIAL AND METHODS

For the investigation, five (A-E) different multi-stage EATS (Dr. Siemers Umwelttechnik GmbH, Eydelstedt, Germany) at pig housing facilities in the county of Vechta and Cloppenburg in Lower Saxony (Germany) were sampled during fattening periods. Bioaerosol measurements were performed in the EATS (i) before and (ii) during the cleaning of their corresponding filter systems. Meanwhile the ventilation of the exhaust air into the EATS was kept constant. Figure 1 illustrates the general assembly of a filter which consisted of a plastic packing material with humidifying action.

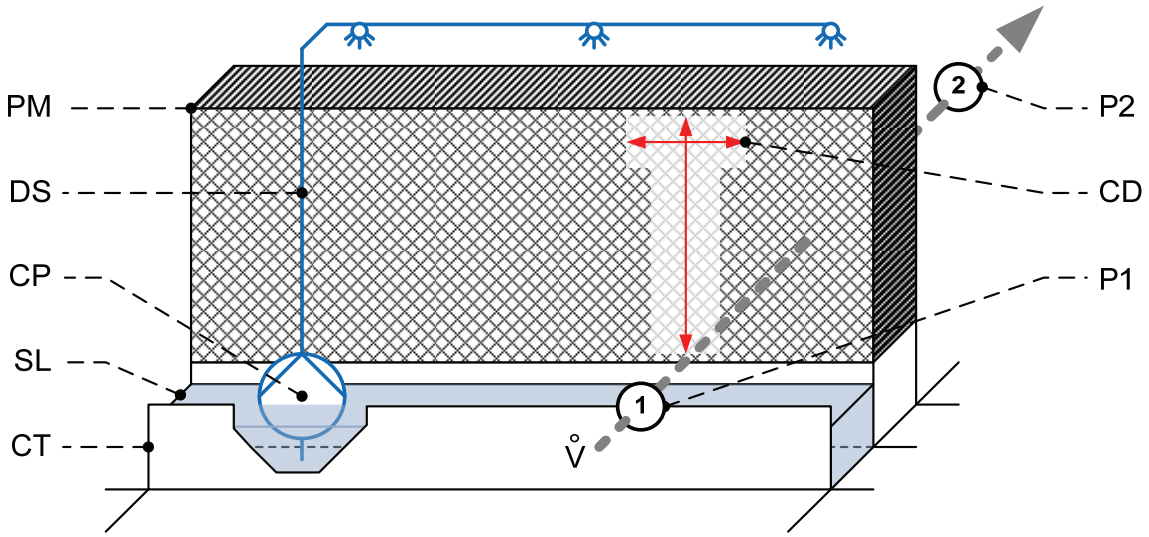

Figure 1: Schematic assembly of a bio-scrubber with packing material (PM), distribution system (DS), circulation pump (CP), scrubbing liquid (SL), collection tank (CT), cleaning direction (CD), air flow volume (V) and cleaning positions in front (P1) and behind (P2) the filter.

Sampling of bioaerosols was performed with SKC® air sampling pumps (type "224-PCXR DELUXE", Analyt-MTC GmbH & Co. KG, Mülheim, Germany). Each pump was connected to a sampling head using a plastic flexible tube. Each sampling head contained a sterile filter cassette with a polycarbonate filter (diameter = 3.5 cm; pore size = 4 μm). The air flow of each pump was set to 3.5 liter per minute. The raw gas of EATS "E" was sampled additionally with "*All-Glass Impinger*" (AGI-30 with a air flow of 12 liter per minute, [1]) as a control measurement to the sampling pumps. To achieve standardized cleaning conditions, cleaning of the filters was performed by the same person each time with a high-pressure cleaner type "HDVAR 7.5/25-150" (Stadiko, Dinklage, Germany) with 1500 liter per hour delivery volume and the same sprayer equipment (1 m length). For the measurements of bioaerosols (i) before the cleaning (in the raw gas, respectively), two pumps were placed in the EATS: in the left and right part with a distance of 1.50 to the filter in 1.75 m height. The sampling time was approximately 45 min. For the measurements of bioaerosols (ii) during the cleaning, the two pumps were wearied by the cleaning person as personal samplers. The sampling head was placed at the right shoulder of the cleaning person with the aperture in viewing direction. Additionally the cleaning person wearied a half-mask filtering face pieces (FFP) type 3 with a blow-off-valve to the left shoulder. The sampling time was approximately 10-30 min, depending on the filter size (length). After the bioaerosol measurements the polycarbonate filter were stored at 4 °C for maximum 24 h. The filtration residue (bioaerosols) were diluted in sterile phosphate buffered saline (PBS), added to the corresponding culture medium and incubated at the optimum temperature depending on the organism. Each sample was analyzed for (a) total bacteria counts, (b) streptococci, (c) staphylococci, (d) MRSA, (e) enterococci, (f) *Pseudomonas* spp. and (g) xerophile moulds. Results are expressed in colony forming units per cubic meter (CFU m^{-3}).

RESULTS

The scrubbing liquids, which irrigated the biofilms on the wet filer walls, contained high inorganic nitrogen salt concentrations (0.4 to 4.3 g-NH_4-N l^{-1}, 0.1 to 3.7 g-NO_2-N l^{-1}, 0.1 to 1.0 g-NO_3-N l^{-1}). The concentration of total organic carbon (TOC) ranged from 0.2 to 0.6 g-TOC l^{-1}). More than 80% relative humidity was measured in the raw gas and more than 95% after the first wet filter, respectively. The measured average of the total bacterial count in the raw gas of all five pig housing facilities ranged from 10^4 to 10^5 CFU m^{-3}. Moreover, in EATS no. "C" generally high bacteria counts were observed. During the cleaning process total bacterial counts were almost in the same range. Staphylococci could be found in the raw gas of the EATS, which ranged between 10^3 and 10^4 CFU m^{-3} as well during the cleaning process (up to 10^5 CFU m^{-3}). MRSA could be detected in three EATS (10^2 to 10^4 CFU m^{-3}). Streptococci were present in all plants in the raw gas (except for "A" = not measured) as well as during the cleaning process (10^3 to 10^4 CFU m^{-3}). Furthermore, enterococci could be detected in the raw gas (10^2 CFU m^{-3}) but not during the cleaning process. Conversely, no pseudomonades could be found in the raw but during the cleaning of the filter walls (10^2-10^4 CFU m^{-3}). Molds were (except for ETAS no. "D") found in the raw gas (10^2 CFU m^{-3}), but not always during cleaning operations (10^2 to 10^4 CFU m^{-3}), although the wet filter walls partly showed slight fungal growth. Overall, no significant differences could be established between the number of germs in the cleaning processes and in the raw gas.

DISCUSSION

The recorded total bacterial counts in the raw gas were approximately in the same range as results of other authors [9, 11] but showed great variations (maximum and minimum values). This count could be explained by the fact that during the cleaning process also larger drops (instead of water vapor) passed the filter sampling head and contaminated a larger filter surface. The alignment of the sampling head was towards the filter wall (equivalent of the face of the cleaning personnel), so that beside the average bacteria count, the maximum values should be considered. However, tests with AGI-30 at EATS no. "E" showed 17% higher total bacterial counts in comparison to the filtration technique. Similar differences were observed for example by [8]. The reason might be due to the higher volumetric flow of the AGI-30 which makes them "less sensitive" to air turbulence and laminar flows in the EATS. Furthermore mechanical disruption of aggregates with bioburden by the higher air flow of the AGI-30 could lead to increased total bacterial counts [11]. Surprisingly the bacterial counts did not show a difference between the cleaning "in front of" and "behind" the filter wall. This might be explained by the fact that the cleaning was performed from the front of first filter to the backside of the last filter. Thus, during the cleaning of the front side, the water of the high-pressure cleaner simultaneously removed parts of the biofilm on the back side (through the filter body). The environmental conditions (aerobic and lower temperatures) of the wet filter walls seemed not suitable for the growth of enterococci (e.g. *Enterococcus faecalis*) which grow in the (anaerobic) gastro-intestinal tract of pigs [2]. In contrast streptococci (maximum: $8 \cdot 10^4$ CFU m^{-3}) and staphylococci (maximum: $7 \cdot 10^5$ CFU m^{-3}) were detected during all cleaning processes. These organisms are well known to affect health due to their extracellular gene products such as toxins, adhesins etc. [4, 8, 13]. On the wet filter walls, usually at the side facing away from the exhaust air flow, a white fungal growth could be often found, indicating molds. The high humidity, oxygen- and nutrient-rich environment support the growth of molds [10] in EATS. However, moulds could not be detected in all measurements. A preventative employer protection, which minimizes the secondary microbiological burden, is personal protective equipment: wearing of waterproof protective clothing and/or disposable clothing, respirators (filtering face pieces mask type 3 with exhalation valve), eye protection (goggles) and ear protection (ear plugs) are required. Similar recommendations are also given in the Technical Rules for Biological Agents [12]. A cleaning should always be performed with the back to the exhaust air flow. A cleaning between filter walls should be performed with a longer (1.5 m) high-pressure cleaner lance containing a pivoted nozzle head (e.g. 90°).

CONCLUSIONS

The cleaning of packing material of filter walls in EATS lead to the formation of secondary bioaerosols (including staphylococci, some MRSA and moulds) which may negatively affect the health of personnel. Therefore personal protect equipment is essential for farmers or personnel during the cleaning process of those microorganisms accumulating systems.

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DETECTION OF SACCHAROPOLYSPORA RECTIVIRGULA BY QUANTITATIVE REAL TIME PCR

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SUMMARY

Saccharopolyspora rectivirgula is described as one causative agent of exogen allergic alveolitis (EAA). EAA is caused by the inhalation of high amounts of *Saccharopolyspora rectivirgula*, which were found in different agricultural environments, like damp hay or duck houses. For the detection of this healthy relevant bacteria we designed a *real-time* PCR primer system targeting the 16S rRNA gene of the type strain *S. rectivirgula* DSM 43747^T and six other *S. rectivirgula* reference strains. To prevent overestimation of *S. rectivirgula* we investigated the number of possibly occurring 16S rRNA operons. Our investigation showed that *S. rectivirgula* presumably own four operons of the 16S rRNA gene, which has to be considered for estimation of cell equivalents. Furthermore our results regarding the DNA recovery efficiency showed values between 7-55% and the recovery rate of DNA in a mixture with non target DNA resulted in approximately 87%. For *real-time* approach high amplification efficiency was detected. The estimated concentrations of *S. rectivirgula* revealed cell numbers of 2.7 x 10^5 cells per m^3 in bioaerosol- and 2.8 x 10^6 cells per g fresh weight in material samples from a duck house. Altogether the results of two clone libraries from bioarosol samples, from a duck house and a composting plant clearly show the specificity of the established q PCR assay that guaranteed the detection of *S. rectivirgula*.

INTRODUCTION

Saccharopolyspora rectivirgula (Corbaz et al., 1963, Krassilnov and Agre, 1964, Korn-Wendisch et al*.,* 1989) a thermophilic bacterial strain belonging to the phylum *Actinobacteria* is associated with the exogen allergic alveolitis (EAA). EAA is an inflammation of the alveoli caused by hypersensitivity to inhaled organic dusts or here in detail by the inhalation of high amounts of spores of *S. rectivirgula*. Because of the medical relevance of *S. rectivirgula,* a reliable detection system is needed. Therefore the aim of the study was to develop a species-specific primer system for *real-time* PCR targeting the 16S rRNA gene.

MATERIAL AND METHODS

Initially we designed a 16S rRNA-gene PCR primer system, based on current sequence information from primary public databases, for the type strain *S. rectivirgula* DSM 43747^T. Furthermore six *S. rectivirgula* (DSM 43113, DSM 43114, DSM 43371, DSM 43755, DSM 43169, DSM 43163) and twelve other *Saccharopolyspora* strains (DSM 44350^T, DSM 45019^T, DSM 45119^T, DSM 40517^T, DSM 44575^T, DSM 45244^T, DSM 43463^T, DSM 44795^T, DSM 43856^T, DSM 44771^T, DSM 44324^T, DSM 44065^T) were investigated to analyse the specificity of the qPCR assay. All strains were either grown on the medium M65 (www.dsmz.de) or TSA (tryptone soy agar). Genomic DNA from bacterial strains was extracted after disruption of cells by a 30 sec. bead-beating step (Precellys 24, Peqlab, Erlangen) with 1g of 0.1 Ø Zirconia beads (Carl Roth GmbH+Co, Karlsruhe) at maximum speed, with the GenElute™ Plant Genomic DNA Kit (Sigma) following the instructions of the manufacturer. For direct DNA extraction from environmental samples (bioaerosol samples from compost plants and duck houses and straw material from a duck house) the FastDNA®Spin Kit for soil (MP, Biomedicals) was used following the manufacturer's instructions. In addition the DNA recovery efficiency of the type strain was tested in combination with bioaerosol or material samples as well as the influence of non target DNA from environmental samples to the recovery rate. To prevent overestimation of *S. rectivirgula* the amounts of 16S rRNA operons occur in *S. rectivirgula* was estimated using southern hybridisation and cloning approach. Specificity of the primer system was analysed by generation of two clone libraries from bioaerosols (one from a composting plant and one from a duck house).

RESULTS

Southern hybridization and cloning approach suggest the occurrence of four copies of the 16S rRNA operon in *S. rectivirgula*. Southern hybridization revealed the presents of 3-5 bands per lane depending on used enzymes. In four lanes, which mean the digestion by PstI, PvuII, SacI, RsrII four distinct bands were visible. Therefore our results lead to the presumption that *S. rectivirgula* own four operons of the 16S rRNA gene, which were later on included in calculation of cell counts.

Furthermore the results showed a recovery DNA efficiency of 7-55% dependent from age of culture. The recovery rate of DNA in a mixture with non target DNA was approximately 87%.

In summary a high amplification efficiency (~ 98%) using real time PCR was found. A linear correlation (r^2=0.99) of C_T- values and corresponding target numbers was observed for concentrations between 10^3 and 10^8 targets μl^{-1}.
The specificity of the quantification system was shown by generation of two clone libraries from bioaerosol samples, from a duck house and a composting plant in which all obtained sequences (n=96, each clone library 48) were most closely related (>99%) to 16S rRNA gene sequences from *S. rectivirgula*.

The application of this detection system on bioaerosol and straw material samples from a duck house revealed cell numbers of 2.7 x 10^5 cells per m^3 and 2.8 x 10^6 cells per g fresh weight and up to 1.0 x 10^7 cells per m^3 in bioaerosol samples from composting plant.

DISCUSSION

Current detection methods for bacteria based on cultivation approach, which often resulted in underestimation of the concentration because e.g. loss in cultivability of the bacteria (Staley & Konopka, 1985; Heidelberg et al., 1997 Zinder and Salyers, 2001; Oliver, 2005). Therefore we developed a molecular detection system targeting the 16S rRNA gene. For example Ranalli and co-workers (1999) detected up to 5.2 x 10^3 cfu m^{-3} air thermophilic actinomycetes in diary barns, where they also detected similar amounts of thermophilic actinomycetes in hay samples (3,3 x 10^3 g^{-1}). In contrast within the present study the estimated amount of *S. rectivirgula* cells using qPCR assay was 1.0 x 10^7 cells per g^{-1} straw material and 2.8 x 10^6 cells per m^{-3} in bioaerosol samples from duck houses. However our findings tend to result in clear higher *S. rectivirgula* concentrations. Furthermore in consideration of the estimated loss of DNA the real concentrations of *S. rectivirgula* in investigated samples seems to be 2-10 folds higher (Fallschissel et al., 2009).

The primer system used in the present study targeting the 16S rRNA gene sequence, which possibly occur multiple (Arcinas, 2004). Results of southern blot and cloning analyses supporting the hypotheses of four operons therefore in the present study calculations of cell counts were made in presumption that *S. rectivirgula* exhibit four 16S rRNA operons.

CONCLUSIONS

Quantitative *real time* PCR generally seems to be a potential method for species specific quantification in bioaerosols and presumably this method is suitable for standardization in occupational exposure measurements in future. Totally the results clearly show the specificity and practicability of the established quantitative real time PCR assay for detection of *S. rectivirgula,* a non infectious but a well known causative of extrinsic allergic alveolitis.

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AERIAL DISSEMINATION OF *CLOSTRIDIUM DIFFICILE* SPORES INSIDE AND OUTSIDE A PIG FARM

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SUMMARY

In both human and veterinary medicine Clostridium difficile infections are increasingly reported. The observation that aerial dissemination occurs in a hospital environment and can play a role in the transmission of human C. difficile infection, resulted in the present study to the occurrence of airborne C. difficile in, and nearby a pig farm with a high prevalence of C. difficile. Airborne C. difficile was detected in all farrowing and weaned piglets' wards, and up to 20 m distant from the farm. A decrease in airborne C. difficile colony counts was observed parallel to aging of the piglets.

INTRODUCTION

Clostridium difficile has been described as a pathogen for piglets since the beginning of the 21st century (10). In North America, C. difficile infection (CDI) is now considered the most important cause of neonatal diarrhea in piglets (11). In humans, the bacterium is known since decades as a major cause of nosocomial infections. Recently, CDI is increasingly reported as a community acquired infection (1, 5). The majority of the community acquired infections are caused by C. difficile PCR Ribotype 078 (1, 8, 13) . The 078 strain is also predominantly present in piglets with CDI (7). The genetical similarity of the human and pig strains gave rise to the concern that zoonotic transmission of this strain is likely to occur (3, 9, 12). Nonetheless, until now there is no evidence of a zoonotic transmission (12) and there is still little knowledge on possible transmission routes from animals to humans (6).

Aerial dissemination of C. difficile was suggested to play a role in the transmission of human CDI in hospitals (2). This prompted us to study whether aerial dissemination of C. difficile also occurs in pig farms and whether aging of piglets is related to a change in spore counts. An additional goal of this study was to determine whether C. difficile could be detected in the air in the close vicinity of the farm.

MATERIAL AND METHODS

2.1. Farm

Air sampling was done at a pig-breeding farm with a known high prevalence of Clostridium difficile in the pigs. The ventilation in the pens is based on a negative pressure system. Fresh air enters the pens from the hallway through slotted air inlets in the doors. The air leaves the pens through a fan, at a height of four meter, which directs the air into an airshaft or directly into the outside environment.

2.2. Sampling procedure

A MB1 MICROBIO Air Sampler (Parrett Technical Developments) was used for collection of airborne Clostridium difficile. The air was directed on commercially prepared C. difficile agar plates (CLO-agar, BioMérieux). Following sampling the agar plates were kept in a refrigerated box, until the laboratory was reached.

2.3. Sampling strategy

2.3.1. Inside air sampling

Sampling of the farrowing ward, the juvenile sow ward and the boar ward was performed in the ventilation shaft of the building. Sampling of the weaned piglets ward, pregnant sow ward and insemination ward took place in the pens self at a height of 1.50 m. This was because air from these pens is directed immediately to the outside environment, i.e. not first directed into an air shaft.

2.3.2. Outside air sampling

Outside air sampling was performed above roof exhausts and at distances 20, 40, 80 and 140 meter downwind from these exhausts at a height of 1.5 m. Sampling time was set on 5 minutes. Data of the Dutch Meteorological Institute was used to determine wind speed and temperature. Control sampling was performed at an upwind point 20 meter distant from the nearest exhaust to exclude any other sources of airborne Clostridium difficile.

2.4. Analysis procedure

Samples were incubated on the CLO-agar plates at 37 °C for 48 h under anaerobic conditions. Using Gram staining the isolates with morphology typical of C. difficile were identified. Per ward and experiment two isolates were randomly chosen, both to be ribotyped. All Isolates from the outside samples were ribotyped as well. Colony counts were calculated per m3.

2.5. Statistical analysis

Data from the continuous sampling experiments were analyzed using the t-test to investigate the correlation between personnel activity and colony count.

RESULTS

3.1. Inside air sampling

Clostridium difficile was detected in the air of all of the wards, except in the air of the pregnant sow unit. The numbers of colonies ranged from 2/m3 to 625/m3, with the lowest numbers found in the weaned piglets ward and the highest numbers found in the air of the farrowing ward. However, once corrected for the number of pigs and piglets, the highest numbers of colonies were found in the boar ward; 66 colonies/m3/pig, compared to 4.9 colonies/m3/piglet as the highest number found in the farrowing ward.

The weaned piglets ward, with piglets ranging from 35 to 65 days of age, and the insemination ward, with 14 sows, were tested negative the first time these wards were sampled. When these wards were sampled again three weeks later, low numbers of colonies were found; 2 colonies/m3 in the weaned piglets ward (100 piglets) and 20 colonies/m3 in the insemination ward (16 sows).

3.2. Outside air sampling

Air from all four exhausts on the top of the building (consisting of air coming from farrowing, boar and young sow ward) tested positive for C. difficile, the numbers ranged from 6 colonies/m3 to 120 colonies/m3. Outside air tested positive 2 out of 4 times at a distance of 20 m downwind from the building. No colonies were found 40, 80 and 140 meter distant of the building. Outside temperature ranged from 2 °C to 8 °C, airspeed ranged from 0.83 m/s to 5.3 m/s. Positive air samples were obtained with the highest airspeeds (5.3 and 3.2 m/s). All upwind air samples were negative for C. difficile.

3.3 PCR Ribotyping

In most air samples within the farm and at 20 m distance from the farm C. difficile was detected. A collection of this share was ribotyped. All 20 C. difficile isolates were identified as PCR ribotype 078.

DISCUSSION

The aim of this study was to detect C. difficile in the air of a pig farm and to relate colony counts of C. difficile in air samples to aging of pigs. Samples taken in different pig wards indicated a decrease in the numbers of colonies found as piglets' age. Highest numbers of colonies were found in air from pens with neonatal piglets, lowest numbers were found in the air from pens with weaned piglets. These results are in accordance with the significant decrease in colonization over time found with cross-sectional and longitudinal fecal sampling of piglets by Weese (2010). However, the finding of high numbers of colonies in the air from the boar ward and the juvenile sow ward is in conflict with the latter results. An explanation can be the minimal cleaning frequency (once a year) of these wards, which allows a continuous build-up of excreted C. difficile. This minimal cleaning frequency is in contrast to the situation of the weaned piglets and farrowing wards, where cleaning takes place before animals move in (every five to six weeks).

In outside air, colonies were detected up to 20 m distant from the farm. The large decrease in colony count immediately outside the building is a logical consequence of the dilution by outside air, and generally applies to the total bacteria concentration (4). Limited dispersal of airborne Clostridium difficile to the outside environment could implicate a low risk of human exposure to airborne Clostridium difficile.

CONCLUSION

This study indicates a correlation between age of pigs and airborne C. difficile colony counts. The widespread aerial dissemination of Clostridium difficile in the farrowing wards may have implications for aerial transmission of Clostridium difficile between piglets. The finding of C. difficile in limited numbers at a 20m distance in the air needs further research to the spread of C. difficile in the environment of pig farms, to determine its significance for human health.

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MAXIMISING HEALTH WITH MINIMUM INTERVENTION: DIAGNOSING FROM THE INSIDE OUT

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Diagnostic techniques used in population medicine are somewhat unconventional when compared to those employed in individual animals. This is especially evident in the poultry industry where it is common to deal with flocks as large as one hundred thousand birds. There are two primary reasons for this. Firstly, it is essential for the diagnostician to detect and address even the slightest hint of subclinical disease within these flocks, in order to maximise biological and hence economic efficiency. This is something that is exacted by intense pressure from public opinion, which requires that rearing conditions be acceptably humane, which is a rather ambivalent demand. When attempting to limit sub-clinical disease, the diagnostic focus shifts from clinically-ill, to apparently healthy individuals, something that makes conventional diagnostic techniques rather superfluous. Secondly, sacrificing a small sample of apparently healthy animals to identify subtle shifts in flock health becomes a realistic and effective option. In large populations of animals, the economic value of each individual is relatively low, which makes necropsy a very cost effective diagnostic technique.

Diagnostic methods have evolved with the development of rapid and cost effective molecular techniques. Unfortunately, their emphasis has been on detecting the presence of an etiological agent, or identifying chemical changes associated with disease-induced aberrations of physiological or biochemical pathways. Routine monitoring of flocks for signs of a shift from normal to abnormal are difficult. A plethora of feedback mechanisms prevent detectable systemic changes from occurring, until the body's homeostatic coping mechanisms are exhausted. In contrast, signs of subtle changes in intestinal health are visible at necropsy.

The physical nature of both the intestinal lining and its content, display detectable changes in the early stages of disease. Ratios of villus height to crypt depth, have for example been used to indicate intestinal integrity. This is possible because the length of an intestinal villus is kept constant by continuous enterocyte replacement. The delicate cells lining the intestinal tract are continuously exposed to potentially damaging luminal content and not surprisingly they require frequent replacement. It has been shown that the life span of a typical enterocyte is 3 to 4 days and consequently complete replacement of the intestinal epithelial lining occurs in this period of time by a process of cell division in the crypt area, sequential migration of the enterocyte to the tip of the villus and finally extrusion from the tip into the lumen. The body's first homeostatic response to accelerated enterocyte attrition is enhanced cell division in the crypt area, and to achieve this, the crypt increases in size. It stands to reason that a slight decrease in villus height to crypt depth ratio in the abscence of any change in villus height is the first indicator that the conditions within the intestinal tract have changed sufficiently to increase the rate of enterocyte attrition. This level of challenge seldom manifests as a change in nutrient assimilation or clinical disease, because intestinal surface area is not affected, but it does however indicate a shift from normal. While it is impossible for even an experienced clinician to detect the change in the thickness of the intestinal wall induced by an increase in crypt depth, there are other changes that give insight into what is happening. Since even minor cell damage induces an inflammatory response, cell debris and inflammatory mediates, including mucus, begin to accumulate in the lumen faster than normal. Apart from causing the villi to stick together and lose optimal alignment (visible to the naked eye) the mucus and cellular debris accumulates to the point where orange coloured mucus forms aggregates or strings within the lumen.

As the severity of the intestinal challenge escalates, so too does the rate of enterocyte attrition. Villus height starts to decline when the rate of enterocyte destruction exceeds the maxim capacity for replacement. At this point the intestinal wall becomes detectably thinner and the intestine loses muscle tone and tensile strength. The mucosal lining of the intestinal wall appears pale and dull giving it a parboiled appearance because of the plethora of dead or dying cells on the luminal surface. The inflammatory exudate makes the shortened villi clump together and their typical zigzag alignment is lost. At this stage there is sufficient reduction in surface area and enough villus damage to compromise intestinal function. There is a net efflux of water into the intestinal lumen, causing what is referred to as a watery enteritis. If the irritation persists of worsens, the enteritis becomes more chronic. There is an influx of inflammatory cells causing the gut associated lymphoid tissue to appear congested and the luminal content becomes dominated by mucus, giving rise to a typical mucoid enteritis. At this stage enzymatic digestion and nutrient absorption is sufficiently compromised for bacterial fermentation of undigested nutrient to result in gas accumulation. Initially the intestinal content become foamy but as the ecology of the intestinal lumen deteriorates the destabilization of the microbiota manifests as the accumulation of free gas.

Changes in the composition of the intestinal microbiota have been associated with deterioration in intestinal function, as measured by feed conversion efficiency. Dysbacteriosis, as it is commonly referred to in the poultry industry, became common place after the moratorium on in-feed antibiotics was introduced in the European Union. These undefined shifts in the intestinal microbiota are difficult to diagnose, even with advanced molecular techniques and yet they appear to be associated with visible intestinal changes. There are changes in the thickness, appearance, muscle tone and tensile strength of the intestinal wall. Signs of inflammation are evidenced by a parboiled appearance of the mucosal surface, the accumulation of inflammatory cell aggregates, congestion and the development of a watery to mucoid exudate in the intestinal lumen. Gas by-products of bacterial fermentation provide confirmation of ecological disturbance.

Efforts to nurture and stabilize a favorable intestinal microbiota with alternative approaches have shown promise in addressing the negative impact of both RSS challenge and in-feed antibiotic removal. While there are several opportunities and product options to achieve this, there are three simple interventions that have demonstrated particular promise. By *seeding* the hatchling gut with favorable organisms, for example with All-LacXCL (Alltech Inc.); *feeding* these organisms with an appropriate organic acid, such as Acid-Pak 4Way(Alltech Inc.); and *weeding* out the unfavorable competitors with a type-1 fimbriae blocker, in the form of Actigen(Alltech Inc.): it is possible to improve performance by accelerating the evolution and maintain the stability of a favorable intestinal microbiota.

Astute observation on the part of the clinician can provide enough information to detect and diagnose subclinical disease in apparently healthy birds at very little cost, if necropsy is performed on a small sample of individuals on a regular basis.

MANAGING MYCOTOXINS - A VETERINARIAN PERSPECTIVE

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SUMMARY

This paper reviews the occurrence of so-called mycotoxicosis in animal production from a veterinarian perspective. According to the World Health Organisation most cereal plants are contaminated with fungal metabolites and there are more than 300 different molecules described as mycotoxins. Traditionally, the occurrence of mycotoxicosis was associated with specific geographical regions; depending on environmental conditions required by the fungi to grow and produce toxic metabolites. However, due to global cereal trade, mycotoxicosis in animals can now occur in any geographic region in the world. The challenge for veterinarians is the lack of a definitive diagnosis for this disease, as feed analysis does not always reveal the true level of mycotoxin contamination. In this situation, diagnosis of mycotoxicosis tends to be based on: clinical history; clinical signs; observation of organ lesions and/or poor animal performance. Preventive control is the only way to avoid the economic and health problems caused by mycotoxins.

INTRODUCTION

Mycotoxins are a large group of fungal metabolites that are toxic to animals, plants and humans. They cause different pathologies according to the structure of the individual mycotoxin. Currently, 300 different mycotoxins have been identified, which are present in more than 30% of cereals produced worldwide [3]. From an epidemiological point of view, fungal growth and mycotoxin production is possible in various animal production systems, throughout the production chain. Fungal invasion and mycotoxin production can occur: in the seeds before harvest while the crop is still in the field; during storage at the feed mill and/or farm; during feed processing; or at feeding if feed bins are not adequately clean and provide conditions for fungal growth.

It is important to know the effects of each individual mycotoxin, as well as the interaction between mycotoxins. The greatest implication of mycotoxin interaction is the effect of immunosuppression due to genotoxic and cytotoxic effects. Poor quality cereal will increase the severity of clinical signs resulting in clinical or subclinical mycotoxicosis; where contamination levels in feed are above those stated in the literature as 'safe' for each mycotoxin.

Fungal growth also causes physical damage and loss of nutritional quality in grains, compounding the severity of the mycotoxicosis. Moreover, the fat content of grains is markedly reduced in contaminated material, which is associated with a reduction in available energy. This could be particularly important with regard to the severity of the disease if not detected and corrected by a nutritionist. For this reason, the concept of the "Poor Quality Cereal Syndrome" is perhaps more applicable than simply mycotoxicosis. This syndrome describes the reduction in health and performance of animals due to consumption of nutritionally poor quality feed from cereals damaged by fungal growth.

Fungal growth and mycotoxin production

Mould growth in grain is a normal occurrence both in the field and during storage. It can spoil the grain and produce secondary metabolites that are highly toxic to animals, humans and plants. Genus of fungi in grain can be divided in two groups according to moisture content: field fungi and storage fungi. However, this nomenclature suggests that the respective groups only grow in either the field or during storage. However, this division is more concerned with moisture conditions than location. For example, field fungi are more prevalent when rainfall is above the average during grain production and harvest; since the field fungi need high moisture conditions (20-21%). But, growth can also occur during storage if moisture conditions are appropriate. This group includes species of *Fusarium*, *Alternaria*, *Clodosporium, Diplodia, Gibberella* and *Helminthosporium*.

Storage fungi (also called storage moulds) need moisture levels lower than field fungi to grow, between 13-18%. The development of storage fungi is influenced by: the moisture content; the temperature; the length of time of this grain is stored; and the amount of insect or mite activity in the grain. The most common storage fungi are species of *Aspergillus* and *Penicillium*.

Losses resultant from fungal growth can be due to physical injury in the grain, losses in nutritional quality of the grains and production of toxins by the fungi. Fungi can affect the coloration, smell and fat content of the grain. When mould grows, the colour of the grain changes from the *sui generis* yellow colour to either: white, pink, red or green, according to fungal species [9]. Mould growth significantly reduces the dry matter and fat content of grain and grains infected with fungi can be half the weight of healthy grains [5]. The metabolic activity of the fungi is related to aerobic respiration. Therefore, grain deterioration results from oxidation of fat and carbohydrates, in the presence of oxygen, resulting in

carbonic acid, water and heat [2]. Moreover, grains infected by fungi have reduced fat, cysteine, lysine and arginine content; which will have subsequent effects on the nutritional quality of the feed [4].

Even if mycotoxins have not been produced, the losses from fungal growth can significantly reduce the nutritional value of infected grains and feed, consequently reducing the performance of animals. Conversely, mycotoxins are an important consequence of fungal growth. Fungi produce mycotoxins in response to stress, for example extremes of temperature, moisture, aeration and, of course, the presence of aggressive agents.

Clinical and subclinical mycotoxicosis

Clinical mycotoxicosis is the ingestion of large amount of mycotoxins in a short time (acute mycotoxicose). It is often associated with situations where the animal is exposed to feed or cereal that was poorly stored. Normally, information regarding the situation that allowed fungal growth, as well as detection of high levels of mycotoxins in the feed material is available for the diagnosis of clinical mycotoxicosis. The clinical signs are generally associated with mycotoxin structure: for example zearalenone in sows will cause reproductive problems and fumonisin in horses will cause leukoencephalomalatia.

However, the majority of mycotoxicosis cases are subclinical. Subclinical mycotoxicosis is associated with either the chronic ingestion of low levels of mycotoxins or varying levels of mycotoxins over a shorter time. Clinical signs, in this case, will be associated with immune suppression and poor performance. For example, mycotoxins can cause regression and cellular depletion of lymphoid organs [6, 7, 8]. In the case of aflatoxin and ochratoxin, the most likely effect is on protein. The immune system relies on an ability to increase protein synthesis and when this is compromised immunosuppression occurs. Mycotoxins also reduce mitotic cell numbers in the bursa of broilers, as well as reducing the humoral immune response to the Newcastle disease virus (NDV) vaccine [8]. This means that, practically, the presence of mycotoxicosis can reduce the efficacy of the vaccine. The discovery that fumonisins are potent inhibitors of the enzyme sphinganine N-acyl transferase revealed that cellular membranes may be a principal target for fumonisins *in vivo*, which is an important factor with regard to immune cells [1].

However, although down-regulation of the immune response is possibly the most well known aspect of mycotoxicosis, an interesting study [10] showed a mechanism of cellular and molecular up-regulation of the immune system with DON and other trichothecenes. In this study exposure to a low dose of trichothecenes, during transcription and post-transcriptionally, up-regulated the expression of cytokines, chemokines and inflammatory genes; which was concurrent with immune stimulation. Whereas, high dose exposure, promoted leukocyte apoptosis, when it was concomitant with immune suppression. These results require closer evaluation to fully understand their implications. If low doses of trichothecenes stimulate the immune response, this does not necessarily mean a positive effect on animal health or performance. In fact, activation of the immune response is a metabolically costly exercise for an organism [6] and numerous 'over-stimulations' could reduce feed conversion rate and overall performance. In light of these results, the effect of mycotoxins with regards to all aspects of health and production should be evaluated. Field cases of subclinical mycotoxicosis associated with immune suppression are often related to: a reduction in vaccine titres; an increase in the occurrence of opportunistic infection (as *E. coli* or *Clostridium sp*); and an increase in losses at the slaughterhouse due to septicemic lesions. On the other hand, increases in non-specific reactions in the intestinal and oral mucosa, or a strong reaction to vaccines, as well as poor feed conversion rates could be associated with up-regulation of immune system.

Prevention of mycotoxicosis

The first point to control mycotoxin problems in poultry is to avoid fungal growth on feed ingredients or feed. Therefore, the detection of contaminated feedstuffs is the primary defense against mycotoxins. The physical condition of the feed can also be evaluated and affected ingredients can be rejected. Good practice is to analyse grains before use and try to include lower levels of damaged grains in diet or, preferably, eliminate them from the diet entirely. To establish a safe level of damaged grains at the field mill it is important to follow the results of an integrated monitoring program (damaged grains versus problems in animals). When the percentage of damaged grain is higher than acceptable is important to correct the formulation of diet according the lower nutritional value of these grains.

Humidity and temperature inside feed bins are important factors to consider with regards to storage. The inclusion of fungal inhibitors, such organic acids, will suppress fungal growth and their toxins. Dilution of contaminated grain with clean grain is often used to reduce mycotoxins levels to below the point of toxicity. This practice presents an obvious risk in that the actual levels of mycotoxins being fed to the animal, and the mycotoxin 'threshold' for that animal's situation, will not be accurately known.

During feed processing, it is important to regularly clean equipment to avoid accumulation of dust. If this is allowed for a long enough periods, fungal growth and posisblely mycotoxin production can occur.

If mycotoxins are present, or suspected, a good strategy is to use an efficient mycotoxin adsorbent, such as Mycosorb (Alltech Inc.,). Nutritional manipulation using antioxidant compounds (like vitamin E and selenium) have been used to improve the animal's defense against mycotoxins: or to decrease their clinical consequences, as they often cause an increase in intracellular reactive oxygen metabolites [11]. Combinations of gluco-manan-oligosaccharides as mycotoxin binders, as well as organic selenium (Sel-Plex, Alltech Inc.) have been proven to be the most effective in the control of T-2 toxicosis in poultry

[12]. Selenium has also been also implicated in reducing the effect of T-2 toxin in human chondrocyte [13].

CONCLUSIONS

Mycotoxicosis is a global problem for veterinarians and the most difficult challenge is to diagnose it using onlymycotoxin analysis of feed. Most mycotoxicosis cases are subclinical and it is often not possible to detect mycotoxins in feed; due to sampling variation and analytical errors. In this situation, it is important for veterinarians to be aware of subclinical aspects of the disease, such as immune suppression and the economic losses associated with it. As there is no treatment for mycotoxicosis a prevention programme is necessary. Control of critical points throughout the animal production chain such as: cereal quality; cleaning and disinfection of the feed mill and bins; as well as the use of anti oxidants and good quality adsorbents in feed, is key to minimising losses associated with mycotoxicosis.

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RESPONSIBLE ANTIBIOTIC APPLICATION IN THE DUTCH DAIRY SECTOR; INITIATIVES OF VETERINARY PRACTICES

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SUMMARY

This paper describes the latest developments in the Dutch bovine veterinary and dairy sector concerning the on-farm usage of antibiotics issue. Both communication on responsible medicine application (i.e. workshop) and providing farmers with the insight into their on-farm antibiotic usage, motivates and encourages farmers to reduce antibiotic usage and discuss on and/or change management that increase the risk of the usage of antibiotics.

INTRODUCTION

In human and veterinary medicine, the use of antibiotics is important for the treatment of bacterial infections in both animals and humans. Most of the antibiotics used in veterinary practice are identical to human therapeutics and may play a role in the development of co- or cross-resistance. The concern of the development of antimicrobial resistance caused by veterinary use of antibiotics, has been risen over the last years. Especially the overlap in critically important antibiotics for both human medicine and veterinary, highlights the importance of prudent use.(FAO/WHO/OIE, 2008)

The WHO already in 1997 recommended that national policies on the use of antibiotics should be reviewed, and also that resistance surveillance programmes and antibacterial drug usage monitoring in the livestock sector should be established. A good example of a herd-level monitoring program is VetStat for all drug usage in production animals implemented by the Danish Government in 2000. (Stege et al., 2003)

The Dutch livestock sector reached in 2008 a voluntary agreement to act on the issue of antibiotic resistance. Currently, although the use of antibiotics in the Dutch dairy sector is relatively limited, dairy farmers are facing those reduction targets of 20% in 2011 and 50% in 2013 (2009 reference year). The goal of our study was to determine whether a reduction of the use of antibiotics could be achieved via communication on effective and responsible use of antibiotics in the first place and secondly by giving farmers easy and clear insight into their antibiotic usage.

MATERIAL AND METHODS

In the end of 2009 and begin 2010 , workshops "Responsible Medicine Application" for farmers were organised in the veterinary practice Van Stad tot Wad dierenartsen (Loppersum, The Netherlands). We tried to motivate farmers to participate in the workshops in different ways by highlighting the importance of the subject "responsible medicine application" in different news letters, conversations at farm visits and during consults by phone.

The key focus points of the workshops were: practicality, easy accessible and bottom up approach at the level of the farm(er). Nearly 120 out of 220 dairy farmers in our practice area attended voluntarily the commercially workshops (farmers had to pay), which were giving by 2 different vets.

Following the workshops a practical tool has been developed on our website to enable farmers to evaluate their usage of antibiotics on farm-level over a period of 2 years. The usage of antibiotics is evaluated using the parameter: "animal daily doses (ADD) per year" for the categories milking cows, young stock aged between 2 weeks and the age at first calving and calves aged 0-2 weeks and the "Total sum" (see also figure 1 below). 1 ADD represents the therapeutic daily dose that a standard animal receives for the treatment according to the label (weights: milking cow 650 kg; young stock 150 kg; calves within 2 weeks of age 50 kg. The "Total sum" is a new defined parameter that differs from the Total ADD-figure that is calculated for dairy cows on the national level.

For example; 8,5 ADD per year for milking cows means that such an animal receives antibiotics for 8,5 days on average per year. The "Total sum" is calculated as the sum of the antibiotic use in all categories over one year and represents a rolling year average.

The ADD figures are based on sales data instead of real data on on-farm usage. In order to calculate ADD figures

we have categorised the antibiotics as follows: an antibiotic with a withdrawal period for milk and meat is used in milking cows; an antibiotic with a withdrawal period for just meat (not registered for milking cows) is used in young stock and a couple of oral antibiotics is used for calves.

By the end of 2010 the new website of our practice was launched, giving farmer clients the insight into their antibiotic usage in their own private login area. From the 120 farmers that has been registered on our website by May 1st 2011, the data of 98 farmers turned out to be reliable for statistical analysis. (48 farmers have been attending the workshop and 50 have not been attending in the workshop on responsible medicine application). The statistical program R was used to analyse the data. A linear mixed effect model was used to identify the effect of "participant" in the workshop or not and if "month" did differ or had an effect.

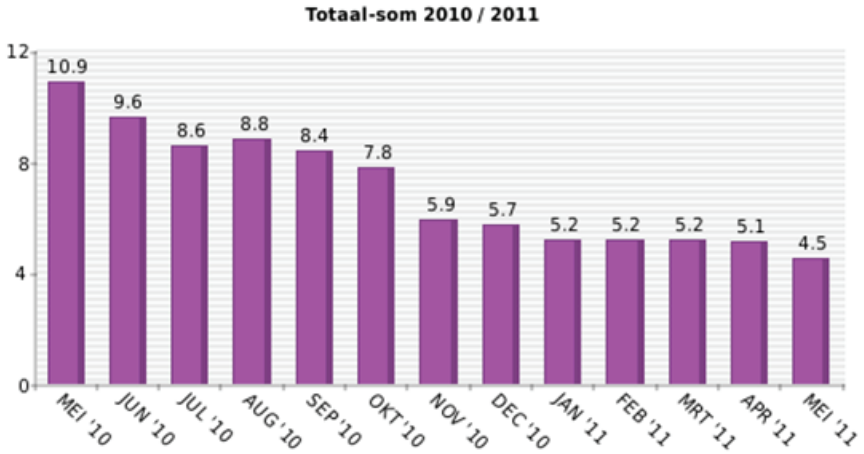

Figure 1: Example of a trend in ADD on a farm. The bar "Mei10" represents the "Total sum" of the antibiotic usage of June '09 - May '10 in total (ADD of milking cow, young stock and calves summed up)

RESULTS

In our practice, 120 out of 220 farmers attended the workshops so far. The main reasons for attendance were: easy accessible (low cost and open atmosphere that allowed asking "stupid questions") and relevant practical information.

From the data we can identify large differences in antibiotic use between farms (Total sum of ADD for individual farms varies from 2 – 30); both in the participant group as well as in the non-participants group. The average antibiotic use per month for participants and non participants can be seen in Table 1.

Table 1: Average antibiotic usage per month in Animal Daily Doses (ADD) for participants and non participant of the responsible medicine course.

	MEI10	Jun 10	Jul 10	Aug 10	Sep 10	Okt 10	Nov 10	DEC 10	Jan 11	Feb 11	MRT1 1	Apr 11
Non - Participants	6,5	6,6	6,5	6,7	6,7	6,7	6,9	6,8	6,5	6,6	6,7	6,2
Participants	8,2	8,1	8,2	8,5	8,5	8,7	8,8	9	10,2	8,8	8,9	8,2

The farmers which have not been participating in the workshop are likely to differ from the ones who have been participating and have significant lower antibiotic usage (p=0,0261) but here was no significant difference between months (p= 0,1976).

DISCUSSION

The reliability of the ADD-figures of the farmers in our practice-area depends on the fact whether they buy their antibiotics at our practice, or not, or partly. For 95% of the farmers it is clear that they only order antibiotics at our practice, but no guaranties, since the concurrence with other practices on the market of veterinary therapeutics is rising.

Another possibly bias in the data has been caused by the categorization of the antibiotics per animals group. Some antibiotics are used as well in milking cows as in calves or young stock. So, in some farms the figures of calves and young stock compared to figures from milking cows are an underestimation of the real situation and vice versa. This problem can be solved when real antibiotic usage data out of farm management programmes. The most reliable data source would be the management programme of the

farmer; note that the reliability of these data depends on how strictly the farmer is recording the application of antibiotics. Unfortunately an easy and cheap connection with the management programmes from farms is not yet accessible for us.

The calculation of the parameter "Total sum of ADD" we defined in our practice, differs from the ADD which is used to measure the usage of antibiotics in the Dutch dairy sector. In the national ADD figure for dairy cattle, the antibiotic usage in total kilograms in young stock and calves, is converted into ADD for milking cows. This standard assumption of 0,2-0,3 ADD is added to the ADD of milking cows. We think that in this way the antibiotic usage in calves and young stock is underestimated by the farmers, since they have no insight into the real ADD of calves and young stock.

By means of the "Total sum of ADD" (rolling year average) the buying peeks in the separate categories are leveled out and the trend in antibiotic usage over time gets visible. The next step is to divide the Total sum of the ADD in the usage per therapeutic indication (e.g. mastitis, drying off, lameness etc.)

From the results can be seen that non participants had a significant lower antibiotic use, but we must be cautious with causality here. An explanation why participants have a higher antibiotic usage could be that this group of farmers are more eager to learn more on this subject because they have a higher usage of antibiotics in the first place. Or that higher usage of antibiotics and participation in the workshops are both results of a not yet defined combined cause. Other parameters like herd size, animal health status, mastitis incidence, replacement rate should be taken into account. More data over a longer period is needed to compare trends in the usage of antibiotics of participants and non-participants.

The reduction of the use of antibiotics on dairy farms can not be an target by itself. The use of antibiotics is a result of the animal health status of individual cows in the first place. If animals (i.e. cows) stay healthy is influenced by the management of the farmer, environment conditions the animals live in and the infection pressure of the most important cattle diseases. In order to change the management that leads to the usage of antibiotics, farmers first need to gain or up-date practical knowledge about how to create a situation that prevents the need for antibiotics. And secondly they need to know what their level of antibiotic usage is, in order to provide them with a monitoring and benchmarking parameter to measure the effect of changed (preventive) management and motivate them to do better. We already notice in practice that insight in the ADD of the farm, triggers farmers to discuss the causes (farm management) which lead to antibiotic usage on the farm. Further, this initiative, the workshop "Responsible Medicine Application", is now adopted by Royal Friesland Campina (RFC), and will be rolled out for RFC farmers in 2011. Our practice will be responsible for the accreditation workshops for Dutch bovine veterinarians in which they learn about our experiences in training and motivation of dairy farmers.

CONCLUSIONS

The bottom-up approach starting at the level of the farmer (news letters, conversations at farmvisits, consults by phone) motivates farmers to attend to workshops. This forms one of the two pillars to change or optimise their medicines application routines. More effective and responsible medicines application routines should result in a diminishing use of antibiotics in the dairy sector, contributing to less resistance to antibiotics in general (public health concern). So far we have not been not able to demonstrate this. However we did learn that farmers are highly motivated by their ADD-figures and more open to discuss the causes (farm management) which lead to antibiotic usage on the farm.

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IMMUNOMODULATION, GROWTH PERFORMANCE, NUTRIENT UTILIZATION AND DIGESTIBILITY INDUCED BY INNACTIVATED CELLS OF Enterococcus faecalis AND MANNAN OLIGOSACCHARIDES SUPPLEMENTED AT A LOW LEVEL (0.5% AND 0.25%) IN A SINGLE OR COMBINED FORM.

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SUMMARY

Seven diets were supplemented with *Enterococcus faecalis* (*E. faecalis*) and Mannan oligosaccharides (MOS) in a single or combined form. Fish were fed experimental diets near satiation level for 12 weeks. At the end of the feeding experiment the fish were submitted to a duplicated furunculosis challenge test. Growth performance, nutrient utilization, protein digestibility and immunological parameters were assessed. Within these factors, significant differences were recorded among experimental groups. Additionally, the supplementation of *E. faecalis* and MOS at different levels, significantly reduced the presence of *Aeromonas salmonicida* in the survival fish after the challenge test.

INTRODUCTION

Population numbers are increasing towards nine billion by 2050. On top of that, a necessity to consume more protein is increased year by year. Aquaculture has dramatically raised its production to satisfy this growing necessity. This fact has conducted to overstressed systems and emergence of diseases which until now have been controlled by antibiotics. The concept of "functional diets", supplemented with natural and environmentally friendly ingredients called prebiotics and probiotics, has been recently introduced in aquaculture not only as prophylactic and prevention method against epizootics, but also as growth promoting ingredients. The current aquaculture challenge is clear, produce more fish sustainably - more from less - . Feed efficiency is key , fish protection the target and respect to our environment the commitment. Thereupon, the aim of this study was to determine the effects of single or combined supplementation of one prebiotic: MOS together with the probiotic *E. faecalis* on the growth performance and immune response of rainbow trout .

MATERIALS AND METHODS

MOS and *E. faecalis* were supplemented in two inclusion levels (0.5 and 0.25%) in a single or combined form. 490 fish (average weight: 36.27±0.42 g) were equally distributed into one of the fourteen 60/ glass tanks. The fish were fed experimental diets near satiation level during 12 weeks. At the end of the feeding experiment, the fish were submitted to a duplicated furunculosis challenge test by intraperitoneally injecting 0.1ml of 2.4 X 10^3 of *Aeromonas salmonicida*. The experimental period was held during 14 days. Mortality number was recorded in a daily basis.

RESULTS

Growth performance, nutrient utilization and apparent digestibility coefficient of protein.

The fish fed the experimental diets showed efficient growth performance in the following experimental groups: E0.25%, M0.25%, M0.5% and EM0.5% which showed significantly (P<0.05) better performance compared to the rest of the experimental fish. Additionally the best growth was obtained in the fish fed EM0.5%. In a closer comparison, weight gain and SGR were significantly different between E0.25% and E0.5% experimental groups. In contrast growth between M0.25% and M0.5% groups was not different. Meanwhile a combined supplementation of MOS and *E. faecalis* in EM0.25% and EM0.5% groups recorded better values in the later one (Table 1). On the other hand, E0.5% and EM0.25% groups did not show significant (P<0.05) difference compared to those shown in fish fed the control diet (C group). The supplementation of MOS and *E. faecalis* in a single or combined supplementation form did not significantly (P<0.05) affect the FGR. However, compared to the C group, this parameter was significantly improved (P<0.05) in fish fed EM0.5% (Table 1).

On the other hand, nutrient utilization and apparent digestibility coefficient of protein showed meaningful or minimum differences among experimental groups.

Immunological parameters and challenge test.

No significantly different immune response (phagocytosis, hematocrit value and mucus production) was shown between the experimental groups fed two single supplementation levels of E.

Table 1. Growth performance of the feeding experiment for 12 weeks

Diet	Weight gain (g)	SGR (%/day)	FGR*
C	67.65 ± 7.89^{ab}	1.84 ± 0.14^{ab}	1.09 ± 0.00^{a}
E0.25%	93.73 ± 9.28^{c}	2.23 ± 0.15^{d}	0.96 ± 0.01^{ab}
E0.5%	63.16 ± 1.24^{a}	1.79 ± 0.00^{a}	1.03 ± 0.02^{ab}
M0.25%	87.92 ± 5.50^{c}	2.16 ± 0.05^{cd}	1.01 ± 0.07^{ab}
M0.5%	81.54 ± 4.36^{c}	2.05 ± 0.08^{cd}	1.06 ± 0.14^{ab}
EM0.25%	72.44 ± 7.08^{bc}	1.91 ± 0.14^{abc}	1.04 ± 0.06^{ab}
EM0.5%	97.52 ± 6.85^{d}	2.30 ± 0.04^{e}	0.90 ± 0.04^{b}

*Dry matter basis

faecalis (E0.25% and E0.5% groups). In contrast a higher single supplementation level of MOS (M0.5% diet) resulted in a significantly (P<0.05) better immune response compared to the lower one (M0.25% diet). On the other hand a combined supplementation of *E. faecaalis* and MOS (EM0.5% experimental group) showed a significantly (P<0.05) increased immune parameters compared to the rest of the experimental fish. In contrast the immune status of control group (C diet), recorded lower immunological values compared to the rest of experimental groups (Fig. 1). The skin mucus production was significantly higher in all the experimental groups than that of the control. However among the single supplementations, there was no significant difference between the two levels of *E. faecalis*. In contrast higher supplementation of MOS (0.5%) recorded a better mucus production than that recorded in the fish fed a 0.25% (Fig. 2). This immune status data was correlated to the furunculosis challenge test results where higher supplementation levels of *E. faecalis* and MOS in either a single or combined form resulted in a significantly (P<0.05) better protection against *A. salmonicida* (Fig 3). On the other hand, fish fed control diet showed the highest mortality rates in this test. Additionally any of the two inclusion levels of *E. faecalis* and MOS in a single or combined form significantly (P<0.05) decreased the presence of *A. salmonicida* in head kidney (HK) of experimental fish, while EM0.5% experimental group showed the lowest presence of this pathogen in HK (Fig. 4).

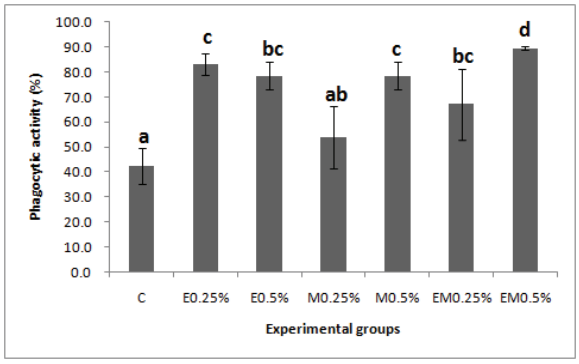

Fig. 1 Phagocytic activity of the fish fed experimental diets at the end of feeding experiment (mean ±SD, n=8). Different letters in a line denotes significant differences (P<0.05).

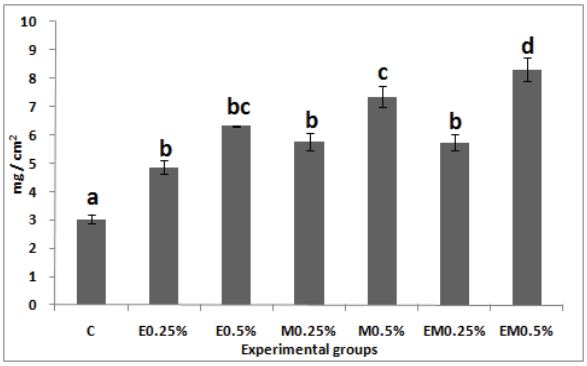

Fig. 2 Mucus production of the fish fed experimental diets at the end of feeding experiment (mean ±SD, n=8). Different letters in a line denotes significant differences (P<0.05).

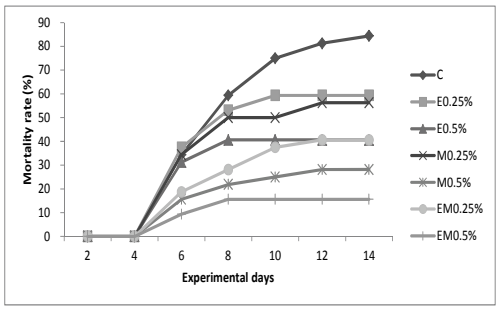

Fig. 3 Mortality curves in a day by day basis during a 2 weeks intraperitoneally injection challenge test with *Aeromonas salmonicida* (mean ±SD, n=32).

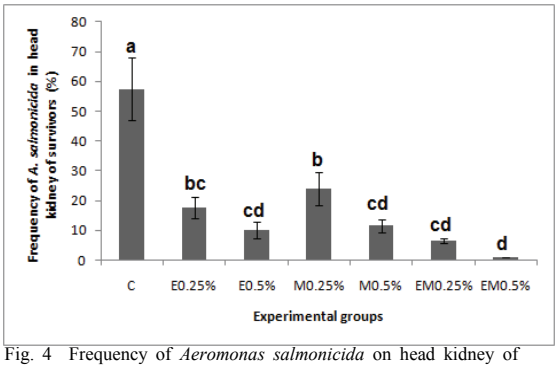

Fig. 4 Frequency of *Aeromonas salmonicida* on head kidney of rainbow trout after a 2 week intraperitoneally injection challenge test (mean ±SD, n=variable number). Different letters in a line denotes significant difference. (P<0.05).

DISCUSSION

Stimulation of the non-specific defense mechanisms by using specific biological compounds, called immunostimulants, enhances the disease resistance and growth of the host (Skjermo *et. al.,* 2006). In this study, immunostimulants supplemented in either single or combined form improved growth and immune response of rainbow trout. The immune stimulant capacity of *E. faecalis* shown in this study could be compared with those of other studies demonstrating that this probiotic was able to improve the non specific immune function of mice (Shimada T., *et. al.* 2009). Similarly these research`s findings can be contrasted with previous studies on the physiological benefits of the genera *Enterococcus* as growth promoter and immune stimulator in animal husbandry (Wang *et. al.,* 2008; Panigrahi A., *et. al.,* 2007), as inhibitory on allergen-induced local accumulation of eosinophils (Shimada *et al.,* 2003; Shimada *et al.,* 2005) as cutaneous anaphylaxis activators (Shimada *et al.*, 2004), as equilibrators of intestinal ecosystem (Shimada *et al.,* 2007), as agents of leukocyte reconstitution (Kanasugi, *et. al.* 1996; Hasegawa, *et. al.* 1993; Hasegawa *et al.,* 1994) as antihypertensive (Shimada, *et al.* 1992; Shimada, *et al* 1996), as blood pressure stimulant (Yamaguchi 1992) and as immune stimulant (Abe *et al.* 1993; Ohashi, *et. al.* 1992; Shimada, *et. al.* 1996).

The growth e immunological effects triggered by MOS can be compared to those of Torrecillas *et. al.* (2007), Sang and Fotedar (2009); Sang and Fotedar (2010); Ringo *et al.* (2010) and Terova *et. al.* (2009) who also observed benefits by administrating this prebiotic (at different levels) in fish. In addition, this oligosaccharide has been experimented in Atlantic salmon (Grisdale-Helland *et al.*, 2008), in channel catfish (Welker *et al.* 2007), in cobia (Salze *et. al.*, 2008), in European lobster (Daniels *et al.* 2006), in European seabass (Torrecillas *et. al.*, 2007), in raibow trout (Staykov *et al.*, 2007; Yilmaz *et al.*, 2007; Rodríguez *et. al.,* 2009), in red drum (Burr *et. al.*, 2008), in sturgeon (Prior *et. al.,* 2003) and in tilapia (He *et. al.,* 2003; Sado *et. al.,* 2008). On the other hand, as will to this research, mucus production was stimulated by MOS in sea bass (*Dicentrarchus labrax*) (Torrecillas *et al.,* 2011).

Few investigations have been conducted in order to assess the effects of combined immune stimulants in fish (Hai and Fotedar, 2009; Ye J. D. *et al.,* 2011). However, the combined results of MOS and *E. faecalis* of this research, can be confirmed with those experiments.

In conclusion, MOS and *E. faecalis* in a single or combined supplementation showed either meaningful or minimum changes in growth performance, nutrient utilization, protein digestibility and immune status of experimental fish. Nevertheless this influence mainly resulted from the inclusion level of MOS or *E. faecalis*.

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Block 4

THE CONTROVERSY OVER CONFINEMENT

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SUMMARY

'Confinement' systems of animal production have triggered an enduring controversy, partly because of conflicting ideas about animal welfare. Some people, including many animal producers and industry veterinarians, tend to emphasize the basic health and functioning of animals. Others, including many organic producers and the public, tend to emphasize the ability of animals to live reasonably 'natural' lives, and/or the 'affective states' of animals such as pain and frustration. The different views of animal welfare reflect different sets of values that have been in conflict since the early debates about human welfare during the Industrial Revolution. Scientific research on animal welfare has been based on the various conceptions of welfare. Such research can help to identify and solve animal welfare problems, but it does not resolve disagreements attributable to the different views of what is important for animal welfare. Resolving the controversy over confinement will likely require improving both confinement and alternative systems so that they achieve good welfare outcomes as judged by the various conceptions of animal welfare.

INTRODUCTION

'Confinement' systems of animal production – whereby animals are confined to limited space, often indoors – have triggered one of the enduring controversies about the welfare of food-producing animals.

On one side of the debate are the many animal producers and industry veterinarians who emphasize the basic health and functioning of animals. Such people are likely to support confinement systems because of hygiene advantages such as cleanable surfaces and separation of animals from manure, together with the ability to exclude pathogens and parasites from high-health facilities.

On the other side of the debate are many citizens and organic producers who emphasize the importance of animals living reasonably 'natural' lives, and/or lives free from pain, frustration and other unpleasant affective states. Such people are likely to oppose confinement systems because such systems are unnatural for the animals, prevent animals from performing much of their natural behaviour, and may consequently lead to unpleasant affective states such as frustration and discomfort.

To explore the controversy in more depth, let us look at one specific disagreement. In 1997 a scientific committee of the European Union reviewed the literature on the welfare of intensively kept pigs. The committee asked, among other questions, whether welfare problems are caused by housing sows in 'gestation stalls' where the animals are unable to walk, socialize, or perform most other natural behaviour during the majority of pregnancy. The review concluded that, 'Some serious welfare problems for sows persist even in the best stall-housing system' (Scientific Veterinary Committee 1997). Based on this review, the European Union passed a directive to ban the gestation stall as of 2013.

Not long after, a group of Australian scientists reviewed much the same literature and asked much the same question, but came up with essentially the opposite conclusion. They concluded that, 'Both individual [i.e. stalls] and group housing can meet the welfare requirements of pigs.' They also cautioned that 'public perceptions may result in difficulties with the concept of confinement housing' but that 'the issue of public perception should not be confused with welfare' (Barnett et al 2001). The swine industry in the United States has used that review, plus a similar one, to argue that there is no scientific basis for eliminating the gestation stall.

Both of these reviews were done by very capable scientists, and both groups likely felt that they had done the best and most objective job possible. What, then, went wrong? How could two groups of scientists review the same scientific literature and come up with opposite conclusions?

Two world views

To answer this question, we need to briefly trace two historic world-views that have been remarkably influential in Western thought and that have coloured our understanding of animal welfare.

During the Industrial Revolution, the so-called 'factory system' became the predominant way of producing textiles and other goods throughout much of Europe. Thousands of factories were erected, and they proved so efficient that traditional, hand production almost disappeared. Workers, often out of economic necessity, moved from villages and rural areas into cities; and instead of working at hand looms in their homes, people operated machinery in the factories. It was a profound social change, and it touched off an intense debate over whether the new industrial system was good or bad for the quality of human life.

On one side of the debate were critics who insisted that the factory system caused people to lead miserable and unwholesome lives. Critics claimed that the cities created cramped, unhealthy living conditions for the workers, and deprived people of contact with nature. The machines themselves caused many injuries, and (critics claimed) they often led to physical deformities because they placed an unnatural strain on the body. Perhaps worst of all, it was claimed that repetitive work with machines made the workers themselves like machines and led to an erosion of their human nature.

But the factory system also had staunch defenders. Instead of imposing unnatural strains, automation (the defenders claimed) relieved workers of much of the drudgery that manual handicrafts required. Far from being unnatural, the factory system represented a step in the natural progression from a time of human labour to a time when automation would make labour unnecessary. Moreover, the wise factory owner would take care to have healthy, happy workers because maximum productivity would not otherwise be achieved. In fact, the productivity of the system was seen as proof that the factories were actually well suited to human workers (see Bizup 2003).

Because the effects of industrialization were so profound, the debate engaged some of the leading intellectuals of the day, and from their writing we can build up a picture of the very different world-views that lay behind their arguments.

The world-view of the anti-industrial critics (Table 1) reflected a set of values that we see extending from the agrarian Latin poetry of Virgil through to the Pastoralist and Romantic writers and painters of the 1600s to 1800s. This world-view (1) values a simple, basic life and rejects the artificiality of cities and factories. (2) It sees nature as an ideal state that we should strive to emulate. (3) It values emotional experience ahead of the cold rationality of science and technology. (4) It values the freedom of the individual ahead of the regimentation of the factory. (5) And it looks back to a Golden Age in the past when people lived better lives in harmony with nature.

The world-view of the pro-industrialists was more a product of the Enlightenment when people looked to reason and science to replace superstition and traditional beliefs. This world-view involved two concepts that were relatively new to Western thought.

One was productivity. Adam Smith opened his book The Wealth of Nations by claiming that the quality of life in a nation depends on the goods that are available to supply the citizens with what they need and want. Increasing the productivity of workers, and thus increasing the supply of goods, should therefore improve the lives of a nation's people. Hence the factory system, where automation and specialization lead to greater productivity, would ultimately make life better (Smith 1776).

The second idea was progress – the idea that human history moves irreversibly in the direction of improvement. As historian Sydney Pollard (1968) points out, belief in progress began with science, because in science each generation was seen as building on the work of earlier generations so that knowledge constantly improves. But during the 1700s the idea of progress took wing, and by 1800, in the words of Pollard, 'firm convictions had been expressed about the inevitability of progress in wealth, in civilization, in social organization, in art and literature, even in human nature and biological make-up.' And a belief that change represents progress, and is therefore both good and inevitable, has remained a common theme in Western thought.

Thus, the pro-industrial world-view was very different from that of the critics (Table 1). (1) Instead of valuing a simple, basic life, it valued a life improved through science and technology. (2) It viewed nature not as an ideal state that we should emulate, but as an imperfect state that we should control and improve. (3) It valued rationality rather than irrational emotion. (4) It valued the productivity of the well organized enterprise more than the freedom of the individual worker. (5) And it looked forward to a Golden Age in the future when progress through science would lead to a better life.

The debate over human welfare during the Industrial Revolution has obvious parallels with the debate over animal welfare in confinement production systems. In fact, much of the disagreement over animal welfare can be traced to the continued influence of the contrasting world-views.

People who lean toward the anti-industrial world-view tend to see a good life for animals as (primarily) a natural life, to be achieved by emulating nature through such means as free-range systems and access to the outdoors. They emphasize the emotions of animals (are they suffering? are they happy?), and attach importance to their freedom. For these various reasons, people who favour an anti-industrial world-view are likely to see confinement systems as inherently incompatible with a high level of welfare, and they may look back to traditional, non-confinement systems as an ideal that we should try to return to.

In contrast, those who lean more toward a pro-industrial world-view tend to see a good life for animals as (primarily) a healthy life, to be achieved by preventing disease and avoiding the hardships of nature. They tend to value the rationality and scientific basis of the system more than the emotions and the freedom of the animals, and they will see a high level of productivity as evidence that the animals are doing well. Such people are likely to see confinement systems as a form of progress that improves animal welfare, and they may look upon older, non-confinement systems as outmoded models that need to be improved.

Animal welfare and science

When the controversy over confinement systems began to emerge, many people looked to the scientific study of animal welfare as a way to settle the dispute by providing an objective, value-free means of assessing the welfare of animals. What actually happened was much more interesting.

Some scientists, roughly in line with a pro-industrial world-view, used the basic health and

functioning of animals as a basis for assessing and improving animal welfare. As one classic example Ragnar Tauson and co-workers improved the welfare of laying hens by studying the basic health of birds in cages of different types and then developing cage designs that would prevent the various health problems they observed. The scientists found that the birds developed foot lesions if the floor was too steeply sloped, and neck lesions if the feed trough was too deep and installed too high for comfortable access. There was often feather damage that could be reduced by using solid side partitions, and overgrown claws that could be prevented by installing abrasive strips. Thus, just by focusing on injuries it was possible to make large improvements in both animal welfare and productivity, and the results formed the basis of regulations on cage design in Sweden and the European Union (Tauson 1998).

Other scientists, more in line with anti-industrial thinking, tried to improve animal welfare by allowing animals to live in more 'natural' ways. For example, as a basis for designing better housing for pigs Alex Stolba and David Wood-Gush (1984) began by observing pigs that they had released in a hilly, wooded area. They found that the pigs showed certain characteristic types of behaviour: they rooted in the soil, exercised their neck muscles by levering against fallen logs, built nests in secluded areas before giving birth, and used dunging areas well removed from their resting areas. Stolba and Wood-Gush then designed a complex commercial pen that allowed the animals to behave in these ways. It included an area with peat moss for rooting, logs for levering, an activity area with a rubbing post, a separate dunging area, and secluded areas where a sow could be enclosed to farrow. The authors claimed that the animals' welfare was significantly improved by the complex pen. However, because some aspects of basic health (especially neonatal survival) were not as good in this system as in well run confinement systems, some people disagreed with that conclusion.

In less radical approaches, scientists have incorporated simple elements of natural behaviour into existing rearing systems. On many commercial dairy farms, calves are separated from their mothers on the day of birth, and are fed milk from a bucket, usually twice per day. With such infrequent meals, there is a fear of feeding too much milk at one time, and total intake is generally limited. Under natural conditions, cows stay fairly close to the calves for the first two weeks, and the calf will feed many times per day in smaller meals and consume a larger total amount per day. Although most farmers consider it impractical to leave calves with the cow, feeding systems can still be made to correspond more closely to the animals' natural behaviour. If the calves are fed more frequently (as they are by the cow), then they can drink more milk per day without developing digestive problems; and if the calves suck from an artificial teat rather than drinking from a pail, the sucking action leads to a greater release of certain digestive hormones. Hence, calves fed frequently by teat gain substantially more weight than calves fed twice daily by bucket (Appleby et al 2001).

In yet other cases, scientists have based animal welfare research on the affective states of animals. Many dairy calves are 'disbudded' by the use of a hot iron to cauterize the nerves and blood vessels that allow the horn-bud to develop. In many countries this procedure is done without any form of pain management. A research group in New Zealand used plasma cortisol levels as an indicator of the pain caused by disbudding. They found that disbudding is followed immediately by a large increase in cortisol, but that the reaction is blocked if a local anaesthetic is used to freeze the area. In the treated calves, however, cortisol levels showed a marked increase several hours after disbudding, probably because the injury remained inflamed and painful when the anaesthetic had worn off. If the calves also received an analgesic, the second peak in cortisol could also be eliminated. Thus the research showed that management of the pain of disbudding requires both a local anaesthetic and an analgesic (Stafford and Mellor 2005).

When research on animal welfare began, there was clearly some expectation that scientific research would replace the subjective, value-based views about what is most important for animal welfare. As these examples show, however, the various scientists, being people with values of their own, essentially adopted one or other of the criteria of animal welfare – basic health, natural living and affective states – as the basis of their research. Thus, instead of the science arbitrating among the different conceptions of animal welfare, the different conceptions of animal welfare actually came to underlie and enrich the science.

The practical side of animal welfare

If the science does not give us an objective, value-free way of 'measuring' animal welfare, could it, nonetheless, help to resolve the controversy over confinement?

Virtually all forms of animal welfare research – whether focused on basic health, affective states or natural living – help to identify and solve animal welfare problems (Fraser 1995). In the examples used above, we see this 'practical' side of animal welfare science: developing cages that prevent injuries, finding ways to feed animals that match natural patterns of intake, identifying methods to reduce pain and thus make animals more manageable and less stressed. In these and many other examples, the contribution of animal welfare science lies in identifying and solving animal welfare problems whether they occur in confinement or alternative systems.

But why is it that such basic problems as cage design and feeding systems had not been solved long ago? In the early 1900s, agricultural research and teaching included 'animal husbandry' – a subject that encompassed the feeding, breeding, handling, management and housing of animals. Then branches of science developed in the areas of nutrition, physiology and genetics, and people saw the scope for applying these fields to animal production. Before long, animal husbandry was replaced by 'animal science', and most research was focused on animal nutrition, animal genetics and reproduction. When that happened, however, the other elements of animal husbandry – handling, management and housing – were largely overlooked.

Later, some newer areas of basic science emerged. Animal behaviour became a recognized field. Stress physiology was showing how 'stressors', such as cold, heat, crowding and pain, trigger a response that is beneficial in the short-term but in the long term can reduce growth, fertility and disease resistance. And the new science of epidemiology, which flowered with the advent of computers, allowed people to identify environmental and other risk factors for disease and injury.

As these and other fields developed, people started to apply them to food-producing animals in a field that has come to be called 'animal welfare science'. In a sense, this new field is restoring the missing parts of animal husbandry by studying how animals are affected by their social environment, their physical environment, the handling they receive, and so on. And given the huge advances that have been made by the older fields such as nutrition and genetics, animal welfare science may well be the field that leads to the next generation of improvements in animal management and production.

CONCLUSIONS

Let us return to the dilemma that was created when two scientific reviews arrived at opposite conclusions about the welfare of sows in gestation stalls.

If we look carefully at the reviews, we see that they were based on different conceptions of animal welfare. The Australian reviewers, largely in line with an industrial world-view, based their analysis almost exclusively on the basic health and functioning of the animals, relying especially on such measures as health, immunology, injuries and growth rate. They did not deny that affective states are involved in animal welfare, but they took the view that all significant risks to welfare would have effects on health and functioning variables. Thus, by presenting evidence that sows in stalls are generally no worse than sows in other types of housing in survival, weight gain, litter size, disease incidence and such variables, they concluded that, 'Both individual and group housing can meet the welfare requirements of pigs'.

The European reviewers took a view of animal welfare that emphasized affective states and natural living as well as basic health and functioning. Thus they included evidence of fear and frustration in their analysis of animal welfare, whether or not the basic health of the animals was affected. They also considered that the opportunity for 'exploration of a complex environment, rooting in a soft substratum and manipulation of materials such as straw' is relevant to animal welfare because of its link to natural behaviour. Using such criteria they concluded that 'Some serious welfare problems for sows persist even in the best stall-housing system'.

In this example, what appeared to be a scientific disagreement – the sort of disagreement that might be resolved by more experiments or better statistical analysis – was actually due to a difference in values traceable to one of the historic debates in Western thought.

What are the implications for the controversy over confinement? All three conceptions of animal welfare – basic health, affective states and natural living – are deeply rooted in Western thought. Hence, practices designed to ensure good animal welfare are not likely to achieve widespread support unless they take account of the different conceptions of animal welfare at least to some degree. Animal producers are not likely to convince their critics that high-health confinement systems are good for animal welfare if these systems cause frustration and prevent animals from carrying out most of their natural behaviour. Similarly, free-range producers are not likely to convince their critics that seemingly natural systems are good for animal welfare if the animals suffer from harsh weather, parasites and other problems. Thus, perhaps we are seeing two practical approaches to the controversy over confinement: one that retains the health and hygiene advantages of confinement housing while meeting other animal welfare concerns, and one that uses more natural environments but with appropriate standards and practices to ensure good health and comfort.

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Table 1. Two world-views

Anti-industrial	Pro-industrial
• values a simple, basic life	• values a life improved by scientific progress
• sees nature as an ideal state	• sees nature as an imperfect state
• values emotional experience	• values rationality
• values the freedom of the individual	• values the productivity of the enterprise
• looks to a Golden Age in the past	• looked to a Golden Age in the future

ASSESSMENT OF ANIMAL WELFARE RISKS IN DIFFERENT TYPES OF ANIMAL HUSBANDRY

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SUMMARY

To demonstrate and test methods to utilize expert opinion for estimation of risks of poor animal welfare (AW) in different types of animal husbandry, opinions were collected from 36 researchers at an international workshop. For each of 13 husbandry categories, participants were asked to individually provide values related to (1) the probability of non-compliance with current AW legislation found at a fictitious control visit to a randomly selected operation in their home country, and (2) the probability of one or several severe AW deficiencies at a randomly selected operation in their home country during a period of one year. Responses were aggregated and predicted probabilities were estimated by three different methods. The probability of non-compliance varied between 15 and 38%, and of severe deficiencies during one year between 25 and 59%. The highest values were obtained for pork, piglet and broiler production, differences between husbandry categories being significantly different. Variation between respondents was large, reflecting both individual and national differences, but predicted probabilites were similar irrespective of the method. It was concluded that it is possible to aggregate experts' individual responses and so reliably assess AW risks.

INTRODUCTION

According to Article 3 of Regulation (EC) No. 882/2004, EU Member States shall ensure that official controls are carried out regularly, on a risk basis and with appropriate frequency, taking account of identified risks that may influence animal health or welfare. Methods to classify animal operations for the risk of poor AW have been investigated in a Swedish research project (*Risk-based animal welfare assessment, RAWA*) aimed at facilitating Swedish official AW control. The present study aimed to demonstrate and test methods for utilizing international expert opinion to estimate risks of poor AW in different types of animal husbandry. It was based on the protocol designed to collect and process opinions of Swedish experts regarding welfare risks in different types of animal husbandry operations.

MATERIAL AND METHODS

Participation and data collection

Forty-five persons attending a workshop in connection with the International Society of Applied Ethology conference in 2010 participated in the study. The respondents, residents of Austria, Brazil, Canada, Denmark, Finland, Germany, The Netherlands, Northern Ireland, Norway, Spain, Sweden, Switzerland, the UK or the USA, were asked to assess individually AW risks in 13 categories of animal operations or holdings in their respective home country. The categories were beef producer, milk producer, lamb producer, piglet producer, pork producer, broiler producer, egg producer, riding-school, kennel or dog rearer, pet dog owner, pet cat owner, cattle and sheep abattoir, and pig abattoir.

For each husbandry category, the participants were asked to provide values related to two different probabilities, expressing two aspects of AW: (P1) the probability of non-compliance with AW legislation, found at a fictitious inspection visit to a randomly selected operation of the specified type in the country, and motivating some kind of corrective action by control authorities, i.e. excluding minor complaints; and (P2) the probability of one or several severe AW deficiencies at a randomly selected operation of the specified type in the country during a period of one year. For each probability type, three values were requested from participants: the lowest imaginable, the most likely and the highest imaginable probability. Respondents were also asked to rate their own certainty of the values given by choosing between three optional shapes of the corresponding probability distribution curves. The respondents also scored their level of expertise separately for each husbandry category, as low, moderate or high. It was possible to use 36 reply forms in the final analysis.

Elicitation of respondent opinion

The values given by participants were used to define individual probability functions, describing the uncertainty of the respondents. Using @Risk 5 (Palisade Corp., Ithaca, NY,
USA) for Microsoft Excel (Microsoft Corp., Redmond, WA, USA), a *modified* PERT distribution was fitted to each respondent's values for each husbandry category and probability type, i.e. totally 26 distributions per respondent. The ordinary PERT [1] has been used to describe probability distributions based on e.g. expert opinion. It is a special case of the beta distribution and has three parameters: minimum value, most likely value (mode) and maximum value. The more general *modified* PERT was suggested by [5] and introduces a fourth parameter (γ) to allow for variation in curve shape; γ was set to 1 for a flat, 3 for an intermediate (ordinary PERT) and 6 for a pointed curve shape. For each individual function, the mean of the distribution was computed.

The association between P1 and P2 among different respondents and husbandry categories was analysed by rank correlation and the difference between the two probabilities within respondent and husbandry category by a matched pairs *t*-test. To estimate the agreement between respondents, the so-called random set reliability (intraclass correlation, ICC[2,1]) for individual means was calculated using the %INTRACC macro [4] in SAS 9 (SAS Institute Inc., Cary, NC, USA) for the two probability types separately.

The predicted probabilities in different husbandry categories were estimated in three different ways. First, a linear mixed model of individual probability means (P1 or P2) was constructed, estimating the fixed effect of husbandry category, probability type and the interaction between these two using the Mixed procedure of SAS. Respondent identity was included as a random effect and 847 observations could be used. Country and stated level of expertise were tested for inclusion but found non-significant (P>0.05) and therefore not retained. Least-squares means and their standard errors were computed for different combinations of fixed effects. The difference between husbandry categories was tested by ANOVA for summary statistics.

Second, for each husbandry category and probability type, the individual probability distributions were aggregated over all contributing respondents and bootstrapping [2] was applied. The distributions were added on top of each other, giving equal weights to the respondents, thus forming a so-called linear opinion pool [3]. Regarding the respondents as a random sample of all possible respondents, samples were taken repeatedly by drawing at random from each linear opinion pool aggregate (*complete* random sampling), with a sample size *n* equal to the number of respondents contributing to the data for that particular probability (*n*=30–36). The overall mean and its standard error were calculated from the resulting sampling distribution. Third, the respondents were instead assumed to have been selected with the intention of having different views on AW represented in a balanced way in the group, and each sample was formed by drawing a random value from all respondents (*restricted* random sampling). Sampling distributions were formed as before. All sampling was done by Monte Carlo simulation in @Risk with 10,000 iterations per sampling distribution.

RESULTS

The overall mean of the individual means (and inter-quartile ranges) was 25% (12 to 44%) for P1 and 41% (22 to 63%), for P2. Out of 468 husbandry category by respondent combinations, the level of expertise was stated in 411 cases, of which 43% were described as low, 34% as medium and 23% as high. There was a strong correlation between means of P1 and P2 (Spearman r=0.57 with *n*=406; P<0.0001). P2 was on average 14.3 percentage units higher than P1 within respondent and husbandry category (*t*=13.2 with 405 d.f.; P<0.0001). The random set reliability was 0.069 for P1 and 0.16 for P2, indicating a generally low agreement between respondents, and a considerably lower agreement for P1 than for P2.

Figure 1 shows predicted probabilities according to the three different methods. The differences between husbandry categories were significant by both complete (F=2.08, P=0.017 for P1 and F=4.92, P<0.0001 for P2) and restricted random sampling (F=12.0, P<0.0001 for P1 and F=30.6, P<0.0001 for P2). Pork, piglet and broiler producers scored highest for non-compliance and welfare deficiencies, while pet cat and dog owners scored lowest. In the linear model of individual P1 and P2, both husbandry category (13 categories) and probability type (P1 or P2) had highly significant effects (P<0.0001), while the interaction was non-significant (P=0.12). The coefficient of determination (R^2) for the model was 0.54. All 26 linear opinion pool aggregates spanned from almost 0 to almost 100%, indicating large variability both within and between respondents, i.e. overall a low level of certainty.

DISCUSSION

The composition of the group, with several countries being represented, was probably decisive for the results. The individual distribution represented the respondent's beliefs of the situation for his/her country. The fact that estimated mean probabilities ranged from 15 to 59% suggests a relatively low trust among respondents in the AW status of their countries.

The agreement between respondents was considerably lower for P1 than for P2, suggesting that differences between country legislation or awareness of such legislation prevail. The variation between respondents was generally great, indicating a low level of agreement and certainty. The observed variation could be due to differences between the countries of concern, but also to differences with respect to views on AW, interpretation of assessed probabilities, level of expertise, and degree of self-confidence. We would have been more confident in the estimates if the sample size had been larger or the respondents had agreed more, even if each one of them was uncertain. However, even in a hypothetical situation where each individual distribution is extremely uninformative (flat), a distribution of sample means would still be unimodal and reasonably Normal, provided the sample size is not too small (according to the Central Limit Theorem). For this reason we argue that it is possible to aggregate expert's probability distributions to achieve reliable estimates.

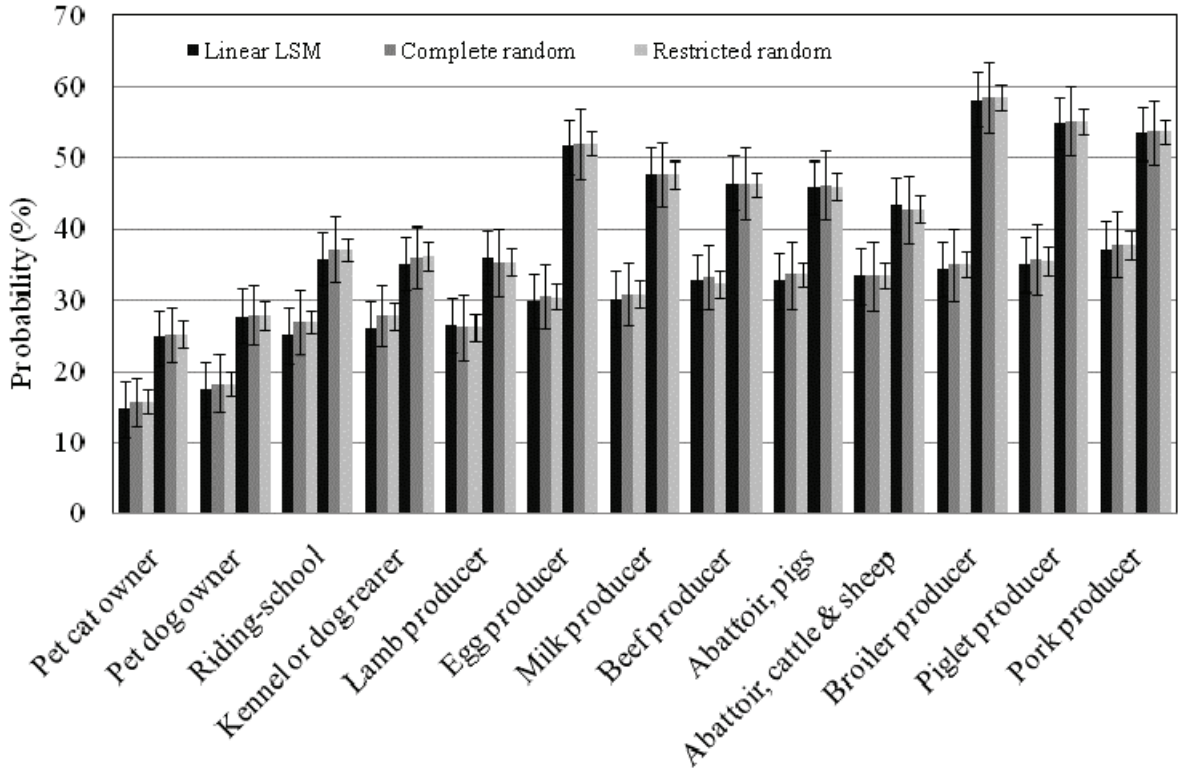

Figure 1. Predicted probabilities (mean ± SE) of non-compliance with AW legislation at a fictitious inspection visit (left) and of one or several severe AW deficiencies during one year (right) at a randomly selected operation of 13 animal husbandry categories, estimated by 36 scientific experts from 14 countries in 2010. Values obtained by linear mixed models of individual distribution means (Linear LSM), and by two different strategies to random sampling from individual distributions (complete and restricted).

CONCLUSIONS

It is possible to calculate and aggregate experts' individual probability distributions describing the perceived probability of poor AW or non-compliance with legislation in a population of operations of a given animal husbandry category. At each occasion, the elicitation of expert opinion should be adapted to the context and the way data were collected.

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STUDIES ON HYGIENE AND BEHAVIOUR OF MINKS (NEOVISON VISON) USING OPEN WATER SYSTEMS

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SUMMARY

The mink (Neovison vison) is a semi-aquatic mustelid whose living habits in the wild are often associated with various types of water systems such as streams, riverbanks, etc. The animals are skilful swimmers and divers. Farmed mink, in contrast typically don't have the opportunity to swim as their cages lack water pools.
40 American mink from a commercial farm were housed in two identically constructed free-range enclosures. Each enclosure housed 20 mink. In each of the enclosures, the mink were offered three different water basins. There was a rectangular "swimming pool", a round "pond" and a running "creek" available.
The mink visited each of the three water basins. The pool had the most visits within each observation week compared to the pond and the creek. In total, the mink spent most time at the pool. Both the results of the direct and the video observation showed that the mink generally accepted all three water basins and used them from the beginning to the end of the study. During the course of the study, an overall increase in frequency and duration of use of the basins was observed. The animals were in good health and the quality of the water was very good. The animals also developed preferences for certain nest boxes. Some were used over a long period of time, others only very shortly. The mink chose to share nest boxes very often.
This fundamental study found that farm mink still use water basins for swimming voluntarily, willingly and extensively, although the animals are considered to be domesticated and this is often used as an argument that the behavior of wild mink can not be compared to the behavior of farmed mink.

INTRODUCTION

The mink (Neovison vison) is a semi-aquatic mustelid whose living habits in the wild are often associated with various types of water supplies such as streams, riverbanks and lake shores (Dunstone, 1993). The animals are skilful swimmers and divers (Wenzel, 1990). They are predators, who mostly live on fish, amphibians and crustaceans but also hunt rabbits and other prey on land (Wiepkema und de Jonge, 1997).
Farmed mink, in contrast typically don't have the opportunity to swim or dive as their cages lack water pools (Wenzel, 1990; Wiepkema and de Jonge, 1997). The claim, that swimming is an essential behaviour pattern for mink has not yet been proven and many studies have dealt with this question (Cooper and Mason, 2000; Hansen and Jeppesen, 2001; Mason et al., 2001; Vinke et al. 2006). However, based on the biology of wild mink, European animal welfare recommendations suggest that swimming is an essential behaviour pattern for mink and therefore a swimming basin should be provided for farmed mink (European Convention, 1999). The aim of this fundamental study was to investigate which sizes, shapes and layouts of water basins in mink husbandry are suitable to allow mink to perform their characteristic swimming and diving behaviour to a large extent.

ANIMALS, MATERIAL AND METHODS

The study was carried out at a research station of the Ludwig-Maximilians-University of Munich, Germany. 40 American mink (Neovison vison) from a commercial mink farm were housed in two identically constructed free-range enclosures (Group A and B), with a size of approx. 300 sqm each. In order to be able to identify the individual animals, all mink were microchipped with a transponder (HDX – Transponder, Texas Instruments). In each of the two identical enclosures, the mink were offered three different water basins, which differed in shape, depth and surface area. Following water basins were available to the mink: A rectangular "swimming pool" (surface area approx. 20.5 sqm, depth approx. 30 cm), a round "pond" (surface area 4.9 sqm, depth approx. 80 cm) and a running "creek" (length approx. 10 m, depth 3-4 cm, which contained two pools/hollows along its length) available. The animal behavior of both groups was assessed by direct, as well as video observation. All observations took place on an approximately monthly basis. Each time, both enclosures were observed simultaneously for a total of seven consecutive days. The scan sampling method (Martin and Bateson, 1993) was used for the direct observation. The behavior patterns "at" (at least one paw at the edge of the water basin) and "in" (all four paws in the water basin) were recorded for one hour at sunrise and for one hour at sunset on each observation day every 2.5 minutes. For the video observation, three cameras were installed in each enclosure, one for each of the three water basins. The real-time recordings were carried out on seven

consecutive days from sunrise until sunset in each of the five observation weeks. Two hours in the main activity time were analyzed similarly to the direct observation.
To achieve information about the use of the nest boxes and the activity rhythm of the mink, all animals were micro-chipped and all nest boxes of Group A were equipped with an automatic registration device (developed at the Bavarian State Research Center for Agriculture, Institute for Agricultural Engineering and Animal Husbandry). Using the automatic registration device, it was possible to assess to the second for each individual mink whether it was in the nest box, in the tube leading to the nest box or outside in the enclosure. It was therefore possible to evaluate the resting and activity patterns including their daily lengths and variations.
The statistical analysis was performed in cooperation with the Statistical Consulting Unit, Department of Statistics at the LMU Munich, Germany. P-values of ≤ 0.05 were considered significant.

RESULTS

Behaviour observation

For the analysis of the direct observation the behaviour patterns "in" and "at" the pool, the creek and the pond were summed up respectively for each observation day. The mink visited each of the three water basins, but the numbers of visits were not evenly distributed. The pool had the most visits within each observation week compared to the pond and the creek. The pond was frequented more often than the creek. Most visits were recorded in the last two observation weeks, when the mink were 26 and 30 weeks of age respectively. These findings are coherent with the results of the video observation. In total the mink spent most time at the pool. The duration of stay at the creek was the lowest. The analysis of variance showed that the total times spent on the pool, the pond and the creek differed significantly from each other. There were no significant differences between group A and group B. It should be noted, that the three water basins were deemed as fixed units in the statistical analysis, disregarding that they differed from each other by several factors, e.g. circumference, surface area, water volume and distance from the nest boxes.

Automatic registration device at the nest boxes

In the first period until the mink were 17 weeks of age, they distributed their time quite evenly over the 20 nest-boxes. The boxes on the "feeding" side were used slightly longer. In the following periods clear preferences for certain nest boxes could be seen, which changed over the course of the study. Nest box 6, for example, had increasing lengths of stay from approx. 500 hours in the first period, 900 and 1200 hours in periods 2 and 3 up to nearly 1400 hours in the last period. The reverse situation was found for nest-box 14, which was used for approx. 100 hours in the first and second period, but hardly visited any more in the third and fourth period. In total the duration of stay in the nest boxes on the feeding side (No. 1 – 10) was significantly ($P < 0,05$) longer than in the nest boxes on the water side (No 11 – 20). The mink chose to share their nest-boxes very often over the whole course of the study. Very frequently two and three animals could be found together in one box. Until the end of the study multiple occupancy of up to 6 and more mink in one nest box occurred. On the other hand, individual animals did not develop preferences for certain nest boxes. The mink used every or nearly every nest box in the four periods.

DISCUSSION

The results of the direct as well as the video observation showed that the mink in both groups (A and B) generally accepted all three water basins and used them from the beginning to the end of the study. These general observations are consistent with the behaviour of semi-aquatic living wild mink described in the literature (e.g. Dunstone, 1993, Wiepkema and de Jonge, 1997). The outcome of this fundamental study was that farm mink still use water basins for swimming voluntary, willingly and extensively. During the course of the study (from August to December) an overall increase in frequency and duration of use of the basins was observed. This does not comply with the results of Hansen and Jeppesens study (2001), who found a higher swimming activity of their mink in the warm summer months. This could be due to the fact that in the present study the animals were observed for only one season from 14 up to 30 weeks of age. It seems possible that an increased swimming activity can only be found when the mink grow older. As it was not possible to distinguish the individual animals in the direct and the video observation, we were not able to tell whether all mink used the swimming facilities or whether only several animals caused the majority of the water contacts, as described by Hansen and Jeppesen (2001). They found that mean duration of stay at the bath and the amount of swimming bouts per day differed greatly between the animals. At the analysis of the video recordings in the present study at least half of the animals in each group (10) could be seen at the same time, which allows the conclusion that at least a majority of the animals actually used the water basins.

CONCLUSIONS

As this study showed, it was successful to keep young mink in groups with free access to swimming water basins, an approach that should be followed up. On the basis of the present study, it is now possible to suggest the use of an approx. 30 cm deep swimming pool, sized one square meter per animal for a subsequent study using a husbandry facility similar to farm conditions. Running water does not seem to be necessary according to the results of this study. These findings are largely consistent with the requirements of the current valid German "Farm Animal Welfare Directive 2006" (Tierschutz-Nutztierhaltungsverordnung 2006), which specifies a 30 cm deep water basin and a minimum size of one square meter.

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INFORMATIONAL STRESS AND INFORMATIONAL PATHOLOGY IN ANIMALS: DISCUSSION PAPER

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SUMMARY

The demonstration of mental experiences in animals has led to the necessity of assessing the effects of informational flow on animal welfare. Also, general interest towards these aspects has increased due to progresses in human medicine research on concepts such as *informational deprivation*, *informational overload*, *informational stress, informational aggression syndrome*, which were studied both on human subjects and on animals, in experimental settings. The present paper analyses the current knowledge in this field. It emphasizes both the importance of information perceived by animals from their environment, and the informational value of food, which ultimately determines its nutritional and biological value. These aspects are analysed considering recent progress in a new scientific field, called *nutrigenomics.* The paper discusses the necessity of integrating recent knowledge from the domain of informational pathology in *pre-established welfare assessment systems.* Eventually, a new classification of known stress types could be attempted based on these ideas. Accurate studies on animal informational pathology may of paramount importance for the development of animal welfare research.

INTRODUCTION

One of the most comprehensive definitions of welfare is the one suggested by *Webster*: '*a state of body and mind as the sentient animal attempts to cope with its environment*'/15/. *The state of body* depends mostly on the physical environment and the access to resources, while *the state of mind* depends mostly on the type and amount of stimuli from the external and internal environment. These stimuli may be perceived differently in the light of the animal's own experience. There are frequent incompatibilities between physiological necessities of animals and their living conditions in current zootechnical systems, which lead to stress and adaptation difficulties. All stress factors, regardless of their nature, act through the same reaction pattern /5**/**. There is currently no classification of stress in animals which grades the severity or importance of stress. Various studies use the terms *stress, eustress, distress, understress* sau *overstress* without precise definitions and general consensus /4/. Generally, stress is classified according to the cause: transport stress, caloric stress, microbial stress, emotional stress, isolation stress, overcrowding stress etc. *Restian* /12; 13/ has described a new type of stress, called *informational stress*, which may be induced both by *informational aggression* and by *informational deprivation.* This type of stress has been described in people and thereafter studied on laboratory animals /3;14/. Considering that laboratory animals and farm animals do not differ essentially in their reactions to stress, we consider that informational stress may occur in farm animals as well, under certain circumstances.

DISCUSSION

Animal organisms maintain their integrity through a continuous exchange of substance, energy and information with their surrounding environment. Information plays a key role in determining the way nutrients and energy are used /1/. Animal organisms use information both to create their own structures in a highly orderly manner, and to regulate their innate behaviour. All the elements of the environment contain information, because matter cannot be separated from information, and the amount of information is bigger in more complex systems. For each category of animals, information must present certain characteristics in order to regulate their behaviour, especially in what concerns social contact, vocalizations, visual and olphactive recognition etc. Behavioural problems occur first whenever the animal's environment does not fulfill physiological requirements, followed by alterations of main body functions and pathological states. *Jones* /6/ has shown in donkeys transferred to different climate and vegetation conditions that mental/psychological adaptation is more difficult than nutritional and physical adaptation. This emphasizes the importance of specific stimuli, which generate necessary information for the adaptation and survival of animals.

The informational value of food is considered by some authors /8/ as the most profound and important characteristic of food quality, determining its nutritional and biological value. One molecule of glucose contains 135 bits, while a protein's molecule may contain $10^6 – 10^7$ bits /12/. Although information is indelibly related to substance, molecular information must not be mistaken for the substance or the energy of that molecule. Information depends on the spatial configuration of molecules and one molecule will only be „recognized" by a molecule that has a complementary spatial configuration.

Such is the case of cellular receptors that are architecturally fit to recognize only the molecules belonging to a very specific type of chemical messenger. Therefore, molecules of the same substance, having the same energy, may in fact carry different information depending on the way those molecules are configured spatially. Also, reversely, different substances may convey similar molecular information just because they present themselves through a similar spatial configuration at one molecular end. Such is the case of morphine and β-endorphin, targeting the same receptors /13/. Different foods, bearers of information, are ingested and then processed by digestion, absorption and metabolic degradation, just to be finally integrated as information proper to the organism that has consumed them. Given the fact that the products being eliminated as waste by living organisms have a simpler and less orderly structure, it has been suggested that living systems thrive on *negentropy* (an acronym for negative entropy) - that is, they feed on the order taken from the environment. Healthy organisms have a „thirst" for information, which they try to satisfy in the same way hunger is satisfied by food. Information gathered from food interacts with an organism's own informational matrix and influences the expression of genes. Studies of the effect of food constituents on gene expression make the object of a new science, called *nutrigenomics*. It appears that nutrients behave as signals that are detected by a sensor system in the cells and inform the cells about the diet. It has been established that inadequately processed food, with altered bioinformation, as well as some food obtained from genetically modified organisms, may contain information which is not familiar to a certain species' organization pattern, thus leading to informational stress. An example of alteration of information with biological significance is represented by *stereoisomers.* These molecular species have the same chemical composition, but differ by the relative spatial arrangements of atoms and groups of atoms that compose them and therefore have an entirely different biological activity /8/.

Lack of information or reduced information supply are often not well tolerated by humans and animals and may lead to certain informational deprivation symptoms that occur slowly and differently, depending on species, age and sex, but also on the level of information decrease or distortion. A state of frustration, anxiety, agitation, tachypnoea, tachycardia, blood pressure elevation, vocalizations, orientation disorders are the most common manifestations of informational deprivation. Understimulation elicits increased levels of urinary adrenaline, noradrenaline and cortisol, as well as increased heart rates in male and female university students /7/. In highly social animals, such as sheep or pigs, prolonged isolation in a confined environment, with high solid walls that impair visual and olfactory exploration of surroundings, as well as communication with other conspecific animals whose presence is felt in the nearby, will most likely lead to stress. These animals show a multitude of endocrine, hematological and biochemical alterations, stereotypic behaviors and a marked increase in heart rate and respiration rate as indicators of stress /9; 10; 11/. Visual isolation is less well tolerated in comparison to spatial isolation when visual contact is maintained, which is expressed by an increased intensity and duration of vocalizations, and by an increase in heart rate and metabolic rate of up to 15% /2/.

Informational aggression is as harmful as other types of aggression, such as: phonic, photonic, nutritional and antigenic. In humans and in animals, information overload causes anxiety, poor decision-making, memory deficit and reduced attention span. Noises exert negative effects upon health and animal behaviour through irritation exerted on the acoustic analyzer by some sound characteristics like frequency, intensity and depth. Informational aggression may be transient and reversible, but through repetition it may generate persistent pathological states, with various clinical manifestations. The *informational aggression syndrome* in humans is characterized by fatigue, anxiety, irritability, insomnia, precordial pain, tachycardia, hypertension, nausea and anorexia /12/. *Restian* et al. /14/ have demonstrated that rats subjected to optic and acoustic signals supplying 400 bits per minute, for 24 hours, had increased blood levels of norepinephrine, 17-ketosteroids, creatinine, glucose, cholesterol and lipids. However, large variations between subjects were recorded during the chronic experiment, showing that the changes had been attenuated by the adaptation of rats to the experimental conditions. Starting from the observation that the *informational aggression syndrome* in animals is similar to *the opioid abstinence syndrome*, *Cristea* and *Restian* /3/ studied the implication of endogenous opioids in experimentally induced informational aggression syndrome. They induced this syndrome in rats using specific sound signals and examined the animals' behaviour by applying a test used in the study of the opioid abstinence syndrome. The animals' reaction to thermal stimulation was recorded as well. They demonstrated a significant increase in endogenous opioids in the first stage, while specific symptoms of the opioid abstinence syndrome developed during the second stage. These symptoms can be prevented with clonidine, that has been used in the treatment of the exogenous opioid abstinence syndrome.

Published studies concerning the impact of information on humans fail to clarify the difference between *'informational stress'*, on one hand, and *'overstimulation'* and *'understimulation'*, on the other hand*.* This type of clarification requires further research. One can only assume that the main difference is that *'informational stress'* is characterized by a biochemical / hormonal response typical for stress conditions, as described in the first part of this paper. Human states of *understimulation* and *overstimulation* may or may not be accompanied by the typical biochemical/hormonal stress indicators. In humans, *understimulation* may be either physical or mental in nature, but generally refers to intellectual boredom. We believe that in animals the phrase *'informational deprivation'* is more appropriate than *understimulation*, because sensorial infomation is of paramount importance for animal welfare*. 'Informational deprivation'* is frequently associated with an animal's inability to express an instinct of environmental exploration and with the absence or the insufficiency of the stimuli / information regarding a strongly motivated behaviour. We also believe that terms like *overstimulation* and *information overload* that are used for humans, could be replaced in animal studies by the phrase *'informational aggression'*, which refers to aggression of any type

eliciting a state of acute or chronic stress. In our opinion, stress through *'informational deprivation'* and through *'informational aggression'* do not necessarily represent new stress categories, others than those already described, but rather a different interpretation of these pre-established categories. Eventually, a classification of known stress types could be attempted, some types being included in the *informational deprivation* stress category (e.g. isolation stress), and others in the *informational aggression* stress type (e.g. overcrowding).

CONCLUDING REMARKS

Further studies regarding informational pathology may contribute to the progress of animal welfare in intensive animal breeding systems, with particular aplications in *precision livestock farming.* Such studies may lead to a better understanding of the reactivity of animals and may serve as an experimental basis for the understanding of informational deficiency syndrome and informational aggression syndrome in humans.

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AUTOMATIC LAMENESS DETECTION OF DAIRY COWS AT THE FEED STATION BY LEG WEIGHT DISTRIBUTION

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SUMMARY

This paper describes the possibility to use the static load of each leg to monitor dairy cow leg disorders.

Four strain-gauge scale platforms were installed into the feed station floor to measure the point of support of each leg. The Spider measurement system (HBM, Germany) was used to transmit the information to a computer. Fifty-five cows were used for the experiments. According to gait assessment the animals were divided into five groups: normal gait (score 1, by Sprecher et al. [9]); one rear leg moderately lame (score 3); one rear leg severely lame (score 4-5), one front leg moderately or severely lame (score 3-4); both rear legs moderately or severely lame (score 3-4). Leg load data were recorded over a period of five minutes. The leg load index (LLI) for each leg, which indicates the partial load of a leg in relation to the body weight, was calculated and used for multiple comparisons. Results from using gauge scales in an automated feeding station may provide valuable information, especially about rear leg disorders in dairy cows.

INTRODUCTION

Lameness causes serious welfare, health and economic problems in dairy cattle management. The loose house system, and increasing sizes of dairy herds, reduces time and possibilities for observation and visual detection of lame animals. This has created a need for the automatic monitoring of leg disorders. For this purpose many different instruments and algorithms for the early identification of lameness in dairy cattle have been created and tested. The basic indicators for the automatic detection of lameness have been the body weight distribution between the legs using special weight scales, and results of analyses of walking gait, using walkthrough scales or video recordings [6,7,8].

Promising results have been found using the automatic measurement of the static load of each leg in a milking robot, where the animal's movement is significantly restricted [2].

The aim of this study was to examine the possibility of using this method at the feed station, where the animals are automatically identified, but their movements are not as constrained as they are in a milking robot.

MATERIAL AND METHODS

Fifty-five cows were used for the experiments. After milking, each cow's gait was assessed using a five-numbered rating score system [9]. Cows were then grouped according to the lameness score:

1) cows with normal gait (rating score 1) – 12 animals;
2) one rear leg moderately lame (rating score 3) – 14 animals;
3) one rear leg severely lame (rating score 4-5) – 17 animals;
4) one front leg moderately or severely lame (rating score 3-4) – 4 animals;
5) both rear legs moderately or severely lame (rating score 3-4) – 8 animals.

An assessment station, with an area of 1.95×1.05 m, was used. On the station floor four strain-gauge scale platforms (each with an area of 710×520 mm) were installed, taking into account the point of support of each leg. The Spider measurement system (HBM, Germany) consisting of an amplifier, an analogue-digital converter and a controller was used to transmit information to a computer. The load measurement had an accuracy of ±1.0 kg and the measurement frequency was 2 Hz. Leg load data were recorded over a period of five minutes while the cows were fed a concentrate feed. The position and activity of animals were observed and recorded on videotape. Special attention was paid on the placement of the legs. The leg load index (LLI) for each leg, which indicates the partial load of a leg in relation to the body weight, was calculated as $LLI_n = L_n/W$, where L_n is the load of n^{th} leg, and $W = \Sigma L_n$ is the body weight.

ΣLLI was calculated as the sum of paired front or rear leg LLIs. The data of less and more heavily loaded front and rear legs were tested for differences of LLIs using the Wilcoxon signed-rank test within each cow group, and the Wilcoxon rank-sum test between cow groups. The Bonferroni correction was used for adjustment of multiple comparisons. The maximum body weight of each cow was found (the sum of four legs' load). If the body weight differed from the maximum by more than 10%, caused by *e.g.* the single leg lifts or the cow's position not fully on the scale platforms, the data were removed as unreliable.

RESULTS

The results of the experiments are presented in table 1.

Table 1: LLI of animals (mean ± standard deviation)

Cow groups	No of ani-mals	Maxi-mum weight (kg)	LLI less loaded front leg	LLI more loaded front leg	LLI less loaded rear leg	LLI more loaded rear leg	Sum of front leg LLIs	Sum of rear leg LLIs
Normal	12	635	0.232± 0.031	0.295± 0.017	0.202± 0.013[a]	0.224± 0.018[a]	0.527± 0.024	0.426± 0.025
Moderately lame (rear leg)	14	658	0.248± 0.034	0.312± 0.041	0.143± 0.025[bc]	0.236± 0.038[ab]	0.560± 0.051	0.379± 0.041
Severely lame (rear leg)	17	610	0.239± 0.030	0.295± 0.030	0.099± 0.044[b]	0.307± 0.059[c]	0.534± 0.044	0.406± 0.041
Lame (front leg)	4	636	0.169± 0.068	0.298± 0.037	0.215± 0.016[ab]	0.244± 0.020[abc]	0.466± 0.063	0.459± 0.028
Lame (both rear legs)	8	614	0.254± 0.012	0.290± 0.037	0.178± 0.020[ac]	0.214± 0.029[ab]	0.544± 0.043	0.392± 0.041

[a,b,c]means with different superscripts within columns are significantly different: $p<0.05$, Wilcoxon rank-sum test with Bonferroni correction within the columns of LLIs; in columns without superscripts were no significant differences.

Table 2 represents tested differences between LLIs within each cow group. There were significant differences between more and less loaded front and rear legs in groups of cows with a normal, moderate and severely lame rear leg. Also, differences between the ΣLLIs of the front and rear legs were significantly different in these cow groups.

Table 2: Mean differences of LLIs (within each group)

Tested difference	Normal	Moderately lame (rear leg)	Severely lame (rear leg)	Lame (front leg)	Lame (both rear legs)
More loaded front leg - less loaded front leg	0.063*	0.064*	0.056*	0.129	0.036
More loaded rear leg - less loaded rear leg	0.022*	0.093*	0.208*	0.029	0.036
Front legs-rear legs	0.101*	0.181*	0.128*	0.007	0.152

*$p<0.05$, Wilcoxon signed-rank test with Bonferroni correction

During the time when the cows were eating concentrates, the front legs of healthy cows were more heavily loaded than the rear legs (ΣLLIs 0.527 and 0.426, respectively; $p<0.05$). There were significant differences between the LLIs of the two front legs (0.063, $p<0.05$). The difference between the LLIs of the rear legs was significantly smaller (0.022, $p<0.05$).

In the case of one rear leg disorder (rating score 3) the front legs were loaded more than the rear legs (ΣLLIs 0.560 and 0.380, respectively). There was a significant difference between the LLIs of healthy and damaged rear legs (0.236 and 0.143, respectively; $p<0.05$), between a damaged leg and a less loaded rear leg of a healthy animal (0.143 and 0.202, respectively; $p<0.05$).

When the rating score of one rear leg was between 4 and 5, the difference between the load of the front and the rear legs was similar to the leg load of the healthy cows (ΣLLIs 0.534 and 0.406, respectively; $p<0.05$). The difference between the LLIs of healthy and damaged rear legs was significant (0.307 and 0.099, respectively; $p<0.05$). The severely lame animal leant on the healthy leg more than a moderately lame animal (LLIs 0.307 and 0.236, respectively; $p<0.05$).

When one front leg was damaged, the difference between the LLIs of the front and rear legs was not significant (ΣLLIs 0.466 and 0.459). The damaged front leg was less heavily loaded than the healthy front leg (0.169 and 0.298, respectively).

Of the animals with both lame rear legs, the front legs were loaded more equably (difference of LLI of the front legs = 0.036) compared with the animals from all other groups. Non significance might have been the result of the low number of animals used.

DISCUSSION

In addition to milking robots, automated feeding stations are being included in housing design. A four balance system for measuring dairy cow leg load distribution during milking in a milking robot, in order to detect lameness, has given promising results [4]. The neural network model, worked out on the basis of continuous monitoring of leg weights, was able to classify 96.2% of the measurements correctly as sound or lame cows [3,4]. According to Pastell et al. [5], the asymmetry of weight distribution within a pair of contralateral legs was a sensitive measure for detecting visibly lame cows, and cows suffering from relatively severe sole ulcers. Chapinal et al. [1] pointed out that the time since milking and pregnancy need to be considered when using measures of weight distribution to detect lameness, and that the conditions affecting cows' weight distribution must be understood before this method can be used in commercial conditions. The movement possibilities at feeding stations are not as restricted as in milking robots. According to our experience, using foreleg weight distribution for lameness detection at the feeding station is complicated. At the time of concentrate feeding, healthy cows' front legs were loaded unequally (table 1), because the animals are often excited and they try to find a more suitable position for apprehending the feed. Analysis of leg load distribution gave encouraging results for the detection of visible weight-bearing disorders (supporting-leg disorders) of the rear legs.

CONCLUSIONS

Using strain gauge scales in an automated feed station may provide valuable information, especially about rear leg disorders, in cows as the asymmetry of body weight distribution within a pair of rear legs is significant.

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SENSOR BASED LAMENESS DETECTION IN DAIRY COWS THROUGH MEASURING PEDOMETRIC ACTIVITY AND LYING BEHAVIOR

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SUMMARY

The aim of this study was to assess the efficiency of sensor based measurement of activity and lying behavior by ALT-pedometers to determine different behavior patterns between non-lame and lame cows and to verify whether combined measurements of activity and lying behavior can be used as a diagnostic tool for lameness.

The ALT-pedometers recorded activity and lying behavior of 30 lactating Holstein dairy cows at 15-min intervals and selected at the beginning of the study based on their 5-point Numerical Rating System (NRS ≤ 2). Two threshold NRS values (3 and 3.5) were used for lameness detection. For classification purpose, Support Vector Machines with an RBF-Kernel were used to determine the deviation from normal behavior.

According to the results of this study, it can be concluded that the difference in activity and lying behavior among individual cows was higher than the change in daily behavior caused by lameness. The deviation in cows' behavior happened both with negative and positive sign. The combination of activity and lying behavior led to a mean accuracy of 76% and a mean precision of 77% to predict lameness on-farm.

INTRODUCTION

Lameness in dairy cows is an economic and welfare concern and considered to be the third most important health related economic loss facing the dairy production, following infertility and mastitis [1]. Traditionally, the locomotion score method represents a subjective possibility for assessing lameness. With the increasing size of farms there is an interest in automated means for lameness detection.

Lameness is a very painful condition that disrupts the cow's ability to express normal behavior [2]. Therefore, measuring animal's behavior may provide information on detection cows with abnormal behavior such as altering their locomotor's activity.

Several studies have documented the topic of measuring lameness by automatic recording the behavior of dairy cattle, such as evaluate gait and weight distribution of individual cows in walking or standing [3,4,5].

The pedometer technology is usually applied for oestrus detection in cows but can be also used to detect lameness in dairy cows. For example, O' Callaghan [6] used daily activity level and value of posture scoring as indicators of pain resulting from lameness. They found that the posture scores and activity levels correlated significantly and the presence of foot lesions was associated with reduced activity levels. In addition, abnormal lying behavior through increases or decreases the total lying spending time, have been shown to be a predictive of lameness risk [7,8].

Further research into behavior of cows would be very useful to the dairy farming, especially if electronic systems for monitoring activity and lying behavior were available and could recognize specific cows that require closer examination.

In this research, a new type of pedometer, called ALT-pedometer (recording activity, lying time, and temperature), have recently served to detect estrus and locomotive activity in dairy cows [9,10] were used to determine whether combined measurements of activity and lying behavior could be improved reliability for an on-farm lameness assessment.

The work so far has led to the following hypotheses: With the help of ALT-pedometers a pattern of locomotion activity and lying behavior of every single animal within a defined period can be recorded. The deviation from their 'normal' behavior patterns as they occur could be expected as onset of lameness. One can predict that lame cows show activity and lying behavior differences to non-lame cows. Hereby it is important to take into account that individual behavior in that activity can differ substantially between cows.

MATERIAL AND METHODS

Two experiments were conducted at "Frankenforst"research station (University of Bonn) with 62 lactating Holstein dairy cows. The investigations were done in two periods. Between Febrauary 2010 and May 2010 and then repeated between June 2010 and October 2010. In the first phase the 30 cows housed indoors and were divided into 24 primiparous and 6 multiparous (parity 2), in the second phase into 12 and 18 (parity 2) with pasture allowance; respectively.

In both phases, a total of 30 cows were selected based on their locomotion score (NRS ≤ 2).

Once weekly, cows' gaits were scored by visual observation during walking along a 16-m grooved concrete alley between milking parlor and stall,

immediately after the afternoon milking using the system described by 1 to 5 numerical rating system (NRS; where 1 = sound and 5 = severely lame) Flower and Weary [11]. Two threshold NRS values (3 and 3.5) were used for lameness detection.Within the two periods, the observation was done by the same person.

Six features were extracted from the ALT-pedometer measurements, step impulses and lying time as derived features. The numbers of bouts and the median of the duration for one bout period were used as features as well. Feature five and six were the minimal and maximal duration for a single bout. To compare lame and non-lame cows, all cows were selected where an NRS of 3 or 3.5 occurred and subsequently all days where this condition was observed were consolidated into the dataset for lame cows. We have build up a dataset for non-lame cows by using days from the same cows where no movement disorder could be observed (NRS ≤ 2). This resulted in a dataset of 549 labelled days from eleven cows in total, with approximately the same amount of lame and non-lame days.

RESULTS

We observed that the difference between the mean of lame days and non-lame days is rather small compared to the high standard deviation of both classes. We found also huge differences between individual cows, which made it difficult to find universal thresholds valid for differentiation between lame and non-lame cows using activity and lying behavior measures. Yet, in the current research, lameness (indicated by locomotion score NRS=3 and 3.5) caused significant disturbances of normal behavioral patterns. The effect of lameness on daily activity may be bidirectional: some lame cows had an increase in daily activity counts, whereas others decreased. The deviation in cows' behavior happened both with negative and positive sign, so that it is for instance more likely that the lying time for lame cows increases. However, even if a clear tendency can be observed, there are always cases where extreme values in the opposite direction occur for lame cows. The overall performance of Support Vector Machines with an RBF-Kernel by combination of activity and lying behavior was 76% accuracy and a mean precision of 77% to predict lameness on-farm.

DISCUSSION

In this research, measurements from ALT-pedometer that measure both activity and lying behavior were used to determine whether combined measurements of activity and lying behavior could be improved reliability on-farm lameness assessment.

The results presented here have shown that thresholds may not be sufficient in order to separate between non-lame and lame cows. This was because of the very individual behavior pattern among the cows. Therefore, a discriminating approach is used to determine the deviation from normal behavior rather than using absolute values.

In contrast to the previous studies, 54% of lame cows had an increase in daily activity, probably because lame cows tend to show shorter strides [12,13] and thus needed more steps to reach the same distance.

Lame cows mostly had longer lying bouts compared with non-lame cows. This supports results from other studies [14,15,16], probably because lame cows are reluctant to stand up. Although the Alt-pedometer data showed that lame cows had longer bouts, only slight differences were observed between lame and non-lame cows in the frequency of bouts.

In contrast to the results of [8], lame cows tend to have longer lying times than non-lame cows. This results support previous finding of [14,17,18,15], who reported an increase in daily lying time in lame cows compared with sound cows. This can be explained that lying is a mechanism by which cows alleviate pain from weight bearing.

The number of bouts was slightly increasing too, but was not as significant for classification as other features. Maximum bout duration, as step impulses, was equally likely to differ with positive and negative sign from normal behavior. Minimum bout duration was the weakest feature and hardly showed any deviation between lame and non-lame cows.

In consequence, understanding the behavioral differences in term of activity and lying behavior between lame and non-lame cows may contribute to develope an on-farm electronic lameness detection tool to determine changes in behavior associated with lameness.

CONCLUSIONS

We were able to record activity and lying behavior using ALT-pedometers. Together with a model for classifying the lameness status, the ALT-pedometer data can indicate different behavior pattern of sound and lame cows. Furthermore, we found that a combination of several features with machine learning methods lead to an increased likelihood of detecting lameness in dairy cows.

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SELECTION OF A GOLDEN STANDARD FOR VISUAL-BASED AUTOMATIC LAMENESS DETECTION FOR DAIRY COWS

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SUMMARY

Lameness detection in dairy cow production systems is still a difficult and time consuming task. Therefore the Marie Curie Initial Training Networks (ITN) project "BioBusiness" seeks for the development of a visual based automatic lameness detector. In order to validate this new technological method it must be compared with a golden standard (GS) that must be selected from one of the many subjective locomotion scoring methods for lameness detection. In order to select a GS a review of 66 articles related with lameness was performed to determine an approximate number of locomotion scores existing and their respective characteristics. We found 24 different locomotion scoring methods. The most used in the literature was the 5 points discrete locomotion score developed by Sprecher et al.. This method fits most of the requirements because it is widely known by possible customers, it uses the uneven gait and arched-back as lameness indicators and it is versatile. The lameness scoring must be assessed by at least two trained observers in order to obtain high inter and intra observer reliabilities.

INTRODUCTION

Lameness is one of the most common health issues in dairy cattle. It is associated with a decrease of the productive performance of cows and it is, probably, the biggest unsolved problem of animal welfare in dairy farms [2]. Nowadays, lameness detection is done by means of subjective diagnostic methods usually called Locomotion Scores (LS). The different existing LS methods are based on the observation of the characteristics of the gait and/or posture of a cow and scoring them according to a reference image. The weakest point of these LS lies in the fact that different observers may give a different score of lameness for the same cow, which consequently might lead to a poor reliability of lameness diagnosis.

One of the objectives of the BioBusiness project is the development of an objective automatic visual-based and reliable lameness detector for dairy cows, in order to improve the early detection of lameness in dairy farms. However, the development of such a system requires understanding of the lameness diagnosis methods in order to choose the best alternative as a Golden Standard (GS) for the labelling and the validation of the automatic lameness detection method. Thus, the objective of this paper is to explain the criteria used for the selection of a GS for the development of a visual-based automatic lameness detector for dairy cows.

MATERIAL AND METHODS

To obtain insight in the existing LS systems, a review of articles related with lameness and different productive and epidemiologic topics was performed. The literature research was conducted in two specialized databases: ISI Web of Knowledge and SciVerse Scopus. The inclusion criterion for articles was that they must indicate which LS method was used for lameness detection. In total of 66 articles were included, published between 1988 and 2010. The LS methods found in the articles were categorized by type (continuous or discrete) and number of points of the used scale. The percentage of utilization (**PU**) was calculated for the type of the score and the number of points of the score (for discrete LS). Moreover, characteristics of the gait and posture observed in each LS method were individualized in order to establish which of them are used for lameness detection. The tables and descriptive statistics were made by using Microsoft Office Excel 2010 (Microsoft Coorporation, Schiphol, The Netherlands).

RESULTS

From 66 articles, 95.7% used LS with discrete scales (dLS) while just 4.3% of the articles used LS continuous scales (cLS). Regarding the dLS, 21 different methods were found (Table 1). If the dLS methods are sorted by number of points present in the scale, it can be seen that 2, 3, 4, 5, 6, 7 and 9 points dLS methods were used. Almost half

of the articles included in the review used a 5 point dLS (45.7%), followed by 9 and 4 points dLS with a PU of 22.9% and 12.9%, respectively (Table 1). The most important dLS was the 5 points-score developed by Sprecher et al. [11] used in 13% of the articles (data not shown). In summary, 9 different characteristics of gait and posture are used in the 21 dLS methods found in the review. As expected, the most widely used sign for lameness detection was the uneven gait, followed by the arched-back.

Table 1: Type, Score range, observation elements, number of scores (N LS) and absolute (N) and relative (R) number of papers for different locomotion scores found in 66 articles sorted by type and number of points of the Score.

Locomotion score (Type)	Score Range (-)	Gait/Posture observed [a] (List)	N LS[b] (-)	N[c] (-)	R[d] (%)
Continuous	0 - 1, 10, 100		3	3	4.3
Discrete					
9 Points scores	1 - 5 with 0.5[e]	UG, AB, HB, Trn, Ris, Beh	2	16	22.9
7 Points scores	0 - 6	UG, HB, Ris, Dis	1	1	1.4
6 Points scores	0 - 5 or 1 - 6	UG, AB	2	3	4.3
5 Points scores	0 - 4 or 1 - 5	UG, AB, Sp, HB	7	32	45.7
4 Points scores	0 - 3 or 1 - 4	UG, AB, Sp, HB, HH, Trn	5	9	12.9
3 Points score	1 - 3	UG, AB, Sp, HB, Tnd	3	4	5.7
2 Points score	0 - 1		1	2	2.9
Total Discrete			21	67	95.7
Total			24	70[f]	100

[a]: UG = Uneven Gait, AB = Archer Back, HB = Head Bob, Trn = difficult turning, Ris = Difficult rising, Beh = Behaviour, Sp = Diminished Speed, HH = Hip Hick, Dis = Discomfort when moving.
[b]: N LS = Number of different locomotion scores founds according sorting criteria.
[c]: N = Number of times in which a locomotion score is used in papers.
[d]: Percentage of utilization.
[e]: Range from 1 to 5 with half points.
[f]: from 66 articles, 4 article used 2 locomotion score for lameness evaluation.

DISCUSSION

One of the final objectives of BioBusiness project is to develop a commercial solution for lameness detection. The PU is an important factor to take into account for the selection of a GS, in order to develop a product with which customers might be familiar. On the other hand, the quality in the assessment of animal behaviour (e.g. lameness) performed by subjective measurement can be estimated by means of its inter- and intra-observer reliability [8]. Thus, the selection of the GS will be based mainly on both indicators.

The huge difference in the PU of both types of scales (dLS or cLS) might be explained by the perception of the observer that dLS are easier to use than cLS. On the other hand, the type of the scale does not have a great influence on the reliability of lameness scoring [12]. Another important factor that affects the reliability of lameness diagnosis in the case of the dLS, is the number of points of the scale. In this regard, it has been described that the inter-observer reliability has a negative correlation with the number of points of the score [7]. Almost 75% of the articles included in the review used ≥ 5 point scores in order to provide a good description of the lameness degree. Recently, two big attempts were made to create a standard dLS (Dairy CO and Welfare Quality). However, they decided to diminish the number of points of the scores to 3 and 4 point dLS, respectively [1] [6]. The aim probably was to make the dLS more easy to use by the observer and to improve the reliability without losing too much resolution in the description of lameness.

The most used and reliable signs for lameness detection are the uneven gait and the arched back [5]. However, the number of characteristics of gait or posture used for lameness detection goes from 1 to 6 depending on the LS. The sign observed for lameness detection is an important factor in the selection of the GS. In a first approach for the development of an automatic visual-based lameness detector, Pluk et al. [9] reported a large variation between individual cows in the measurement of step overlap and recommend not to use it as a single classifier for lameness. Recently, Poursaberi et al. [10] reported that the measurement of the arched spine line shows promising results for an automatic visual-based lameness detection. Thus, the chosen LS to be used as a GS must include, at least in the first stage, the observation of the uneven gait and the arched-back as signs for lameness diagnosis.

According several authors, the experience of the observers is an important factor for the reliability in lameness diagnosis. It has been proved that the inter- and intra-observer reliability has a positive correlation with the experience of the observer [3] [7]. For the Sprecher et al. score an inter-observer reliabilities of 0.77 (kappa) and a percentage of agreement of 86.6% has been reported for trained observers [4]. Typically percentages of agreement and kappa for the inter-observer reliability must be above 75% and 0.4, respectively as the minimum agreement to consider the measurement as clinically useful [3].

CONCLUSIONS

The large amount and diversity of LS reported in the literature makes the selection of a GS for the development of a visual based automatic lameness detector difficult. However, based on the PU and the factors that might improve the reliability of lameness diagnosis, the selected GS must be a 5 points dLS which also includes the uneven gait and the arched-back. The advantage to select a 5 points dLS instead of a score with fewer amount of points lies on the versatility of the 5 points dLS, may be transformed into a 4, 3 or 2 points score according to the requirements of the experiments and the product. The LS that fits the previously stated requirements was developed by Sprecher et al. [11]. The lameness scoring must be assessed by at least two trained observers.

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AUTOMATIC MONTIORING OF MILKING ORDER IN AN LARGE LOOSE HOUSING COWSHED

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SUMMARY

The aim of this study was to investigate dairy cows' entrance order to milking parlour in large loose housing cowshed where automatic identification of cows is used. Milking of 692 cows in seven different feeding groups was monitored automatically in this study during six months. Quite strong ranking in milking order inside the groups existed. Newly added cows and cows with health problems stayed more backward in groups. Changes in milking order could be a low-cost way to indicate some diseases and discover those in early stage. No association between milking order and milk production and age of cow was found at the same time.

INTRODUCTION

Precision livestock farming (PLF) is becoming widespread for loose housing of dairy cows. PLF has a great potential in developing the technology for continuous automatic monitoring and improvement of animal health, animal welfare and quality assurance at farm level [7].

Since in Estonia and all over Europe the number of farms is decreasing [1] while the number of cows per herd is increasing, the possibilities to discover individual animal welfare and health problems are lower. Therefore automated methods for detection of these problems should be elaborated.

In commercial farming practice, cost reduction is often achieved at the expense of animal welfare, which inevitably can reduce individual productivity [5]. Voluntary movements of the cattle have been studied in different situations, one is entering to the milking parlour. In dairy cows, order of entry into the milking parlour is fairly consistent [3]. Possible applications of detailed knowledge of the milking order in farming practice could arise from several aspects, like an uncharacteristically late entry into the parlour can often be related to health problems [6] and can therefore be used as an indicator, prompting further examination. The objective of this study was to analyse milking order in a large loose housing cowshed and propose the automatic monitoring as a tool to detect health problems of dairy cows based on entrance order to milking parlour.

MATERIAL AND METHODS

A commercial loose housing cowshed with 600 dairy cows was used for the current investigation. Cows in milk were kept in 7 feeding groups (60–80 cows each) and milked in 2×12 DeLaval tandem milking parlour three times per day. Walking distance varied from 50 to 300 m depending on the cows' group location within the cowshed. Before milking the whole group entered into the waiting area (with the size of 171.5 m^2) next to the milking parlour equipped with moving crowd gate to drive cows gently (Figure 1A).

At the entrance to the milking parlour each cow was identified automatically by passing identification gate. Cows' number with entrance time and individual milk amount were stored in the memory of the milking parlour controller. From the controller cows' data were transferred to the computer of the management information system (MIS ALPRO) of the cowshed. Cows' health data were entered manually by the veterinarian. Data from MIS were regularly copied to the project server connected to local area network (LAN) of the cowshed and transferred daily via Internet to the computer network of the University for analysis (Figure 1B).

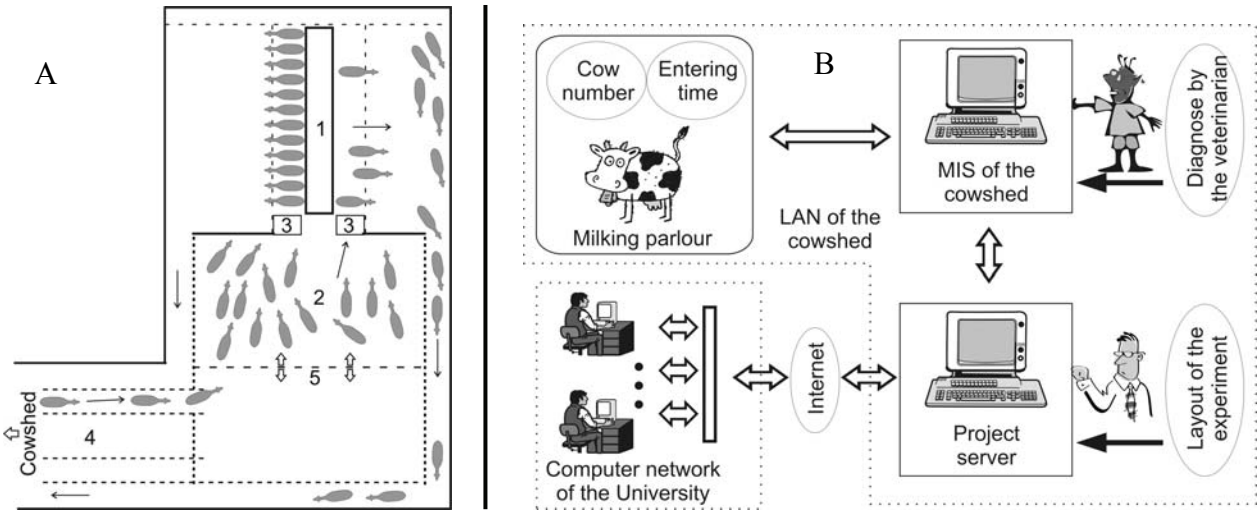

Figure 1: A – cows traffic during the experiment: 1 – milking parlour, 2 – waiting area, 3 – identification gates, 4 – walking allays, 5 – crowd gate; B – scheme of data exchange

In the present study the milking order and time lag regarding the beginning of the milking were analysed. The dataset was collected from January to June 2006 with a total of 81 550 milkings of 692 dairy cows. Health problems of cows were also recorded.

To study the stability of milking order and time lag in following days at cows' single milking level (separately for three milking times), the 1st to 10th order autocorrelations between milkings at following days were calculated for each cow. To summarize the results the average autocorrelations over all cows separately for three milking times were evaluated. The same analyses were repeated based on the average day values (evaluated as the average milking order and time lag per cow's day). To study the stability among three milking times inside the day, the Spearman rank correlation coefficients between milking times were calculated. At first the analyses were performed based on single milkings and further based on the cows' average values (evaluated as the three average milking orders and time lags corresponding to the different milking times per cow). Data was managed and analysed with MS Excel and statistical package SAS 9.1.

RESULTS

The stability of milking order and time lag regarding the beginning of milking at following days separately for three milking times was relatively low – the average first order autocorrelation coefficients for the three milking times were 0.14–0.16 for milking order and 0.16–0.24 for time lag. Also the correlations of milking orders and time lags between three milking times in a day varied from 0.45 to 0.82. Considerably more stable were the average day values of three milking times. The average first order autocorrelation coefficients per cow were 0.29 for milking order and 0.27 for time lag. Compared with the milking duration or milk yield, the milking order and time lag were less stable (Figure 2). But as the values corresponding to the following days were still positively correlated a cow specific milking order seems to exist there over days. The correlations of average milking orders and lag times per cow between three milking times in a day were 0.86–0.97 (Figure 3 and 4). Therefore, even if on single milking time the milking order was quite variable, on average there was quite strong order inside the groups.

Our preliminary analysis showed also that there is no association between milking order and milk production and age of cow, but the newly added cows and cows with health problems stay more backward.

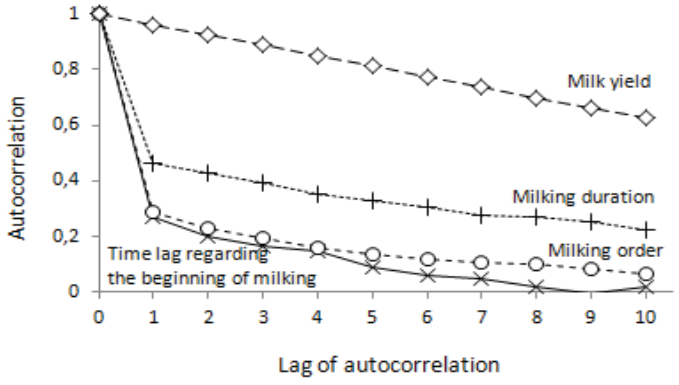

Figure 2. Autocorrelation plot of average day values of milking order and time lag regarding the beginning of milking. For comparison also the autocorrelations of average day values of milking duration and milk yield are shown.

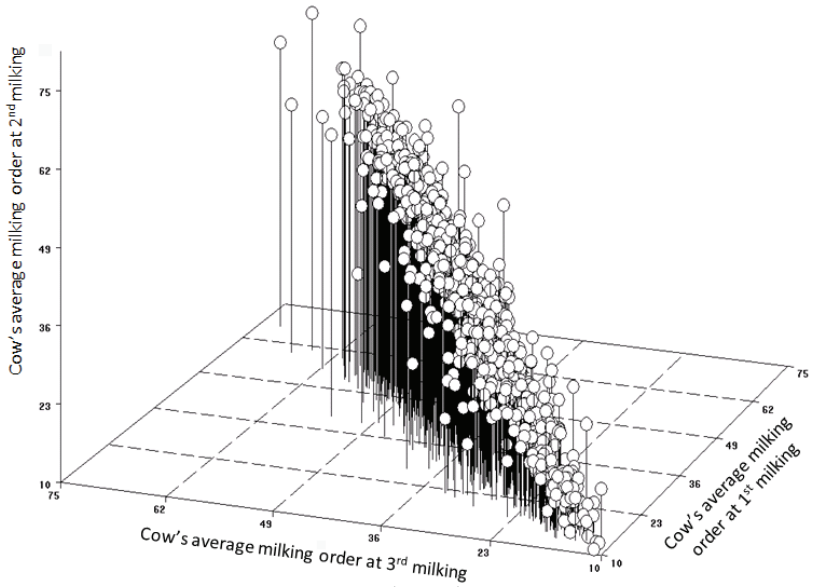

Figure 3: Cow's average milking order at 1st, 2nd and 3rd milking. Each circle marks one cow.

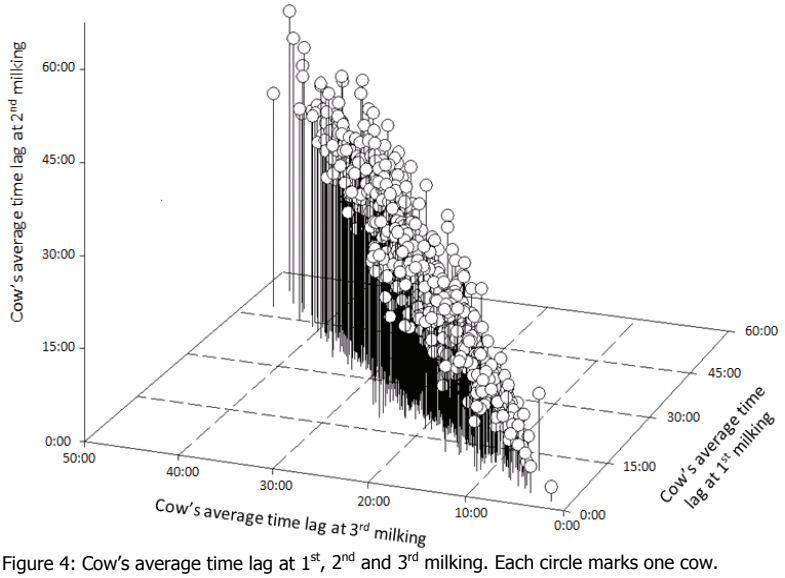

Figure 4: Cow's average time lag at 1st, 2nd and 3rd milking. Each circle marks one cow.

DISCUSSION

Our results indicated that cows had a consistent order of entry into the milking parlour. This behaviour is considered a prominent feature of the social system of dairy cattle that could have implications in farming practice e.g. speed of throughput of cows [6, 3].

Reinhardt [6] found that entrance order is positively correlated with the social rank. This would mean that entrance is in relation with the motivation for food ingestion (concentrate). Nevertheless in this specific farm cows were not fed during the milking. Rathore [5] found that high-yielding cows voluntarily entered into the milking parlour earlier than low-yielding cows, suggesting that the relief in udder pressure brought by milking is a reward for the cow. In our case preliminary analysis showed that there is no association between milking order and milk production and age of cow. Grasso *et al.* [3], notes that positive correlation was observed in primiparous animals between milking order and milk production although it was not very high (0.22).

The cows newly added into the group and cows with health problems stay more backward probably because those animals which are moved from one group to another often upset the old established social order and after a while a new social order is created [4]. Cows with health problems could stay backwards in milking order because they are slower than others and are also fearful of getting hurt during moving to the parlour or during milking [2].

CONCLUSIONS

In this study a consistent entrance order to the milking parlour was found. On single milking time the milking order was quite variable, but on an average there existed stable ranking inside the groups. Preliminary analysis showed that cows with health problems stay more backwards in milking order, so this lets us assume that automatic monitoring of milking order could be a good and low-cost way to indicate some diseases and discover those in early stage. In this way monitoring of milking order could be a good PLF tool for large loose housing cowshed management and needs additional studies in this field.

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EFFECTIVENESS OF SLIGHTLY ACIDIC ELECTROLYZED WATER FOR IMPROVEMENT OF HYGIENIC CONDITIONS OF TEAT LINERS OF AUTOMATIC MILKING SYSTEM (AMS)

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SUMMARY

This study was to evaluate the effects of slightly electrolyzed water (SAE) on cleanliness and bacterial counts of teat liners of automatic milking system (AMS). The SAE water (HOCl, 12-15 ppm, pH 5-6) was supplied to AMS on 2 dairy farms. Swab samples were collected from the inner surface of teat liners of AMS to measure the cleanliness and bacteria counts on the teat liners of AMS using SAE water or tap water which were used for rinsing teat liners after milking. The optical density (OD) values, a parameter for cleanliness, in samples from teat liners after using SAE water were significantly ($P<0.05$) lower than those of water supplied. The number of viable bacteria in samples from inner surface of liners of the AMS was significantly ($P<0.05$) decreased after rinsing with SAE water compared to that of tap water used. The ratio of viable to non-viable bacteria on the inner surface of the AMS was markedly decreased by SAE water supply. The use of SAE water supply for rinsing the teat liners of the AMS proved to be effective to cleanliness and reduce the bacterial counts without disinfectants.

INTRODUCTION

Milk quality is a critical concern on dairy farms. The increase in bacteria counts and the spread of contagious pathogens were commonly recognized on dairy farms after the introduction of the AMS (1,2,3,6), compared with that of the milk quality before introduction of the AMS. The hygienic status of AMS appears to be dependent strongly on the environmental conditions of dairy housing and could not ignore the spread of contagious pathogens via teat cup liners of AMS (3).

The inner surface of teat cups of AMS after milking is basically treated by a cleaning system with compressed air and clean water mixture without disinfectants. This process may involve not only the risk of transferring mastitis-causing contagious pathogens via teat liners but also increase in bacteria counts in milk due to the lowering the hygienic conditions of teat liners of AMS. Slightly acidic electrolyzed (SAE) water has been used to sanitize milk plants in milking companies (5). The aim of this study was to evaluate the hygienic conditions of teat liners of AMS by employing a system supplying SAE water.

MATERIALS AND METHODS

Slightly acidic electrolyzed (SAE) water

SAE water (HOCl,12-15ppm, pH 5-6) produced by the system (PuresterR, Morinaga) was used for washing the teat brushes and rinsing the inner of the teat cup liners of AMS.

Experimental Farms

This study was performed in 2 dairy farms that use two types of AMS. Farm A, the milking herd consists of 70 Holstein cows milked in a parlor system and 27 cows milked with a AMS (Lely Astronaut). Farm B, the milking herd consists of 150 cows milked in parlor systems and 43 cows milked with a AMS (Delaval).

Automatic milking system (AMS)

The milking procedure for AMS starts with brushes teats or cleaning individual teats by washing depending on the equipments. After milking, the milking units are rinsed with tap water supply as unit flush. Teat cleaning systems include brushes or rollers (Lely), and with washing teats in the teat cup (Delaval). The AMS wash the teat cups with water (without disinfectants) after every milking.

Swab sample from the inner surface of teat liners of AMS

Samples were collected from inner surface of AMS after rinsing with tap water or SAE water supply at each pre-milking event from randomly selected cows. Count data of bacterial cells were expressed as colony-forming unit of swabbed (CFU/ml), and CFU was normalized by log10 transformation.

Evaluation of cleanliness of teat liners of AMS

The cleanliness of inner surface of teat liners of AMS was evaluated by a commercially available hygiene monitoring kit (Swab'N'Check). The samples were measured and the results were expressed as optical density (OD) at 562 nm.

Bacterial counts

Bacteriological analysis was carried out according to the guidelines of National Mastitis Council. The number of viable, non-viable and total bacteria in swab samples

collected from inner surface of AMS before milking was measured by the bacteriological counter (Bioplorer R).

RESULTS

The cleanliness of inner surface of teat cup liners of two types of AMS before milking was compared with tap water and SAE water supply. The OD values, parameter for cleanliness, of inner surface of teat cup liners of AMS after rinsing with SAE water were significantly ($P<0.05$) lower compared to those of water supply. Values were markedly decreased after whole system cleaning together with SAE water than that of tap water used.

The number of viable bacteria in samples taken from the inner surface of teat cup liners of AMS was evaluated. The number of viable bacteria in samples from inner surface of teat cup liners of both AMS were significantly ($P<0.05$) decreased after rinsing with SAE water, compared to those with tap water supply. The viable bacteria counts in inner surface of teat cup liners were markedly decreased after milking system cleaning together with SAE water. The ratio of viable to non-viable bacteria was significantly ($P<0.05$) decreased by SAE water supply, compared with that of tap water supply.

DISCUSSION

SAE water was supplied to the AMS and the effects of SAE water on the cleanliness and bacteria counts of the teat liners from both AMS were compared in the study. The chances for bacterial growth and contamination of milk increase under poor hygienic conditions (1,2,6). In order to alleviate such conditions in AMS, this study was focusing on the improvement of hygienic status of the inner surface of the teat cup liners of AMS on dairy farms. To avoid the chemical residues in raw milk which has been strictly regulated, the use of disinfectants is not allowed in many countries, tap water supply is used for flushing and cleaning the teat cup liners of AMS before and after milking. Under such conditions, inner surface of teat cup liners has not been sanitized properly.

The effects of two different automatic teat cleaning devices, cleaning within teat cups vs. cleaning by brushes, were compared of the effectiveness of SAE water. The principle of making SAE water is based on the reaction that 2 molars of hydrochloric acid produce each 1 molar of hydrochlorous acid (HOCl) and hydrochloric acid. The properties concerning the SAE water such as bactericidal activity, safety, stability, influence on metals, costs have been well characterized (4,5). The perishing speed of SAE water against microorganisms is 10 times faster in comparison with that of sodium hypochlorite solution (4). The number of bacterial counts in swab samples taken from inner surface of teat liners of AMS was significantly decreased by using SAE water than that of tap water supply. This finding demonstrated that SAE water reduce the total bacterial counts and proved to be effective to kill bacteria. Not all bacteria were killed, significant reduction of bacteria was found in samples taken from the teat cup liners after both unit flush and enforced system cleaning.

CONCLUSIONS

The use of SAE water for rinsing the teat liners after milking in AMS proved to be effective to sanitize the teat liners. The SAE water is contributable for improvement of hygienic status of AMS system.

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CUBICLE SURFACES FOR GROWING-FINISHING BULLS

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SUMMARY

Different alternatives in cubicle flooring were explored in this study, comparing a control surface with elevated longitudinal slope and a draining surface thus indicating a variation in self cleaning options and properties of surfaces which also act as the lying area of the animals. The effects of these surfaces were assed on the hygiene of the animals, the surfaces and the time budgets the animals. Fifteen 13-15 month old growing-finishing bulls of the Holstein breed were allotted to three groups of five individuals each. The animals were subjected to three different designs of cubicle surfaces, in a Latin square design experiment for about one month per treatment and group. The treatments were: CONTROL (rubber mat and 2% inclination, rubber slatted surface in the back half of the cubicle (DRAINED) and elevated longitudinal inclination of a rubber mat surface (SLOPE 6%). Hygiene of the animals was assessed by a linear scoring representing the percentage of the different parts of the hind legs (the thigh, the hock including the meta-tarsal) covered with urine and fresh and dried manure. Hygiene of the back 80 cm of the cubicle was done by linear scoring of the surface covered with urine and manure together with recordings of dung piles. The time budgets (lying, standing and activity) of the animals were recorded with anima activity loggers. The statistics was done using a GLM model. The contamination of fresh dung on the thighs and the hock including the meta-tarsal of animals were significantly (or had a strong tendency) lower when animals were on the DRAINED or SLOPE 6% floor compared with the CONTROL. However, no differences were found in the assessment of the cubicle surface hygiene. Time budgets revealed no differences between treatments in lying time per day for the different flooring in the cubicles. Animals were generally cleaner in the both the rubber slatted (DRAINED) and elevated inclination (SLOPE 6%) of the surface of the cubicle than CONTROL. The treatments did not seem to affect total time spent lying per day between treatments

INTRODUCTION

An increasing awareness of the importance of the design of the cubicles affects the hygiene, welfare and work consumption as well as work safety of cattle. Concerning growing-finishing bulls kept in cubicles, both the fact that bulls urinate under their bodies and that it is a great risk to enter the animal's compartment to manage the surface of the lying area which makes demands on the self cleaning properties of the surfaces. Additionally, floors in cubicles or tie-stall must be solid for 80% of the area as regulated by provisions of the Swedish animal welfare act. But new developments in flooring can meet requirements of the animals regarding softness, reducing injuries and hygiene (Schulze Westerath *et al.,* 2007; Friedl *et al.,* 2004; Hultgren, 2001).

A control cubicle rubber surface was compared with a rubber surface where the back half allowed drain and with a surface which had an elevated longitudinal inclination. The effects of these surfaces were assed on the hygiene of the animals, the surfaces and the time budgets the animals. The aim of the trial was to generate an understanding of how floors in cubicles can be modified in order to improve hygiene of the animals without compromising welfare.

MATERIAL AND METHODS

Three different floor surfaces in cubicles were studied: CONTROL, which was standard rubber mat and a longitudinal slope of 2 %, increased slope of 6 % (SLOPE 6%) including a standard rubber mats and slatted rubber floor in the back half of the cubicle surface (DRAINED)which was a rubber mat with 40 X 200 mm slots and 125 mm slats. Some bedding, saw dust, was provided once a week.

Fifteen 13-15 month old growing-finishing bulls of the Holstein breed were allotted to three groups of five individuals each. The animals were subjected to the three different designs of cubicle surfaces, in a Latin square design experiment for about one month per treatment and group. The animals were kept together for about a month in cubicles with standard surface before being allotted to the treatments. Formation of the treatment groups was done by blocking the animals by age, three by three, prior to random allotment. The composition of groups is given in table 1.

Table 1: The average age (months) of the growing-finishing bull at the start of the experiment

	Mean age, months	Standard Deviation	Minimum age	Maximum age
Group 1	13.3	0.64	12.6	14.2
Group 2	13.1	1.06	11.1	14.2
Group 3	14.1	0.51	13.3	14.8

The animals were accustomed to each of the surfaces for a minimum of two weeks before any registrations were made

Animal hygiene

Hygiene of the animals was scored separately on the different parts of the hind legs (the thigh, the hock including the meta-tarsal) by linear scoring (0-100), representing the percentage covered with urine and fresh and dried manure respectively. Both sides of the animal was scored and then a weighed score was recorded.

Cubicle surface hygiene

Hygiene of the back 80 cm of the cubicle was done by linear scoring of the surface covered with urine and manure separately (0-100). Recordings of dung piles were done by recording numbers of piles of about 10 cm in diameter. Included were also smaller piles of dung, recorded as parts of a full pile of dung, e.g. 0.5 or larger, e.g. 2.5.

Behaviour

The time budgets (lying, standing and activity) of the animals were recorded with animal activity loggers, IceTags (IceRobotics, Edinburgh, Scotland). Only 3 loggers were available during the first period which only allowed data from 2 full days to be used. But during the following experimental periods more loggers were available wich allowed data from seven days per animal to be used. Means for one full 24 hour day was calculated. One logger malfunctioned which was found during period 3 which means that the values of one animal was left out for period 2.

Statistics

The data was compiled and comparison was done using a GLM model (MiniTab) with the following model:

$$Y = \mu + A_i + P_j + B_k + C_l + E_m$$

Where
Y was the overall mean
μ was the mean
A_i was the effect of the i treatment
P_j was the effect of period
B_k was the effect of animal or cubicle number
C_l was the effect of group (cubicle surface hygien)
E_m was the residual

RESULTS

The contamination of fresh dung on the thighs and the hock including the meta-tarsal of animals were significantly (or had a strong tendency) lower when animals were on the rubber slatted or elevated inclination cubicle floor compared with the control (Table 2.)

Table 2: Hygiene linear scores on thighs on growing-finishing bulls on different cubicle flooring. LS-means ± SE

	Urine	Faeces	Dung, dried
CONTROL	8 ± 1,3959	8^{a} ± 1,2642	1^{a} ± 0,1975
SLOPE 6 %	4 ± 1,3959	$4^{(b)}$ ± 1,2642	0^{b} ± 0,1975
DRAINED	5 ± 1,3959	1^{b} ± 1,2642	0^{b} ± 0,1975

Columns with different superscript differ significantly (P<0.05); Superscript within brackets differ with a tendency (P<0.1)

The hocks and the meta-tarsus were cleaner when animals were in the SLOPE 6% and DRAINED cubicle surfaces than in the CONTROL (Table 3).

Table 3: Hygiene linear scores on hocks and meta-tarsus on growing-finishing bulls on different cubicle flooring. LS-means ± SE

	Urine	Faeces	Dung, dried
CONTROL	2^{ab} ± 0.6988	10^{a} ± 1.4087	1 ± 0.3158
SLOPE 6 %	3^{a} ± 0.6988	5^{b} ± 1.4087	1 ± 0.3158
DRAINED	1^{b} ± 0.6988	2^{b} ± 1.4087	0 ± 0.3158

Columns with different superscript differ significantly (P<0.05).

No differences were found in the assessment of the cubicle surface hygiene.

Time budgets revealed no differences between treatments in lying time per day being 64.8%, 67.1% and 64.6 % for CONTROL, SLOPE 6% and DRAINED flooring in the back of the cubicle.

DISCUSSION

The experiment gave a good indication of importance of cubicle for the hygiene of the animals. Differences appeared mainly on the animals and less on how the floor base looked like. It was peculiar that it was not possible to see differences in hygiene on the surfaces reflected in the hygiene in the animals which we have previously seen in studies on dairy cows. There was a large variation between scores of the floor surface hygiene which may have contributed to difficulties in finding statistical differences. However, numerical differences were indicated in the data. Of course, more observations, i.e. more animals in the study and longer registration periods will improve the statistics.

Because of the short study periods and the experimental set up in which all animals were subjected to all treatments, it was not considered useful to study e.g. swelling and hairlessness on hocks and legs. At slaughter, these injuries were minor.

It would be desirable to follow animals from an early age until they go to slaughter in the cubicles floor surface alternatives tried in this study in order to evaluate the long term impact. Also, further evaluation of self cleaning properties of cubicle floor surfaces has to be investigated.

CONCLUSIONS

Animals were generally cleaner in the both the rubber slatted and elevated inclination of the surface of the cubicle. The treatments did not seem to affect total time spent lying per day between treatments

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VACCINATION INDUCES EFFICIENT AND SAFE PROTECTION AGAINST HISTOMONOSIS IN TURKEYS

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INTRODUCTION

Histomonas meleagridis is the aetiological agent of Histomonosis, a parasitic disease in poultry (Tyzzer, 1920). Prevention or therapy is no longer possible within the EU (CEC, 1995; CEC, 2002) and very much limited in the USA because all effective chemotherapeutics are banned to address concerns with regard to consumer protection. As a consequence very serious outbreaks are reported all over Europe, some of them with high losses of birds (Esquenet *et al.*, 2003; Lévêque, 2007). Every possible prophylaxis and therapy would be welcome in order to minimize the number of outbreaks. A recently evaluated aminoglycoside antibiotic paramomycin sulphate was critically evaluated by the European Food Safety Authority (EFSA) due to the selection for antimicrobial resistance (EFSA, 2009). Therefore, efficient vaccination would have the highest priority considering all the above mentioned constraints with the licensing of drugs. In a few previous studies the application of an inactivated vaccine was found non-effective (Hess *et al.*, 2008; Bleyen *et al.*, 2009) confirming earlier results that the transfer of immunoglobulins won't protect the birds (Clarkson, 1963). Contrary to this we were able to demonstrate recently that turkeys are fully protected, following vaccination with an *in vitro* developed live vaccine (Hess *et al.*, 2008). These promising results promoted several studies concentrating on efficacy and safety of this new vaccine.

ANIMALS, MATERIALS AND METHODS

A set of experimental infections was performed to address the different issues of vaccine development. For this turkeys were vaccinated at different ages and challenged at different time points to investigate the onset of immunity. Various newly developed diagnostic tools like immunohistochemistry, PCR and ELISA were applied in these experiments to investigate the effect of virulent and attenuated histomonads. Most of these techniques were developed recently in the context of a broader research project. All trials were discussed and approved by the institutional ethics committee and licensed by Austrian law. The detailed description of every experiment can be found in the publications listed in the references.

RESULTS AND DISCUSSION

Following micromanipulation to obtain a highly defined culture, *H. meleagridis* was gradually attenuated through continuous *in vitro* passaging and evaluated *in vivo* at different stages (Hess *et al.*, 2008). Whereas in the initial experiments birds were vaccinated cloacally it could be demonstrated later on that oral vaccination is possible (Liebhart *et al.*, 2010). Moreover, the route of vaccine application had no influence on production data, like body weight gain. In all these experiments none of the vaccinated and non-challenged birds showed any adverse clinical signs. Vaccinating day-old birds was also possible, even though it took 4 weeks to achieve complete protection, as some of the birds challenged 2 weeks post challenge died.

Immunohistochemistry and PCR confirmed the presence of attenuated histomonads in the caecal lumen of vaccinated turkeys and chickens which did not show clinical signs or lesions (Liebhart *et al.*, 2011). This underlines the safety of the newly developed vaccine. Altogether, the efficacy and safety of a live vaccine to protect poultry against histomonosis was shown in a series of experiments which paved the way to proceed with this innovative approach.

CONCLUSIONS

For the first time it could be demonstrated that live vaccination based on clonal cultures is a new and suitable concept to prevent fatal histomonosis in turkeys, a strong demand to cover aspects of animal welfare accordingly. The avoidance of any residues in meat products obtained from such animals is an important issue in the area of veterinary public health. Furthermore, vaccination complies fully with the EU animal health policy "Prevention is better than cure".

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EXPERIMENTAL COCCIDIOSIS INDUCED IN GUINEA FOWLS FOR SCREENING OF COCCIDIOSTATS

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SUMMARY

An experimental model of induction of clinical coccidiosis was developed for guinea fowls in order to test the efficiency of anticoccidial feed additives used in broiler production. The infected birds had a strong decrease in weight gain and developed clinical signs with morbidity and diarrhoea, compared to uninfected birds. The anticoccidial feed additives tested were salinomycin, diclazuril and monensin. In this study, and with the *Eimeria* isolate used, salinomycin was fully efficient to maintain growth performance, but it did not block completely parasite development, and a few signs were observed. Monensin was partially efficient on growth, but marked clinical signs were observed. Furthermore, a high dose of monensin without coccidial infection induced growth lowering, compared to birds receiving no additive. Diclazuril was not efficient at all on the parasite isolate tested, with no enhancement of growth or clinical signs compared to infected birds receiving no additive. Salinomycin seems to be the best candidate for coccidiosis control in view of the results obtained in the present study.

INTRODUCTION

Coccidia are protozoan parasites frequently encountered in guinea fowl farms. They develop in the gut and can cause severe economic losses and diseases with digestive signs in the flocks. Four species of coccidia infecting guinea fowls have been described. They belong to the genus *Eimeria: Eimeria grenieri* [7], *Eimeria numidae* [5], *Eimeria gorakhpuri* [2] and *Eimeria khobahensis* [1]. *Eimeria grenieri* appears to be the most frequent species, and it is also the best known. Two articles precisely describe its morphology, its life cycle and its pathogenicity [4,7]. The other *Eimeria* species remain uncertain and difficult to identify as no other article confirms clearly their existence.

Coccidiosis in guinea fowls can be very severe, sometimes causing death, but typical gut lesions are not described, unlike the coccidia infecting chickens. Liquid content is described, and sometimes caseum can be found in the caeca but these findings are inconsistent. Thus, coccidiosis outbreaks are uneasy to diagnose in the field, and they are mainly based on poor health and high quantities of oocysts in the gut or in the faeces.

Guinea fowl producers have no longer anticoccidial feed additives at their disposal in the European Union since 2002 (Clinacox has just been authorized in March 2011, but it is not used yet in the field). Only treatments are available: they can limit economic losses but they do not prevent the coccidial development and the disease.

In order to investigate the pathogenicity of coccidia infecting guinea fowls and to test the efficiency of methods of control of coccidial development, we developed an experimental model of coccidiosis in guinea fowls. Using this model, we investigated the efficacy of three coccidiostats with a field isolate of coccidia collected in 2009.

MATERIAL AND METHODS

A battery model was developed, based on the model used for battery anticoccidial sensitivity tests for chickens. 400 keets were taken at a commercial hatchery and placed at one day of age in batteries with controlled temperature, light and filtered air. They had free access to adapted feed and water. At the age of 19 days, birds were individually identified with a wing band and weighed. 192 birds were kept to form eight groups of 24 birds with homogenous weight. They were placed the same day in cages with four birds per cage. Two days later, they were orally infected with a suspension of sporulated oocysts using an oesophagus cannula. Two groups served as control: uninfected (NINS) and infected (INS). Five groups were infected and received the following coccidiostats: salinomycin at the two doses of 50 (IS1) and 60 (IS2) mg/kg of feed, diclazuril at the unique dose of 1 mg/kg (IS3), and monensin at the two doses of 50 (IS4) and 70 (IS5) mg/kg. The last group (NIS6) received monensin at 100 mg/kg of feed but was not infected. Zootechnical (weight gain and FCR) as well as pathological criteria (morbidity from D+4 to D+7 post-infection, faeces aspect from D+4 to D+7 post-infection, oocyst output from D+4 to D+7 post-infection, gut lesions on D+8 after infection) were followed up. Morbidity was evaluated according to the following scale: 0: normal behaviour – 1: ruffled feather, especially on the neck – 2: ruffled feathers and apathy – 3: marked apathy – 4: total prostration. Faecal degradation was measured according to the following scale: 0: normal droppings – 1: soft droppings – 2: very soft but not diarrhoeic – 3: diarrhoeic but no mucus – 4:

watery with mucus. Birds were weighed on D+8 and humanely euthanasied before examining guts. Feed intake was measured globally for each group, and feed conversion ratios (FCR) were calculated using weight gain on the period.

The field isolate intended for the trial was collected in Western France in 2009 and maintained by regular passages on guinea fowls. Before the trial, the pathogenicity of the *Eimeria* isolate was evaluated on birds receiving doses of 50,000, 100,000, 150,000 and 200,000 oocysts. Behaviour and droppings were followed up in order to use a sufficient amount to cause clinical signs, but not too much in order to preserve bird welfare. An infective dose of 100,000 oocysts per bird was defined.

RESULTS

Results of weight gain and FCR are presented in table 1. The infection led to a significant decrease of growth parameters in the infected control group INS compared to the uninfected group NINS. Salinomycin prevented growth slow down (IS1 and IS2) and there was no statistical difference with group NINS. Diclazuril (group IS3) was ineffective on this isolate: weight gains were not significantly different between groups INS and IS3. Monensin led to an improvement in growth in infected groups, which was intermediate (significant differences with group NINS and with group INS). In the group NIS6, monensin had a negative effect on growth when used at 100 mg/kg of feed with no coccidial infection (significant difference between NINS and NIS6). FCR were in accordance with weight gains. It ranged from 2.63 to 2.75 in the groups NINS, IS1 AND IS2. In the groups receiving monensin, it ranged from 3.58 to 3.85. It was very high, with values of 5.93 and 6.86 in the groups INS and IS3.

Pathological criteria are summarized in table 2. No death occurred during the study. Birds in groups INS and NIS6 had normal behaviour during the observation period. In the other groups, morbidity appeared on D+5 post–infection. The highest morbidity scores of 3 were observed on day 5 or day 6 in groups INS, IS3, IS4 and IS5. In the IS5 group, the score was 0 on the other days. In groups INS, IS3 and IS4, the score was 2 the two other days. In the IS1group, morbidity score was only 2 but from D+5 to D+7. Finally, in the IS2 group, morbidity was noted 1 on D+5, and 0 the following days. Faecal aspect was not altered in groups NINS and NIS6. In groups IS1, IS2 and IS5, alteration was slight, from soft to very soft, but not diarrhoeic. In the IS4 group, diarrhoea occurred on D+4 and D+5, but improvement was observed on D+6 and D+7. Finally, in groups INS and IS3, faeces were diarrhoeic with mucus during the four days of observation.

Oocyst output was detected on D+4 in all infected groups, but it peaked on D+5 except for the INS group where it peaked on D+6. Global oocyst output was reduced of more than 50% only in groups IS1 and IS2, but high levels remained in these groups.

Gut changes were investigated on all birds on D+8. Jejunum and ileum colour was normal in groups NINS and NIS6, and about half of the birds had pale jejunum mucosa in groups IS1, IS2, IS4 and IS5. Most of the birds had a pale small intestine in groups INS and IS3. Caecal contents were normal in all birds in group NIS6. In groups NINS, IS1, IS2 and IS5, about half of the birds had liquid caecal contents. More than two third in group IS5 had liquid caecal contents, and all the birds in groups INS and IS3 had liquid content, sometimes with caseum in the caeca.

DISCUSSION

The *Eimeria* isolate used at the dose of 100,000 oocysts per bird was pathogenic and induced a weight gain decrease above 60% in the INS group. Clinical signs were also strongly marked: morbidity from D+5 to D+7 and watery diarrhoea from D+4 to D+7. However, no typical lesion was observed: pale intestine was not observed in all birds, and liquid caecal content was not typical, although observed on all birds in the group INS, as it was also observed in uninfected birds in group NINS.

Salinomycin gave the best results of weight gain and FCR, with no significant difference on weight gain with the uninfected birds. The two doses tested were efficient, but with the lower dose of 50 ppm, clinical signs were higher than with 60 ppm. In the two cases, coccidial development was not completely inhibited, but the economic impact was totally erased.

Diclazuril showed no effect at all on the coccidia isolate used here. However, we performed a similar test in 2003 [6] and the results with diclazuril were the best obtained, with no difference compared to uninfected birds. This result suggests that there are sensitive and resistant populations of coccidia in the field. The isolate used here came from a farm where guinea fowls are reared continuously, and where toltrazuril is frequently used. The resistance observed in this study might be explained by description of cross-resistance between toltrazuril and diclazuril [3]. Monensin showed some toxicity at the dose of 100 ppm without infection (NIS6). Thus, it is difficult to conclude on the effect on coccidia at 50 and 70 ppm: an effect on coccidia is obvious as weight gain and FCR are improved, but the benefit might have been decreased due to a possible toxicity also at those doses. In order to know better the effect of monensin, groups given 50 and 70 ppm but uninfected should have been included in the study. However, the interest of this molecule for guinea fowls is doubtful due to its toxicity at 100 ppm.

CONCLUSION

Coccidiosis was successfully induced in the infected control group, validating our model for screening candidates to the control of these parasites. The results obtained with this isolate showed that salinomycin can be

considered for the prevention of coccidiosis. Diclazuril was not efficient in this case, but a previous study on a different isolate proved its interest for coccidiosis control in guinea fowls. Monensin had an effect on coccidial development consequences, but its negative effect on growth at the dose of 100 ppm suggests a toxicity for guinea fowls which limits its interest.

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Table 1: body weight gain and feed conversion ratio (FCR)

Group	NINS	INS	IS1	IS2	IS3	IS4	IS5	NIS6
Weight gain	166[a*]	61 [c]	173 [a]	157 [a]	58 [c]	113 [b]	105 [b]	123 [b]
FCR	2.75	5.93	2.63	2.71	6.86	3.58	3.85	3.78

Weight gain was measured between D-2 prior to infection to D+8 post-infection
*: values with same letter are not significantly different
For group identification, refer to material and methods

Table 2: mortality, morbidity and faecal aspect changes

Group	NINS	INS	IS1	IS2	IS3	IS4	IS5	NIS6
Mortality	0	0	0	0	0	0	0	0
M D+4	0	0	0	0	0	0	0	0
M D+5	0	3	2	1	2	2	0	0
M D+6	0	2	2	0	3	3	3	0
M D+7	0	2	2	0	2	2	0	0
F D+4	0	4	2	2	4	3	1	0
F D+5	0	4	1	1	4	3	2	0
F D+6	0	4	1	1	4	2	1	0
F D+7	0	4	2	1	4	1	0	0

For group identification, refer to material and methods
M: morbidity – scoring is given in material and methods
F: faecal aspect – scoring is given in material and methods

Detection of oxytetracycline (OTC) residues in chicken exposed to cadmium as stress factor

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ABSTRACT

A total number of 450 Ross chicks one day old were used and divided into two equal groups A and B, which were fed on OTC free ration and tap water for 31 days. At the age of 31 days, birds of group A were divided into three sub-groups each contained 75 birds: Sub-group A1 was kept as control while A2 and A3 were exposed to OTC 3 and 6 g/l in drinking water for 5 successive days respectively. Birds in group B were exposed to OTC free ration and tap water containing 0.1 ppm cadmium chloride for 31 days. At the day 31, they were divided into three sub-groups (B1,B2,B3) each contained 75 chicks. Sub-group B1 was kept as positive control while B2 and B3 were exposed to 3g and 6 g OTC/L in drinking water for 5 successive days respectively. Cd administration was continued till the end of experiment. At the age of 37th days the birds were slaughtered, pectoral, thigh muscles and liver samples were taken every 48 hours till the end of experiment. Samples were kept in deep freeze at -20°C till used for OTC and cadmium analysis. The total reduction rate of OTC residues in the pectoral muscles was 98.3% in group A2; 70.26% in group A3; 95 % in group B2 and 71.1% in group B3. In thigh muscles it was 64.8% in group A2 ;79.9% in group A3 ; 82.9 % in group B2 and 48.% in group B3. The total reduction rate in the livers was 48.4% in group A2; 56.4% in group A3; 97.9 % in group B2 and 35.3% in group B3. The total reduction rate of OTC in muscles and livers was evident in the pectoral muscle at 11- 21 days post exposure and was within the permissible limits. The significance of these results will be discussed. In conclusion, the protection of human consuming animal products (meat and liver) depends on the assessment of residual withdrawal duration of drugs and the use of recommended hygienic regime.

INTRODUCTION

Antibiotics are used in food producing animals for treatment or prevention of diseases and for increased production performance or increased efficiency of use of food consumed by animal for growth, product output or modifying the nutrient composition of animal product. Tetracyclines (TCs) are large family related compounds developed after the sulfonamides and are widely used therapeutically in humans, animals and fish.

TCs such as oxytetracycline (OTC), tetracycline, chlortetracycline, and doxycycline, have for decades continued to play an important role in veterinary medicine and feed additives because of their broad spectrum activity and economical advantages **(Cherlet et al.,2003).**

In poultry industry, too much antibiotics were used that have a diverse effect on the health of poultry with possibility that human could getting much antibiotics residue in poultry tissues. OTC is the most common used antibiotics in Assiut poultry farms.

Cadmium has been shown to suppress antibody formation in animals and has been epidemiologically linked to respiratory cancer **(Koller et al., 1975).** It effects on a variety of tissues and biological system and has been associated with such diverse maladies as hypertension and carcinogenesis. In several epidemiological studies cadmium exposure in the work place has been linked to carcinogenesis in various tissues including the lung, prostate, kidney and stomach **(IARC, 1993 and Waalkes, 2000).** Cadmium induced renal lesions that are not reversible, which may interfere with OTC clearance leading to high residual levels in chicken tissues.

Brander et al. (1991) reported that OTC could be used in treatment and prophylaxis of infectious diseases of poultry either in the feed by dose of 10 – 60 g/100 kg of feed. Also, oxytetracycline antibiotic could be used in the drinking water by dose of 0.1 – 0.3 g/liter.

The present study was aimed to study the withdrawal time of OTC residue in broiler chickens tissues under the stress effect of cadmium presence in their administered water and both therapeutic and overdosed of OTC.

MATERIALS AND METHODS

A-Materials

1-Birds: Four hundred and fifty Ross chicks of both sexes (one day old) were used. These chicks were housed on floor, administered feed with balanced OTC free ration and drinking tap water. The routine system of feeding, watering, warming, ventilation and vaccination was applied allover the period of experiment.

2-Drug: Oxyteteracycline hydrochloride powder (100%) concentration, water soluble was obtained from (Pharco Company, Egypt).

3-Chemicals: a- Cadmium chloride (Merck). **b-** Natt and Hericks solution, prepared as follow: NaCl 3.88g, Na_2So_4 2.5g, $Na_2HPO_4.12H_2O$ 2.91g, KH_2PO_4 0.25g, Formalin (37%) 7.5 ml and Methyl violet 2B 0.10g (El-salam for chemical Industries).In the order listed, the above ingredients are dissolved and brought to volume with distilled water in a 1000 ml volumetric flask. The solution is allowed to set overnight and then filtered. The final pH should be 7.3. **c-** Sodium nitrate (El-Salam for chemical Industries, Egypt). **d-** HCL (El-Salam for chemical Industries, Egypt). **e-** Sodium hydroxide pure 96% (El-Salam for chemical Industries, Egypt). f- Nitric acid 55% ((El-Salam for chemical Industries, Egypt).

4-Experimental design: Birds were divided into two groups (A and B), each contain (225) broiler chickens.

a. Group (A): Birds were fed on OTC free ration and drinking tap water from day one till the end of experiment. After 31 days age, these birds were divided into three sub-groups (A1, A2 and A3) each contained 72 birds and treated as follow:
1-Sub-group A1: Left as control till the end of the experiment.
2-Sub-group A2: Given therapeutic dose (3g OTC/L water) for 5 successive days.
3-Sub-group A3: Given 6g OTC/L (Double therapeutic dose) for 5 successive days.

b. Group (B): These birds were fed the same OTC free ration and tap water as group A in addition to 0.1 ppm cadmium chloride, which added to drinking water from day one till the end of experiment. These birds were also divided into three sub-groups (B1, B2 and B3) after 31 days age; each contains 72 chicks and treated as follow:
1-Sub-group B1: Kept as control for group B.
2-Sub-group B2: Given 3g OTC/L water (therapeutic dose) for 5 successive days).
3-Sup-group B3: Given 6g OTC/L (Double therapeutic dose) for 5 successive days.

6-Sampling: Before administration of OTC, 9 chickens were slaughtered from group A and B, pectoral muscles, thigh muscles and liver were obtained. After stopping of OTC administration, 6 chickens from all groups (A1, A2, A3, B1, B2 and B3) were slaughterd day after day at age of 37^{th} days till the end of experiment and samples from pectoral muscles, thigh muscles and liver were obtained.

B-Methods

1- Estimation of OTC residue by HPLC:-
a. Extraction of samples: Samples were extracted according to the method of **Ueno et al. (1999)** as follows: One g of muscle or liver sample was put into a 10 ml centrifuge tube and 5 ml of cold 30% methanol containing 0.5% EDTA was added. The sample was homogenized and centrifuged at 4000 rpm for 5 min. The supernatant was filtered through a syringe filter unit (0.2 µm). Aliquot samples (20 µL) of the filtrate were injected into the chromatograph.

b. Measurement of OTC: OTC residues were measured by HPLC (Perkin-Elmer series 200, USA) according to the conditions listed by **Martinez and Shimoda (1988)** as follows: **(1) Conditions:** LC column: C18 (10µm Phenomenex, USA) reversed - phase deactivated silica packing, 47mm×250mm. LC mobile phase: pH 2.0, methanol- acetonitrile-0.01 M oxalic acid (aqueous) (1+ 15+ 6.5). Flow rate: 0.75ml/mint Detector: UV Wave length: 360 nm**.** Operating conditions: Injection volume 20 µl; runtime 10 min, manual injector. **(2) Calculation:** Measure peak area for OTC standard solutions and for test samples. Prepare standard curve of OTC standard solutions concentrations versus peak areas by using data from the same standard solutions. From measured peak areas of test samples, calculate OTC concentrations from regression equation as follows**:** $y = mx + b$**,** Where: y = peak area x = OTC concentration (jig/g) and m =slope of curve b = intercept of y.

2- Statistical analysis: Firstly, general linear model Analysis of Variance (GLM-ANOVA) was performed on the pooled data using SPSS 10 software package (SPSS, Chicago, IL) according to **Borenstein et al. (1997**). The Least Squares Means (LSM) were compared with comparison-wise standard error rate after significant F-tests to assess the all-mean values

RESULTS

Oxytetracycline (OTC) residues (ppm)

Means with different superscrept letters in the same raw within each group are significantly differ at $P<0.05$.

From the present study the recovery of oxytetracycline was 80% while in the liver was 80.06%.

Table 1: Mean concentrations of OTC residues (ppm) in pectoral muscle, thigh muscle and liver of the studied broiler chickens (n=6).

Gr.	Pectoral Muscle			Thigh M.			Liver		
	1day	11days	21days	1 day	11days	21days	1 day	11days	21days
A2	1.20 [a] ±0.041	0.03[b] ±0.002	0.02[b] ±0.001	1.22 [a] ±0.052	0.48 [b] ±0.016	0.43 [b] ±0.023	38.29 [a] ±1.224	11.76 [b] ±2.110	8.00 [c] ±0.642
A3	1.14 [a] ±0.036	0.34 [b] ±0.011	0.34 [b] ±0.017	3.43 [a] ±0.051	1.15 [b] ±0.021	0.69[c] ±0.0426	182.74[a] ±4.665	115.5 [b] ±5.661	79.74[c] ±3.642
B2	1.20 [a] ±0.022	0.54 [b] ±0.009	0.06 [c] ±0.008	2.10 [a] ±0.063	0.64 [b] ±0.009	0.36 [c] ±0.012	68.39 [a] ±3.516	42.99 [b] ±2.335	1.43 [c] ±0.013
B3	1.21 [a] ±0.029	0.52 [b] ±0.014	0.35 [c] ±0.021	10.71[a] ±0.241	6.86 [b] ±0.163	5.53 [b] ±0.149	224.0 [a] ±8.115	161.7 [b] ±6.542	144.9 [c] ±8.021

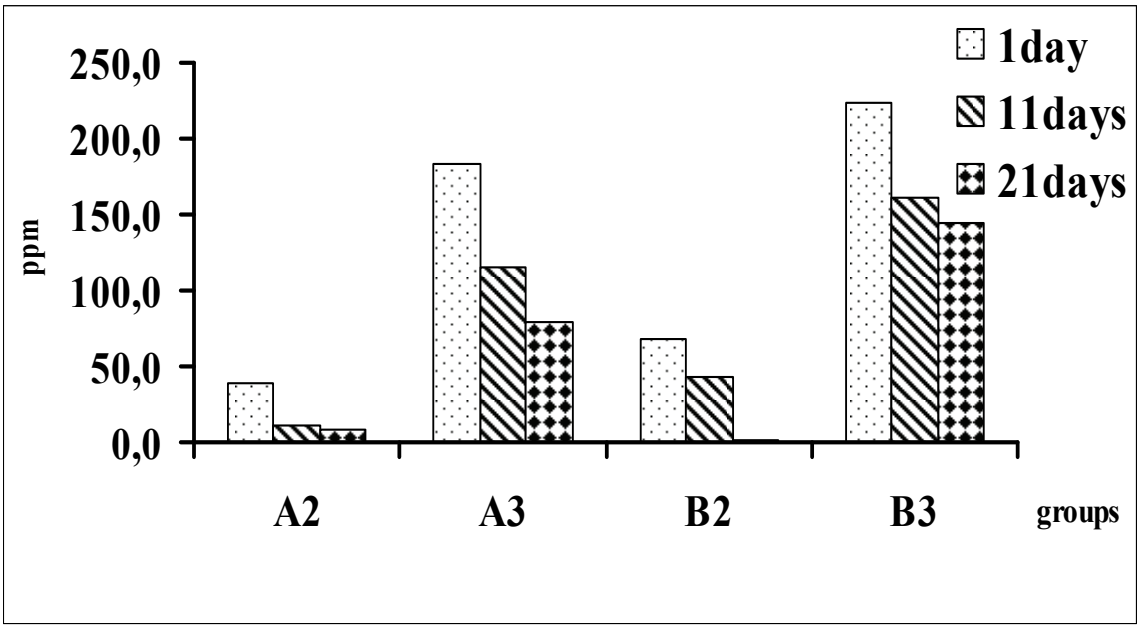

Figure 1: Concentrations of OTC (ppm) in the pectoral muscle of the broiler chickens

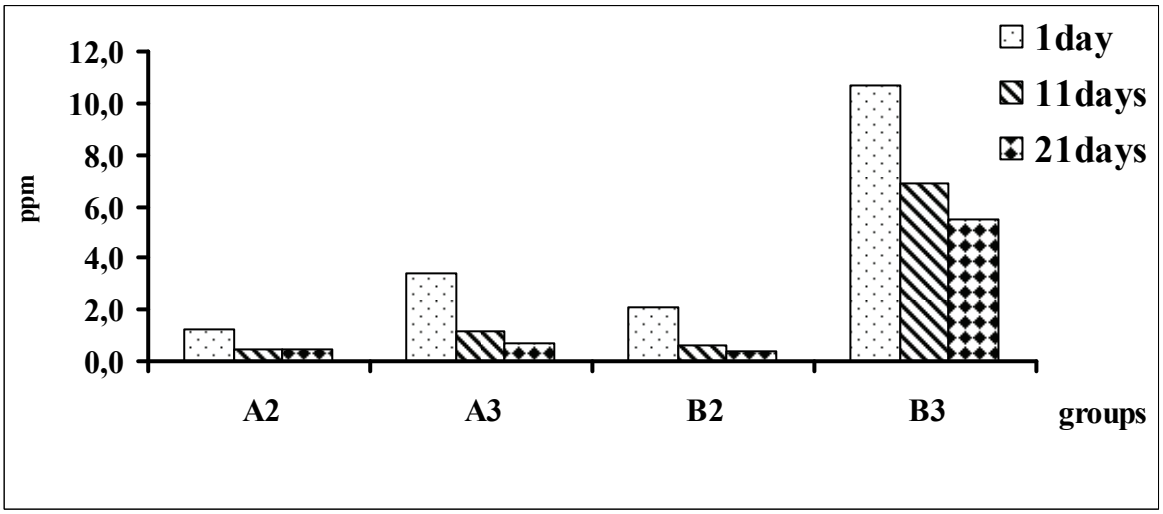

Figure 2: Concentrations of OTC (ppm) in the thigh muscle of the broiler chickens

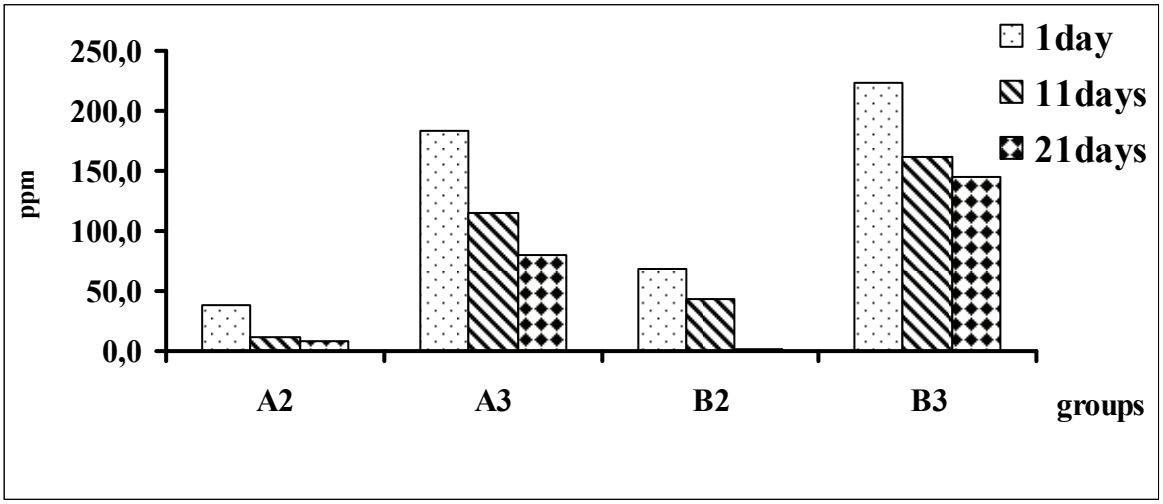

Figure 3: Concentrations of OTC (ppm) in the liver of the broiler chickens

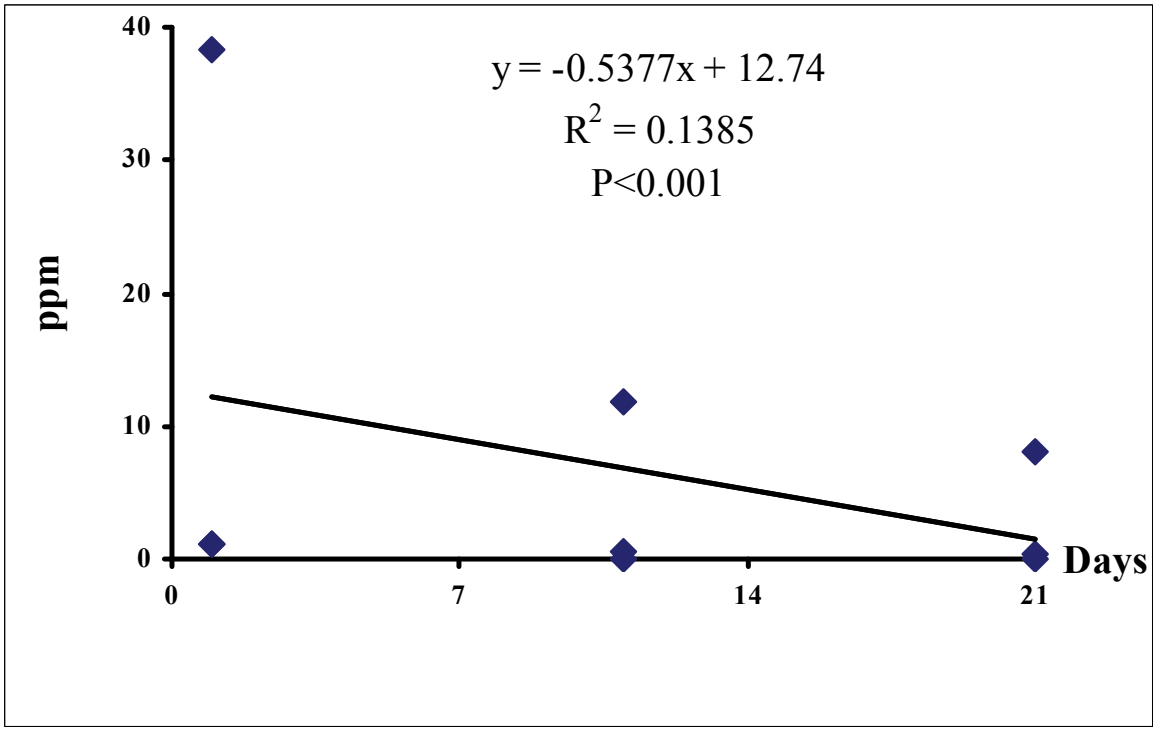

Figure 4: Linear regression analysis, regression equation, R^2 value and the level of significance (P-value) of the OTC withdrawal in relation to time in group A2

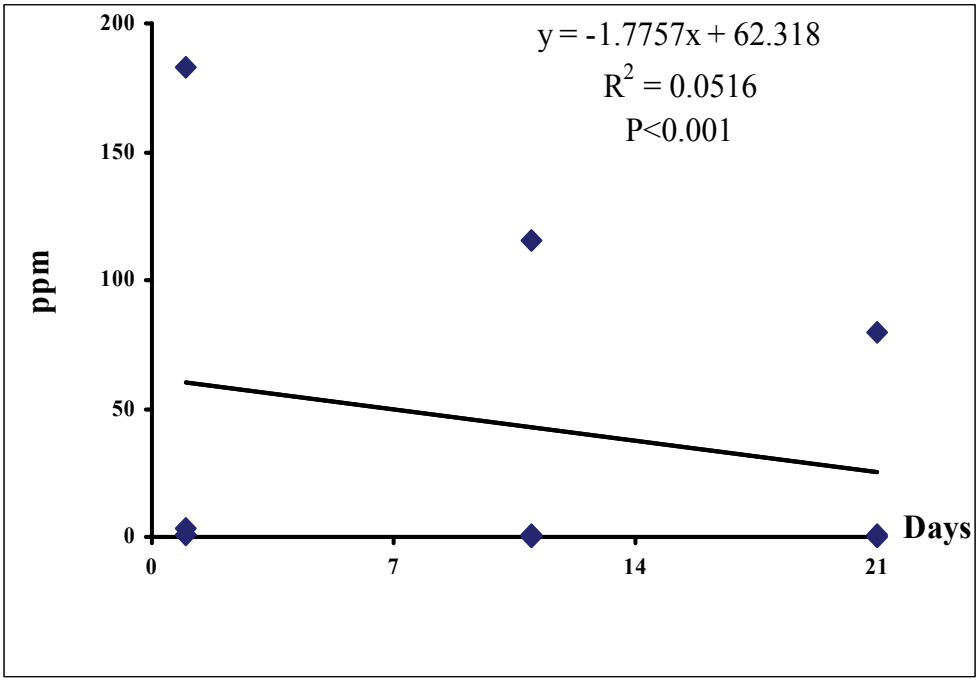

Figure 5: Linear regression analysis, regression equation R^2 value and the level of significance (P-value) of the OCT residues in group A3

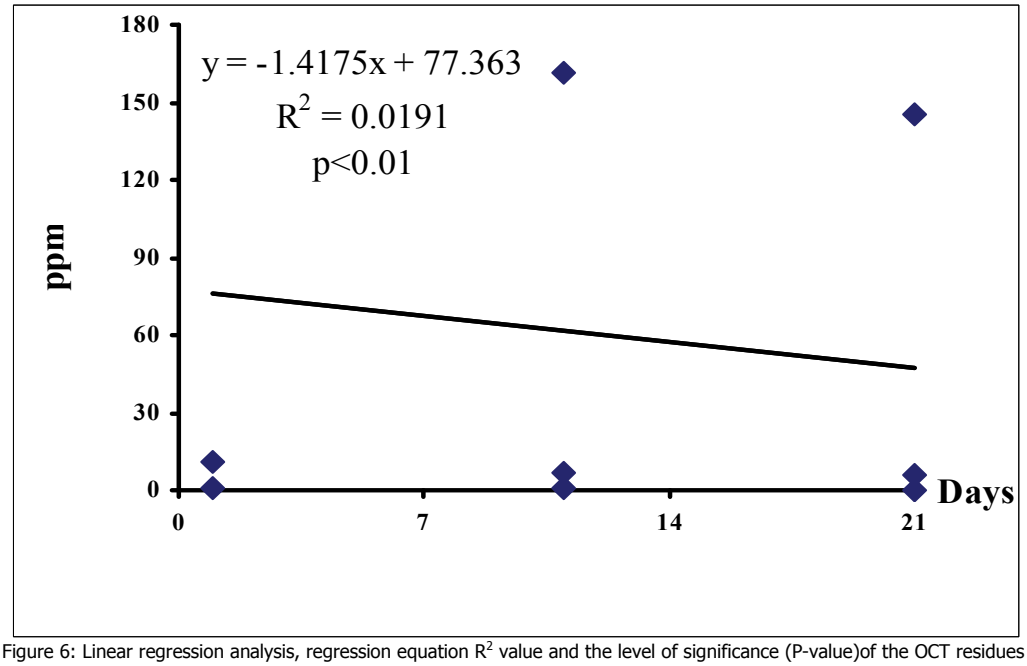

Figure 6: Linear regression analysis, regression equation R^2 value and the level of significance (P-value)of the OCT residues in group B3

The regression analysis, curvilinear data and regression equation showed a significant increase in OCT withdrawal in all treated groups with time. In group A2, the regression equation was y = -0.5377x + 12.74 and regression factor (R^2) = 0.1385 which was highly significantly correlated with time (P<0.001). In group A3, the regression equation was y = -1.7757x + 62.318 and regression factor (R^2) = 0.0516 which was less than that in group A2 but it was highly significantly correlated with time (P<0.001). In group B2, the regression equation was y = -1.164x + 25.883 and a powerful regression coefficient between OTC withdrawal and time than in groups A2 and A3 was denoted by the regression factor (R^2) = 0.1628 (P<0.001). In group B3, the regression equation was y = -1.4175x + 77.363 and regression factor (R^2) = 0.0191 (P<0.01) which was the lowest value between the other treated groups.

DISCUSSION

Broiler chickens constitute important source of animal protein in Egypt, however chicken industry facing numerous number of field problems as infectious diseases and heavy metal toxicity. Tetracycline like oxytetracycline

plays an important role in veterinary medicine and feed adititives because of their broad-spectrum and their economical advantages. Oxytetracycline was approved by the US food and drug administration (FDA) for use in animal feeds to control bacterial infections (Huang et al., 1997).

Oxytetracycline (OTC) is the most common antibiotic used in assiut poultry farms. The uncontrolled use of antibiotics in poultry industry has adverse effect on health of poultry. The possibility that human could get antibiotic residues in poultry tissues when some feed animal drugs can be purchased over the counter without veterinary prescription. Drug residues in food products of animal origin are an important consideration for consumers.

The goal of the present study was to determine the relation and what is the kind of this relation between the interactions of different doses of OTC (therapeutic and over dose) and 10 times of the permissible limit of cadmium in water (0.1 ppm).

1. Recovery rate of oxytetracycline residues in pectoral, thigh muscles and liver in broiler chickens

The present study revealed that the recovery rate of OTC residues in broiler muscles as well as liver spiked with different OTC concentrations was 80%. Such result was in agreement with those recovery rates obtained by Furusawa (1999) and Hussien (2001) in broiler chicken muscles. However, many investigators such as Martinez and Shimoda (1988), Mulders and Lagemeet (1989), Wu et al. (1994), Kawata et al. (1996) and Ruyck et al. (1999) reported lower recovery rate rather than that of the present results. On the other hand, Fujita et al. (1997) and Huang et al. (1997) obtained higher recovery rate where they recorded a rate of 90.4 and 92.9 %, respectively.

2. Withdrawal periods of oxytetracycline residues in broiler pectoral, thigh muscles and liver

The present study showed that the withdrawal period of OTC (Tables 1 and figures 1-6) from pectoral, thigh muscles and livers decreased significantly in all treated groups A2, A3, B2 and B3. Concerning withdrawal days of OTC residues in broiler pectoral, thigh muscles and liver it decreased from 1st day to 11th day significantly in pectoral muscle (1.2 ± 0.04 to 0.03± 0.002 ppm) in group A2 and in group A3 (1.14± 0.036 to 0.34± 0.011 ppm), in group B2 (1.20± 0.022 to 0.54± 0.009 ppm), in B3 (1.21± 0.029 to 0.52± 0.014 ppm) as well as in thigh muscles and liver (Table 57).However, this recorded decrease in pectoral muscle of group A2 was within the maximum residual limits (MRLs=0.1 ppm) by EU which is in pectoral muscle only of group A2.

At 11th to 21st days the decreased in level of OTC residue in tissues of pectoral, thigh muscles and liver of all studied groups not within the (MRLs) except in the pectoral muscle in group A2 (0.02± 0.001 ppm) and in group B2 (0.06± 0.008 ppm). This may be resulted from the significant interaction effect between the therapeutic dose of OTC and cadmium (0.1 ppm) in-group B2.

Depending on the finding of the present study (Table 1) about the OTC residues in the studied raw tissues of pectoral, thigh muscles and livers and with regards to (MRLs) it is not recommended to use livers from all treated groups (A2, A3, B2 and B3) for human consumption. The residual levels of OTC in thigh muscles of all studied treated groups showed a level, which slightly exceed the (MRLs=0.1 ppm according to EU) in-group A2 (0.43± 0.023 ppm), and B2 (0.36± 0.012 ppm) at 21st day. in group A3 (0.69± 0.0426 ppm) and in B3 (5.53± 0.149 ppm) which exceed the (MRLs) that may be due to the toxic interaction between overdose of OTC group (A3 and B3) and prolonged exposure to cadmium in-group B3. The higher residual levels which exceed the (MRLs= 0.3 ppm according to EU) observed in livers of group B3 (144.9± 8.02 ppm) at 21st day may be due to the toxic interaction between overdose of OTC and prolonged exposure to cadmium.

According to the above mentioned results broiler chickens pectoral and thigh muscles and livers should be examined for OTC residue levels before marketing and it is recommended to discard livers because of the intensive and uncontrolled usage of OTC in broilers chickens farms as growth promoters and the improper monitoring programs to evaluate the safety of them to human consumption. .

3. Reduction rate of (OTC) residues in broiler tissues (pectoral, thigh muscles and liver)

The obtained findings revealed that the total reduction rate of OTC residues in pectoral muscles was 98.3% in-group A2; 70.26% in-group A3; 95 % in-group B2 and 71.1% in-group B3. In thigh muscles it was 64.8% in group A2 ;79.9% in group A3 ; 82.9 % in group B2 and 48.% in group B3. In livers, the total reduction rate was 48.4% in-group A2; 56.4% in-group A3; 97.9 % in-group B2 and 35.3% in-group B3.

In Egypt, OTC withdrawal time was variable, depending on route and dose which given to broiler chickens, where OTC residues were detected 20 hours post injection in both pectoral and thigh muscles and completely disappeared from all the examined samples after 48 hours from last injection (EL Mossalami et al., 1985). Also (OTC) residues in thigh, pectoral muscles and livers of broiler chickens could not be detected after 36 hours (Moustafa et al., 1988), 3 days (Youssef et al., 1993), 4 days (Abd elhamid, 2000) and two weeks (Amin et al., 1977) and in broiler meat after 17 days (Hussien, 2001).

The longest withdrawal period was observed in the liver (above 21 days from stopping intake of OTC) and to some extent in thigh (between 11 and 21 days) then pectoral muscles (about 11 days). The presence of a relatively higher concentration of residues in the tissues and the relatively long withdrawal period reported in the present study are scientific points of considerable importance, as it constitute a potential public health hazard.

Moustafa et al. (1988) could detect OTC residues in 14 experimentally injected chickens with OTC (20mg\kg). The birds were slaughtered in equal groups at 6, 12, 18, 24,

30, 36 and 48 hours, and samples from thigh, breast and liver were collected. The experimental study did not succeed to detect the OTC residues in the examined tissues 36 hours after dosing. The incidence of antibiotic residues in the thigh, breast and liver samples was 5, 8, and 13%, respectively.

Donkova (2005). indicated that increased drug loads on broiler chicks and laying hens have (the prolonged oral use of (OTC-HCL) in a dose of 50 mg/kg or the single administration of its long-acting formulation-nitox in a dose of 200 mg/kg) lead to the accumulation of residues in the poultry flesh, by-products, and eggs even provided that the poultry exposure schedule before slaughtering is kept. The highest levels of the antibiotic are detectable at the site of injection and in the eggs after injection of nitox, a long-acting formulation of (TCs) The detection of the residues of the antibiotic in the poultry flesh, by-products, and eggs may be associated with the development of cytotoxic effects and with the reduced functional capacities of the organs that are responsible for the detoxification and excretion of medicinal xenobiotics.

CONCLUSIONS AND RECOMMENDATIONS

The present study allow to conclude the toxic effects of OTC on broiler chickens muscles (pectoral and thigh) and livers due to administration of therapeutic and over dose according to the design of the experiment which simulate the conditions in poultry farms in Assiut governorate .In addition to evaluate the toxic effect of OTC under previous condition associated with stress factor of environmental pollutant in water as cadmium and. to study the withdrawal time of OTC residue in broiler chickens tissues.

Many researchers have shown that microbial resistance in people can developed from drug used in animals. Also allergic reactions have been also reported following the ingestion of feed stuff containing antibiotic residues. Antibiotics residues are on the top of priority for the public health. Antimicrobial drugs have been used widely in animal production for treatment and prevention of disease and for growth promotion. (OTC) is the preferable, widely used antibiotics in broiler chickens farms in Assiut.so its use as feed additives must be evaluated.

From the obtained data it could be concluded that: Average recovery rate of OTC residues in spiked tissue were 80% in muscles (pectoral and thigh) and 80.06% in liver. Addition of OTC to the drinking water of the broiler chickens at a dose of 3g /L as a (therapeutic dose) and 6g /L as an (overdose) for 5 days at age of 31 days old indicated low residual levels in muscles (pectoral and thigh muscles) and very high residual levels in livers specially in groups taken overdose of OTC with or without cadmium (0.1 ppm) in drinking water group (B3 and A3) respectively.

Application of OTC in broiler chicken husbandry is improved when procedures follow industry quality-assurance guidelines and with the assistance of veterinarians, document the instances of drug use and the practices associated with the drug use. It is of great concern that sufficient withdrawal times must be observed to avoid the presence of OTC residues in broiler meat.

- Antibiotics which are used according to veterinarian prescription are assumed to be in general highly accountable.

- The heights residue levels and the longest withdrawal period which exceed the marketing age of this broilers for OTC were observed in the livers of all treated group.
The toxic interaction between the overdose of OTC and cadmium increase their levels of this group (B3).

-The withdrawal period detected in the present study was 16 days post-exposing between 11-21 days. It is not recommended for livers in all studied treated groups, it is suitable for the pectoral muscles of group A2 (which take therapeutic dose of OTC in tap water for five days) and group B2 (which take therapeutic dose of OTC in tap water for five days and cadmium during the time of experiment in tap water). However for other studied muscles of the other groups are not suitable.

-The levels of OTC and the length of the withdrawal period in different studied muscles and livers were dose related and was also correlated with the length of period of toxic interaction with cadmium.

-Broiler chickens producers used to employ oxytetracycline as antimicrobial growth promoting factor or for combating poultry diseases must be informed to apply accurately the obtained withdrawal periods. A strong regulatory action against violators is recommended and a regulatory enforcement in reducing illegal residues is also urgent.

-overdosing of OTC and prolonged administration of cadmium must be avoided as it has a deleterious effect on health performance and immune mechanism of the broiler chickens. It also resulted in increased of OTC residues in studied tissues and organs as well as prolonged withdrawal period which well constitute a hazards effect for public health.

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MOLECULAR ANALYSIS OF EMISSIONS FROM BROILER SHEDS

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SUMMARY

This paper describes investigations into the characterization of bacterial emission from broiler sheds.

INTRODUCTION

Poultry processing plants are relevant sources of emission from microorganisms [1]. These microorganisms from live stock buildings and their environmental impact are hardly characterized. In particular residents in surroundings of poultry processing plants are increasingly interested in this characterization because of a potential negative health effect [2]. Therefore we investigated the microbial load and the bacterial diversity in emission samples from broiler sheds by cultivation independent analysis.

MATERIAL AND METHODS

The broiler shed comprised ~ 40000 animals. Distributed over 2.5 fattening periods emissions samples from this broiler shed were collected by impingement into isotonic cell free NaCl solution. On the one hand total cell count after DAPI staining and on the other hand DNA extraction for generating 16S rRNA gene sequence clone libraries and qPCR analysis were done.

RESULTS

Concentrations of microorganisms in emission samples clearly increased during the fattening period from 4×10^7 cells per m^3 at the beginning to 9×10^8 cells per m^3 at the end (after ~ 40d). Depending on the ventilation rate a number of > 10^{10} microbial cells were emitted from the broiler shed per second. The most abundant sequences (60%) of 16S rRNA gene clone libraries could be assigned to the genus *Staphylococcus.* All together 28 different bacterial species within 11 different genera were detected. The most frequently detected sequences are those which are most closely related to bacteria of the risk group 1. However, sequences which are most closely related to *Staphylococcus saprophyticus, Aerococcus viridans, Enterococcus hirae, E. faecium* and *Escherichia* spp. indicating the emission of risk group 2 bacteria as well. Between 4 and 11% of sequence in 8 of 12 investigated clone libraries could be assigned to the genus *Jeotgalicoccus.* This high abundance was verified by a *Jeotgalicoccus* specific quantitative real-time PCR. A remaining of about 21% from all analysed sequences was next related to yet uncultured bacteria.

DISCUSSION

It was confirmed in this study that broiler sheds are a potential source for microbial air pollution. The abundant bacterial genus was *Staphylococcus*. But the cloning and real time PCR approaches of this study show that *Jeotgalicoccus* may be a potential detection target for emission and ambient air measurements from broiler sheds.

CONCLUSIONS

Against the background of increasing numbers of poultry fattening plants, both from ecological and medical point of view the environmental impact of these emissions should be considered in further investigations

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AIRBORNE MICROORGANISMS AND DUST FROM LIVESTOCK HOUSES

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SUMMARY

The objective of this study was to evaluate the efficiencies and suitability of samplers for airborne microorganisms and dust, which could be used in practical livestock houses. Two studies were performed: 1) Testing impaction and cyclone pre-separators for dust sampling in livestock houses; 2) Determining sampling efficiencies of four bioaerosol samplers for bacteria and virus.

Study 1. The overloading problem of the EU reference impaction pre-separator (IPS) was tested in layer houses and compared with cyclone pre-separators (CPS) for sampling PM_{10} and $PM_{2.5}$. Study 2. Physical and biological efficiencies of Andersen 6-stage impactor, all glass impinger (AGI-30), high air flow rate sampler OMNI-3000, and MD8 with gelatin filter were investigated for collecting aerosolized bacteria, *Enterococcus faecalis*, *Escherichia coli*, *Campylobacter jejuni* and *Mycoplasma synoviae* and live Gumboro vaccine virus. A tracer (uranine) was used to determine physical efficiencies and bioaerosol deposition. The study was done in a HEPA isolator (volume: 1.3 m^3).

The results show the PM_{10} IPS did not become overloaded in 24 h measurements in layer houses, whereas $PM_{2.5}$ IPS became overloaded within 1 h. CPS did not become overloaded during 48 h sampling of both dust fractions. The OMNI-3000 (62%) had lower physical efficiency than the MD8, while the other samplers had similar efficiencies as MD8. All the bioaerosol samplers had high biological efficiencies for all four bacterial species, except for *C. jejuni* (1%) when measured with the OMNI-3000 and for *E. coli* (38%) and *C. jejuni* (2%) when measured with the MD8. The biological efficiencies of the Andersen impactor (61%), the AGI-30 (90%) and the MD8 (163%) were not significantly different from 100% for collecting the aerosolized virus. However, the biological efficiency (23%) of the OMNI-3000 was significantly lower than 100%.

INTRODUCTION

Airborne microorganisms in livestock houses attach to dust particles. They can emit to the ambient air through the ventilation exhausts. Emission of pathogenic microorganisms pose infection risk to animals in other nearby livestock units and/or to humans. Lab-scale experiments have confirmed short distance airborne transmission of some microorganisms from animal to animal: it was found that healthy animals kept physically but not aerially separated from infected animals became infected (Berthelot-Herault et al. 2001; Brockmeier and Lager 2002). Also, the porcine reproductive and respiratory syndrome virus (PRRSV) was collected kilometers away from the source farm (Otake et al. 2010). However, to date these findings still have not incontrovertibly been linked to long distance airborne transmission of microorganisms between farms. Furthermore, there is still lack of knowledge about the role of dust in airborne transmission. Knowledge gaps in airborne transmission need to be filled and effective transmission control technologies require to be developed (Zhao et al. 2011a). Therefore, investigations on airborne microorganisms and dust should be carried out, such as source identification, suspension, physical and biological decay in airborne transportation, deposition in respiratory tracts, and infection in recipients. Almost all the above mentioned investigations cannot be performed without accurately and precisely measuring airborne microorganisms and dust from livestock production systems.

Measurements of airborne microorganisms is performed with bioaerosol samplers applying different principles, including impaction, impingement, cyclone forces and filtration. Because airborne microorganisms may either be physically miss-collected or biologically inactivated by various sources of stresses during sampling, the efficiencies of these samplers are generally known to be imperfect. To date, the efficiencies of the samplers for collecting different microbial species have not been well established. Notably, there is no standard protocol for sampling airborne microorganisms that specifies the requirements for hardware and also the procedures immediately prior, during and after the sampling. The lack of a protocol makes it difficult to interpret and compare the results of different studies. The sampling protocol for collecting PM_{10} and $PM_{2.5}$ in ambient air has been legislated by the EU commission and US EPA (European Commission 1998; 2005a; US EPA 2010). These sampling techniques, however, might not be suitable to sample dust in livestock houses, because the concentrations and particle sizes of dust in livestock houses are profoundly different from those in the ambient air, and this may compromise the efficiency and accuracy of the sampling. There is therefore an urgent need to develop a technique and eventually a protocol suitable for sampling dust in livestock houses.

The objective of this study was to investigate the suitability and efficiency of bioaerosol and dust samplers for measuring airborne microorganisms and dust from livestock production systems. In details, experiments were carried out to:

- investigate the overloading problem of EU reference dust sampler with an impaction pre-separator (IPS), when used for measuring PM_{10} and $PM_{2.5}$ in the dusty environment of layer houses; evaluate the cyclone pre-separator (CPS) as a reference equivalent pre-separator for PM sampling in the dusty environment of livestock houses following the EU standard procedure.
- assess the sampling efficiencies of four bioaerosol samplers (Andersen 6-stage impactor, AGI-30, OMNI-3000, and MD8 with a gelatine filter) on measuring aerosolized *E. faecalis*, *E. coli*, *M. synoviae*, *C. jejuni*, and Gumboro vaccine virus.

MATERIAL AND METHODS

Evaluation of IPS and CPS in livestock houses

Sampler and pump

EU reference dust sampler consists an IPS and a filter holder. In the IPS, a flat impaction plate was rubbed with grease and placed under eight impaction nozzles. Larger particles strike the plate at speed and are retained on the impaction plate because of their inertia. The smaller target particles (PM_{10} or $PM_{2.5}$) are carried along in the air stream and are collected on the downstream filter. The airflow rate through the inlet head of an IPS is 2.3 m^3 h^{-1}. More detailed descriptions of the EU sampler can be found in EU documentations (European Commission 1998; 2005a).

The candidate sampler consists of an air inlet head, a CPS (URG corp., US) and a filter holder. The CPS uses the centrifugal principle to separate large particles trapped in a dust collector. PM_{10} or $PM_{2.5}$ are conveyed in the air stream and collected by a glass fibre filter in the filter holder. The airflow used for a CPS is set at 1 m^3 h^{-1}.

Charlie HV pumps (Ravebo Supply b.v., Brielle, the Netherlands) were used to suck air through the two types of samplers. These pumps are able to maintain constant airflow (< 2% nominal value) during dust sampling.
Mass of each filter before and after sampling was measured. The PM_{10} or $PM_{2.5}$ concentration was calculated by dividing the mass difference by the total volume of air passing through the filter. The unit of dust was expressed as μg m^{-3}.

Overloading of IPS and CPS

When a pre-separator becomes overloaded, it's greased plate (of IPS) or dust collector (of CPS) is no longer able to separate larger particles from the incoming air stream. Therefore, dust particles in the whole size range are transported to the downwind filter. This results in an overestimation of PM_{10} or $PM_{2.5}$ concentration. Understanding above mentioned phenomenon, the overloading of a pre-separator was determined in this study by comparing the PM concentration collected with a sampler without cleaning the pre-separator during sampling (control) to that collected by a sampler with regular cleaning the pre-separator (treatment). When the dust concentration measured by a control pre-separator is higher than that measured by a treatment pre-separator, the pre-separator was overloaded. See the study by Zhao et al. (2009) for details.

Validating CPS

To be qualified as the reference equivalent device, CPS should be able to perform precise and accurate measurements. The equivalent test was carried out following the EU standard procedure as required (European Commission 1998). Ninety-six pairs of 24 h measurements, 48 for PM_{10} and 46 for $PM_{2.5}$, were conducted in various environments: livestock houses (three fattening pig houses, one broiler house and one dairy barn); an industrial workplace; and in the ambient air. For each pair of measurements we used one IPS (as the reference sampler) and two CPSs (as the candidate sampler).

Sampling efficiency of bioaerosol sampler

Sampling efficiency includes physical and biological efficiency. The physical efficiency of a sampler reflects how well the sampler aspire, transport and retain the airborne particles from the ambient air to its collection medium. The biological efficiency reflects how well the viability of the microorganisms is maintained during sampling. In this study, the physical and biological efficiencies of four bioaerosol samplers (Andersen 6-stage impactor, AGI-30, OMNI-3000, and MD8 with gelatin filter) on collecting five microbial species (*E. faecalis*, *E. coli*, *C. jejuni*, *M. synoviae*, and Gumboro vaccine virus) were investigated. This was done by aerosolizing the microbial suspensions (with or without a physical tracer) in an HEPA isolator, and by collecting the aerosolized microorganisms with the samplers. The physical efficiency was calculated based on the amount of tracer collected; and the biological efficiency was calculated based on the microorganisms/tracer ratio. More details can be found in Zhao et al. (2011b; 2011c; 2011d).

RESULTS AND DISCUSSION

Dust sampler

Overloading of IPS and CPS

The results show that $PM_{2.5}$ IPS was overloaded within 8 h when used for sampling dust in a layer room. The overloading of $PM_{2.5}$ IPS was not solved even with a 1 h plate cleaning interval (Zhao et al. 2009), thus it cannot be used for PM sampling in such a dusty environment. Compared to IPS, $PM_{2.5}$ CPS was more resistant to high dust concentrations. It is shown that the $PM_{2.5}$ CPS did not become overloaded during 24 h sampling. Both PM_{10} IPS and PM_{10} CPS had no overloading problem.

Validating CPS

The results show that both PM_{10} and $PM_{2.5}$ CPSs were qualified as the reference equivalent pre-separator for the EU IPS, when these candidate samplers were used in environments with low dust concentrations (<100 μg m^{-3} for PM_{10}, and working place/ambient air for $PM_{2.5}$). The relative two side 95% confident interval (CI_{95}) of PM_{10} CPS (6%) is almost within the required value (5%); and the PM_{10} concentrations measured by the CPS was within the acceptance envelope: ($y = x \pm 10$) μg m^{-3}, where y is the PM concentration measured by CPS and x is the PM concentration measured by IPS. The absolute CI_{95} of $PM_{2.5}$ CPS (2.3 μg m^{-3}) is within the required value of 5 μg m^{-3}; and the $PM_{2.5}$ concentrations measured by the CPS was within the acceptance envelop: ($y = x \pm 10$) μg m^{-3}.

The PM_{10} concentrations measured by PM_{10} CPS were systematically lower than those measured by IPS in less dusty environments, and were higher in dusty environments. Therefore, the PM_{10} concentration measured by CPS should be corrected (Equations 1 and 2).

$y = 1.09x$ (x ≤ 223 μg m^{-3}) (1),

$y = 0.83x + 57.5$ (x > 223 μg m^{-3}) (2)

y: is the calibrated concentration, μg m^{-3}; x: is the concentration measured with CPS, μg m^{-3}.

Bioaerosol sampler

Physical and biological efficiency

The physical efficiencies of the Andersen impactor and the AGI-30 were not different from the high efficient sampler - MD8. However, the physical efficency of the OMNI-3000 (62%) was signficantly lower than that of the MD8. The biological efficiencies of the samplers on collecting all microbial species were not different from 100%, except for C. jejuni (1 ± 1%) and Gumboro vaccine virus (23 ± 10%) when sampled by OMNI-3000, and for *C. jejuni* (2 ± 1%) and *E. coli* (38 ± 10%) when sampled by MD8. The significant lower efficiencies suggested that these microbial species were inactivated due to sampling stress from samplers.

The total sampling efficiency (combination of physical and biological efficiencies) and the detection limit were calculated from the efficiency data and are listed in Table 1. This information may be helpful for selecting samplers suitable for practical measurements. The Andersen impactor and the AGI-30 are suitable for sampling all microbial species because their total efficiencies are high. The MD8 is suitable for sampling *E. faecalis*, *M. synoviae* and Gumboro vaccine virus, but not *E. coli* and *C. jejuni*. Although the OMNI-3000 has low sampling efficiencies of 62% for *E. faecalis*, *E. coli* and *M. synoviae*, and 14% for Gumboro vaccine virus, it could still be a suitable sampler because its high air flow rate gives low detection limits. The OMNI-3000 cannot be used for *C. jejuni* because this species would be seriously inactivated by sampling stress. The Andersen impactor has high sampling efficiency on Gumboro vaccine virus (100%), however, its detection limit (4.1 $\log_{10}$ EID50 m^{-3}) is highest among all samplers, because virus was lost in air sample handling (Zhao et al. 2011b).

Table 1. Total sampling efficiency and detection limit of bioaerosol samplers.

	E. faecalis	*E. coli*	*C. jejuni*	*M. synoviae*	Gumboro
Sampling efficiency (%)[1]					
Andersen[2]	100	100	100	100	100
AGI-30	100	100	100	100	100
OMNI-3000	62	62	0.6	62	14
MD8	100	38	2	100	100
Detection limit[3]					
Andersen[2]	3.9	3.9	3.7	3.8	4.1[4]
AGI-30	3.9	4.2	3.8	4.0	3.3
OMNI-3000	2.5	2.5	4.5	2.7	2.5
MD8	4.1	4.4	5.4	4.3	2.9

[1] 100% means that in our study the measured efficiency was not significantly different from 100%.

[2] Physical and biological efficiencies of the Andersen impactor were set to 100% because it collected similar amounts of viable microorganisms as the AGI-30.

[3] Detection limit was calculated based on a 2 min sampling duration. The unit of DL is $\log_{10}$ CFU m^{-3} for bacteria, and $\log_{10}$ egg infective dose 50% (EID_{50}) m^{-3} for virus.

[4] Detection limit was calculated by assuming agar plates of Andersen impactor were rinsed 1 h after sampling.

CONCLUSIONS

EU reference $PM_{2.5}$ IPS cannot be used for dust sampling in livestock production systems because of overloading. PM_{10} and $PM_{2.5}$ CPSs are equivalent to IPS when used in environments with low dust concentrations, and are more resistant to dusty environments. Therefore, CPSs are promising devices for dust sampling in livestock production systems.

The physical and biological efficiencies of the bioaerosol samplers vary. In order to perform accurate measurement of airborne microorganisms, the efficiency of a sampler should be investigated beforehand. In this study, we found OMNI-3000 was not suitable for sampling *C. jejuni* and MD8 was not suitable for *C. jejuni* and *E. coli*.

LIVESTOCK-RELATED MICROBIAL IMMISSIONS IN THE VICINITY OF A POULTRY MEAT PROCESSING FACILITY

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SUMMARY

Particulate emissions from livestock buildings support the dissemination of airborne micro-organisms in the environment. Discussions about their direct health effects in men are going on but little is known about their impact when deposited on foodstuff. A prospective case study was undertaken to calculate the aerial dispersion pattern of microbial emissions (mesophilic total bacteria, *Enterobacteriaceae*, *Clostridium perfringens*, *Bacillus cereus*) from a planned pig barn and the related receptor concentrations and depositions around neighbouring poultry meat processing plant. The presumed emissions from the planned piggery added yearly mean extra concentrations of mesophilic total bacteria (mtBac) between 25 and 78 CFU per m³ and of *Enterobacteriaceae* (Entero) between 1 and 4 CFU/m³ at three locations around the meat plant. Deposition rates reached there 0.78 CFU/m² x sec (mtBac) and 0.04 CFU/m² x sec (Entero) in maximum. The predicted immissions for *Clostridium perfringens* and *Bacillus cereus* were low and very low, respectively (< 1 KBE/m³ and << 1 KBE/m² x sec). Despite of these rather small concentrations it can not be fully excluded that zoonotic pathogens are among the microbial emissions from the piggery and reach the food processing. Therefore, mitigation techniques should be taken into consideration in order to protect the process hygiene and the products in the plant.

INTRODUCTION

Bioaerosols are a major fraction of substances emitted from livestock houses. The potential risks for public health are under discussion since decades. Little is known about the possible influence of bioaerosols from animal houses on the hygienic quality of food stuffs. The importance of such an impact is actually outlined in the EC regulation no. 852/2004, because the siting of food premises must avoid or minimise airborne contamination, for example [1]. On the other hand environmental influences caused by livestock operations must also be limited. This intention is normally guaranteed by environmental protection acts and regulations. However, for some specific local situations an individual case study is required to decide whether a direct neighbourhood between sites of livestock farming and food processing units is permissible or not. This paper presents such a case report, which outlines the possible hygienic risks which may arise from a planned pig fattening unit with 1490 animal places in close vicinity to a poultry meat processing plant, which produces mechanically separated meat (MSM) from poultry carcases.

The aim of the study is to assess the potential food hygiene risk by applying a numerical dispersion model, which predicts the expecting receptor concentrations and depositions (=immissions) at the food premise caused by the planned livestock building.

MATERIAL AND METHODS

Firstly, a selection of specific micro-organisms was defined as hygiene indicators, which may negatively influence the hygiene of the poultry processing plant: mesophilic total bacteria (general hygiene parameter), *Enterobacteriaceae* (faecal indicator), *Clostridium perfringens* (indicator for potential toxi-infections) and *Bacillus cereus* (indicator for potential intoxications). To derive suitable emissions factors for these micro-organisms a field investigation has been conducted in already existing pig fattening units of the same type on the same farm. During these investigation All-Glas-Impingers (AGI-30) were operated in three pig houses, in which animals of different body weights (50, 75 and 100 kg) were kept. The sampling time was 20 min at an adjusted flow rate of 10.5 l/min. The fluids of the AGI-30 were cooled during transport (4° C) to the laboratory and analysed on the mentioned groups of bacteria. Assuming that probably not all relevant micro-organisms can be detected by impingement due to loss of viability during sampling, deposited dust was additionally collected at different sites in the barns, analysed on bacteria and compared to literature values [2]. By means of known sedimentation rates [3], it is then finally possible to calculate the related concentrations in the air. This procedure was applied for *Bacillus cereus*. In case of mesophilic total bacteria and *Enterobacteriaceae* the found concentrations were compared to literature-related concentrations and then finally associated to corresponding emission strenghts [4]. Airborne

concentrations of *Clostridium perfringens* in pig barns [5] were combined with an annual average ventilation rate typical for pig confinement houses in the northern hemisphere.

A computerized Langrangian particle dispersion model (LASAT 2.9, Janicke Consulting Environmental Physics, Germany) was used to calculate the yearly mean concentration in 2 m height and the deposition on the ground within a grid of 400 m x 300 m (x/y). According to the construction plan of the pig barn, bioaerosols were emitted via 18 chimneys in 8.2 m height. Both facilities are located within the grid together with other buildings, which were only aerodynamically considered by the computer program (e.g. generation of turbulences). The grid resolution was 2 x 2 m. Site-specific meteorological data were obtained from the German Weather Service (DWD). In Figure 1 a scheme of the local conditions is shown and the relevant openings (in and out transportation of goods and materials) in the food premise are indicated, where contaminants may enter the inner space of the poultry meat processing plant.

RESULTS

The calculations revealed that the highest concentrations of bacteria from the piggery can be expected at the three doors and gates of the food processing plant, where food stuff is transported in or out of the building and where bioaerosols can easily enter the building via air movements. The yearly mean concentrations of mesophilic total bacteria (mtBac) ranged at these three locations between 25 and 78 CFU per m³ and for *Enterobacteriaceae* (Entero) between 1 and 4 CFU/m³. Deposition rates reached 0.78 CFU/m² x sec (mtBac) and 0.04 CFU/m² x sec (Entero) in maximum. For *Bacillus cereus* and *Clostridium perfringens* only marginal receptor concentrations and apparently no depositions (if values are limited to 2 digits only) could be calculated (Tab. 1).

DISCUSSION

Emissions from the pig barn would certainly cause surplus concentrations in the ambient air and depositions of micro-organisms in the vicinity of the food premises. Assuming that the calculated additional concentrations of bacteria in the ambient air (immissions outdoors) can reach the rooms with the meat products inside the meat processing plant some operational uncertainties have to be considered: (i) Air exchange rates of the food premise is varying and is sucking from time to time more or less numbers of micro-organisms into the building, (ii) doors and gates are only temporarily open, (iii) wind induced fluctuations may randomly trigger the magnitude of bacterial loads reaching the facility, (iv) during the transmission of the livestock-related bacteria, impactions on solid surfaces may reduce survival of bacterial loads in the production rooms, (v) the open exposure time of the meat products is limited, because of packaging and storage in cooling cells.

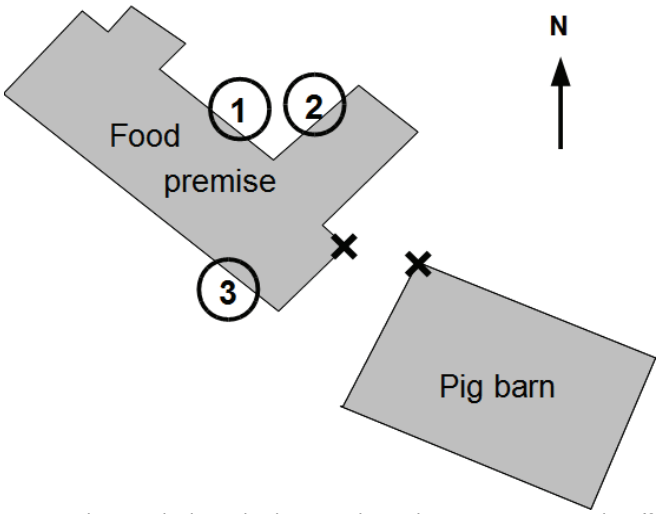

Figure 1: The spatial relationship between the poultry meat processing plant (food premise) and the planned pig barn (not true to scale). Circles 1 to 3 are indicating the relative position of doors and gates around the building. Door no. 1 consists of two single doors (1a, 1b), which are nearly side by side. The distance between the crosses is approximately 15 m giving an impression of the narrowness between both buildings.

Table 1: Concentrations and depositions of selected micro-organisms close to the doors and gates of the poultry processing plant

	Door/gate no.	Concentration	Deposition
Microorganisms		CFU/m³	CFU/m^2 x sec
Mesophilic total bacteria	1a,b	25.06	0.25
	2	31.17	0.32
	3	77.86	0.78
Enterobacteriaceae	1a,b	1.18	0.01
	2	1.47	0.02
	3	3.66	0.04
Clostridium perfringens	1a,b	0.01	0.00
	2	0.01	0.00
	3	0.03	0.00
Bacillus cereus	1a,b	0.04	0.00
	2	0.06	0.00
	3	0.14	0.00

If these limitations are not considered, the absolute deposition amounts can be calculated as follows: Taking into account a regular work shift of 18 hours (2 x 9 hours) in the food premise, the additionally microbial depositions can be extrapolated up to approximately 5, 0.3, 0.002 and 0.009 CFU/cm² for mtBac, Entero, *Clostridium perfringens* and *Bacillus cereus*, respectively. The most relevant questions in this context is how these burdens may impair the hygienic status of the processed poultry meat, because the mechanically destroyed muscle tissue is acting as an excellent nutrient for microbial growth. In the EC regulation no. 2073/2005 microbial limits for foodstuffs are defined [6]. For aerobic colony counts and *Escherichia coli* as an indicator of faecal contamination alert limits of 5 x 10^6 CFU/g and 500 CFU/g, respectively, are given at the end of the manufacturing process. However, such process hygiene criteria are not related to surface contaminations of MSM and therefore it is difficult to come up with a clear interrelationship between surface and mass. Only for meat preparations in combination with *Escherichia coli* an upper limit of 5000 CFU/cm² exists [6]. In comparison to the additional deposition of the here predicted 0.3 CFU of *Enterobacteriaceae* per cm² the risk seems to be very low to negligible. On the other hand each initial microbial contamination has the potential to be a starting point of microbial growth. This is particularly true, when zoonotic agents get into foodstuffs. Therefore, avoidance strategies are needed for a long-term establishment of a good hygienic practice. Measures like operations of air treatment technologies (eg. filtration, UV radiation) or a more distant siting of the planned pig barn can help to alleviate or solve the conflict.

CONCLUSIONS

Deposition rates and airborne concentrations around the food premise are relatively low under the conditions defined for the dispersion modelling. However, specific weather conditions such as downwash effects or peak emissions from the piggery can considerably increase short term bacteria deposition. Therefore, it can not be fully excluded that food hygiene relevant bacteria (e.g. Salmonella) may reach the food processing rooms of the plant. For similar cases it is recommended to perform early planning procedures (as demonstrated here) and consider mitigation techniques or an alternative siting of the emitter or receptor building, in order to prevent legal conflicts later.

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SLURRY REMOVAL: A SIMPLE WAY TO REDUCE NH3, GHG AND ODOURS EMITTED BY PIGGERIES

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SUMMARY

During two batches of fattening pigs (B1 and B2), measurements of ammonia and GHG were achieved on the exhaust air of the two identical rooms which only differed in manure management. In the first room (Reference room), the slurry was stored in the pit during the whole fattening period. In the second (SR room), a fine layer of water was discharged into the pit before pigs entered. The day of the feed change, the pit was emptied and an additional layer of water was discharged. No effect of the treatment was observed on animal performance. In the SR room, the N_NH_3 daily emission per pig was reduced by 21% (B1) and 24% (B2) in comparison to emissions from the Reference room. In the treated room, the N_N_2O daily emission per pig was slightly lower than in the Reference room. Odours emitted by the SR room was 25% lower than the Reference room.

INTRODUCTION

Ammonia, greenhouse gases and odours emitted by pig housing originate mostly from the slurry stored in the pit. Reducing the duration of time that the manure is stored inside the buildings would be an effective way to reduce emissions from pig housings. The main problem is to find a technique adding a high efficiency, a low cost and which can easily be transposed in all kind of housing. In this project, a fine layer of water was discharged in the pit before the pigs entered. The aim was to limit the sedimentation of the solid fraction in the bottom of the pit, which is commonly known as being highly concentrated in very odorous components.

MATERIAL AND METHODS

Two batches of crossbred (PPxLW)x(LWxLD) pigs were fattened at the IFIP experimental farm from October 2009 to February 2010 (B1) and September 2010 to January 2011 (B2) in two identical rooms, which only differed in the management of slurry. In the first room (Reference), slurry was stored underneath the pit for the duration of the whole fattening period. In the second room (SR), before pigs entered, the pit was emptied with 2.5 m^3 of water (41.5 litres per pig). Slurry was removed the day of the feed change and an additional 2.5 m^3 of water was discharged into the empty pit. Until the departure, slurry was stored in the pit. In both rooms, 60 pigs were group-housed in 6 pens on fully slatted floor. Fresh air entered via a ceiling of perforated plastic sheeting and the air exhaust was an under-floor extraction chimney. The set-point temperature was fixed at 24°C during the whole period. Animal performance (weight, average daily gain, feed conversion ratio and carcass characteristics) were recorded per room. Pigs were individually weighed at the beginning of the growing period, thereafter at the change of feed and the day before slaughtering. The feed intake was recorded weekly on a pen basis. All pigs were slaughtered on the same day. Temperature and hygrometry were continuously monitored inside and outside the two fattening rooms. The ventilation rate was continuously recorded by measuring the rotation speed of a full-size free-running impeller unit coupled with the exhaust fan of the buildings. Gas concentration in the exhaust air of both rooms and outside were measured by photoacoustic infrared absorption spectrometry using a gas analyser (Innova 1412) coupled with a sampler dosimeter (Innova 1303). Emission factors were validated by the mass balance method. Only for B1, air samples for odour measurements were achieved and analysed to determine the odour concentrations using dynamic olfactometry in accordance with the European CEN standard.

The mass balance method was applied for nitrogen (N), carbon (C) and Water (H_2O) including the calculation of inputs (piglet carcass, feed consumption) and outputs (pig carcass, slurry composition, gaseous emissions). An analysis of variance (SAS 1998, proc GLM) was performed to test the effects of sex (X) and treatment (T) on animal performance.

RESULTS

Growth performance

Table 1 summerizes the growth performance of the two batches involved in this study. For both rooms, the fattening duration was 104 and 100 days for B1 and B2 respectively. No significant effect of the treatment has been outlined on live weight, Average Daily Gain (ADG), Feed Conversion Ratio (FCR), nor on carcass weight and muscle content. Performance of pigs reared in both rooms were in accordance with previous data obtained through similar conditions.

Table 1: Growth performance

Rooms			Ref. room	SR room	RSD	Stat.[1]
Live weight (kg)	At the beginning	B1	23.9±2.7	23.8±2.8	2.75	-
		B2	24.6±3.9	25.1±3.9	3.9	-
	At slaughtering	B1	113.5±11.9	110.3±11.9	11.59	X**
		B2	108.7±9.6	106.5±8.3	8.7	X**
ADG (g/j)	Growing period	B1	835.3±116.1	809.0±106.1	109.7	X*
		B2	891.4±94.1	875.7±85.0	85.7	X**
	Finishing period	B1	840.2±121.0	809.3±143.3	128.3	X***
		B2	843.2±102.7	803.8±94.2	96.7	X* T*
	Total	B1	837.9±99.9	809.2±103.1	97.8	X***
		B2	858.2±84.2	830.8±73.2	75.5	X**
FCR	Growing period	B1	2.47±0.18	2.43±0.29	0.24	-
		B2	2.21±0.11	2.42±0.41	0.30	-
	Finishing period	B1	3.25±0.32	3.21±0.22	0.28	-
		B2	2.97±0.16	3.20±0.39	0.29	-
	Total	B1	2.88±0.23	2.84±0.17	0.21	-
		B2	2.59±0.12	2.79±0.39	0.29	-
Carcass weight (kg)		B1	90.8±9.9	87.7±9.9	9.5	X**
		B2	87.8±7.7	88.1±8.5	7.9	X*
Muscle content (%)		B1	59.7±2.9	60.0±5.7	2.5	X***
		B2	60.2±2.3	60.2±2.8	2.3	X***

[1] Analysis of variance including sex (X) and treatment (T) as main effects, ***: P<0.001, **: P<0.01, *:P<0.05

Ambient parameters

During the whole fattening period, the average outside temperature was 5.4±4.9°C for B1 and 7.3±5.7°C for B2. The average ambient temperature was 24.8±1.1°C and 24.8±0.4°C inside Reference and SR rooms, respectively for B1 and 25.1±0.4°C and 24.7±0.4°C for B2. During the first batch, in the Reference room, the average ventilation rate was 18.3±4.1m^3 per hour per pig and 22.3±4.8 m^3 per hour per pig in the SR room. For the second batch, the average ventilation rate was 23.1±5.5 and 30.3±5.4 m^3 per hour per pig for the Reference and the SR rooms, respectively.

Slurry

For both batches, an intermediary emptying was done the day of the feed change in the SR room while the slurry was stored during the whole fattening period in Reference room. The lower dry matter of slurry sampled in the SR room is the result of the dilution by the additional water emptied at the beginning of the fattening period and the day of the feed change (table 2). The composition of slurry produced by pigs kept in the reference room was in accordance with literature and previous studies achieved through similar conditions [1, 2].

Table 2 : slurry composition

Room	Reference room		SR room	
Batch	**B1**	**B2**	**B1**	**B2**
pH	7.4±0.1	7.4±0.1	7.3±0.1	7.5±0.1
Dry Matter (%)	4.1±0.7	5.9±0.8	2.1±0.3	3.0±0.5
Total nitrogen (g/kg)	3.8±0.3	4.5±0.2	2.7±0.3	3.9±0.2
Ammonium nitrogen (g/kg)	2.5±0.2	3.2±0.3	2.0±0.2	2.9±0.4
Organic Carbon (g/kg)	14.5±2.9	19.3±3.0	6.2±0.9	9.7±1.3

Input-Output mass balances

For nitrogen, the mass balance deficit per room for B1 was 3.9 and 4% of the input of nitrogen for the Reference and SR rooms respectively and 2 and 2.7% for B2. For carbon, the mass balance deficit, for B1, was 17 and 11% of the input of carbon for the Reference and SR rooms respectively and 5 and 11% for B2. For water, it represents 3 and 12% of the input of water for the Reference and SR rooms during the first batch and 1 and 17% for the second.

Gaseous emissions

Nitrogen emissions (NH_3 and N_2O) measured for the Reference and the SR rooms explained between 73 and 70 % of the nitrogen losses by volatilisation calculated by the input-output mass balances for the first batch. For B2, nitrogen emissions explained 69 and 85% of the mass balance deficit. For the whole period of fattening, ammonia emission was 6.5 g N_NH_3 per pig per day in the Reference room and 5.2 g N_NH_3 per pig for the SR room for B1, that is a reduction of 20%. For B2, ammonia emission was 10.4 and 7.9 g N_NH_3 per pig in the Reference and SR rooms respectively, that is a reduction of 24%. The N_2O emission was 0.2g N_N_2O per pig per day for both rooms during B1 For B2, the Reference room emitted 0.95 g N_N_2O per pig per day vs 0.93 g $N\text{-}N_2O$ per pig per day for the SR room. Values of the first batch were totally in agreement with data obtained by Philippe et al. (2007) [3].

Carbon emissions (CO_2 and CH_4) measured during the whole fattening period represented 76 and 70% of calculated emissions for Reference and SR rooms, respectively, for the first batch. For the second, carbon emissions explained 92 and 85% of the carbon losses by volatilisation calculated by the mass balance. For CH_4, emission during B1 was 8.6 g C_CH_4 per pig per day in the Reference room and 8.1 g C_CH_4 per pig per day in the SR room. During B2, CH_4 emissions were 5.4 and 5.1g C_CH_4 per pig per day for the Reference and the SR rooms, respectively. According to Gallman et al. (2003) [4], CH_4 emissions ranged between 6 and 9 g C_CH_4 per pig per day for animals reared in winter on totally slatted floor with ambient temperatures between 19 and 23°C. For CO_2, for B1, emissions were 583 and 626 g $C\text{-}CO_2$ per pig per day for the Reference and the SR room, respectively. For B2, CO_2 emissions were 706 and 750 g $C\text{-}CO_2$ per pig per day for the Reference and the SR room, respectively. For both batches, our values were lower than those obtained in the literature [3,4] and no effect of the treatment was observed on C_CH_4 and C_CO_2 emissions.

Odours

During the first batch, in both rooms, samples for odour measurements were realized 26, 83 and also 103 days after the pigs entered. For the Reference room, the average odour emission was $1.0\ 10^8 \pm 4.5\ 10^7$ vs $7.5\ 10^7 \pm 4.5\ 10^7$ odour units per day per pig for the SR room leading to an odour reduction of 26%.

DISCUSSION

Previous studies concerning slurry removal once during the fattening period have already been achieved, showing a slight reduction of ammonia emitted but an increase in odour emission [5]. The main reason was the sedimentation of the solid fraction of the slurry kept in the bottom of the pit, contributing to the volatilization of ammonia. Moreover, some odorous compounds like p-cresol and phenols were present in the solid fraction contributing to the increase of odour emissions during and after the slurry removal. The addition of a fine layer of water limits this sedimentation, especially at the beginning of the fattening period, where the slurry volume is very weak and then can easily be deposited into the bottom of the pit. During the first removal, 50 days after the beginning of the study, the water layer facilitated the slurry emptying. This pit was cleaner than the reference pit. During the finishing period, the increase of slurry volume produced per pig reduced the positive effect of the water layer. Globally, the effect of the treatment is around 20-25% on ammonia and odour. A higher volume of water discharged at the beginning of the finishing period or an additional discharge during the finishing period could perhaps increase the treatment effect.

CONCLUSIONS

In our study, the addition of water to the pit twice during the fattening period led to a reduction of more than 20% of ammonia and 25 % of odours. This technique can be implemented in all kinds of pig farms without making changes to the building's structure or pig breeding. Associated with others techniques, this technique should permit to a large number of pig farms to reduce their impacts on atmospheric pollution. Nevertheless, the increase in the volume of slurry has to be taken into consideration for manure management on the farm scale.

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FATE OF PATHOGENS IN A SIMULATED BIOREDUCTION SYSTEM FOR LIVESTOCK CARCASSES

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SUMMARY

The aim of this work was to validate the efficacy of bioreduction in reducing the numbers of pathogens in a laboratory-scale system. The outcome should help evaluate whether bioreduction represents a biosecure method of containing fallen stock prior to disposal.

INTRODUCTION

Since the implementation of the Animal By-Products Regulations (EC/1774/2002) in 2003 [2], the options available to most farmers to dispose of fallen (dead) livestock have been effectively limited to either rendering or incineration. The regulations have led to animosity within the agricultural industry due to the considerable costs and biosecurity concerns associated with centralised collection and rendering or incineration of fallen stock [3,9]. Indeed, there is call for both a change in legislation and the development of alternative methods of disposal [3,13].

Bioreduction is a novel technology has shown potential as a viable option for storing and pre-treating fallen stock prior to disposal [13]. Bioreduction is the aerobic biodegradation of animal by-products in a partially sealed vessel, where the contents are mildly heated (approx 40 °C) and aerated and ultimately disposed of via the permitted route for 'Category 1' material within the EU ABPR [2]. Bioreduction has been shown to reduce the volume of waste and hence the frequency of collection and associated disposal cost, as well as being a practical method for industry [13]. The active aeration coupled with the competitive and antagonistic effects of the prevalent microbes are hypothesised to reduce zoonoses levels [13]. For bioreduction to be approved under the revised EU ABPR (EC/1069/2009) as an alternative method of storing fallen stock prior to disposal, the fate of pathogens within the system must be elucidated and the evidence presented to the European Food Safety Authority (EFSA), which then decide whether to ratify the system for industry use [4]. EFSA stipulate that novel disposal methods should lead to a 5-log reduction in the numbers of two indicator organisms representing bacterial pathogens, *S.* Senftenberg and *Enterococcus faecalis* [4]. EFSA guidelines state that simulated systems can be used as a proxy of field-scale systems provided that they are representative of actual conditions [7].

MATERIALS AND METHODS

Laboratory-scale bioreduction vessels were constructed using 5 l polypropylene containers; 19 cm high × 13 cm wide × 26 cm long. Three were inoculated with pathogens and two were used as controls. These mini bioreduction vessels (MBVs) were placed within a darkened incubator set to 40 °C (± 2 °C) and the contents continuously aerated at a rate of approximately 6 l min^{-1}.

A total of 231 g of sheep carcass components were added to each MBV, comprising of muscle, bone, fat, pelt, blood, stomach contents, wool and liver, in proportions representative of a sheep carcass. A commercial catalyst was added at the recommended dose of 1 g catalyst to 1 kg of carcass [10,13]. The MBVs were just under half filled with water and the level of water was maintained so that the animal contents were two-thirds covered for the duration of the study [10].

S. Senftenberg (NCTC13385), *S.* Poona (NCTC4840), *E. faecalis* (ATCC 29212), *C. jejuni* (6035), *C. coli* (6168), a *lux*-marked strain of *E. coli* O157 (3704 Tn*5 luxCDABE*) and an environmental strain of *E. coli* O157 (#3704) were grown from frozen stock. All *Campylobacter* media were incubated microaerobically. The final concentration of each inoculum was obtained by serially diluting and plating onto selective agar; *Salmonella* on XLD agar; *E. coli* O157 on CT-SMAC; *E. faecalis* on Slanetz and Bartley Medium (SBM) and *Campylobacter* spp. on mCCDA. Both SBM and mCCDA were incubated for 48 hours at 37 °C and 41.5 °C respectively. All other plates were incubated at 37 °C for 24 hours. Each treatment MBV was inoculated so that the final concentration of micro-organisms per ml of liquid was as follows: 7.91 log_{10} CFU of *Salmonella*, 7.89 log_{10} CFU of *E. faecalis*, 7.5 log_{10} CFU of *E. coli* O157 and 6.81 log_{10} CFU of *Campylobacter.*

Liquor samples (25 ml) were recovered on days 0, 3, 23, 56 and 84 and homogenised in a stomacher for 1 min at 230 rev min^{-1} with 225 ml maximum recovery diluent (MRD), then serially diluted. Samples were enumerated as described previously whilst a further 1 ml sample from each MBV was placed into a plastic luminometer cuvette and its luminescence [relative light units (RLU)] determined using a SystemSURE 18172 luminometer.

Where *Salmonella, E. coli* O157 and *Campylobacter* were not detected by enumeration, enrichment was used to confirm the absence of these bacteria. Enrichment of samples for *Salmonella* spp. was based on ISO standards 6579:2002 and *Campylobacter* samples were enriched based on the ISO 10272-1:2006(E) method. For the enrichment of *E. coli* O157, 20 ml of mTSB containing VCC supplement was added to 5 ml of liquor and shaken in an orbital shaker for 6 hours (37 °C, 150 rev min^{-1}) after which time 0.1 ml of the enriched culture was streaked onto duplicate plates of CT-SMAC. Plates were incubated and presumptive colonies confirmed. Presumptive colonies were sub-cultured onto nutrient agar and incubated at 37 °C for 24 hours, whilst presumptive *Campylobacter* colonies were incubated at 41.5 °C for 48 hours. *E. coli* O157, *Campylobacter* and *Salmonella* spp. were confirmed using latex agglutination with further biochemical tests using Microbact™ GNB 12A for *Salmonella* spp. Confirmation of *E. faecalis* was performed using glucose and bile aesculin agars.

Bioaerosol samples were taken on days 0, 24, 57, and 85 using selective agar plates in an Andersen Air Sampler 2000. The pump was activated for 30 min at a flow rate of 10 l min^{-1}. Brilliant Green Agar (BGA) was used instead of XLD for *Salmonella* and plates were incubated as described previously whilst the BGA was incubated at 37 °C for 24 h.

Data was $\log_{10}$ (y +1) transformed before normality of the microbiological data was tested and means analysed using either related Samples T-Tests if normal or Wilcoxon signed rank test if non-normal.

RESULTS

The controls were found to contain natural populations of *Salmonella* spp., *E. faecalis*, and *Campylobacter* spp. but no *E. coli* O157 were detected (Table 1). Survival of the introduced *Salmonella* spp. and *E. faecalis* in the treatment MBVs followed similar survival patterns to natural populations in the controls. Although numbers of both *Salmonella* spp. and *E. faecalis* reduced markedly over the three month trial, the dynamics of survival differed between both micro-organisms. Specifically, *Salmonella* spp. numbers remained relatively stable until day 54, after which they significantly declined (P <0.05) so that they could only be detected by enrichment at the end of the trial period. Numbers of *E. faecalis* generally decreased more steadily throughout the trial, although had recovered somewhat in the control MBs towards the latter stages (P >0.05). A significant (P <0.05) decline in both the numbers and activity of *E. coli* O157 were seen in the inoculated MBVs, culminating in a 5-log reduction by day 84 and luminescence values falling to below background levels. *Campylobacter* spp. numbers declined significantly (P < 0.05) within the first three days and none were recovered at the latter stages of the trial.

Table 1: Initial and final concentrations of pathogens in the inoculated and control mini bioreduction vessels. ND = not detected by enumeration, T = time in days.

Pathogen	Initial Concentration (T0) ($\log_{10}$ CFU ml^{-1})		Final Concentration (T84) ($\log_{10}$ CFU ml^{-1})	
	Inoculated MBV	Control MBV	Inoculated MBV	Control MBV
Salmonella spp.	7.91	4.01	1.41	1.41
E. faecalis	7.89	2.52	3.75	1.97
E. coli O157	7.50	ND	0.38	0.00
Campylobacter spp.	6.81	1.71	0.00	0.00

No pathogens were recovered as bioaerosols from the control bioreducers. Low numbers of *Salmonella* spp. and *E. faecalis* were detected as bioaerosols in initial stages of the trial from the inoculated MBVs; although no *Salmonella* were detected after the first sampling date and numbers of *E. faecalis* decreased considerably with each sampling date until they were undetectable (data not shown). Although this mimicked the decline in mean concentration of *E. faecalis* within the liquor, the relationship between bioaerosol and liquor counts was not statistically significant (P >0.05; data not shown). Neither *E. coli* O157 nor *Campylobacter* were detected within any bioaerosol samples

DISCUSSION

By applying the criteria stipulated by EFSA for ratifying novel disposal methods to a simulated storage process that is bioreduction, this study will help verify whether the system is biosecure. Over the three month trial period, all pathogens, with the exception of *E. faecalis* had reduced by 5-log values, although *E. faecalis* had also notably decreased by over 4-log values. The work also showed that the cellular metabolic activity of *E. coli* O157 also decreased significantly within the bioreduction system. Pathogens were only rarely detected in bioaerosols, at low numbers, and only in the initial stages of the trial.

Williams et al. (13) hypothesised that microbial competition and predation reduce the population of pathogens within bioreduction vessels. Numerous other studies have showed the reduction of pathogens in a range of wastes is augmented by competition from naturally present antagonistic microbes [5,11]. On the other hand, *E. faecalis* has shown the ability to survive in

stressful environments [8]. This may explain why numbers of *E. faecalis* decreased less within the simulated bioreduction system; although it too may have decreased further had the trial period been extended.

Many pathogens can enter a viable but non-culturable state (VBNC) when under environmental stress and this may lead to underestimation of numbers when using culturing methods. However, bacteria containing the *lux* gene that have entered a VBNC state can also be detected in real-time by measuring bioluminescence [6]. Luminescence directly reports on bacterial metabolic activity which represents a prerequisite for host infection [12]. Bioluminescence measurements showed that there was a concomitant decrease in both numbers and metabolic activity of *E. coli* O157; hence conditions within the vessels were not conducive to the organism's proliferation.

Only low levels of bioaerosols were detected from the simulated bioreduction systems. Although Andersen samplers recover only the culturable fraction of micro-organisms they are the preferred industry choice for sampling bioaerosols from other types of organic wastes. Adkin et al. [1] concluded that were was negligible risk for airborne transmission of prions to operators from bioreduction systems. Although our study involved bacterial pathogens and not prion material, collectively the findings indicate that bioaerosols are unlikely to be of concern from bioreduction systems.

CONCLUSIONS

This work provides evidence to show that bioreduction is efficient at removing pathogens from carcass material and hence that the system could potentially be suitably secure to store fallen stock prior to ultimate disposal. Further investigation at field-scale level is required so that the system can be soundly considered for industry use and incorporation into the revised EU ABPR (1069/2009).

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ENHANCEMENT ANIMAL MANURE COMPOST VALUE BY USING EFFECTIVE MIRCROBIAL

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SUMMARY

Intensive pig farming uses to generate excessive animal manure under a confine space. This excessive animal manure can be managed by composting process to utilize it as an organic fertilizer. Duration of composting process can be shortening by using effective microbial, Effective microbial (EM) are defined as group of beneficial microorganisms that are claimed to enhance microbial activity in compost and soil, which may increase their nutrients value. In view of that a study on evaluation the effective of EM composting process was conducted on pig manure from an intensive pig farm. Two compost piles were set up based on 2 different treatments; (I) Compost without EM or as a control, (II) Compost with EM. The ingredients (manure, sawdust, charcoal, limestone and EM) were well mixed under shed on concrete flooring. Each treatment was turned over weekly to allow aeration. The samples of compost piles were collected every 5 subsequent day for their nutrients analysis, and in situ parameters were measured daily. In terms of EM efficiency, it was found that temperature and pH of compost treated with EM were higher than the control result. The result showed that compost with EM had better nutrient values than compost without EM in terms of nutrient contents. Total nitrogen content was increased about 4 % and 7% gain for TI and TII, respectively. However, a decrease of P and K content about 16% and 8%, respectively found in TI. A decrease result of P about 2.5% and no change of K content in TII. The C/N ratio was decreased about 13.5% and 23% for TI and TII, respectively.

Keyword: Animal Manure, compost, effective microbial

INTRODUCTION

Intensive pig farming uses to generate excessive animal manure under a confine space. If this excessive manure is improper managed, will result in loss of nutrients to environment that can cause air, water and soil pollution. The magnitude of the nutrients loss highly depends on the manure nutrient elements, the manure management system and environment conditions. One way to manage the animal manure is by composting process to utilize it as an organic fertilizer, which can directly apply to crop [1]. Applied under proper conditions at crop nutrient requirements, animal manures are a valuable fertilizer and soil conditioner [2].

Effective microbial (EM) is widely used in many systems pertaining to agriculture and environmental management especially, in waste management nowaday in Malaysia. Commonly, EM is not toxic or pathogenic and is safe for humans, animals and the environment. It was defined by Sekeran *et al.* (2005)[3] that effective microbial as group of beneficial microorganisms can be claimed to enhance microbial activity in compost and soil, which may also increase their nutrients value. Duration of composting process by using effective microbial can be shortening through reaching a stable temperature earlier. In view of this, study on effectiveness of EM on composting process and pig manure nutrients was conducted for an intensive pig farm in Malaysia.

MATERIAL AND METHODS

An experiment was conducted to convert the pig manure into fertilizer by composting. Two compost piles were set up based on two different treatments, namely, (TI) Compost without EM or as a control, (TII) Compost with EM. In this experiment, the commercial EM type was obtained from commercial feedlot farm. The compost pile ingredients (manure, sawdust, charcoal, limestone and EM) were well mixed under shed on concrete flooring. Each treatment was turned over weekly to allow aeration. The samples of both compost piles were collected every 5 subsequent day for their nutrients (e.g. Nitrogen, Phosphorus and Potassium) analysis, and in situ parameters (pH, temperature) were measured daily. The manure was composted for a period of 30 days.

RESULTS

Mean daily temperature and pH for TI and TII of the compost pile are presented in Figure 1 and Figure 2. Both TI and TII show the same blue shape changes of temperature and pH. TII reached a higher mean maximum of about 36 °C and 8.2 compared to TI of about 34.5 °C and 7.9 for temperature and pH, respectively. However, temperature and pH of TI and TII dropped off sharply thereafter to a mean temperature and pH of about 25 °C and 6.3 at day-30.

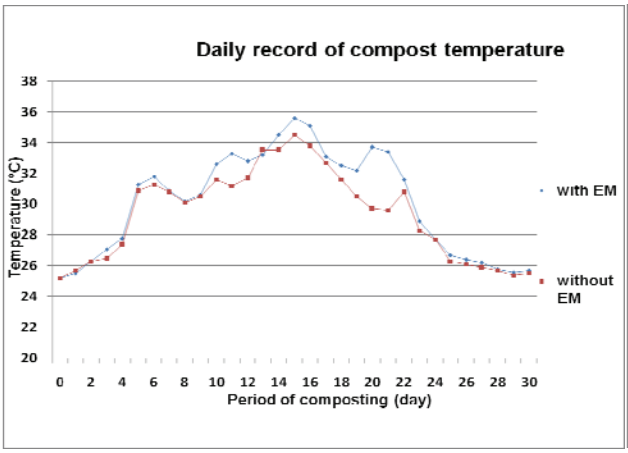

Figure 1: Mean daily temperature for TI and TII

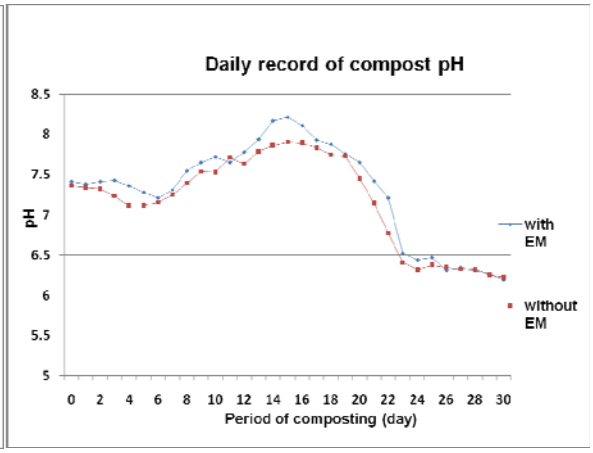

Figure 2: Mean daily pH for TI and TII

Table 1 shows the comparison mean compost nutrietns values of day 0 (initial) and day 30 (end) for TI and TII.

The both treatments were initiated with high moisture content, reflecting by low dry matter (DM) content of compost piles. DM increased one fold at the end of composting. Total nitrogen content was increased about 4 % and 7% gain for TI and TII, respectively. However, a decrease of P and K content about 16% and 8%, respectively found in TI. In TII, a decrease result of P about 2.5% and no change of K content. The C/N ratio was decreased about 13.5% and 23% for TI and TII, respectively.

Table 1: Mean values of compost nutrients for TI and TII on initial (Day 0) and end of composting period (Day 30)

Parameters	Day 0		Day 30	
	TI	TII	TI	TII
DM (%)	26.97^{a}	24.90^{b}	52.89^{a}	50.95^{b}
TKN (%)	1.57^{b}	1.64^{a}	1.64^{b}	1.75^{a}
TOC (%)	45.33^{b}	52.4^{a}	41.12^{a}	43.22^{a}
P (%)	1.52^{a}	1.61^{a}	1.27^{b}	1.57^{a}
K (%)	0.24^{b}	0.27^{a}	0.22^{b}	0.27^{a}
C/N (%)	28.93^{b}	31.95^{a}	25.02^{a}	24.70^{a}

Note: TI: Treatment without EM, TII: Treatment with EM, EM: Effective Microbial, DM: Dry Matter, TKN: Total Kjeldhal Nitrogen, P: Phosphorus, K: Potassium, C/N: Carbon Nitrogen Ratio, $p = 0.05$

DISCUSSION

Pile temperater and pH measurements were collecte to monitor the composting process. Temperature is a common measured indicator of composting activity [4]. However, pH used as chemical parameters to indicate the compost stability[5]. Compost piles of both treatments were in the mesophilic range during active composting (Day-5 to Day-22). The two treatments compost pile only range in mesophilic of 31.4 °C to 35.6 °C because of aeration by turning weekly. Also the piles size of compost with 1m x 1.5m influenced by the surrounding ambient temperature. The TII indicated rapid organic decompostion by reached mesophilic range of composting earlier than TI,. This may due to effect of adding commercial EM in the treatment. It resulted in increasing the effective microbial population in the compost and make it more efficient in decomposition process.

At the intiation of composting (Day 0), both TI and TII had same initial pH values near 7.4, reflecting neutral condition of compost piles. During the first 15 day of composting, TII pH was about one pH unit higeher than TI. The difference may attribute to the destruction of organic acids under more effective microbial and aerobic environment found in TII pile. A sharply dropped of pH values after day 19 was observed during curing, reaching pH values near 6.0. This indicated accumulation of organic acids prior to the end of curing.

In comparison of compost nutrients, there was significant difference (p = 0.05) between TI and TII for all nutrients values, except the TOC and C/N ratio at the end of compost (Day 30). The decreased of C/N ratio throughout the composting process due to the C losses as CO_2 [6] and then stabilizes in value nearly 25%. The TKN changed slightly with the composting time, in this study, a significant reduction of this TOC (9% and 17.5% of TI and TII, respectively) in composting active period. Low C/N ratio at the end was mainly due to the depletion of easily degradable carbon compounds found in pig manure in TI and TII. The final C/N ratio about 25% of two compost piles indicated that the finished product not yet reached the soil humus C/N ratio 12:1. The increased of TKN value may result of N released from biosolids organic matter, that may be taken up by microbial and converted back to organic forms. Alternatively, composting process reduces the available nitrogen content (e.g. mineral N: NH_4^+ and NO_3^-) of organic matter by immobilizing it and converting it to a slow release form.

CONCLUSIONS

The addition of EM in TII presented a significant difference (p = 0.05) between TI and TII for all nutrients values, effectively increased the TKN contents. It may accelerate the production of higher quality compost from manure. The compost pile with EM showed higher temperature and pH values compared to compost without EM addition during composting process. However, the study showed that the addition of EM did not shorten the duration of composting process.

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COMPARISON OF IMMUNOGOLD AND POLYMERASE CHAIN REACTION IN DETECTION OF RABIES INFECTION IN CLINICAL SAMPLES

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SUMMARY

Diagnosis of rabies depends on clinical signs *i.e.* hypo or aerophobia and laboratory methods like IFT. Rapid and accurate diagnosis of rabies is essential for the post-exposure treatment and vaccination. In the clinical laboratory immunodiagnosis can be performed on fresh tissue specimens, which stored at desired temperature. The specimen selection depends on the test and the stage of infection in human and animals. The objective of this investigation was to evaluate the specificity and sensitivity between immunogold assay and PCR for accurate detection of rabies in the clinical samples. For this exercise, an immunogold assay for detecting rabies virus antigen was developed, using colloidal gold nano particles labeled with rabies virus antibodies developed in rabbits.
In another hand RT-PCR was used to amplify the N gene of the rabies virus genome in order to confirm the presence of rabies virus in the clinical samples. For amplification, viral RNA was extracted from the clinical sample by using TRI reagent and the reverse Transcriptase-PCR (RT-PCR) was performed. Two pairs of primers were designed with the sequence of forward primer being 5'-CTACAATGGATGCCGAC-3' and that of reverse primer being 5'-TGGGGTGATCTTRTCTCCTTT-3'.
The result of RT-PCR was visualized using Agarose Gel Electrophoresis. The PCR product is loaded along with 100 bp ladder on 1% Agarose gel and a clear band of about 320 bp was observed. The results of the present investigation showed that the immunogold assay was found sensitive and able to detect upto 92.3% and PCR result showed the 99.7% sensitivity and 100% specificity. PCR is considered as more accurate, sensitive, specific and time saving tool for the detection of rabies virus from the clinical samples than immunogold assay. However, later can be used under field conditions also, if the reagents are available.

INTRODUCTION

Rabies is responsible for approximately 60,000 annual deaths worldwide in humans, making it the tenth most common deadly infectious disease [3]. It is a serious public health and economic problem in Asia and Africa. The incidence of rabies is particularly high in Bangladesh and India followed by moderate incidence in Nepal, Myanmar, Bhutan, Thailand and Indonesia. Its prevalence has been documented from 20-50% in different species of domestic animals [17, 11, 7].
For rabies diagnosis, the direct fluorescent antibody test most frequently used and the gold-standard test approved by both WHO and OIE for rabies diagnosis [2, 9, 15, 16]. This test is performed on brain tissue from animals suspected of being rabid. The test can only be performed post-mortem. Rapid and accurate laboratory diagnosis of rabies in humans and other animals is essential for timely administration of post-exposure prophylaxis [4]. If the animal is not rabid, prompt diagnosis may save a patient from unnecessary physical and psychological trauma, as well as financial burden [6]. The objective of this investigation was to evaluate the specificity and sensitivity between immunogold assay and PCR for accurate detection of rabies in the clinical samples.

MATERIAL AND METHODS

13 rabies virus infected dog brains were collected. Cut a small piece of hippocampus (0.5-1 cm) and placed it on a spatula/filter paper with cut surface facing upwards. Pressed a clean microscope slide on the tissue piece to get an impression smear. After then placed the slides in cold acetone and kept it in the refrigerator for 45 - 60 minutes to fix the impression smear. Air dried the slides and added a drop of Conjugate and incubated in a humid box, at 37^0C for 45–60 min. Finally washed the slides in running tap water and dried the slides. Slides were then observed by applying a drop of 10% glycerol/saline solution under the fluorescent microscope.

Antiserum was produced by immunizing rabbits with purified viral protein. Rabbits were given a series of purified viral protein injections with a time interval. All the injections were given subcutaneous and intramuscular route. The immunization period lasted for 49^{th} days and the first bleeding was carried out 28^{th} days later. Preparation of Conjugated Colloidal Gold was followed as per Slot and Geuze (1985).

RT-PCR

The viral RNA was extracted using TRI reagent (Sigma). A set of RT-PCR primers corresponding to two regions with constant sequences, both of which flank most variable

sequences of the N gene, was designed from the Pasteur rabies virus strain genome sequence [14], (sense primer, 5□-CTACAATGGATGCCGAC-3□, at position 66-82, and antisense primer 5□-TGGGGTGATCTTRTCTCCTTT-3□, at position 365-385). Five ml of RNA (1 to 2 µg) was added to 95 µl of RT-PCR cocktail containing 100 pmole of each primer, 0:2mM deoxynucleoside triphosphate, 10mM Tris-HCl, pH 8.9, 1.5mM $MgCl_2$, 80mM KCl, 10U of AMV reverse transcriptase (QIAGEN), 2U of DNA polymerase (QIAGEN) and 10U of RNase inhibitor (QIAGEN). The mixtures were incubated for 30 min at 42^0C, and then proceeded to PCR (94^0C for 60 sec, 45^0C for 60 sec, and 72^0C for 60 sec) in a DNA thermal cycler (Whatman Biometra). After completion of the PCR reactions, 5µl of each amplicon was run along with 100 bp ladder on 1% agarose gel contains ethidium bromide and visualized in gel documentation system under UV light.

RESULTS

Clinical samples were tested for the comparative evaluation of FAT, Immunogold assay and PCR. On rabies infected brain samples 11 samples out of 13 samples were found positive for FAT assay 12 for Immunogold Assay and all 13 samples were found positive by RT-PCR on the agarose gel a single PCR product with the expected molecular size of 320 bp was clearly amplified. The results of the present investigation showed that RT-PCR was found more sensitive and specific than FAT and Immunogold assay.

DISCUSSION

Clinical diagnosis is difficult in the early stages of rabies and it can easily be confused with other nervous disorders or with normal aggressive behavior of the animals. The rapid identification and confirmation of rabies suspected infection is essential in animals to allow specific control strategies. The diagnostic method PCR has proven to very useful diagnostic tool [13]. Many diagnostic methods are being used to detect rabies virus antigen. In this study, FAT, PCR and Immunogold assay were used as the diagnostic techniques. FAT is the OIE recommended test for rabies diagnosis as it is sensitive, specific and easy to perform. It became a standard diagnostic procedure and is the preferred test for rabies diagnosis [2]. RT-PCR was reported to be the important test for diagnosis and epidemiological studies of rabies by various workers [10]. It was found sensitive and specific particularly in decomposed brain samples, where the sensitivity of FAT is decreased. The nested RT-PCR was reported to be more successful in highly decomposed brain samples of animal and human origin [8]. David *et al* employed RT-PCR on decomposed brain samples which were tested negative on direct FAT.

In cases of rabies suspected in humans antemortem diagnosis may be achieved by several techniques. Isolation of the virus from the patients' saliva, tears, cerebrospinal fluid, or urine by mouse inoculation is possible but requires at least 2 week and is unreliable. More-sensitive techniques such as PCR have produced satisfactory results for detection of rabies virus antigen in brain [8] and in cerebrospinal fluid and saliva specimens [1].

As results show that RT-PCR is more sensitive and accurate diagnostic tool comparison to FAT, Immunogold assay. A study conducted by Kang et al, they found that RT-PCR was 16-fold more sensitive than immunodiagnostics. RT-PCR can be used as confirmatory test instead of fluorescence antibody or mouse inoculation tests in the rabies diagnostic laboratories [5]. Based on this study, it was found that the accurate diagnostic technique plays an important role for the early clinical diagnosis of rabies even in animals not showing symptoms of the disease. Based on the study, the one-step RT-PCR was found to very rapid and more sensitive technique (100%) for rabies diagnosis which can be used on a variety of clinical and morbid samples.

CONCLUSION

In this investigation PCR is found more sensitive and specific diagnostic tool than FAT and immunogold assay for the clinical and morbid samples. Therefore, RT-PCR as an alternative to the currently recommended method of diagnosis with use of brain biopsy specimens for confirmation.

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DETECTION OF BACTERIOLOGICAL CONTAMINATION IN FROZEN BUFFALO MEAT IN SYRIA AND IRAQ

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SUMMARY

The research was carried out on 100 samples of frozen Buffalo meat, that collected from different meat sell shops and these samples divided into two groups, first group consisted of 60 samples from meat sell shops in Hama city/ Syria and second group contained 40 samples from shops in Mosul city/ Iraq. Bacterial and Biochemical tests were carried out on these samples and the results of the first group revealed detection of *Escherichia coli, Staphylococcus aureus , Klebsiella pneumoniae, Clostridium perfringens , Micrococcus and Tetrads*, while the results of the second group showed isolation of *Staphylococcus aureus, Corynebacterium spp., E. coli, Bacillus cereus., Staphylococcous coagulase negative , Klebsiella pneumoniae , Enterococcus feacalis, Streptococcus, Proteus vulgaris and Pseudomonas aeruginosa*. These bacteria consid- ered as main species food borne bacteria that were causes food poisoning and diarrhea in human and the Iraqi samples were more contaminated than Syrian samples. Viable bacterial count also done and revealed that the total bacterial numbers in the first group ranged between 23.9 $x10^6$- 13.6$x10^8$ cfu/ g and in samples of the second group about 23.1$x10^6$ – 65.2 $x10^8$ cfu/g. According to these counts the total numbers were higher than world standards and made these Imported frozen buffalo meats unfit for human consumption

INTRODUCTION

Buffalo Meat is one of the most important sources of meat in India, which has 96.9 million of buffaloes that accounts for 58% of the total buffalo population in the world. India produce 1.49 million tons of buffalo meat contributing 45.56% of world buffalo meat production (1). Major quantity of meat is export in frozen form from India to Malaysia and Gulf countries (2). Meat and meat products are being called among the important cause of food-borne diseases. Limiting the contamination and subsequent inactivation of occurring pathogenic bacteria will be decisive to the safety of meat and their products (3).

In some Middle Eastern, African, and Asian countries, most of carcasses are commonly marketed at refrigerated condition. However, many undesirable changes can be occurred during storage time as a result of microbial growth and /or lipid oxidation resulted in quality reduction and economical loss (4).The object of this study was to investigate the bacterial contamination of the Indian frozen buffalo meat in Syrian and Iraqi markets.

MATERIALS AND METHODS

The 100 collected meat samples included 2 divisions, 60 samples of frozen Buffalo meat collected from different meat sell shops in Hama city/ Syria and 40 samples collected from shops in Mosul city/ Iraq. These samples consisted of meat pieces from different parts of the carcass, transported directly to the laboratory under the cooling conditions. The samples saved in 4°c for 24h.

One piece of each sample was cultured aseptically within Nutrient broth and thioglycolate broth and incubated at 37°c for 24h, after that cultured on blood, MacConkey agar and Mannitol Salt Agar at 37°c for 24h for aerobic growth, and on Clostridial agar and SPF agar at 37°c for 48h within anaerobic jar with specific gas pack for anaerobic growth. Colony characteristics and bacterial morphology were studded and diagnosis confirmed by Gram's stain, KOH test, Coagulase test and Biochemical tests(Catalase, Oxidase, Nitrite reduction, Methyle Red, Voxproskawer , Indole, Urease, H2S production and citrate utilization) and Enterplory strips.

Viable Bacterial count was made by weighed 25g from each samples, then homogenized in a blender containing 225 ml sterile nutrient broth. After that serial decimal dilutions were carried out for each sample and plate counting method was used to determined the bacterial numbers by spread 0.1 ml from each suitable dilution(103-109 dilutions) on plate surface and repeated 3 times for each dilution ,after incubated at 37°c for 24h calculated the bacterial number as mentioned by(5)

RESULTS

The results of bacterial culture for Syrian samples revealed isolation and identification of Escherichia coli, Staphylococcus aureus , Klebsiella, Clostri- dium perfringens , *Micrococcus and Tetrads* (table 1). While results of Iraqi samples indicated isolation and diagnosis of *Staphylococcus aureus, Corynebacterium spp., E. coli, Bacillus spp., Staphylococcus coagulase negative, Klebsiella, Enterococcus feacalis, Strepto-coccus, Proteus and Pseudomonas* (table 2) . Some Iraqi samples revealed isolation more than one bacterial type (table3). Results of Bacterial count was manifested in tables (4, 5).

DISCUSSION

Intact tissues from healthy animals are sterile, but when these animals are slaughtered bacteria from the hide, the gut, or the processing environment may contaminate the surfaces of meat. Some of these bacteria are spoilage bacteria while others are pathogenic to humans. Results of this study were revealed isolation of many bacterial types from the samples that taken in both countries with more variation in Iraqi samples. The major pathogenic bacteria isolated in Syrian samples included Clostridium perfringens, Staphylococcus aureus and E. coli, while in Iraqi samples Corynebacterium, Staphylococcus aureus and E. coli isolated. Clostridium perfringens has not been isolated from Iraqi samples. Many researches were referred to isolation of same bacteria excepted Clostridium perfringens (6, 7, 8, 9). Contamination of Meat products with these bacteria lead to rapid spoilage, then food poisoning and diarrhea may be occurs (3, 10).

Results of viable bacterial count showed high averages of bacterial numbers in sam-ples of both countries (23.9x106- 13.6x108 cfu/ g, 231x106 – 65.2x108cfu/ g). These numbers were higher than the numbers mentioned by other studies, (6,11,12) that showed total number of mesophilic aerobic bacteria about 4.4x104-3.6x106cfu/g (6) and 105-106 for each 3 samples from five samples (12). These numbers that were upper than 107 cfu/g of meat considered as unacceptable meat for human consu-mption (Niemand et al,1981), and same meat products must not be permit to import.

CONCLUSIONS

1. Isolation of many important pathogenic bacterial types included Staphylococcus ,E. coli , Clostridium perfringens, Corynebacterium, that are food borne bacteria and causes food poisoning and diarrhea in Humans.
2. According to the viable bacterial count these samples in the two countries are unfit for human consumption and higher than world standards.
3. Iraqi samples showed high variation in bacterial types more than the Syrian samples.
4. Imported frozen buffalo meat must be under quality control.

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Table 1: Bacterial types isolated from frozen meat buffalo in Syria

Bacterial Isolates	Samples No.	Percentage 100%***
E. coli	26	43.33
Staph. aureus	21	35
Klebsiella	14	23.33
C. perfringens	11	18.33
Tetrads	4	6.66
Micrococcus	3	5

*** Percentage of bacterial isolation according to the samples number.

Table 2: Bacterial types isolated from frozen meat buffalo in Iraq

Bacterial Isolates	Samples No.	Percent. 100%***
Corynebacterium	14	35
Staph. aureus	11	27.5
E. coli	9	22.5
Klebsiella	8	20
Bacillus spp.	4	10
S. Coagulase -	3	7.5
En. feacalis	2	5
Pseudo. aeruginosa	2	5
Proteus	2	5
Streptococcus	1	2.5

*** Percentage of bacterial isolation according to the samples number.

Table 3: Isolation of mixed bacterial types from Iraqi samples

Samples No.	Bacterial isolation	Percentage 100%***
5	*Corynebacterium + E. coli*	12.5
4	*Staph + Corynebacterium*	10
2	*Staph+ Proteus+ Bacillus*	5
2	*Klebsiella+ Enterococcus*	5

*** Percentage of bacterial isolation according to the samples number

Table 4: Bacterial count in Syrian samples

Bacterial type	Cfu/g	Bacterial type	Cfu/g
E. coli	$48x10^6$ - $24x10^7$	*C. perfringens*	**19.1**$x10^6$-$43x10^8$
Staph aureus	$11x10^6$- $23x10^7$	*Tetrads*	**8**$x10^6$-$10x10^7$
Klebsiella	$8x10^7$- $11x10^8$	*Micrococcus*	$21x10^5$-$13x10^6$

Table 5: Bacterial count in Iraqi samples

Bacterial types	Cfu/g	Bacterial types	Cfu/g
Corynebacterium	$8x10^7$-$11x10^8$	*S. coagulase -*	**8**$x10^6$-$10x10^7$
Staph. aureus	**19.1**$x10^6$-$43x10^8$	*En. feacalis*	$8x10^7$-$11x10^8$***
E. coli	$15x10^6$-$23x10^8$	*Pseudo. aeruginosa*	$8x10^7$-$11x10^8$
Klebsiella	**25**$x10^6$-$34x10^7$	*Proteus*	$8x10^7$-$11x10^8$
Bacillus sp.	$21x10^6$-$13x10^7$	*Streptococcus*	$21x10^6$-$13x10^7$

***counted as a samples with mixed bacterial contamination

SURVEY ON INFESTATION TO DICTYOCAULUS FILLARIA IN SLAUGHTERED SHEEP IN TABRIZ(NORTHWEST OF IRAN)

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SUMMARY

Dysfunction of respiratory tract in sheep and goats is one of the most important causes of economic and hygienic losses in Iran. This disease can be due to bacteria, viruses and parasites. In ruminants pulmonary worms are the most prevalent cause of this disease. *Dictyocaulus fillaria* is one of the most important in lungworms which produce pathogenic signs in lungs. The worms are diagnosed by inspection in slaughterhouse. This study was conducted to diagnose the infestation rate of slaughtered sheep to *Dictyocaulus fillaria* and seasonal changes in Tabriz (Northwest Iran).Two hundred thirty five respiratory tracts of slaughtered sheep in four seasons of one year were inspected carefully and trachea and lungs were incised and adult worms of Dictyocaulus *fillaria* were isolated in laboratory and were recognized by diagnostic keys. Totally 63(26.8%) lungs were infested to Dictyocaulus *fillaria*. Most infestation area was in apical lobes of lungs. It was not significantly difference in infestation to *Dictyocaulus fillaria* in different seasons, but infestation in autumn was higher than other seasons. Based on this results preventation and therapies were recommended due to high rate of infestation of sheep to *Dictyocaulus fillaria* in this area.

Key words: *Dictyocaulus fillaria* , slaughtered sheep, Tabriz .

INTRODUCTION

Lungworm of small ruminants are limited to two super families such as Dictyocaulidae and Metastrongylidae. *Dictyocaulus fillaria* (in Dictyocaulidae) is one of the commonest lungworm in the ruminants such as sheep and goats (2). Helminth parasites of ruminants are ubiquitous, with many tropical and subtropical environments of the world providing nearly perfect conditions for their survival and development. Although these parasites are widely prevalent, the clinical signs they showed in infected animals can be less obvious than signs of other livestock diseases (5). Infestation to lungworms in goats is characteristics by dyspnea, sneezing and cough due to pneumonia. *Dictyocaulus fillaria* is one the most important in lungworms which produce nodules and brownish spots in lung. This spots are diagnosed in slaughterhouse (3). The aim of present study was to determine the prevalence of *Dictyocaulus fillaria* infestation in slaughtered sheep and its changes in different seasons.

MATERIAL AND METHODS

Study area

The study was conducted in Tabriz, that is located in the East-Azarbaijan (36°43′ 39°25′N and 45°3′–48°19′E) .The city is located at an elevation of 1351/4 meters above sea level and the climate is temperate. Summers are relatively hot and dry while winters are cold.

Sample collection and postmortem examination

235 respiratory tracts of slaughtered sheep in four seasons of one year were inspected carefully and in susceptible cases trachea and lungs were incised. The trachea and main bronchi were opened longitudinally, carefully examined, after placing them under running water; the lavage was poured into a container to collect adult worms. A visual inspection of dorsal and ventral surfaces of the lungs was achieved and presence of verminous nodules was recorded. The pulmonary parenchyma, in particular affected areas, was dissected under a stereomicroscope to extract adult nematodes. Adult worms were made with lactophenol and were recognized by diagnostic keys (2,6).

RESULTS AND DISCUSSION

A total of 235 respiratory tracts were observed through postmortem examination of Which 26.8% were positive for *Dictyocaulus fillaria*. (Table 1)

Table 1- The prevalence of *Dictyocaulus fillaria* in respiratory tract

Number of tested	Number of positive	% of positive
235	63	26.8%

The prevalence of *Dictyocaulus fillaria* by season was recorded. It was not significantly difference in infestation to *Dictyocaulus fillaria* in different seasons, but infestation in autumn was higher than other seasons.

Table2- The prevalence of *Dictyocaulus fillaria* by seasons

Season	Number of examined	Number of positive	% of positive
Spring	60	16	26.6
Summer	63	18	28.57
Autumn	59	22	37.28
Winter	53	7	13.2

The results of this survey were showed that the overall infestation of sheep in Tabriz to *Dictyocaulus fillaria* was 26.8%. Different prevalence rates reported in previous studies in Iran. The prevalence of *Dictyocaulus fillaria* was reported 27.4% in Urmia, 32% in Tabriz and 16% in Tehran (4, 7). Different prevalence rates reported between this study and previous studies should be related to factors such as intermediate hosts density, and environmental temperature (5). In adjacent countries in Turkey and Iraq infestation to *Dictyocaulus fillaria* was reported 32.5% and 64. 2% respectively (1,8).

The results of this study revealed that was not significant difference between seasons in infestation to *Dictyocaulus fillaria* in sheep in Tabriz. This finding is not similar to other studies in Iran such as Tavassoli et al (1999) and Golezardy (2001) findings (4,7). This agreement is probably due to different whether condition and inbreed managements. In conclusion infestation to *Dictyocaulus fillaria* in sheep of Tabriz is not high, this phenomena probably is due to orderly treatment of small ruminants and other prevention procedures. This research serves as baseline for future studies for treatment or prophylaxis of lungworms in Tabriz.

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CLINICAL, HAEMATOLOGICAL, AND BIOCHEMICAL CHANGES IN NATURALLY TICK AND MANGE MITE INFESTED CATTLE (Abstract)

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INTRODUCTION

Tick and mange mite infestations in cattle are belonging to the most destructive enemies for animal health and economical thrifty especially in tropical and subtropical countries. They play an important role in reducing animal production through sucking of blood, injection of toxins and skin damage. The present study was designed to throw light on the effect of natural infestation of cattle with tick and mange mite on general health condition with a special regard to haemogram and some biochemical parameters.

ANIMALS, MATERIALS AND METHODS

Clinical investigation of total 49 cows aged from 2-4 years old revealed that 23 cows were tick infested and 11 were mange mite infested, while 15 appeared clinically healthy. Two blood samples from both clinically healthy and infested cattle were collected from jugular vein, one in heparinised vacutainer tube for examination of haemogram and the second without anticoagulant for obtaining clear non haemolysed serum for biochemical analysis of ALT, AST, GGT, Total proteins, albumin, glucose, iron, copper and total iron binding capacity (TIBC) using commercial test kits.

RESULTS

Clinical examination revealed that tick infested cattle showed poor general health condition in the form of rough shaggy appearance of coat, mild itching, spectacle appearance and presence of ticks in groins, perineum and withers regions. While mange mite infested cattle were thin and weak with presence of alopecia, pruritis, fissures, crakes and blood oozing. Parasitological examination indicated infestation of cattle with *Boophilus spp.* and *Psoroptic spp*. Haemogram indicated normocytic normochromic anaemia with eosinophilia ($P<0.001$) in ticks and mange mite infested cattle. Furthermore, mange mite infested cattle showed relative increase in total leucocytic count ($P<0.01$), and significant decrease in neutrophils ($P<0.01$). Biochemical serum analysis in tick and mange mite infested cattle showed decreased serum levels of albumin and total proteins ($P<0.01$) with increased serum globulins ($P<0.001$). The serum level of glucose decreased ($P<0.001$), while AST increased ($P<0.01$) in both cases. ALT and GGT were insignificantly changed ($P>0.05$). Serum levels of both iron and copper were significantly decreased in tick and mange mite infested cattle ($P<0.01$), while TIBC was decreased only in mange mite infested one ($P<0.01$).

CONCLUSIONS

It can be concluded that trace elements should be substituted and general tonics can be recommended along with the specific management of tick and mange mite infested cattle.

Epidemiological Study about Prevalence and Distribution of Sheep and Goats Gastrointestinal Parasites in Duhok Province

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SUMMARY

Four hundreds and seventy six samples of feces had been collected directly from the rectums of various animals, sheep and goats, of both sexes, of different ages and areas in Duhok Province (Duhok, Zakho, Imadia, Shaikhan, and Aqrah) during the period between September 10, and October 10, 2010. The samples were placed in clean plastic sacks were transferred into the laboratory of Duhok veterinary Directorate-Duhok for parasitic examinations. Parasitic tests included flotation and sedimentation techniques. The finding showed that the overall percentage of intestinal parasite infection had reached 15.85% in Duhok Province .The results had also shown difference in the infection rates from one area to another. The highest rate of infection was noticed in Aqrah with a percentage of 26.16% while the lowest one was seen in Imadia with a percentage of 2.71% . Moreover, the study results proved that the flotation method was the best in finding out parasites from examined feces.

INTRODUCTION

Sheep and goats are of a prominent standing among animals in many countries in the world for being domesticated and able to survive in various circumstances and on different types of fodder [1]. Furthermore, sheep and goats are milk and meat producing sources, in addition to the use of their wool, hair and leather in diverse scopes of industry . The number of sheep and goats in the developing countries is more than twice their numbers in the developed countries but their production of milk and meat is less than the half of that in the developed countries for many reasons [2]. The most important factors influencing the production involved the diseases, infections and malnutrition. However, such helminth infection a crucial role [1]. Internal parasites are small creatures lodge the Lumina of intestines of animals and sometimes can be seen through a naked eye. Also, fecal examination using microscope may reveal the existence of the helminth or their ova [1]. Economic losses due to helminthisis is may represent by weakness, lack of production, and death of animals [1]. Indirect losses are invisible wastes gain, drugs used in treatment, measures applied in control and therapy of animals [2]. Additional hidden economic damages may be represented by weight loss, lack of food conversion, reduction of milk production and impact on wool. It was mentioned that in Ethiopia 5-7 millions of sheep and goats die annually as a result of helminthic infection [3]. More cases of helminth infection occur during warm and wet climates because such environment keeps parasites' vitality [4] .

Due to the lack of studies about distribution and prevalence of certain parasites in Duhok Province / Iraq, this study was planned to identify some of epidemiological aspects of parasitic infection and to demonstrate their species in sheep and goats .

METHODS AND MATERIALS

Four hundreds and seventy six fecal samples had been collected from different areas in Duhok Province (Duhok, Zakho, Imadia, Shaikhan, and Aqrah). These samples were taken directly from the rectums of sheep and goats. The animal were of both sexes, of different ages and areas in Duhok Province during the period between September 10, and October 10, 2010. Ten to fifteen gm feces were collected which were placed in clean plastic bags, and were transferred into parasite laboratory. The following parasitic techniques were conducted on the samples :

1- Flotation Method :

This method was done by mixing a quantity of feces with saturated sugar. Then, the mixture was filtered and placed in a test tube covered with a slip. Later, the sample was left for 10-15 minutes followed by the microscopic examination of the slip after its placingon the slide.

2- Sedimentation Method :

This method was done by mixing a quantity of feces with physiological salt solution in a beaker. The solution mixture was filtered in test tubes and were placed in a centrifuge at a speed of 1500 rpm for five minutes . Later, the sediment was transferred into a glass slide to be examined using the microscope [5].

RESULTS

The overall percentage of intestinal parasitic infection had reached 15.85% in Duhok Province. The results had also revealed difference in the infection rates from one area to another. The highest rate of infection was noted in Aqrah with a percentage of 26.16% whereas the lowest one was seen in Imadia with a percentage of 2.71% as shown in table No. 1 .

Table (1) : Infection rates of parasites species obtained from sheep and goats

Area of Study	Total Incidence%	Incidence%		
		Nematode	Trematode	Protozoa
Duhok	8.51	92.31	5.10	2.5
Zakho	10.26	93.65	6.34	0
Imadia	2.71	89.90	10.90	0
Shaikhan	14.14	79.77	20.22	0
Aqrah	26.16	83.30	16.60	0
total	15.85	85.99	13.66	0.39

The study had also shown an increase in nematode infection in Zakho with a percentage of 93.65% and the trematode infection in Shaikhan which had reached (20.22%). While protozoa infection percentage in Duhok was (2.5%). Besides, the results had clarified that nematode infection percentage amounted to (85.99%), whereas infection percentages of trematode and protozoa were (13.66%) and (0.39%), respectively. Furthermore, it was found that the flotation method was the best in identification of egg parasites from feces as shown below in table No.2 .

Table(2) : Identification percentages of egg parasites in different methods

Area of study	% for Different Identification Methods	
	Flotation	Sedimentation
Duhok	79.45	0
Zakho	79.36	0
Imadia	74.54	0
Shaikhan	50.56	26.96
Aqrah	40.99	36.03

DISCUSSION

The overall parasitic infection rate amounted to 15.85 which was considered somewhat high since the study was carried out in autumn. The identification rates of egg parasites among the study areas were variant. This may be due to many reasons such as the study season, geographical and climatic differences. It was seen that the highest infection rate was recorded in Aqrah. Such high infection rate could be to the fundamental factors represented by the availability of convenient helminthic environment like temperature and humidity in addition to the existence of the intermediate hosts e.g. snails as well as the abundance of animals raised there. Whereas the lowest parasitic infection rate was seen in Imadia which was regarded as cold mountainous area. Which is unfavorable for survival, growth and perpetuation of the parasites. Such findings were in agreement those reported by other researchers [4],[6].

Fitch [4] and Radostits *et al*,[6] mentioned that watershed on the way of grazing animals is considered as a parasitic contamination source. The water of the current study is formed as a result of its gathering and coming from many areas which perhaps being polluted with animals feces containing eggs and parasitic larvae. Furthermore, such water may be contaminated through its passage among green pastures. Consequently, watering of these animals by with contaminated water as well as bringing the green fodder from another areas or provinces result in contamination of both water and feed. Another possible source of infection may occur through transporting of fodder when there is a feed scarcity or shortage influencing the local markets. These factors support spread of parasitic infections among the provinces all over the country [7].

The reasons behind the parasitic infection could be attributed to the decline in host immunity as a result of malnutrition which usually occurs in the poor agricultural lands. In these fields more than one kind of animal is grazing such as cattle, sheep, and goats which is a usual practice. Also, predominant suitable climatic factors like temperature and humidity promote parasites growth and development especially in autumn during the study period [1], [3].

Among other important things is the development of parasites resistance to drugs as a result of indiscriminate and frequent use of many types of antiparasitics agents which is applied by many farmers and animal owners [3].

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OCCURRENCE OF FASCIOLOSIS IN SLAUGHTERED CATTLE IN ESPÍRITO SANTO STATE - BRAZIL

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SUMMARY

Brazil is one of the largest cattle producers and the largest exporter of cattle meat in the world. The fasciolosis is a disease of great importance in veterinary medicine, both because of damage to livestock and to cause economic losses, as well as to be a zoonosis. A study was carried out to determine the prevalence and to assess the economic significance of bovine fasciolosis due to liver condemnation in slaughterhouse located in north of Espírito Santo state, southwest of Brazil. Data were collected in 2007, and the total number of animals slaughtered during this period was 10,234. Postmortem findings of liver lesions showed a high rate of liver condemnation, especially due to the presence of *Fasciola hepatica* in 25.9% of them. The results obtained describe for the first time the occurrence of fasciolosis in the north of Espírito Santo state, indicating that this infectious disease may be spreading to other areas of Brazil, since it was restricted only in the south region of this country. Fasciolosis has a significant economic importance as the resultant liver condemnations caused an average loss of approximately 24,000 USD per annum to producers and slaughterhouses in Brazil, represents a potential risk of transmission to the consumers of meat products, could hinder exports of meat and cause serious damage in all countries that import beef from Brazil.

Keywords: 1. Liver condemnation; 2. Fasciolosis; 3. Cattle.

INTRODUCTION

Fasciola hepatica (Linnaeus, 1758) is a trematode that causes a parasitic disease named fasciolosis, which affects the liver and bile ducts of cattle, sheep, goats and horses, causing an injury known as liver rot (7). The fasciolosis is a disease of great importance in veterinary medicine, both because of damage and cause major economic losses to livestock, as well as constituting a zoonosis in which man may be accidental hosts of this trematode (6).

The economic damage attributed to fasciolosis is due to both direct and indirect causes, such as the fall in production and quality of milk, meat and wool, weight loss, abortions and decrease in fertility, stunted growth and high mortality rates of parasitized animals (3, 7). Although often these aspects go unnoticed by the producers, slaughter plants conviction in the livers of animals infected as a result of official sanitary inspection performed by veterinarians, and following the rules described in the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin (RIISPOA) occurs due to the injuries found in these organs, which are sent to the slaughterhouse's rejection section and may not be intended for human consumption (2). Statistics provided by the federal inspection service of the Ministry of Agriculture, Livestock and Supply of Brazil show that the occurrence of this parasite and the condemnation of the liver fasciolosis have been increasing gradually over the past year, and that the areas most affected are located in the states of Rio Grande do Sul, Santa Catarina, São Paulo, Rio de Janeiro, Minas Gerais and Paraná (7).

Whereas to date there are few reports of the presence of this parasite in other states than those previously mentioned, the purpose of this study is to report the occurrence of *Fasciola hepatica* in cattle livers from animals slaughtered, from January to October 2007, in the north of Espírito Santo state, Brazil.

MATERIAL AND METHODS

This work was carried out using data obtained from post-mortem inspection of slaughtered bovines, from various localities at north of Espírito Santo state. Data were collected between January to October 2007, the total number of animals slaughtered during this period was 10,234, giving an approximate average of 102.34 head/day. The condemnations made during the post-mortem inspection was performed following the standards described in RIISPOA (2) and recorded on nosographic maps, whence they were removed from all data related to them for this work. In parallel, were obtained some photographs of injuries observed during this study in order to illustrate the same and provide scientific support for future studies.

RESULTS

The result of the number of cattle slaughtered and condemnation of livers by *Fasciola hepatica* from January to October 2007 were obtained from the nosographic maps of inspection service of Espírito Santo state and are presented in Tables 1 and 2.

Table 1: Number and percentage of liver condemnations in slaughtered cattle, from January to October 2007, in the north of Espírito Santo state, Brazil.

Month	Slaughtered animals	Number of liver condemnations	Percentage
Jan/07	962	44	4,57
Feb/07	913	41	4,49
Mar/07	1122	31	2,76
Apr/07	1014	49	4,83
May/07	1055	44	4,17
Jun/07	938	23	2,45
Jul/07	1028	72	7,00
Aug/07	1174	56	4,77
Sep/07	928	75	8,08
Oct/07	1100	66	6,00
Total	10,234	501	4,89

As can be seen in Table 2, the fasciolosis was identified in all months of the study, with a higher number of condemnations in July 2007, indicating that it became endemic in this region.

Table 2: Causes of liver condemnations in slaughtered cattle, from January to October 2007, in the north of Espírito Santo state, Brazil.

Causes of liver condemnations	Jan/07	Fev/07	Mar/07	Apr/07	May/07	Jun/07	Jul/07	Aug/07	Sep/07	Oct/07	Total
Abscess	22	19	10	13	7	5	11	19	13	5	124
Cirrhosis	0	2	0	5	5	3	4	4	0	0	23
Contamination	1	0	0	0	1	2	0	0	0	12	16
Cysticercosis	5	6	2	6	1	0	1	3	5	0	29
Fasciolosis	8	6	14	8	17	6	36	5	11	19	130
Tuberculosis	0	0	0	0	0	0	1	0	0	0	1
Others	8	8	5	17	13	7	19	25	46	30	178
Total	44	41	31	49	44	23	72	56	75	66	501

The percentage of liver condemnation in slaughtered cattle during the study period due presence of the *F. hepatica* was 25.95% (Table 3).

Table 3: Percentage of liver condemnations due to the presence of *Fasciola hepatica* in slaughtered cattle, from January to October 2007, in the north of Espírito Santo state, Brazil.

	Number of liver condemnations	Presence of *Fasciola hepatica*	Percentage of liver condemnations
Jan/07	44	8	18,18
Feb/07	41	6	14,63
Mar/07	31	14	45,16
Apr/07	49	8	16,33
May/07	44	17	38,64
Jun/07	23	6	26,09
Jul/07	72	36	50,00
Aug/07	56	5	8,93
Sep/07	75	11	14,67
Oct/07	66	19	28,79
Total	501	130	25,95

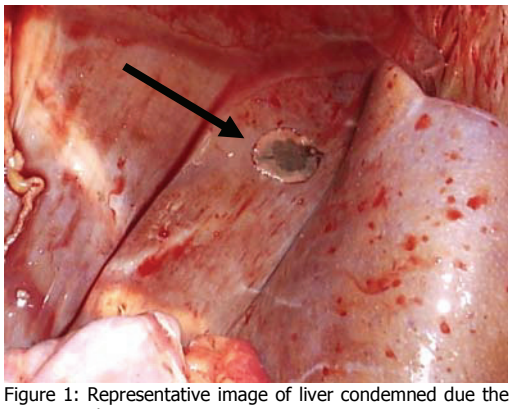

Figure 1: Representative image of liver condemned due the presence of *Fasciola hepatica* (arrow) in slaughtered cattle in the north of Espírito Santo state, Brazil.

Figure 1 shows the presence of *Fasciola hepatica* in the liver of cattle slaughtered in the slaughterhouse where this study was conducted.

DISCUSSION

According to the study conduced in the Rio Grande do Sul state, southern region of Brazil, were slaughtered about 460,000 animals in 2003, and 43.5% of liver were sentenced because fasciolosis and hydatidosis, resulting a loss of about US$ 1,5 million (1). Taking into consideration that a bovine liver weighs about 5kg, with an average cost per pound in Brazil to around US$ 2.00, the liver condemnations generating an economic loss of approximately US$ 24,000 to meat producers in Brazil. Thus we emphasize the importance of herd health management as an important measure in preventing this disease that causes economic losses to both producers to slaughterhouses. Moreover, as the fasciolosis an important zoonosis, it reinforces the role of sanitary inspection in official establishments of animal products as a way of maintaining public health.

It is noteworthy that in the scientific literature found no record of *F. hepatica* in the north of Espírito Santo. According to Moreira (5) and Lima (4), there is only notice about fasciolosis in the south region of the Espírito Santo state. Whereas the slaughterhouse where this study was conducted is located in north of Espírito Santo state and receives animals from several neighboring municipalities, including from the state of Bahia, it appears that beyond this disease is advancing within the state. There is the possibility of it being introduced in the state of Bahia, which would also its introduction in the Northeast Brazil region, where to date there is no record of fasciolosis in livestock.

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Block 5

ANIMAL FEED HYGIENE – CHALLENGES AND OPPORTUNITIES FOR THE FUTURE

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INTRODUCTION

We are in a global food crisis. Food prices have reached the highest levels ever recorded. Eighteen million people have died from hunger during the last 3 years. Last year, for the first time in history, global food consumption was greater than food production. The world will need 100% more food by the year 2050 and most experts predict that 70% of it will need to be produced with improved technology using fewer resources. Will food prices and reduced availability of food cause us to change the way we think about feed and food safety risks? Will we become more tolerant of production technologies previously considered too risky to utilize, or will we increase our scrutiny because consumers will be spending a greater portion of their income on food and have even higher expectations of feed safety? It is impossible to separate food security from feed safety.

Food security has been defined in many ways. A recent definition of food security is "fair prices, choice, access through open and competitive markets, continuous improvements in food safety, transition to healthier diets, and a more environmentally sustainable food chain" (Anonymous, 2008). We need new food production technology to feed the growing world population. Perhaps one of the biggest challenges in our global society is embracing the wide diversity of viewpoints of how food should be produced. For example, some countries accept the use of genetically modified grains in animal feeds while others are reluctant. We are at a critical point in history where intelligent decisions must be made to develop and use new technologies, at the same time provide realistic risk assessment of potential short and long-term consequences of these technologies.

Different perspectives, such as opposition to intensive livestock production and use of various feed types and production technologies create sociological questions (e.g. impact of intensive food animal production on rural communities), ethical questions (e.g. acceptability of animal housing conditions, use of castration, and consumption of animal food products), environmental questions (odor, pollution, and carbon footprint), and sanitation questions (e.g. zoonotic and emerging diseases, occupational health, and food safety). These different perspectives result in implementation of different types of animal production systems including organic and natural food animal production. How do we develop feed and food safety systems that are effective for all types of animal production systems?

Many uncertainties impact food production and safety. Some of these uncertainties include: global economic recession, currency fluctuations, political unrest, war, climate change, impacts of emerging technologies, animal disease, high and volatile commodity prices, and governments' role in improving food safety and health. These, coupled with increasing global trade of feed and food, along with different tolerances, standards, and analytical capabilities to assess feed and food safety among countries, add further complexity. Feed and food safety is one component of many complex interrelationships of our global food production system. Given this complexity, our ability to meet increasing global demands for adequate quantities of high quality, safe, and low cost animal food products in an environmentally, economically, and socially sustainable way is a daunting task**.**

Is our current global feed and food safety system adequate?

Societies everywhere expect consistently safe food. However, our current animal feed and food safety systems do not meet these expectations. Feed and food safety recalls continue to occur, and are growing in frequency. For example, during the first three months of 2011, there have been over 20 notifications of feed contamination cases in Europe alone, and the number of reported feed contamination events in Europe has been increasing since 2004. The trend is likely due to better reporting procedures rather than declining feed safety standards. Nonetheless, failure to control feed safety has continued to result in continued recalls of millions of tons of contaminated animal food products, putting further pressure on food supply and prices.

To emphasize the impact of two independent, single events involving feed safety in the food chain, consider the following recent examples:

In August, 2010, chicken feed was tested positive for salmonella on layer farms in Iowa (U.S.) causing a recall of 0.5 billion eggs. The U.S. FDA linked this outbreak to almost 2,400 cases of salmonella-related human illnesses around the country in the largest outbreak since the 1970. The egg recalls came weeks after a new FDA rule took effect requiring large-scale producers to practice better safety and to test for salmonella bacteria in poultry facilities.

In January, 2011, dioxin contaminated feed cost German poultry and pig farmers tens of millions of dollars and

raised questions about the rigor of food testing in Germany and the strength of food safety regulations in the EU. Approximately 4,700 farms were affected and millions of eggs were removed from the retail food supply. South Korea and Slovakia stopped imports of animal food products from Germany, while other countries were investigating the extent of the contamination. The problem occurred when the manufacturer mixed waste (assumed safe) from biofuel production into animal feed.

Events like these create several common consequences:

1. Create fear and panic among consumers.
2. Reduce the amount of food available to consumers.
3. Reduce consumer trust in current feed and food safety prevention, monitoring, regulatory, and control systems.
4. Can cause human illness and death, and lead to future health risks for consumers.
5. Affect the entire food chain from production to consumption.
6. Cost millions of dollars in lost revenue and increased costs.
7. Are often global in nature.

These examples are not new and perhaps not unexpected. However, think about the feed and food safety consequences of the recent unexpected earthquake, aftershocks, tsunami, and radioactive leaks that occurred in northern Japan. Events like this have serious long-term environmental, as well as human health, animal health, and food safety consequences. Japan, perhaps the most prepared country in the world, could not prevent these catastrophic consequences. How should continents, countries, and communities prepare for infrequent, devastating events like this that dramatically affect the quantity and quality of the food supply?

Why doesn't our current feed safety system work?

There are many possible reasons for ongoing feed safety problems. Sometimes, it is as simple as not applying effective, well established, quality control procedures. For example, there are well established GMP's for feed manufacturing quality control. Many feed mills are ISO and HAACO certified. Why aren't all feeds mills certified and using the same standard feed safety and quality control procedures?

Sometimes, it is a case of "everyone has responsibility, but no one is responsible". Poorly defined and implemented roles and inadequate communication of overlapping government agencies continue to be a problem.

Sometimes, feed safety concerns are not easily controlled. For example, salmonellosis continues to be a significant feed and food safety problem. There are approximately 1.4 million of cases of salmonellosis within North America each year. Many feed and food safety agencies have been engaged in on-going attempts to address the risk posed by *Salmonella* and reduce the prevalence of this pathogen in the food chain. However, despite all these efforts the annual number of salmonellosis cases remains high.

We need to re-evaluate approaches to feed/food safety prevention and control. All aspects of global feed/food safety can't be completely understood, there is no single entity or technological solution that can solve these challenges, and there are always going to be unanticipated events and unintended consequences in our global feed/food safety system. Improving our feed/food safety system will require "systems thinking" in the context of all of the interrelationships in our food production system and a holistic approach to bring together multiple perspectives. It will require embracing a "one health" perspective along with collaborations and partnerships with shared leadership.

Assessment of current feed safety hazards

Scientists are generally trained in highly specialized, individual disciplines with the notion that there are single answers to solve problems. Almost all of the feed safety problems today are complex and multi-disciplinary. Because of our "reductionist thinking" scientific culture, we often categorize feed safety hazards into known biological or chemical compounds shown to have adverse effects on animal and human health, and can be directly measured in feed and animal tissues with a high degree of accuracy (Table 1). While this approach is enormously important and effective, we can and must do better.

Global environmental conditions play a significant role in our feed safety system. For example, we have generally ignored the possibility of heavy metal contamination in mineral supplements fed to livestock and poultry. In one of our recent studies, we measured cadmium and lead levels in commonly used mineral supplements. We found cadmium levels to be high in common zinc and cobalt sources, and high lead concentrations in several common mineral supplements. What are the long-term effects of feeding common mineral supplements contaminated with significant levels of heavy metals on animal health, meat product safety, and animal manure as it is applied to arable land?

Human exposure to mycotoxins present in animal feeds has been of marginal concern relative to other feed safety hazards, with the exception of aflatoxins. However, changes in global climate, agricultural practices, and geographic distribution are increasing our exposure to mycotoxins. How should we adapt to these changes from a feed and food safety perspective?

Organic pollutants are a major feed/food safety concern due to their wide prevalence in the environment, ability to accumulate in animal tissues and be secreted in cow milk and human breast milk. How do we prevent them from contaminating the feed supply, and ultimately, the human

food supply, when the environment is the resource used to produce these feedstuffs?

Table 1. Common feed safety hazards.

Biological hazards	Examples
Mycotoxins	aflatoxin B1, deoxynivalenol, fumonisin B, zearalenone, ochratoxin, T2 toxin, ergot
Innate plant toxins and secondary metabolites	saponins, glucosinolates, alkaloids (pyrrolizidine and tropane), gossypol, hydrocyanic acid, theobromine, volatile mustard oil, vinyloxazolidine thione
Botanical impurities	Datura stramonium, Castor oil, Crotolaria spp., (Mowrah, Bassia, Madhuca), Mustard (Indian, Sareptian, Chinese, black, Ethiopian), unhusked beech mast, purghera, croton
Genetically modified plants	maize, soybeans
BSE	ruminant derived animal proteins
Microbiological agents	brucella, salmonella, endoparasites (echinococcus, toxoplasma gondii, cisticercus, trichinella, E. coli O157
Marine biotoxins	
Chemical hazards	
Heavy metals	arsenic, cadmium, lead, mercury, fluoride, nitrites
Organic pollutants	dioxins, dioxin-like PCBs, dibenzofurans, organochlorine pesticides (aldrin, dieldrin, camphechlor, chlordane, DDT, endosulfan, endrin, heptachlor, HCB, HCH, melamine
Animal health agents	antimicrobials, coccidiostats (e.g. ionophores)

Although we know a great deal about current feed safety hazards, we are still limited in understanding how these hazards can be more rapidly and accurately quantified and how the relative risks of each hazard should be incorporated into an integrated, holistic feed safety management system of the future. Furthermore, little consideration has been given to non-targeted identification of potential toxic metabolites in animal tissues using analytical techniques such as mass spectrophotometry and metabolomics. We need to consider emerging (non-traditional) toxicological endpoints for hazard characterization such as delayed neurotoxicity, developmental toxicity, trans-generational effects (endocrine disruptors), immuno-toxicity, and metabolomics technology.

Risk assessment is a critical component of a comprehensive feed safety program and is a multi-disciplinary challenge. Prevention of harmful residues in animal derived food products involves hazard identification, hazard characterization, exposure assessment, and risk assessment. However, there are deficiencies in each of these steps. For example, hazard characterization has been based on a dose-response assessment for each animal species and each age category. However, the typical end-points of toxicity assessment in animals (e.g. reduced feed intake, weight gain, productivity, reproductive impairment, modulation of immune competence, organ specific-lesions) are different than end points for humans (e.g. genotoxicity, carcinogenicity, developmental toxicity, allergenic potential). Other deficiencies include significant biological variability among species in bioavailability of substances and exposure to mixtures of contaminants, air-borne substances, and medications. We need develop diverse, multi-disciplinary, global programs to overcome the deficiencies in each of these risk assessment steps to improve our feed safety system.

Impacts of water supply and quality on food security and safety

Water is sometimes described by nutritionists as the "forgotten nutrient" because we often take water availability for granted. However, reduced quantity and quality of water is becoming a serious issue worldwide, and consequently, is having an increasing impact on animal health and food safety. A clean water supply is the single most important determinant of public health. Destruction of water supplies, along with sewage disposal infrastructure, after major catastrophes (e.g. earthquakes, floods, war, etc.) poses an immediate threat of severe epidemics due to waterborne diseases. Steps must be taken to reduce the scarcity of usable water and water pollution around the world.

Most of the projected increase to the world population by 2050 will be in countries already experiencing water shortages. Unless population growth can be slowed quickly, many fear that there may not be practical, non-violent or humane solutions to the emerging world water shortage.

How will water availability and quality impact feed safety? Drought stress during grain production increases the likelihood of mycotoxin production. Livestock consuming poor quality water may accumulate various toxic compounds in body tissues such as heavy metals, and other types of pollutants. Animal health may be compromised leading to increased disease susceptibility and pre- and post-harvest food safety concerns.

Are there unknown feed safety concerns with some current feed ingredients?

Oxidized feed fats

Although, monitoring and avoiding the use of feed ingredient sources with known contaminants will continue to be at the forefront of keeping animal feed safe, there

are other potentially hazardous components, such as secondary lipid oxidation products in fats used in animal feed that may warrant closer attention.

Oils and fats are significant components of animal feeds. However, our ability to assess feed lipid quality and safety is limited. When lipids are subjected to heating at high temperatures, they undergo oxidation resulting in changes to their chemical and physical characteristics. Highly unsaturated oils are especially susceptible to oxidation at high temperatures because they contain high levels of linoleic and linolenic acid. For example, thermal oxidation of soybean oil results in the formation of 4-hydroxy-2-trans-nonenal (HNE), which is an unsaturated aldehyde and secondary oxidation product shown to have cytotoxic and mutagenic effects, and several studies have associated HNE toxicity to the incidence of athlerosclerosis, stroke, Parkinson's, Alzheimer's, and Huntington's diseases, and liver disease in humans. Our research group at the University of Minnesota is currently evaluating the level of secondary oxidation products in feed fat sources and their potential impacts on pig and human health.

By-products from the biofuels industry

The desire to increase the use of renewable energy sources has led to the development of large ethanol and biodiesel industries in several countries and continents, most notably the U.S., EU, and Brazil. As a result, large quantities of by-products from these industries are produced and used in animal feeds. The predominant by-product from the fuel ethanol industry in the U.S. is maize distiller's dried grains with solubles (DDGS), whereas the by-product from the biodiesel industry is crude glycerol. Because these by-products are relatively new ingredients in the international feed ingredient market, their safety for use in animal feeds has been questioned. The presence and concentrations of mycotoxins and antibiotic residues are the primary concerns in DDGS, whereas methanol is the contaminant of concern in crude glycerol.

We know that if mycotoxins are present in the grain used to produce ethanol and DDGS, they are not detoxified during the production process, but instead, are concentrated by a factor of approximately 3 times. However, unless there is a high prevalence of mycotoxin contamination in grain used for ethanol production during a given crop year, there is minimal concern regarding mycotoxins in the resulting DDGS.

Antibiotics are used to control bacterial infections during fermentation in ethanol production. Virginiamycin and penicillin have been the most commonly used. When antibiotics are used, they are added to fermenters in very small quantities relative to usage rates in animal feeds. In November, 1993, the FDA's Center for Veterinary Medicine issued a "letter of no objection" for the use of virginiamycin in ethanol and DDGS production. In 2007, the FDA expressed concerns related to antibiotic residues in distillers grains and initiated a nationwide survey. Preliminary results (2009) revealed antibiotic residues were detected in 24 of 45 samples (obtained from ethanol plants in several U.S. states) tested thus far. The FDA has not published these results, commented on their health and safety implications, or implemented regulatory action to date.

A new genetically modified maize variety (Enogen, Event 3272) has been developed by Syngenta Seeds, Inc., with the goals of improving ethanol yields while reducing energy costs and greenhouse gas emissions. The use of Enogen grain by U.S. ethanol producers can provide a 100-million gallon ethanol plant, efficiency improvements that save 450,000 gallons of water, 1.3 million KWh of electricity and 244 billion BTUs of natural gas, the equivalent power to heat several thousand homes while reducing carbon dioxide emissions by 106 million pounds. Enogen was approved by FDA for human food consumption in 2007, and in February, 2011, it was cleared for production by USDA. What will be the acceptance of these maize by-products for feed use in countries outside of the U.S.?

Biodiesel is produced by a variety of esterification technologies. In general, the resulting by-product, crude glycerin contains methanol levels which warrant special consideration. Methanol is a potentially toxic compound. Metabolic elimination of methanol is much slower than that of ethanol, and animals differ widely in their ability to metabolize methanol depending upon enzyme activity and hepatic folate levels. In the U.S, the FDA addresses free methanol levels by requiring that levels of methanol in methyl esters of higher fatty acids not exceed 150 ppm (0.015%) or a level shown to be safe for use in animal diets. In Europe, German regulations allow 0.5% (5,000 ppm) methanol in crude glycerin. Will we eventually have a common, global standard for maximum levels of feed safety hazards?

Transfer of antimicrobial residues from animal manure amended soils

"Everything is connected to everything else" is a simple ecological principle often used to describe biology and the ecosystem. The implications of using antibiotics in food animal production have been a subject of debate for decades. Until recently, there were few studies conducted to determine the effects of antibiotics in animal manure on the environment. Significant amounts of therapeutic and subtherapeutic antibiotics given to animals are excreted in feces and urine, and can remain biologically active for long periods of time in manure during storage as well as in soil after manure application. However, the likelihood of direct, toxic effects of antibiotic residues present in manure on soil and plants is low because they are found in low levels and are further diluted when applied to soil. If residual antibiotics from animal manure affect soil bacteria, the nutrient cycling process through decomposition and mineralization can be affected. Studies have shown that antibiotics in animal manure can be found in small quantities in potable water, and if manure containing antibiotic residues continues to be applied to the soil, ground water could become a potential source of antibiotics in the food chain. Antibiotics that tightly absorb to soil particles have limited ability to reach groundwater but can reach surface water due to erosion. Because the transfer of resistant bacteria is not limited to a specific country or continent due to global trade, global regulations are needed to prevent further spread of bacterial resistance via animal products

How do we evaluate the safety of new technologies?

Nanotechnology

Several nanotechnology applications are being developed that will provide multiple benefits to science and society including animal health, veterinary medicine, and various aspects of animal production. Nanotechnology is defined as "understanding and control of matter at dimensions of roughly 1 to 100 nm where unique phenomena enable novel applications" (NNI, 2007). Nanomaterials have greater permeability, reactivity, surface area, and quantum properties due to their small size, resulting in use of less material and new or more efficient chemical and physical reactions compared with larger materials. However, they may pose greater risks to health and environment because greater toxicity and penetration could occur in biological systems. There are currently a limited number of food-related nanotechnology products on the international market, but several potential and future nanotechnology applications in animal production such as pathogen detection and removal, growth hormone and vaccine delivery, detection of contaminants in animal feeds, delivery of genes into cells to improve specific animal traits, and nanobarcodes to trace feed and animals from farm to consumption are being developed.

Nanotechnology has great potential for improving the efficiency of food animal production. However, analytical capabilities to measure and determine the safety of nanobiotechnology compounds need improvement. Additionally, attention should focus on proactively dealing with societal acceptance of these types of emerging technologies.

Impact of climate change on feed safety and security

FAO (2011) reported in a submission to the United Nations Framework Convention on Climate Change that slow-onset climate changes are expected to increase in developing countries resulting in potentially catastrophic impacts on food production. While the focus on short-term climate impacts caused by extreme weather events is necessary, slow-onset impacts are predicted to cause more significant changes in the ecosystem that may result in disastrous consequences to food security in the years 2050 to 2100. Therefore, we need to move beyond short-term perspectives and think about long-term challenges. Undoubtedly, the extent of these changes will affect feed quality and safety.

Part of the solution for dealing with slow-onset impacts of global climate change is to develop comprehensive computer models to use for predicting various scenarios and impacts well in advance of them occurring, so that thoughtful actions plans can be developed and implemented. Some examples of these types of models currently exist, and are used to predict the effects of climate change on mycotoxin production and emerging mycotoxins prior to harvest. However, developing these comprehensive models require greater integrated, multidisciplinary, and internationally coordinated input in order to mitigate these complex biological interactions that will affect the quantity, quality, and safety of grain production resulting from climate change.

Emerging new analytical and assessment technologies

Use of mass spectrometry and metabolomics to detect changes in non-targeted metabolites, and near infrared spectroscopy to monitor feed safety from "farm to fork" are examples of exciting new technologies that may enhance our ability to ensure a safer food supply. Irradiation technologies are improving our ability to remove microbiological contaminants, and interventions to ameliorate the adverse effects of mycotoxins are being developed.

Emerging (non-traditional) toxicological endpoints for hazard characterization include: delayed neurotoxicity, developmental toxicity, trans-generational effects (endocrine disruptors), immuno-toxicity, metabolomics technology.

Data collection, information management, and communication technologies

Computer and communication technology has dramatically changed lives and ability to manage and use information in many ways. New communication and data management technologies have improved data collection, data management, and rapid information exchange. Our challenge is to utilize these technologies in new ways relative to a new global model for feed and food safety, and more fully integrate them with feed safety monitoring and detection technologies.

Time magazine's cover story (February 21, 2011) by Lev Grossman, explains that at the current rate of exponential growth in computing power, computer capabilities will surpass the brainpower of an individual human by the year 2023, and surpass the brainpower equivalent to that of all human brains combined by the year 2045. Whether this level of artificial intelligence and computer technology is actually realized remains to be determined, but it describes a tremendous resource, with almost limitless possibilities, which we could utilize to help us understand and make intelligent decisions related to feed/food safety in a complex global system.

Global standardization

Global trade of feed and food, along with different tolerances, standards, and analytical capabilities to assess safety among countries, add to the complexity of our ability to effectively manage feed safety in the future. Progress will require international cooperation and a "systems approach" to implement new technologies and infrastructure. The SSAFE Food Safety Project is an excellent example of international feed and food companies working together with well established feed and food safety programs in the EU and U.S., to foster continuous improvement and global acceptance of internationally recognized food protection systems and standards.

CONCLUSION

Those of us who work in the feed safety dimension of our food production system, must be diligent in our efforts to become more aware of technology being developed in a wide variety of disciplines both within and outside our domains of expertise. This has never been more essential for building and managing and effective feed safety systems than ever before. There is a vast array of technology and information being developed and currently available that must be integrated and implemented in our current feed safety systems. The computer and communications technologies available today will greatly facilitate these efforts. However, the most critical factor is our ability to engage and utilize our best scientists, practitioners, and other human resources in an international, multidisciplinary, coordinated way to achieve common goals of improving our ability to meet consumer expectations for safe and affordable animal food products in our complex and interconnected global food production system.

MYCOTOXIN CONTAMINATION OF FEEDSTUFFS - AN ADDITIONAL STRESS FACTOR FOR BROILER CHICKENS

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SUMMARY

This study was conducted to investigate the effects of deoxynivalenol (DON) contamination of broiler diet on productive performance and the index of stress in broiler chicken, Heterophil:Lymphocyte ratio (H/L ratio) and other physiological manifestations of stress in poultry. Furthermore, the effects of microbial feed additive (Mycofix Plus) to counteract the adverse effects of DON were also investigated. Thirty two male 1-d old broiler chicks (Ross 308) were divided into four groups (8 birds per group). Each group was fed with one of the following dietary treatments;1) basal diet, 2) diet contaminated with 10 mg DON/kg feed, 3) diet contaminated with 10 mg DON/kg feed and supplemented with Mycofix plus, 4) diet supplemented with Mycofix® Plus. The live body weight, body weight gain and feed to gain ratio were measured for each group weekly. At 5 wk old, 6 birds from each group were slaughtered and blood was collected into heparinized vials. Complete white blood cell counts and differential leukocyte counts were performed and the heterophil to lymphocyte ratio (H/L ratio) was calculated. Contamination of broiler diet with 10 mg DON/ kg diet increased the feed conversion rate (feed to gain ratio) and decreased the weekly body gain at 3, 4 and 5 wk compared to controls. Interestingly, addition of Mycofix counteracts the adverse effect of DON on the feed to gain ratio and body weight gain and it returns to the normal value as control birds. Moreover, addition of mycofix in the absence of DON decreased the feed: gain ratio and increased the weekly body gain in broiler chickens. Contamination of broiler diet with 10 mg of DON resulted in an increase ($P < 0.05$) in the heterophils (H) counts and (H/L ratio) compared with controls. In contrast, the lymphocyte (L) counts were decreased ($P < 0.05$). However, addition of Mycofix to diet in the presence of DON counteracts the toxic effects of DON. From these results, we can conclude that contamination of broiler diet with DON increases the stress responses and decreases the feed intake, live body weight and body weight gain of broilers. Moreover, addition of Mycofix® Plus to diet contaminated with DON counteracts the adverse effects on productive performance and the physiological stress responses produced by DON.

INTRODUCTION

Contamination of broiler diet with mycotoxins exhibit a variety of biological effects in animals such as: liver and kidney toxicity, central nervous system abnormalities, estrogenic responses and others. Mycotoxins exert their effects through reduction of nutrients absorption [1] and suppression of the immune system [2, 3].

Trichothecenes such as DON and T-2 toxin reduce immunity by inhibiting protein synthesis and thus cell proliferation. Some mycotoxins are cytotoxic to lymphocytes in vitro. However, there is no information available regarding the effect of contamination of broiler diet with deoxynivalenol (DON) on physiological stress response. Physiological manifestations of stress in poultry include changes in the number of circulating leukocytes in particular a pronounced heterophilia and lymphocytopenia which is a reliable indicator of stress [4-6].

In addition Gross and Siegel [7] compared plasma corticosterone concentration and H/L ratio responses to various stressors and concluded that the H/L ratio is a better indicator of stress in poultry. In the present study, the heterophil: lymphocyte ratio (H/L ratio) was used as an index of stress in chicken as [8].

Therefore, the present study was conducted to investigate the effect of contamination of broiler diet with 10 mg deoxynivalenol mycotoxin on physiological manifestations of stress in broilers. The effect of feed additive (Mycofix plus, BIOMIN GmbH Industriestrasse 21, 3130 Herzogenburg, Austria) addition to broiler diet in the presence and absence of deoxynivalenol mycotoxin contamination on the heterophil: lymphocyte ratio was also investigated.

MATERIALS AND METHODS

Birds and Housing

Thirty two one day male broiler chicks (Ross 308) were obtained from a commercial hatchery and divided randomly into four groups (8 birds). The birds were housed in battery cages (4 birds per cage, 2 replicates for each group).

Diets

The control group was fed starter and grower diets based on corn, soya HP, soya oil, and a premix with vitamins, minerals, amino acids (Lysine, Methionin, and Threonine), salt, and monocalcium phosphate. Birds of each group were fed with one of the following dietary treatments;1) basal diet, 2) diet contaminated with 10 mg DON/kg feed, 3) diet contaminated with 10 mg DON/kg feed and supplemented with a microbial feed additive, Mycofix plus (2.5 kg/ton of diet), 4) diet supplemented with Mycofix plus (2.5kg/ton of diet).

The chicks were fed with the normal starter diets from days 1 to 13 and low protein grower diet from day 14 – 35. The feed additives were delivered by Biomin® GmbH, Herzogenburg, Austria. The birds had free access to water and feed.

Growth performance

Birds of each group were individually weighed weekly and the body weight gain was calculated. The feed intake for each group was measured weekly and the feed:gain ratios were calculated.

Measurement of stress index

At 5 wk old, 6 birds from each group were bled and blood samples were collected in heparinised tubes and sent to the central laboratory of the veterinary university of Vienna for analysis. Blood films were air dried (unfixed) and stained in concentrated May-Grunwald stain for 6 min, 1:1 May-Grunwald stain-distilled water for 1.5 min and 1:9 Geisma stain for 15 min [9]. To determine the counts of heterophil and lymphocyte, 100 cells per film were examined by light microscopy. All blood counts including granulocytes (Heterophil, Basophil and Eosinophil) and non-granulocytes (Lymphocyte and monocyte) were examined by the same investigator. The results are presented as the percentage of each cell occurring in each film. The H/L ratio was examined by dividing the number of heterophils by the number of lymphocytes [7].

Statistical analysis

Statistic SPSS program version 17.0 was used for data analysis. Kolmogorov Smirnov test was used to test the normal distribution of the data. Results are given as means ± SEM. Analysis of variance (ANOVA) was performed between the four groups followed by Duncan test to find the significance between dietary treatments. Statements of statistical significance were based on $P \leq 0.05$.

RESULTS

Growth performance

Contamination of broiler diet with 10 mg DON/ kg diet decreased the live body weight (996.25 ± 29.71) compared to controls (1205.75 ± 29.71) at 5 wk (P=0.001). Interestingly, addition of Mycofix counteracts the adverse effect of DON on the live body weight of birds (1224.88 ± 29.71) and it returns to the normal value as control birds (1205.75 ± 29.71). Furthermore, addition of Mycofix Plus in the absence of DON increased the live body weight (1293.38 ± 29.71) at 5 wk compared controls (1205.75 ± 29.71).

Furthermore, the weekly body weight gain was low for DON fed group at 3, 4 and 5 wk compared to controls ($P < 0.05$, Table 1). Mycofix addition to the contaminated diet could also counteract the adverse effect of DON on the weekly body weight gain and it returns to the normal value as control birds. However, addition of mycofix in the absence of DON significantly increased the weekly body gain of broiler chicken. Moreover, the feed conversion rate (feed to gain ratio) was higher for DON group at 3, 4 and 5 wk (3.01, 3.38 and 2.37 respectively) than control group (2.90, 2.97 and 1.76 respectively).

Additionally, Mycofix could counteract the adverse effect of DON on FCR and it returns to the normal value (2.49, 3.11 and 1.91 at 2, 3 and 4 wk respectively) as control birds (2.90, 2.97 and 1.76 respectively). However, addition of mycofix in the absence of DON decreased the feed: gain ratio (2.61) at 3 wk than control (2.90) and had a similar feed: gain ratio at week 4 and 5 (2.95 and 1.79) as control birds (2.97 and 1.76).

Table 1: The effect of addition of microbial feed additive to broiler diet contaminated with Deoxynivalenol mycotoxin on the body gain (g) of broiler chicken

Age (week)	Treatments				SEM	P
	Control diet (n = 8)	Deoxynivalenol (10 mg/ kg diet) (n = 8)	Deoxynivalenol Plus Mycofix (n = 8)	Mycofix (2.5 kg/ ton diet) (n = 8)		
W 1	155.50	148.00	159.25	145.25	3.45	0.466
W2	255.75	237.88	253.38	261.50	7.62	0.744
W3	171.13^{ab}	151.13^{b}	182.63^{ab}	191.63^{a}	5.90	0.078
W4	198.88^{ab}	166.88^{b}	199.75^{ab}	224.88^{a}	8.10	0.078
W5	386.13^{a}	241.88^{b}	380.25^{a}	414.13^{a}	24.15	0.044

$^{a, b, ab}$Means within the same row with different superscripts are significantly different (One way ANOVA followed by Duncan test, n = 8/treatment).

Stress responses and Heterophil:Lymphocyte ratio

Contamination of broiler diet with 10 mg of DON resulted in an increase ($P < 0.05$, Table 2) in the heterophils (H) counts (60.33 ± 4.70) compared with controls (44.14 ± 4.24). However, addition of Mycofix to diet in the presence of DON counteracts the effect of DON on H counts (55.50 ± 2.43).

In contrast, Lymphocyte (L) counts were decreased ($P < 0.05$) due to contamination of diet with DON (29.66 ± 3.38) compared with controls (43.29 ± 2.96). However, supplementation of diet with Mycofix in the presence of DON increase ($P < 0.05$) the L counts (34.33 ± 1.78) compared to DON group (29.66 ± 3.38).

Moreover, the heterophil to lymphocyte ratio (H/L ratio) was increased ($P < 0.05$, Table 2) for birds fed diet contaminated with DON (2.37 ± 0.51) compared with controls (1.07 ± 0.15). Interestingly, Mycofix supplementation to diet contaminated with DON decrease ($P < 0.05$) the H/L ratio (1.64 ± 0.13) compared with DON group (2.37 ± 0.51).

Table 2: The effect of addition of microbial feed additive to broiler diet contaminated with Deoxynivalenol mycotoxin on differential leucocytic counts and index (Heterohpil: Lymphocyte ratio) in broiler chicken

Item	Treatments				SEM	P
	Control	DON	DON Plus Mycofix	Mycofix		
H %	44.29^{b}	60.33^{a}	55.50^{ab}	43.67^{b}	2.62	0.044
L %	43.29^{a}	29.67^{b}	34.33^{b}	41.32^{ab}	6.62	0.020
Monocyte %	7.14	6.33	7.33	9.67	0.95	0.674
Basophil %	4.43	3.50	2.93	5.17	0.55	0.502
Eosinophil %	0.29	0.17	0.67	0.50	0.15	0.697
H/L ratio	1.07^{b}	2.38^{a}	1.65^{ab}	1.19^{b}	0.19	0.050

$^{a, b, ab}$Means within the same row with different superscripts are significantly different (One way ANOVA followed by Duncan test, n = 6/treatment).

DISCUSSION

Fusarium species produce a vast array of mycotoxins, many of which are economically important in regard to animal production. Because of mycotoxins can increase incidence of disease and reduce production efficiency. The current study demonstrated clear effects of DON on physiological stress response and immunological functions of broilers.

Most experimental studies [10-16]with poultry show a highly variable effect of DON on performance indicating that zootechnical traits might not be a sensitive indicator of toxicity of this *Fusarium* toxin. However, in the current experiment, feed refusal, reduced weight gain and feed conversion were adversely affected ($P < 0.05$) by inclusion of 10 mg/kg DON in broiler diets. This finding may be ascribed to the inhibition of protein synthesis by this toxin. We may hypothesize that when the birds are fed with low protein diets, the toxic effect of DON will be more evident. However, the supplementation of Mycofix was useful in counteracting toxic effects of DON on performance

Concerning the haematological variables, we observed decreases in total leukocyte count and lymphocyte counts. This can be attributed to that the immune system is very sensitive to DON [17, 18].

To our knowledge, this is the first report demonstrating the effect of 10 mg/ kg purified DON on physiological stress response. Physiological manifestations of stress in poultry include changes in the number of circulating leukocytes in particular a pronounced heterophilia and lymphocytopenia which is a reliable indicator of stress [4-6].

In the present study, the feeding of DON contaminated diets resulted in an increase ($P < 0.05$) in the heterophils (H) counts and (H/L ratio) compared with controls. These responses may indicate predictable physiological changes to the presence of mycotoxin. In contrast, the lymphocyte (L) counts were decreased ($P < 0.05$). However, addition of Mycofix to diet in the presence of DON counteracts the toxic effects of DON. These results indicate that dietary supplementation of Mycofix relatively increased the L counts and relatively decreased the H counts which could help to overcome the stresses of broilers due to exposure for mycotoxin, suggesting that supplementation of broiler diet with Mycofix can modulate the physiological stress responses.

CONCLUSION

In conclusion, it can be concluded that the contamination of broiler diet with DON increases the stress physiological responses in broiler due to the adverse effect on lymphocyte. These effects of DON on the stress responses could explain the DON effects on performance and nutrients absorption. Moreover, addition of Mycofix to diet contaminated with DON counteracts the physiological stress manifestations produced by DON.

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PREVENTION OF SWINE DYSENTERY WITH FITOBIOTICS

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SUMMARY

An effective method was elaborated for extracting the active substances from commercially available Garden thyme and carob seeds. The antibacterial activity of dilutions of thyme and carob extracts and their combinations were studied by taking the minimum inhibitory concentrations (MIC) with agar well diffusion technique. The most effective concentrations of the two herb extracts were used for establishing the best combination of the extracts and on this basis a new feed additive was developed and manufactured for pilot studies. The feed additive efficiently reduced the cost of medications in five pig farms known to be notorious for prevalence of swine dysentery. Feed supplementation also improved weight gain and feed conversion efficiency.

INTRODUCTION

Medicinal plant extracts known as essential oils are fragrant and volatile ingredients of spices and herbs and have long history of use in traditional (folk) medicine. Anti-inflammatory, antioxidant, antimicrobial, antifungal, flavouring, (positive/negative) allelopathic and pesticidal properties of essential oils, herb and spice mixtures have been well documented. Related agricultural research gathered momentum by banning the use of antibiotics as production promoters and by the concern about the ever increasing resistance against antimicrobial drug preparations.

Swine dysentery is one of the management related diseases which cause considerable losses in the pig industry with high mortality and morbidity, depression of growth and feed conversion efficiency, and by the costs of continuous in-feed medication. The causative agent of the disease (Brachyspira /Serpulina/ hyodysenteriae) prevails 45% of the finishing pigs in the Hungarian large-scale farms (Biksi et al., 2007). Control of swine dysentery and other enteric disorders caused by Lawsonia intracellularis, Brachyspira pilosicolli, Salmonella enterica, Clostridiium perfringens A and C, enteropathogenic E. coli needs consistent application of hygienic and biosecurity measures, diminution of stress related risks and strictly supervised implementation of well-designed medication programs. Because efficiency of medication has steadily decreased over the last 10-20 years, research was launched to develop a plant extract combination that might be efficient in combating swine dysentery.

This paper shortly describes the preparation of the plant extracts, discloses the results of the laboratory tests and gives account of the results of the pilot studies conducted with growing pigs in large-scale pig farms.

MATERIAL AND METHODS

Preparation of the experimental material

Several of the available herbaceous plants had been pilot tested and Garden thyme (*Thymus vulgaris L.)* and seeds of carob-tree *(Ceratonia siliqua)* were selected for further studies.

For in vitro study of the antimicrobial effects cold (25 ^{o}C) water-ethanol extracts were produced. Commercially available varieties of Garden thyme were ground to powder of which 3 gm was added to 100 mills of distilled water then shaken for 1 hour in a bolter. The mixture was repeatedly ultra-sieved through ultra-filter membrane columns (KOCH/Romicon WF2, Hollow Fibre; pore size: 1; 10; 100 and 500 kDa). Filtrates were then used for preparing dilutions up to 1:20 000 concentration. Similar procedures were followed with seeds of carob-tree.

Antimicrobial effects of water extracts of thyme and carob seeds were studied separately by determination of the minimum inhibitory concentration (MIC) with *agar well diffusion technique* by using (among others) strains of *B. hyodisenteriae* collected from pigs that showed clinical signs of swine dysentery.

Isolation of strains and preparation of the culturing media. Pigs with dysentery were stunned, exsanguinated and subjected for collection mucosal scrapes from affected parts of the colon. Small amounts of the material were dispersed over the surface of freshly prepared trypton soy culturing media (Scharlau-Aldricht Ltd.) that contained 10% defibrinated bovine blood and 400 µg/ml spectinomycin (Sigma-Aldricht Ltd.). Inoculated plates were incubated for 96 hours at 42 ^{o}C with strict anaerobic conditions which were safeguarded with gas-generating

bags (Oxoid, Gas Generating Kit, Anaerobic system BR0038B) and anaerobic jars (Oxoid, Anaerobic jar). Determination and identification of the primary and secondary biochemical characteristics of the strains were carried out according to standard methods. Until use the strains were stored deep frozen (-80 ^{o}C) in TSB-broth suspension that contained 25% sterile glycerine (Scharlau Microbiology).

Determination of MIC by agar well diffusion technique. Blood agar (modified trypton-soy agar + 10% defibrinated bovine blood) was used for producing proto-cultures from thawed mass of bacteria. Eight/ten agar cubes of identical size (±5% deviation) were cut out from parts of agar plates showing haemolysing cultures. The agar-cube inoculums were spread by sterile glass rod over freshly prepared agar plates of 90 mm diameter. The plates were closed and dried for 5 minutes.

According to pre-determined order a hole of 5 mm diameter was cut centrally into the inoculated plates. Hundred µL of either dilution of thyme or carob seed extracts was measured into the holes. The plates were placed into anaerostat (Oxoid anaerob bags and jars) within 15 minutes after dripping the known dilution of herb extract into the holes and incubated for 4-5 days at 37 ^{o}C.

Sensitivity of Brachyspira strains to given dilution of the herb extract was estimated on basis of diameter of the haemolysis-inhibition ring that formed around the holes. On this basis the strains were assorted into three groups as follow:

- **Sensitive (S)**: Diameter of the inhibition ring is >25 mm;
- **Moderately sensitive (MS)**: Diameter of inhibition ring is between 15 and 25 mm;
- **Resistant (R)**: Diameter of the inhibition ring is <15 mm.

Combination of sensitive dilutions of the above herbs with stabilizers and other materials were used for manufacturing a solid feed additive for further studies.

Pilot studies with the feed additive

Preliminary trials were conducted in one small-, one medium- and three large-scale pig farms. All farms have been notorious for the high prevalence of swine dysentery. In these farms parallel groups of pigs were formed at the beginning of the fattening period. Both groups were housed with identical conditions. Feeds of the control and experimental pigs were identically formulated but the diets of the experimental pigs were supplemented with the above additive at 2 kg/ton concentration.

The effect of the feed additive on swine dysentery was measured by the cost of medication. Other additional effects were analysed by data of daily weight gain and feed conversion efficiency.

RESULTS and DISCUSSION

Antimicrobial activities of various essential oils of medical herbs (including thyme and carob seed) against foodborne pathogenic or spoilage bacteria and moulds, germs related to gynaecological diseases, Salmonella typhi, Staphylococcus aureus, Enterococcus sp. and almost innumerable other germs have been studied. To our best knowledge this is the first report that accounts the in vitro efficient effects of special extracts of Garden thyme and carob-tree seeds on Brachyspira hyodisenteriae.

Within the experimental programme hundreds of in vitro examinations have been carried out showing great variance of MIC according to dilutions of the individually tested herb extracts and their combinations. One sample of these results is disclosed in Table 1. Of the data shown two combinations were effective at as big as 1:5 and 1:10 thousand dilutions (Nr. 89 and 94), while others failed to show any effect at as low as 1:250 and 1:500 dilution.

Agar plate diffusion assays are useful as a qualitative assessment of biological activity of essential oils but are not appropriate in assessing quantitative effects (Donaldson et al. 2005). Because all in vitro tests had been carried out in identical conditions, the MIC data had permitted showing the anti-microbial effects of the herb extracts and combinations and on this basis selecting the best combination for manufacturing an effective feed additive.

Five pilot studies were conducted with the feed additive. Data collected in two of the five farms are disclosed in Table 2. and 3. In both cases the feed additive reduced the cost of medication considerably and improved the data of production parameters substantially. The same applies for the other three pig farms. The improve-ment of medications in farms that produced 500, 1700 and 27500 finished pigs per year was 67; 9 and 9%, respectively. With respect to the average daily weight gain and FCR improvements were similar to those found in the other three farms.

Table 1: Sample from the MIC tests

Herb extract combinations	Dilution	MIC, mm	Evaluation
Nr. 87	250x	20	MS
Nr. 87	500x	20	MS
Nr. 89	5000x	35	S
Nr. 89	10000x	30	S
Nr. 94	5000x	45	S
Nr. 94	10000x	28	S
Nr. 95	250x	10	R
Nr. 95	500x	13	R

S: sensitive; MS: moderately sensitive;
R: resistant

The findings of the present investigation have shown good agreement with other experiments reported in the relevant literature. Herbal extract (based on thyme, clove

and oregano at levels of 0, 700, 1400 and 2100 ppm) decreased the diarrhoea incidence and improved the live weight gain of pigs (Pedrosoet et al., 2005). Piglets fed rations containing sage, coriander, yarrow and thyme were reported to have significantly higher daily gain (by 7%) and feed conversion efficiency (by 3%) than controls (Wagner, 2003). The inclusion of essential oils (at 25 mg/kg) of oregano, or thyme or garlic in the pigs' diet significantly improved the average daily weight gain and feed conversion ratio as compared to control pigs and also had positive effects on the carcase composition (Onibala et al., 2001). Plant extracts composed of pepper, cinnamon, oregano, thyme and aniseed can increase the digestibility of pig rations from 81 to 85%, with a concomitant 5% increase in piglet live weight gain (Mavromichalis, 2003). The promising results need further reinforcements.

Table 3: Main data of the pilot study in a large-scale farm that produces 9800 finished pigs/year

Items	Experimental group	Control group	Change
No. of pigs	396	722	
No. of fattening days	115	129	
Cost of medication*		301.561	
Cost of the feed additive*	138.750		
Total cost of medication	143.456	301.561	-52%
Daily weight gain, g/pig	0.864	0.731	+15.4%
Feed conversion rate, kg/kg	2.82	3.14	-7.7%

*= Costs calculated in the Hungarian currency (Ft).
Please observe the relative changes.

Table 2: Main data of the pilot study in a large-scale farm that produces 9500 finished pigs/year

Items	Experimental group	Control group	Change
No. of pigs	304	305	
No. of fattening days	121	124	
Cost of medication*		134.425	
Cost of the feed additive*	102.250		
Total cost of medication	102.250	134.425	-24%
Daily weight gain, g/pig	0.636	0.583	+8%
Feed conversion rate, kg/kg	3.50	3.77	-7.7%

*= Costs calculated in the Hungarian currency (Ft).
Please observe the relative changes.

CONCLUSIONS

The best combination of the most effective extracts of the active substances of thyme and carob seeds set basis for the development of a feed additive, which is promising for its beneficial effects on medication expenses and production parameters of grooving-finishing pigs. Results of the pilot studies claim laboratory model experiments with growing pigs.

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EFFECT OF PROBIOTIC OR ANTIMICROBIAL TREATMENTS ON THE INTESTINAL BACTERIAL COMMUNITY IN PIGS

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SUMMARY

The aim of this study was to observe the effect of a probiotic, *Pediococcus acidilactici*, or an antibiotic, amoxicillin, on the diversity and structure of the commensal dominant bacteria and Archea at various levels of the pig intestinal tract. Forty-eight Specific Pathogen Free pigs (SPF), 70 days old, were separated into 3 experimental groups at day 0 (D0) i) the negative controls i.e. pigs receiving no product, ii) pigs fed with a diet containing the probiotic tested, and iii) the positive controls including pigs treated with amoxicillin. The antibiotic was used at 0.1g/kg of pig weight, once a day during 3 days (from D42 to D44). The probiotic, *Pediococcus acidilactici*, at 1g (10^{10} CFU)/kg of feed, was given from D14 to D44. The digestive microbiota was studied by a molecular method, Capillary Electrophoresis-Single Strand Conformation Polymorphism (CE-SSCP). Using this fingerprint technique, we investigated the total bacterial population in the ileum, caecum, colon, as well as the *Bacillus-Streptococcus-Lactobacillus* (BSL) group and Archaea in caecum and colon. Probiotic decreased bacterial diversity in ileum whereas antibiotic decreased bacterial diversity in caecum and colon. Antibiotic had a significant effect on the total bacterial structure of caecum, colon and ileum. The effects of both treatments on total bacterial structure were highest in the caecum. The effect of both treatments was higher on BSL structure in colon than in caecum. The archeal structure in caecum and colon remained very stable whatever the treatment.

INTRODUCTION

Antibiotic growth promoters (AGP) have been used the past decades in animal production, introducing the potential risk for a reservoir in food animals of antibiotic resistant bacterial populations transmittable to humans [1, 2]. Therefore the European Union moved towards a ban of AGP. As a consequence, alternative feed additives were used to improve animal health and performance, such as probiotics, thought to establish a balanced gut microflora [3, 4]. This study aimed to look at the effect of one probiotic, *Pediococcus acidilactici* on the diversity and structure of commensal microorganisms at different levels of the pig intestinal tract (ileum, caecum, colon) using Capillary Electrophoresis-Single Strand Conformation Polymorphism (CE-SSCP) targeting all bacteria, the *Bacillus-Streptococcus-Lactobacillus* (BSL) group and Archea. In this study, the antibiotic treatment, administered at a therapeutic dose, was used as a positive control since such a treatment is known to induce changes in the intestinal microbiota.

MATERIAL AND METHODS

Forty-eight SPF pigs of our local flock were separated randomly into 3 experimental groups at D0, i) the negative controls i.e. pigs receiving no product, ii) pigs fed with a diet containing the probiotic tested, from D14 up to D44 (*Pediococcus acidilactici* CNCM MAI 18/5M, at 1g/kg of feed, i.e. 10^{10}CFU/kg of feed), and iii) the positive control being pigs treated with an antibiotic, (amoxicillin at 0,1g/kg of animal weight, orally administered once a day, 3 consecutive days, from D42 to D44), in order to compare changes due to probiotic *versus* antibiotic.

The pigs, 70 days old at the beginning of the study (D0), were fed *ad libitum* a diet for growing pigs, examined daily for rectal temperature, feces aspect, feed refusal and weighed weekly. Samples of contents of colon, caecum and ileum were collected during autopsies and stored at -20°c until DNA extraction. DNA was extracted from 200 mg of each sample using a QIAamp®DNA stool mini-kit according to the manufacturer's instructions. Amplicons of the 16SrDNA (V3 region) were obtained using fluorescent primers w49 and w104, specific of the Eubacteria phylogenic domain [5]. An additional nested PCR was performed for the analysis of the *Bacillus-Streptococcus-Lactobacillus* (BSL) and archeal communities, using universal primers w108 and w96 respectively [5, 6]. The CE-SSCP was then performed on an ABI Prism 3130 analyzer with the PCR products obtained previously. Statistical analyses were carried out using a statfingerprint / R software. The Simpson diversity index of CE-SSCP profiles was estimated ($S= 1- \square Pi^2$, where Pi is the proportion of the peak area). The community structure of the ecosystem was defined as the number of strains (number of peaks) and their relative abundance (comparison of the peak size for each scan of the profile). The ANOSIM-R value indicated the extent to which the groups differed. R close to 1 indicates separated groups. The closer R gets to 1, the more groups are separated, whereas R values close to 0 indicate overlapping groups [7].

RESULTS

No morbidity / mortality was recorded during the survey and no significant differences were observed on the weekly relative weight gain and feed intake of the pigs whatever the treatment.

The dynamics of the bacterial communities in the pig intestinal tract was evaluated by the diversity index and the degree of similarity.

1) Comparison between intestinal contents.

In control pigs, the ileum bacterial diversity was significantly lower than that of caecum and colon (table 1; all p values < 0.005). The CE-SSCP profiles showed a change in bacterial community structures within the 3 intestinal segments.

2) Effect of treatments on the total bacterial structure.

The probiotic had a significant negative effect on the bacterial diversity of the ileum and not on the other compartments (table 1). On the opposite, the antibiotic had a significant negative effect on the bacterial diversity of caecum and colon but not in the ileum (table 1).

Considering the degree of similarity R between CE-SSCP profiles, the antibiotic had a significant effect on the total bacterial structure of caecum, colon and ileum, whereas the probiotic had a significant effect only in the caecum and colon (table 1). The effects of both treatments on total bacterial structure were highest in the caecum.

3) Effects of treatments on *Bacillus-Streptococcus-Lactobacillus* (BSL) and archeal communities

The dominant BSL group, mainly found in the colon, had its structure significantly affected by the antibiotic in both caecum and colon, whereas an effect of probiotic was only observed in the colon (table 2).

No significant effect was observed on the archeal community whatever the treatment.

DISCUSSION

The CE-SSCP method was successful in assessing changes in the microbial diversity of the intestinal microbiota but this method did not allow the identification of specific strains. Our results showed that the bacterial diversity indexes were very similar between colon and caecum whereas the bacterial community structures varied within the 3 intestinal contents. Considering more specific bacterial populations, no significant difference of the BSL community was observed between caecum and colon, which is in agreement with a previous study [8]. The effect of both treatments on the dominant bacterial structure was mainly observed in caecum, whereas it was higher in colon for the changes observed for the BSL structure. This could be explained by more sensitive BSL strains in the colon where the antibiotic effect was about 2.6 higher than that of the prebiotic.

CONCLUSIONS

Although the CE-SSCP method proved to be effective in assessing microflora dynamics in the intestinal contents of growing pigs, further investigations will be needed to identify the bacteria affected (positively or negatively) by the treatments. It would be also interesting to observe the effect of *Pediococcus acidilactici* on piglets with bacterial infections, since probiotic therapy has already made its way in the treatment of various infections.

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Table 1: Effects of antibiotic and probiotic treatments on the bacterial diversity of the intestines

Intestinal content	Treatment	Diversity Index	SD	Significance (p) (T *vs* control)	Degree of similarity R (T *vs* control)	Significance of R (p)
Ileum	Control	5.3	0.4	/	/	/
	Antibiotic	5.3	0.4	> 0.05	0.122	0.04
	Probiotic	4.8	0.3	0.014	0.075	> 0.05
Caecum	Control	6.2	0.5	/	/	/
	Antibiotic	5.7	0.3	0.016	0.463	0.01
	Probiotic	6.5	0.2	> 0.05	0.205	0.01
Colon	Control	6.6	0.3	/	/	/
	Antibiotic	5.9	0.3	0.0002	0.397	0.01
	Probiotic	6.5	0.4	> 0.05	0.172	0.01

Ttreatment; Diversity index was expressed as the mean of 8 individual indexes ± SD (Standard Deviation).

Table 2: Effects of antibiotic and probiotic treatments on the BSL structure

Intestinal content	Treatment	Degree of similarity R (T *vs* control)	Significance of R (p)
Caecum	Antibiotic	0.378	0.002
	Probiotic	0.069	> 0.05
Colon	Antibiotic	0.467	0.001
	Probiotic	0.179	0.03

THE EFFECTS OF MANAGEMENT AND FACILITIES ON THE WELFARE OF CATTLE IN LOCAL DAIRIES

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SUMMARY

The major welfare problems of dairy cattle are malnutrition, lameness and infectious diseases, such as mastitis. The facilities, management system, social environment and the human-animal interaction play a key role in dairy cattle welfare.
During 2008-2009, thirteen dairy farms were evaluated for: state of the facilities, management practices, body condition score, lameness and stereotype occurrence in cattle.
The mean of animals per dairy farm was 151.3 ED 44; the capacity in the parlours for simultaneous milking was 16.5 ED 6.48 cows. 61.5% of roads walked by cattle back and forth, were in a bad condition, 30.8% were regular and 7.7% in a good condition. Of the 305 cows evaluated, 1.3% had mild locomotion problems, 0.3% severe and 98.4% were not lame. Of 320 cows, 65% had unacceptable body condition score (BCS) while for 35% it was acceptable. No stereotypes were registered during the ethological surveys.
Road condition is one of the critical points in dairy facilities, nevertheless this fact doesn't appear to be related to the percentage of lame cows found. BCS results could be related with the adverse climate conditions that affected forage availability during the winter-summer 2008-2009. The absence of stereotypes could be associated with the unrestricted forage behaviour related to the free access to pastures in the Uruguayan extensive production system.

INTRODUCTION

Animal Welfare could be defined as the state of an individual in relation to their attempt to cope with the environment [3]. The environment is a multidimensional space that among other variables includes, facilities, management, social environment and human-animal relationship, as well as animal´s internal medium, which include its nutritional and sanitary condition and its feelings.
The most common issues in the welfare of dairy cattle include lameness, malnutrition, infectious diseases, as well as hypocalcaemia and acidosis.
In Uruguay, research on Animal Welfare is recent, and has been mostly oriented to the study of beef cattle [8, 9].
Today there is little research into welfare of dairy cattle. Nevertheless this is the second livestock activity of the country, involving more than 770.000 animals [9].

Welfare can be evaluated at two levels, the input factors and the output factors. The first ones include everything that is given to the animal like, housing, feeding, management, social environment, among others. The output factors are the "response" of the animal to the input factors, such as the disease prevalence and its behavior among others.

The aim of this work was to survey the general situation of local dairy farms and the cattle condition, taking into consideration some of the principal elements that can influence dairy cattle welfare, like the facilities, management, body condition score, lameness and behavior.

MATERIAL AND METHODS

Thirteen dairy farms located in the departments of Canelones (n=1), San José (n=6) and Colonia. (n=7) were visited during 2008-2009. Selection criteria were the predisposition of the owner to receive the research team, and the presence of a veterinary in the dairy.

Each farm was visited to observe the whole milking process, including the transportation of the animals from the paddocks to the waiting room, and their exit from the parlour.
Data was collected by one researcher previously trained for the task.

Input factors

Management
Herd size and the surface in hectares and were registered. For assessing cattle herding from the paddocks were registered the following items: riding horses, using dogs, using a motorcycle or walking.

Facilities
Facilities were subdivided in several items for its evaluation: roads, the floor of the waiting room, fences of the waiting room, troughs (water availability), and the floor of the milking parlour. A special category to assess the potential flow of cows within facilities was created. For

this, were taken the presence and type of steps, changes in lighting and presence of straight angles within the milking parlour and it´s accesses into account. Each of these items was evaluated as Good (G), Regular (R), and Bad (B).

Output Factors

Clinical indicators

Lameness was evaluated at the exit of the milking parlour, using the next categories.

Severe lameness; the cow has severe difficult in walking, and couldn't stand the injured limb. Mild lameness; the cow barely lean on the injured limb.

Body Condition Score (BCS) was evaluated at the exit of the milking parlour. Edmonson [5] body score chart was adapted to local survey conditions. Non acceptable body condition 0 was assigned for values 1 and 2. Acceptable 1, was matched with values of 3, 4 and 5, of the Edmonson body score chart.

Ethological indicators

In each dairy 15 minute "one zero" samples [1] were taken, in the waiting room and in the parlour, in order to detect the occurrence of two oral stereotyped behaviors; bar biting and tongue rolling

Statistical Analysis.

All data was entered into Microsoft® Excel 2003 charts and processed with the statistical software Minitab 15 (© 2007 Minitab Inc)

RESULTS

Management

Mean surface in hectares of the dairies was 222.7 ED 205 (range 630), mean herd size 151.3 DE 46.2 (range 170), the capacity for simultaneous milking had a mean 16.5 ED 6.47 (range 20). In all the dairies, cows had free access to pasture during the day. Feeding was supplemented with a 2 Kg grain based diet during the milking.

Facilities

Roads were evaluated as Bad in 61.5% (n=8), Regular in 30.8% (n=4) and Good 7.7% (n=1). Floor of the waiting room was Bad in 30.8% (n=4), Regular en el 53.8% (n=7) and as Good in 5.4% (n=2). Fences of the waiting room Bad in 15.4% (n=2), Regular in 38.5% (n=5) and Good in 46.1% (n=6). Droughts Bad in 7.7% (n=1), Regular 61.5% (n=8) and Good 30.8.1% (n=4).

The flow of animals in the miliking parlour and its accesses were evaluated bad in 42.2% (n=6), regular in 23% (n=3) and good 30.8% (n=4)

Management

The herding of the animals to the parlour was evaluated in 8 dairies, 75% (n=6) was done using horses and 25% (n=2) walking. In all the dairies (n=8) a whip to move the animals was used.

Lameness

From a total of 305 cows evaluated, 98.4% (n= 300) were non lame, 1.3% (n=4), were mild lame and 0.3% (n=1) were severe lame.

Body Condition

From the total of evaluated cows for Body Condition Score, 65% (n=209) were scored 0 (non acceptable) and 35% (n=113) were scored as 1 (acceptable)

Ethological surveys

In the "one-zero" samples the expected stereotypes were non presented.

DISCUSSION

Management

The facilities assessment showed that in most establishments the floor of the areas in which cows should move were in inappropriate state. The floor should be adequate to prevent falls and injuries, so a non-slip floor it's required [7].

The status of the walkways can influence the presence of lame cows, even though lameness is a multifactorial event, also associated with poor nutritional status. The fences were in most of the establishments, evaluated as good, this is important because during the stay in the waiting room animals are in large numbers, really tight between them and against fences, that situation can cause injuries to the cows.

Troughs were of insufficient size, difficult access of several animals simultaneously, which may adversely affect water consumption of animals of lower hierarchical positions as the dominant grab the resource, preventing the others for doing it.

The presence of large steps and radical changes in light were the main obstacles to the movement of animals, as cattle tend to move from darker to more lighted areas [7]. Proper lighting is necessary; otherwise the animal will be reluctant to enter the parlour from the waiting room [7]. The presence of straight angles should be avoided, curved spaces should be preferred. The contrasts of lighting on the ground and small water streams should be kept away from the cattle, otherwise they may stop, due to the difficulty to quickly focus in the objects.

Lameness

Lameness acts as a stressor agent for the animal because of the pain [8, 11], lameness is a behavioural expression of suffering, it also affects the social behaviour of cattle, especially regarding the use of space and access to feeding and resting areas [6].

Hernandez-Mendo [8] showed that cattle suffering from lameness recover over a period of four weeks when they are kept on pasture, probably due to more floor softness.

Malnutrition

Evaluation of body condition showed high proportion of animals under the minimum acceptable level. The amount of non acceptable body condition contrasts with similar assessments done previously at the same dairies, where 74% of the animals were evaluated as acceptable [4]. This difference may be due to the fact that the present evaluations were done in late winter, with a probably low quality of pastures. The animals were also not fully recovered from the effects of drought that affected Uruguay during the summer-winter 2008-2009.

Stereotypes

Since animals are in extensive production systems, they may spend long periods with free access to water and pasture. Under this system, foraging behavior of cattle is not restricted. The lack of stereotyped behavior is an expected result if the factors suggested by Bergeron et al. [2] are taken into account, as a cause of this behavior.

CONCLUSIONS

Cattle welfare in local dairies can be considered acceptable, like most of the issues found are related with facilities. The solution to the problems generally found does not require high economic costs. This aspect coupled with the training of the operators in proper handling of animals, can have a positive impact on cattle welfare.

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INTEGRATION INTO THE COW HERD: LONG TERM EFFECTS OF MOTHER CONTACT DURING THE FIRST 12 WEEKS OF LIFE

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SUMMARY

The integration of dairy heifers into the cow herd shortly before their first parturition is a common management practice and is associated with stress. In this study we investigated if the ability to cope with such challenges is affected by experiences during early age. Three groups of heifers that differed with respect to contact to their mother during the first 12 weeks of life were compared. At the age of 25±0.2 months heifers were integrated individually into the cow herd and observed for 33 hours. Heifers reared with contact to their mother used the cubicles quicker and more consistently and also tended to differ in the social behaviour compared to the heifers reared without mother. These results suggest some positive long-term effects of mother-bonded rearing on later challenge response and welfare of dairy cattle.

INTRODUCTION

Without human intervention, calves are running with the mother and are suckled approximately 10 months (Reinhardt & Reinhardt 1981). By contrast in dairy production calves are usually separated from the mother shortly after birth and fed by bucket or automatic milk feeder (artificial rearing). This early separation from the dam can have short and long term effects on the behaviour and stress reaction of the animals. For example cross sucking is often a problem in dairy cattle (Keil & Langhans 2001). Mother-bonded rearing can prevent cross sucking in calves (Roth et al 2009). Furthermore mother-bonded reared calves as compared to artificially reared calves were chronically less stressed with respect to results in an ACTH-challenge test. On the long term heifers having been reared with a foster cow as calves showed a higher activity and, in Saler, heifers had a higher rank in the social hierarchy compared to artificially reared heifers (Le Neindre & Sourd 1984). In our study we wanted to investigate the potential long term effects of mother-bonded rearing compared to artificial rearing in the first 12 weeks of calves' life on later ability to cope with regrouping as heifers. We hypothesised, that mother bonded reared heifers show less stress responses and cope more easily with the social challenge.

MATERIAL AND METHODS

Three groups of heifers that differed with respect to contact to their mother during the first 12 weeks of life were compared. One rearing group was fed via an automatic milk feeder two or six times a day (A, n=10). These calves were compared to two treatment groups that had either restricted contact to their mother outside the cow herd (twice a day 15 min suckling; S, n=9) or permanent access to their mother and to the cow herd via selection gates (P, n=7). After weaning animals were kept in one group with each other and other animals up to this experiment. 25-38 days before expected parturition, heifers were integrated one by one into the dairy cow herd. Each heifer was observed for 33 hours continuously by behaviour sampling. Additionally the nearest neighbour was recorded every five minutes by instantaneous sampling. The behavioural data were analysed with Mann-Whitney-U tests.

RESULTS

Heifers reared with contact to their mother (S, P) used the cubicles quicker ($p<0.01$) and more consistently ($p<0.05$), P heifers also showed more self-grooming ($p<0.05$) compared to A heifers. Concerning social behaviour P heifers showed more submissive behaviour ($p<0.05$) and tended to initiate less aggression ($p<0.1$) than A. One P and one S heifer were close to their mother above chance level ($p<0.05$, nearest neighbour over chance level), for another S heifer there was a tendency ($p=0.06$).

DISCUSSION

Regrouping influences behaviour and milk production of dairy cattle (Hasegawa et al 1997). Regrouping causes reduced feeding time, less and shorter lying behaviour and less allogrooming behaviour in dairy cows (von Keyserlink et al 2007). In our study mother bonded reared heifers laid down in cubicles earlier and more frequently and showed more self grooming suggesting lower levels of stress in these animals (Roth 2008). In cattle lying behaviour has a high priority (Munksgaard et al 2005). If animals can lie down earlier, they may improve their welfare. With respect to social behaviour animals with contact to the mother in their early live phase showed more submissive behaviour and less aggression. It seems that mother-bonded animals have a better ability to adopt appropriate social behaviour and by this a better integration into the dairy cow herd. After integration into a group of cows heifers often obtain a low social status (Boe & Fxrevik 2003). A strategy of low aggression and submission may be more beneficial for the animal.

The results of nearest neighbour observations suggest that at least three animals could identify their mother within a herd of about 40 cows after two years separation. This indicates a strong bond with the mother and good memory, at least for those few animals.

CONCLUSIONS

The results suggest a lower level of stress and enhanced social skills in animals reared with mother contact. Mother contact in the first weeks of life, even if very limited, seems to affect later behaviour of dairy heifers and the ability to cope with stress during regrouping.

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SOCIAL BEHAVIOUR AND INJURIES IN HORNED AND HORNLESS DAIRY GOATS

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SUMMARY

The aim of the study was to investigate the situation with respect to social stress and injuries in dairy goat herds keeping horned or hornless animals and to identify risk and success factors. 45 farms with purely hornless or mixed herds were visited once and social behaviour, injuries and potentially influencing factors were assessed. Success and risk factors were identified by linear regression models.
There was huge variation between farms with respect to animal based parameters (social behaviour, injuries etc.) as well as with respect to potential risk or success factors of housing and management. Prevalence of injuries at the udder was higher in herds with horned animals, but no higher risk of injuries at other body parts existed. The below average occurrence of udder injuries in part of the horned herds indicates that the risks are controllable also with horned animals. Factors lowering the risk of injuries were mainly related to increased social stability, lower competition and higher intensity of contact and care by the caretaker and underlying attitudes. In sum keeping horned dairy goats is possible without a higher occurrence of injuries and stress.

INTRODUCTION

Disbudding of goat kids is a problematic procedure. Due to the extremely thin skull and the relative large size of the horn buds the risk of complications and severe traumata (e.g. cerebral haemorrhage) is relatively high [9]. However, disbudding is common in dairy goats and justified by a high risk of injuries and higher stress for subordinate animals when keeping horned goats as compared with hornless ones. Studies comparing horned and hornless goats or investigating housing factors that may influence social stress used small groups so far [1, 6] The aim of the study was to gain data on the situation with respect to social stress and injuries in large dairy goat herds keeping horned or hornless animals and to identify risk and success factors.

MATERIAL AND METHODS

45 goat farms with a herd size of 78 to 620 dairy goats (mean ± SD: 170 ± 116) were visited for two days each. Farms were selected only if they had at least two years experience with keeping and milking dairy goats and a herd size not below 80 lactating goats at day of first telephone contact. We aimed at comparing farms that did not disbud kids and thus keep horned and (genetically) hornless goats with farms that practice disbudding and thus only have hornless (genetically or disbudded) goats.
During farm visits the social behaviour of the goats was observed for 6 h in total (3*2h). A subsample of goats was examined with respect to injuries, general health signs (swellings of lymph nodes or joints, nasal or ocular discharge) and body condition during two milking times. Potential influencing factors, i.e. housing conditions, management and the human-animal-relationship were recorded in detail by own assessment, structured interview and questionnaires. Success and risk factors were identified by linear regression models with stepwise forward procedure.

RESULTS

15 farms (33%) had purely hornless herds (= no single animal with fully developed horns), the rest (30 farms) had herds mixed of horned and hornless goats (=horned herds). In all hornless herds animals with snag horns were seen (median, minimum-maximum: 15%, 1,9 to 43%), in horned herds the percentage of horned animals ranged from 1,4 to 78 % (median: 48%), but in most herds (21 farms) the percentage was above 33%.
There was huge variation between farms with respect to animal based parameters (e.g. injuries) and influencing factors, mostly independent of horns. Both in horned or hornless herds farms with many problems as well as successful farms with low prevalence of injuries, aggression or health problems existed. Frequency of aggressive interactions tended to be slightly higher in hornless herds (median: 1.06 interactions / animal*10min) compared to horned herds (0.93, $p=0.071$). Occurrence of injuries at the udder was higher in horned herds (horned: 0.43 / animal; hornless: 0.22 / animal, $p<0.01$), but no association existed with the percentage of horned animals. As well, in several horned herds prevalence of udder injuries was low. Horned and hornless herds did not differ with respect to body condition or health.

In the regression model injuries at the udder were lower with a lower number of milkers, if no acquisition of goats from other farms (both $p \leq 0.001$) and no regrouping during the lactating period ($p \leq 0.05$) took place and if management was more adapted to the needs of the goats as indicated by higher scores in the composite factors "special management" (comprising agreement by the farmer that horned animals need adapted management; $p \leq 0.001$) and "welfare friendly practices" (comprising claw trimming at least twice per year or access to pasture) (both $p<0.05$). Total number of injuries also was associated positively with the number of milkers and acquisition of goats from other farms as well as with the frequency of regrouping ($p=0.001$), while negative associations were found for quality of food ($p<0.01$), years of keeping goats, working time in contact to the goats and claw trimming at least 2 times per year (all: $p<0.05$). Prevalence of deep injuries at the udder (observed on 12 of the 45 farms) was lower if palisade feed barriers were used on the farm. The number of social agonistic interactions was lower, for instance, with higher quality of food, lower number of goats per drinker, longer working time in contact to the goats, less stockpeople responsible for the goats, "general management", breeding for socially agreeable animals, later separation of kids from the mother, and "good housing" (comprising access to an outdoor run, and no bottlenecks or dead ends).

DISCUSSION

The large variation between farms with respect to the frequency of agonistic behaviour and injuries independent of presence of horns already points at the underlying influence of the specific situation on the farm. In experimental situations horned goats displayed less agonistic interactions especially with body contact compared to hornless goats [2, 8]. The lack of consistent differences between horned and hornless herds in our study again underlines the importance of environmental conditions for agonistic behaviour. A higher level of social agonistic interactions with body contact increases the risk for injuries by butts, in horned animals the risk for scratches by horns gets higher. Thus factors contributing to more aggression also increase the risk for injuries, matching our results. Factors contributing to low level of agonistic interactions and injuries in the dairy goat herds in our study can be summarized by management practices allowing for a low level of competition and high level of social stability, as well as by higher intensity of stockperson-goat contact and provided care, but also a well designed housing. As well management practices and attitudes of the herd manager more orientated towards the needs of goats were associated with lower level of injuries. These results are in line with previous on farm studies in loose-housed horned dairy cows [7] or experimental studies in goats and other animals [e.g. 1, 4, 5]. Horned and hornless herds did not differ in body condition or general health signs suggesting no essential difference in stress levels. Similarly horned and hornless goats kept in small groups did not differ in heart rate variability, an indicator of chronic stress [3].

CONCLUSIONS

Low level of aggression and injuries both in horned and hornless dairy goats in larger groups can be achieved by respective housing design, management and care. The risk of udder injuries is higher in horned herds. However the below average occurrence of udder injuries in part of the horned herds indicates that the risks are controllable also with horned animals. Thus keeping horned dairy goats is possible without a higher occurrence of injuries and stress.

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EFFECT OF STRAW PROVISION ON THE WELFARE STATUS OF ITALIAN HEAVY PIGS

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SUMMARY

This paper investigates the feasibility to provide straw by manger racks as rooting material for Italian heavy pigs. Results indicate that the use of straw, while causing no management problems, can improve pig welfare reducing serum levels of haptoglobin and cortisol, as well as gastric ulcer susceptibility.

INTRODUCTION

Directive 2008/120/EC [5] requires farmers to allow pigs to express their exploratory/rooting behaviour via the use of straw, wood, sawdust, compost, peat or mixture of these. The ideal enrichment must be edible, odorous, chewable, deformable and destructible: among all the items tested, straw has provided the most remarkable results in reducing aggressiveness [4]. Despite its benefits, straw is never routinely supplied in slatted floor systems (commonly used in Europe), due to possible management problems.

The aim of this work was to evaluate the technical feasibility of straw provision and its beneficial effect on the welfare of fattening Italian heavy pig (slaughter at 9 months of age and 160kg of weight), as no scientific literature was yet available on the issue. The evaluation of stress in both the experimental and control groups considered haematological (serum levels of haptoglobin and cortisol, albumin/globulins ratio) and anatomopathological (gastric ulcer at slaughter) parameters.

MATERIAL AND METHODS

The research involved 672 commercial hybrid pigs (sex ratio 1:1; castrated males) aged 80 to 290 days of age bred in 24 single-sex pens of an open-site fattening farm. Twelve pens were enriched with straw through a grated rack fastened on the side wall (fig.1).

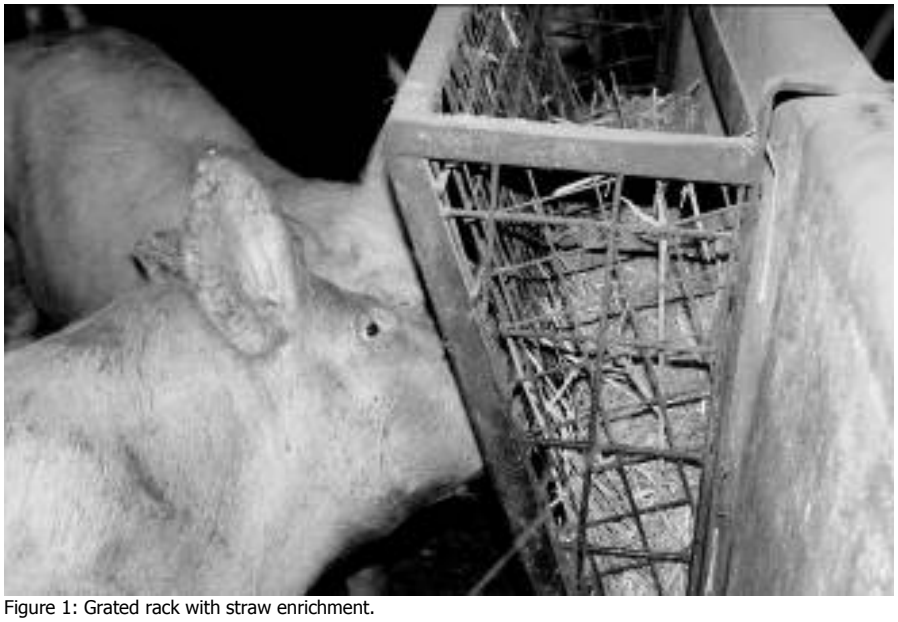

Figure 1: Grated rack with straw enrichment.

Blood samples were stored at 4°C during transportation to the lab, centrifuged at 2400xg for 10 minutes at 20°C and stored at -20°C before analysis.

Serum concentrations of haptoglobin (Hp), albumin, globulins and cortisol were quantified by collecting 3 blood samples from each of 6 randomly selected pigs per pen at 122, 210 and 274 days of age,. Total serum proteins were measured by a colorimetric kit with Biuret reagent (TP, F. Hoffman-LaRoche Ltd, Basel, Switzerland) using a BM Hitachi 911 analyzer (F. Hoffman-LaRoche Ltd) using. The albumin/globulins ratio (A/G ratio) was obtained by determining the concentration of different protein fractions by serum electrophoresis with the Hydrasis LC semi-automated analyzer (Sebia, Lisses, EVRY Cedex, France) on 0.8% agarose gel (Hydragel 30 Protein; Sebia). Hp concentration was determined by an ELISA kit (Phase Haptoglobin, Tridelta Develpoment ltd, Maynooth, County Kildare, Ireland).

Serum cortisol concentration was determined by an immunologic chemiluminescent kit (LKCO1, Medical System, Genoa, Italy) applied to the Immulite One automated analyzer (Immulite One, Medical System).

At slaughter the stomachs of all the animals were scored according to the method proposed by Robertson *et al.* [10]: 0=normal, 1=hyperkeratosis, 2=eroded, 3=severe ulcer (fig.2).

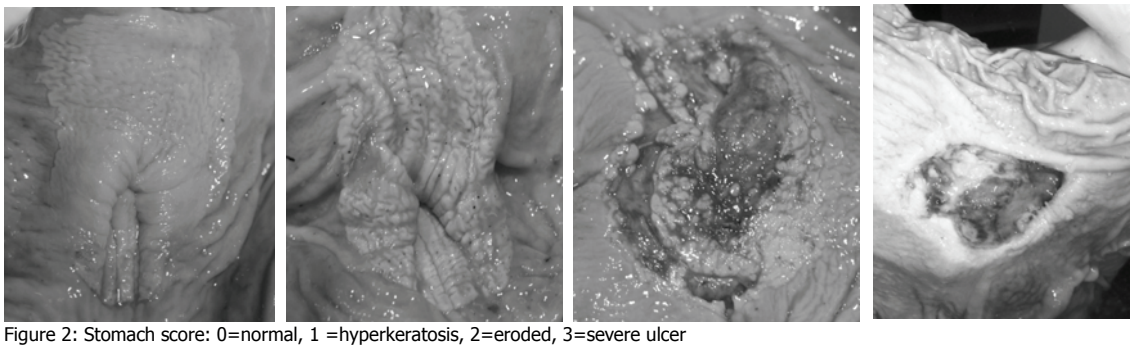

Figure 2: Stomach score: 0=normal, 1 =hyperkeratosis, 2=eroded, 3=severe ulcer

ANOVA model for mixed designs was applied to evaluate significant differences for the blood parameters considering gender (M *vs* F) and straw provision (Y *vs* N). Logistic regression was performed to analyze the gastric scores, after having gathered the scores 0-1 and 2-3, respectively.

RESULTS

Mean results of blood parameters at the three samplings stratified by gender and straw presence are given in table 1. At the first sampling (122 days of age), the mean level of serum Hp did not differ among the groups. At the second sampling (210 days of age), Hp level seemed to be higher in females, but it was also associated to a significantly higher variance, making improper any statement about gender effect. At the same sampling, the presence of straw induced a significant reduction of the mean Hp level. At the third sampling (274 days of age), straw presence still had a significant effect on reducing Hp levels ($P=0.006$) and a considerable gender effect could also be observed ($P<0.001$). At the first and second sampling, the mean level of serum cortisol did not differ among the groups, while at the third one the group provided with straw evidenced lower levels ($P<0.01$). Serum albumin/globulins ratio did not differ among groups at any sampling time.

Gastric lesion scores of all slaughtered animals are given in table 2. Males had higher scores of gastric lesions than females (OR=1.51, IC95%:1.09-2.11); straw proved to be a protective factor (OR: 0.42; IC95%: 0.26-0.64).

The manger cage structure minimized straw waste and avoided blockage of the slatted floor. Straw loading (3 times a week) did not increase farmers' workload considerably.

Table 1**:** Effect of gender and straw provision on physiological parameters in fattening pigs.
*= P value<0.05 **= P value<0.01 ***= P value<0.001

		Age (days)					
		122		210		274	
Item	Strata	Mean	sd	Mean	sd	Mean	sd
Hp (mg/dl)	Male	77.6	52.0	*18.6*	*24.5*	43.7***	44.5
	Female	78.0	53.3	*57.8*	*62.8*	106.6***	60.2
	Straw	68.3	48.4	30.5	34.5	63.1**	56.4
	No straw	87.3	54.9	45.8	63.4	87.2**	64.3
A/G ratio	Male	0.71	0.13	0.82	0.14	0.77	0.14
	Female	0.78	0.13	0.86	0.12	0.76	0.12
	Straw	0.74	0.12	0.84	0.09	0.76	0.10
	No straw	0.75	0.15	0.85	0.16	0.76	0.16
Cortisol (nmol/l)	Male	55.0	25.1	60.7**	31.4	64.0	33.2
	Female	50.7	24.2	84.3**	48.9	64.9	42.1
	Straw	50.8	24.1	73.4	50.0	55.7**	32.5
	No straw	55.0	25.3	71.6	33.9	73.2**	40.8

Table 2: Gastric lesion scores of all slaughtered animals.

Score	Female		Male		No straw		Straw	
	n.	%	n.	%	n.	%	n.	%
0	*58*	*18.30*	*46*	*14.47*	*12*	*3.85*	*92*	*28.48*
1	*124*	*39.12*	*107*	*33.65*	*103*	*33.01*	*128*	*39.63*
0+1	182	57.41	153	48.11	115	36.86	220	68.11
2	*83*	*26.18*	*61*	*19.18*	*95*	*30.45*	*49*	*15.17*
3	*52*	*16.40*	*104*	*32.70*	*102*	*32.69*	*54*	*16.72*
2+3	135	42.59	165	51.89	197	63.14	103	31.89
Total	317	100	318	100	312	100	323	100

DISCUSSION

The values of all considered parameters at the 3 samplings remained within physiological range [7], confirming an overall good health status of the animals. Several studies [9] have shown that serum concentration of Hp may be useful in the assessment of pig welfare: at 274 days, the "straw" group presented a lower level of Hp compared to the "no-straw" one, and this difference may be related to the beneficial effect of straw on reducing fights and other aggressive behaviours that, in turn, may cause ear damages, tail lesions and inflammation. This result was also confirmed by the lower serum level of cortisol.

At 274 days, females presented higher values of Hp than males: this gender gap could be due to a different physiological condition (i.e. hormonal status), in agreement with Lipperheide *et al.* [8], that reported higher Hp levels in sows than in boars and barrows. For this parameter, Italian heavy pigs seem to be physiologically closer to breeding pigs than fattening light pigs, in which no gender differences were observed [8].

Ulceration of the oesophageal region of the stomach is a common, widespread condition in swine [6]. Its multifactorial causes may be related to nutritional [12], environmental [10], [1], health reasons [6], or stress [2]. In our study all the animals were kept under the same husbandry conditions and no significant health problems were evidenced: as a result, the "straw" group's lower scores may be attributed to the experimental variable. The lower scores in females may depend on the protective effect of estrogens: this result was not confirmed in fattening light pigs [3], [10]; but it was demonstrated in female adult mice [11].

CONCLUSIONS

Straw provision did not hinder slurry outflow and was associated with lower serum levels of Hp and cortisol and reduced ulcer susceptibility. For these reasons, it could be recommended to farmers, as it would help improve pig welfare without causing management problems.

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EFFECT OF TRANSPORT DURATION ON DAY-OLD CHICK DEHYDRATION AND ANIMAL MORTALITY, FEED INTAKE, AND WEIGHT DURING REARING PERIOD

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SUMMARY

Decreasing broiler density in farms is foreseen by directive 2007/43/EC [2], especially if mortality results are higher than a fixed threshold. The highest incidence of chicken mortality in industrial production occurs during the first week of rearing. In order to decrease mortality of broiler chickens during the first days of rearing, this experiment studied the effect of different transport durations on day-old chicks' mortality. Also, dehydration and zootechnical performances such as feed intake and body weight gain were assessed during the rearing period. Just after hatching, 7800 chicks (ROSS PM3) from breeders of 35 weeks old were transported to a poultry house in boxes of 100 chicks and distributed into three treatments: the first group was transported in a truck for 4 hours (T4), the second was transported in the same truck for 10 hours (T10) and the last group (T0) was moved directly from the hatchery to the farm (few minutes). During transport air temperature in the truck was 28°C to 29°C with a relative humidity of 34% to 36%. After transport, chicks were reared in the same house and each treatment was divided into 4 pens of 35 m² with 650 chicks each. Hematocrit rates, used as an indication of dehydration status, were assessed by sampling chick blood before and after transport and at day 3. Chicks were weighed before and after transport, and once per week until slaughter (Day 35). Feed intake was measured weekly until slaughter age. No differences between treatments were observed in hematocrit rates, feed intake, or mortality. However, chicks' weights just after transport and until day 21, were significantly lower in transported chicks for 4 (T4) and 10 hours (T10) than those moved directly to the farm (T0), but no effect was observed at Day 28 until slaughter age (Day 35). In conclusion transport of chicks for 4 or 10 hours had no effect on mortality, dehydration, and feed intake but reduced chick body weight until 21 days.

INTRODUCTION

Decreasing stocking density may reduce farmer's income by reducing profitability. European Directive 2007/43/EC [2] laid down maximum stocking density for broilers. The maximum density allowed by the directive is 33 kg/m². If stricter welfare standards are met, farmers are allowed to raise stocking density to 39 kg/m², but not more than 42 kg/m², especially if mortality rates are below the threshold (mortality of 3.52% at 42 days). The highest incidence of chicken mortality in industrial production occurs during the first week of rearing [6]. This mortality is a result of incubation conditions and post-hatch handling in hatcheries, transport of day old chicks, and conditions during the first days of rearing. It was reported that transport distance can increase mortality during the first week of rearing [5]. In order to decrease mortality of broiler chickens during the first days of rearing, this experiment aimed to study the effect of different transport durations of day-old chicks on mortality, dehydration, and zootechnical performances such as feed intake and weight gain during rearing period.

MATERIAL AND METHODS

Just after hatching, 7800 chicks (ROSS PM3) from breeders of 35 weeks old were transported to a poultry house in boxes of 100 chicks and distributed in three treatments: the first group was transported in a truck (in a routine tour of hatchery truck for chicks distribution on farms) for 4 hours (T4), the second group was transported in the same truck for 10 hours (T10) and the last group (T0) was moved directly from the hatchery to the farm (200 m, few minutes of travel). During transport, air temperature was 28°C to 29°C with a relative humidity of 34% to 36% in the truck. After transport, chicks were housed in the same house and each treatment was divided into 4 pens of 35 m² with 650 chicks each. Hematocrit rates, used as an indication of dehydration status, were assessed by sampling chick blood before transport (total of 40 chicks), after transport (32 chicks per treatment) and at day 3 (32 chicks per treatment: 8 animals per pen). Blood samples for hematocrit were collected in heparinized micro-tube of 50 mm long and 1.4 mm wide, sealed by clay, centrifuged in a micro hematocrit centrifuge (Centri Jouan A13) at 15000 g for 5 min, and the results were read using the Janetzki® micro-capillary linear reader. A total of 120 chicks were weighed before and after transport, and then once a week (30

chickens per pen) until slaughter (Day 35). Feed intake per pen was measured weekly until slaughter age; and mortality per pen was checked daily. All results were analyzed by analysis of variance (ANOVA) using General Linear Model (GLM) procedure of SAS [7] for Windows XP Pro Platform, with transport duration as the main effect. Means were compared using Student-Newman-Keuls multiple range (SNK) test contrasts. Homogeneity of data was controlled by SAS [7] and normality of distribution was assessed by SYSTAT® [8].

RESULTS

Results of dehydration, mortality, feed intake, and weight are presented in **Table 1**. No differences between treatments were observed in hematocrit rates ($P>0.05$) neither before nor after transport at Day 0 and Day 3. Also, no significant differences ($P>0.05$) were observed in weekly feed intake and mortality. However, chicks weight was significantly higher ($P<0.05$) in T0 compared to T4 and T10 just after transport and at day 7. At day 14 and 21, weight of chicks in T0 was higher ($P<0.05$) than T10, but no differences was observed when T4 was compared to T0 and T10. During the last two weeks, no significant differences ($P>0.05$) in body weight was observed between the treatments.

DISCUSSION

The experimental hypothesis was that broiler chicks would have a lower performance, as measured by feed intake and weight gain, and higher mortality rates after exposure to long transport duration due to exposure to stressors during load and unload and feed deprivation.

No significant differences ($P>0.05$) were observed in hematocrits rates. However the high variability of results may have been due to methodological problems. After a power test, it was concluded that at least 84 samples are needed per treatment to show whether significant differences exist.

No differences were observed in mortality rates. This result are in accordance with Almeida et al. [1] who suggested that a long fasting time after hatching has no effect on mortality rate when chicks were held for 12 hours after hatch in incubators. On the contrary, Chou et al. [5] suggested that transport of chicks for distances higher than 50 km (fasting time more than 1 hour plus stress of transport) results in higher mortality rates during the first week of rearing.

Almeida et al. [1] suggested that removing chicks directly after hatch, compared to those removed 12 hours later, results in higher feed intake but lower weight gain (poorer feed conversion ratio). In our experiment, no significant differences were observed in feed intake expressed by grams of feed per chick during the rearing period.

However, despite no significant differences in feed intake for T0 chicks compared to T4 and T10, having early access to feed and water resulted in significant higher weights ($P<0.05$) during the first week. Batal and Parsons [4] suggest that early feeding after hatch stimulates early gut development resulting in efficient energy utilization and higher weight gain. During the next two weeks (day 14 and 21), weights were significantly higher ($P<0.05$) in T0 when compared to T10, but not between T4 and the rest of treatments (T0 and T10).

During the last two weeks no differences were observed between treatments until slaughter age. This result confirms results of Baião et al. [3] who suggested that a longer period of hatching associated with a longer interval between hatching and housing, impaired the weight gain of the broilers in the first week of rearing, but did not change the final weight nor feed efficiency at the end of rearing period.

CONCLUSIONS

In the present experiment, transport of day-old chicks for 4 or 10 hours reduced chick body weight up to 21 days, but no effect has been found after 28 days until slaughter age (Day 35). This study found the transport duration had no effect on mortality, dehydration, feed intake and final weight.

Other parameters such as incubation conditions may affect on animal behaviour and health need to be explored in order to improve broilers welfare and decrease young mortality. This will be the main objective of the European BioBusiness Project in which this study forms part.

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Table 1: Effect of transport duration of day old chicks on dehydration, mortality, feed intake and body weight.

	Treatments				
	T0	T4	T10	SEM	P
Hematocrit (%)					
– Day 0	30.66	28.50	29.55	0.78	0.3487
– Day 3	26.78	26.75	27.27	0.16	0.8485
Weight (g)					
– Day 0	39.95[a]	38.65[b]	38.14[b]	0.29	<.0001
– Day 7	158.81[a]	154.58[b]	152.11[b]	1.26	0.0008
– Day 14	422.99[a]	413.99[ab]	409.53[b]	3.38	0.0172
– Day 21	867.63[a]	849.48[ab]	839.74[b]	6.67	0.0117
– Day 28	1426.64	1411.16	1397.96	14.31	0.3724
– Day 34	1841.60	1881.82	1873.65	19.21	0.2953
Feed intake (g/week)					
– Week 1	116.16	125.24	119.05	6.21	0.5909
– Week 2	434.73	434.09	419.45	7.71	0.3300
– Week 3	593.63	571.14	569.83	7.78	0.1031
– Week 4	933.48	932.80	904.99	10.75	0.1571
– Week 5	901.03	927.44	937.26	15.42	0.2786
Mortality (%)					
– Day 1	0.0769	0.0000	0.0000	0.0444	0.4053
– Day 3	0.1154	0.2692	0.0385	0.0821	0.1848
– Day 7	0.8462	1.1923	1.0385	0.2996	0.7238
– Day 14	1.3846	1.5769	1.3846	0.3351	0.8972
– Day 21	2.0000	1.8462	1.7692	0.3333	0.8846
– Day 28	2.5769	2.1923	2.4231	0.3462	0.7391
– Day 35	3.7692	3.6538	3.2692	0.3039	0.5032

[a,b] different letters within the same line denote significant differences (P<0.05).

RISK-BASED MONITORING OF ZOONOSES

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SUMMARY

For consumer protection, it is important to achieve and maintain a good animal health situation regarding zoonotic pathogens, and a high level of safety of food of animal origin. An important tool for this are monitoring and surveillance programmes. However, a large number of zoonotic pathogens exists, and many different animal species and foods need to be included in a monitoring. A good level of protection often requires testing a large number of samples for estimating the prevalence of a disease, or for early detection of a re-introduction of a disease absent in a country. An alternative can be risk-based monitoring and surveillance programmes. A risk-based approach combines methodologies from risk assessment and design of surveillance programmes in order to focus sampling efforts to those animals or food items, where the risk of infection is highest. The objective of the current presentation is to illustrate the concept of risk-based zoonoses monitoring.

INTRODUCTION

Even though several zoonotic diseases such as bovine tuberculosis, brucellosis or rabies have been successfully controlled in many European countries, zoonoses remain a major challenge for public health. Monitoring of zoonoses is an important prerequisite to estimate their public health impact, to detect changes in their prevalence over time, and to verify that a country remains free from a zoonosis after successful eradication. In most cases, monitoring is based on a random sample of the population. While this results in representative data, the number of samples which need to be tested for detecting a positive sample can be rather high, especially for rare diseases. Therefore, it can be beneficial to focus sampling efforts to those animals or food items which are at an increased risk of being positive. Risk-based surveillance has been defined as a surveillance programme in the design of which exposure and risk assessment methods have been applied together with traditional design approaches in order to assure appropriate and cost-effective data collection (Stärk et al. 2006). The objective of risk-based zoonoses monitoring and surveillance programmes is either to achieve a better protection of consumers from zoonoses with the same resources, or to save resources while maintaining the level of protection.

This presentation illustrates the concept of risk-based monitoring and surveillance programmes with two examples: (1) Risk-based monitoring of antimicrobial resistance in meat and meat products with the objective to prioritize products according to their risk of harboring antimicrobial resistant bacteria of relevance for public health (Presi et al. 2009), and (2) Risk-based surveillance of *Trichinella* in slaughter pigs with the objective to demonstrate freedom from infection (Schuppers et al. 2010).

MATERIAL AND METHODS

(1) Risk-based monitoring of antimicrobial resistance in meat and meat products

Four different product types (raw meat, frozen meat, dried or smoked meat products and heated meat products, and four different animal categories (poultry, pork, beef and calf) were compared. The public health risk of each product type was described as the probability for each product to be contaminated with bacteria resistant to antimicrobials, the amount of product consumed in Switzerland, and the potential of each combination of bacteria and resistance to cause adverse health effects in humans consuming the product. Contamination with *Campylobacter* spp., *Enterococcus* spp. and *E. coli*, and resistance against 7 different antimicrobial classes was considered in the model.

In a semi-quantitative risk assessment the prevalence of each combination of bacteria and antimicrobial resistance and the amount consumed for each product (exposure) was estimated. Risk scores for the public health hazard associated with each bacteria-resistance combination were combined with the exposure to obtain a ranking of the different products regarding their public health relevance.

(2) Risk-based surveillance of *Trichinella* in slaughter pigs

The domestic pig population of Switzerland is free from *Trichinella* spp. Nevertheless, wildlife is known to be infected with the parasite, and may be a reservoir for infection of domestic pigs. Pigs kept outdoors and older pigs are therefore at a higher risk of infection compared to slaughter pigs raised indoors. A surveillance system for demonstrating continued freedom of the domestic pig population from infection could therefore gain efficiency by testing mainly the high risk population. Routine examination of all slaughter pigs for *Trichinella* was compared with a risk-based approach which focused on pigs kept outdoors and adult pigs because of their higher infection risk. In a scenario tree model the probability of freedom from infection after testing all pigs negative with the different surveillance methods was calculated. In a stochastic model built in the program @Risk, a surveillance period of 15 years was simulated.

RESULTS

(1) Risk-based monitoring of antimicrobial resistance in meat and meat products

The risk ranking of the four different product types and animal categories is summarized in table 1. Poultry meat was ranked highest for fresh and frozen meat regarding public health relevance. For dried and smoked meat products, pork represented the greatest risk. Heated meat products were in the lowest risk category for all animal categories.

Table 1: Ranking of different product types and animal categories according to a semi-quantitative risk assessment for their risk regarding contamination with bacteria with antimicrobial resistance. 1 represents highest potential public health risk, 4 lowest risk.

	Raw meat	Frozen meat	Heated meat products	Dried or smoked meat products
Poultry	**1**	**1**	4	3
Pork	2	2	4	**1**
Beef	4	3	4	2
Veal	3	4	4	4

(2) Risk-based surveillance of *Trichinella* in slaughter pigs

Routine examination of all slaughter pigs with the digestion method resulted in a sensitivity of the surveillance system of 62%. The specificity was assumed to be 100%. Different scenarios modeled for the risk-based surveillance resulted in a sensitivity of the surveillance system between 51% and 61%. In order to demonstrate with 95% certainty that the prevalence of *Trichinella* infection is below 0.0001%, the traditional surveillance required testing of 2.4 million pigs per year. With risk-based surveillance, the same level of certainty could be achieved by testing 120,000 to 620,000 pigs (depending on the scenario used).

DISCUSSION

Risk-based zoonoses monitoring has a great potential to make better use of the scarce resources available for these programmes. However, the approach also has a number of disadvantages. Designing a valid risk-based monitoring requires good data on the distribution of infection, and on risk factors for infection of a farm or animal. If the risk-based sampling is based on incorrect risk factor information, the level of protection will be decreased compared to random sampling, rather than increased. Therefore, the optimal solution for many monitoring programmes is not either random or risk-based sample collection, but rather a combination of both approaches. This allows maximizing the efficiency of the monitoring programme, while maintaining a minimal level of consumer protection for the worst case of completely valid assumptions regarding risk factors.

CONCLUSIONS

Risk-based monitoring and surveillance systems can achieve an improved level of protection from zoonoses by targeting the available resources to those products where the public health risk is highest. On the other hand, the method can also be used to save resources while maintaining the level of protection.

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ESBL PRODUCING *KLEBSIELLA PNEUMONIAE* ISOLATED FROM DAIRY FARMS - PRELIMINARY RESULTS

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SUMMARY

This paper presents preliminary data regarding phenotypic identification of *Klebsiella pneumoniae* extended spectrum β-lactamases (ESβLs). Swabs were performed on different locations, including milking parlors, pre-milking parlors, free stalls, animal's rectum and hindlimbs. Milk samples were obtained from the animals and the bulk tank. All *Klebsiella pneumonia*e isolates were screened regarding ESβLs production by the double disk synergy method. This portion of the study presents preliminary data regarding phenotypic identification of ESβLs.

INTRODUCTION

Dry cow therapy with β-lactam antibiotics is commonly adopted worldwide [7]. Chronic mastitis cases irresponsive to treatment are increasing in dairy farms. Extended-spectrum β-lactamases (ESβLs) and carbapenemases strains of *Klebsiella pneumoniae* are a major global concern, mainly in human medicine [8]. Multi resistant pathogens may be encountered naturally in the environment. These multi resistant pathogens can, in appropriate conditions, infect the mammary gland. There is a lack of information regarding the presence of these enzymes in veterinary medicine. This study aims to detect and identify these enzymes in bacterial strains isolated from different locations in dairy farms. This portion of the study presents preliminary data regarding phenotypic identification of *Klebsiella pneumoniae* ESβLs.

MATERIAL AND METHODS

Twelve dairy farms participated on the study. 240 swabs were performed on different locations, including milking parlors (two swabs per farm), pre-milking parlors (two per farm), free stalls (eight per farm), animal's rectum and hindlimbs (six per farm for both), totalizing 24 swabs per farm. The swabs were collected in five points of a square meter, in pre-defined locations of the dairy farms (milking parlor entrance and exit; pre-milking parlor top and bottom halves; eight free stall beds on the same locations regardless of the farm) in the case of non animal swabs. For the animals swabs, random animals were chosen during the milking process. In all farms, animals were screened for intramammary infections (IMI) using the *California Mastitis Test*. In two farms, swabs were not performed, only the milk was obtained. A sample of 200 mL of milk was collected per bulk milk tank. Microbiologic exams were performed on both milk and swabs. Identity of *Klebsiella* species was determined based on motility test, citrate utilization, indole production and urease activity.

128 strains of *K. pneumoniae* were isolated from the twelve dairy farms in a three-year period. These strains were isolated from intramammary infections (29.69%), bulk milk tanks (3.91%), free stalls (28.91%), milking parlors (7.03%), rectum (11.72%) and hindlimbs (11.72%) of the animals, and pre-milking parlors (7.03%). The strains were screened to production of ESβL by the double disk synergy test using the following drugs: aztreonam, ceftriaxone, cefpodoxime, ceftazidime, cefoxitime, cefotaxime and clavulanic acid. Briefly, a bacterial suspension (0.5 McFarland) was subcultured onto Müller-Hinton agar plates with a cotton swab, and each β-lactam disk was placed two centimeters distant from the clavulanic acid disk. The presence of "ghost-zones" was considered as indicative of ESβL production.

RESULTS

22.66% of the isolated strains were resistant to cefpodoxime, 21.88% to aztreonam, 38.28% to cefotaxime, 23.44% to ceftriaxone, 17.19 to ceftazidime and 1.56% were resistant to cefoxitime. Table 1 summarizes the resistances profiles according to the location, excluding the cefoxitime profile, due to the low number of observed resistant strains.

Table 1. Number of *Klebsiella pneumoniae* isolates per location (N), absolute frequency (R/S) and its corresponding relative frequency (%) of the *Klebsiella pneumoniae* resistance profile to cefpodoxime (CPD), aztreonam (ATM), cefotaxime (CTX), ceftriaxone (CRO) and ceftazidime (CAZ) according to the place of isolation (Location)

Location*	N	CPD				ATM				CTX				CRO				CAZ			
		R	%	S	%	R	%	S	%	R	%	S	%	R	%	S	%	R	%	S	%
IIM	38	6	16	32	84	5	13	33	87	7	18	31	82	7	18	31	82	5	13	33	87
BT	5	2	40	3	60	1	20	4	80	2	40	3	60	3	60	2	40	0	0	5	100
FS	37	13	35	24	65	11	30	26	70	17	46	20	54	9	24	28	76	10	27	27	73
HL	15	0	0	15	100	3	20	12	80	6	40	9	60	1	6.7	14	93	1	6.7	14	93
PM	9	2	22	7	78	3	33	6	67	4	44	5	56	3	33	6	67	0	0	9	100
MP	9	2	22	7	78	2	22	7	78	6	67	3	33	3	33	6	67	2	22	7	78
RT	15	4	27	11	73	3	20	12	80	7	47	8	53	4	27	11	73	4	27	11	73

* IIM = intramammary infection; BT = bulk milk tank; FS = free stall; HL = hindlimbs; PM = pre-milking parlor; MP = milking parlor; RT = rectum

50% of the strains were resistant to, at least, one antimicrobial agent. 28.13%, 21.88%, 16.41%, 7.81% and 0.78% of the isolated *K. pneumoniae* were resistant to, at least, two, three, four, five and six principles respectively. Table 2 summarizes the differences of the resistance profiles regarding the location.

Table 2. Absolute frequency of *Klebsiella pneumoniae* sensitive to all principles tested (R0), resistant to, at least, one principle (R1), resistant to, at least, two principles (R2), resistant to, at least, three principles (R3), resistant to, at least, four principles (R2=4), resistant to, at least, five principles (R5), and resistant to all six tested principles (R6), and its corresponding relative frequencies (%) according to the location.

Location	R0	%	R1	%	R2	%	R3	%	R4	%	R5	%	R6	%
IMI	27	71.1	5	13.2	0	0	1	2.63	3	7.89	2	5.26	0	0
BT	1	20	2	40	1	20	0	0	1	20	0	0	0	0
FS	14	37.8	9	24.3	3	8.11	2	5.41	5	13.5	4	10.8	0	0
HL	8	53.3	4	26.7	2	13.3	1	6.67	0	0	0	0	0	0
PM	4	44.4	2	22.2	0	0	2	22.2	1	11.1	0	0	0	0
RT	7	46.7	4	26.7	0	0	1	6.67	0	0	2	13.3	1	6.67
MP	3	33.3	2	22.2	2	22.2	0	0	1	11.1	1	11.1	0	0

* IMI = intramammary infection; BT = bulk milk tank; FS = free stall; HL = hindlimbs; PM = pre-milking parlor; MP = milking parlor; RT = rectum

Eight (08) strains (6.25%) yielded positive results to the double disk synergy test, suggesting the production of ESβL. Two (02) strains were isolated from free stalls, one (01) from an animal's hindlimb, three (03) from IMI, one (01) from a milking parlor and one (01) from a bulk milk tank.

DISCUSSION

Penicilin remains as one of the most used veterinary drugs [6]. Excessive and bad conducted treatments with β-lactams are responsible for the increase of β-lactamases producers bacteria [1]. However, a multi resistant pathogen is not necessarily result of an unsuccessful treatment. Coliforms are environmental agents that may have contact with bacterial flora present in the environment, which facilitates the acquisition of genetic material. Systemic treatments that results in antimicrobial residues in feces, milk and urine, may collaborate with pathogen selection in the environment. These multi resistant pathogens can, in appropriate conditions, infect the mammary gland. Multi resistant pathogens may be encountered naturally in the environment, which collaborate for the acquisition of resistance genes by the coliforms [2].

According to table 2, *K. pneumoniae* strains isolated from IMI apparently showed higher susceptibility than the ones isolated from free stalls and milking parlor. Pulsed-field electrophoresis (PFGE) will help to identify the possible environmental sources of IMI.

Previous studies regarding mastitis agents and ESβLs have been conducted recently [2-4], and shows that these enzymes are present in other then nosocomial infections. In the present study, eight ESβL strains were detected by the double disk synergy test among the different locations. It is known that coliforms present in the animal's bedding may be responsible for the IMI onset, and the presence of multi resistant bacteria on the

freestalls may present a potential mastitis risk. The presence of these enzymes in bacterial strains located outside the udder suggests that not only the IMI therapy is to blame for the increasing pathogen resistance observed in the dairy farms.

All ESβL producers were isolated from three different dairy farms, suggesting that these enzymes are spreading in Brazil. These three farms had different management systems. These results are high alarming, in both terms of antibiotic therapy and public health, as one of the isolates was from the bulk milk tank.

Phenotypic methods only suggests the presence of ESβLs, and don't differenciate the subtypes of enzymes that are produced [8]. ESβLs presents distinct prevalence between the studies, but regardless of location, the most common groups observed are CTX-M, TEM and SHV enzymes [5].

CONCLUSIONS

Recent studies are showing that ESβL are an increasing concern in veterinary medicine. The spreading of these enzymes will represent great losses in terms of antibiotic effectiveness. The genotypic identification of these enzymes and PFGE of the *Klebsiella penumoniae* strains, are currently being carried out, which will identify the group responsible for the observed resistance, and the potentital risk locations. To our knowledge, this is the first study regarding ESβL production in coliforms isolated from dairy herds in Brazil, and also the first study that detected an ESβL producing strain in a bulk milk tank.

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COMPARISON OF METHODS FOR DETECTION OF VTEC (VEROTOXIGENIC ESCHERICHIA COLI) IN ANIMAL SAMPLES

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SUMMARY

The EU Zoonoses directive states that zoonoses and zoonotic agents which pose a threat to human health should be monitored. 237 samples of this monitoring programme from cattle and sheep were analysed for *VTEC*. Samples were swabs of rectal mucosa from both animals versus colon contents from cattle and fleece from sheep. After a selective enrichment three commercial ELISA test kits were used for the detection of Verotoxin. After positive results the sediment was cultured on agar plates. Out of this culture Verotoxin 1 and 2 genes were examined using PCR.

The testing of the ELISA showed that the Premier™ EHEC was more sensitive for Verotoxin detection than Novitec®Verotoxin or Ridascreen®Verotoxin.
The comparison of various sample matrices showed clear differences in the number of *VTEC* positive detected animals. Both cattle (57,0%) and sheep (76,4%) swabs of rectal mucosa lead to a higher rate of Verotoxin positive samples and subsequently more *VTEC* isolates (27% in cattle und 68,5% in sheep) than colon contents from cattle or fleece from sheep.

INTRODUCTION

The EU Zoonoses directive states that zoonoses and zoonotic agents which pose a threat to human health should be monitored. Accordingly Salmonellae, Campylobacter and VTEC are monitored in Austria. Different methods for the detection of the prototype of Verotoxigenic E. coli, VTEC O157 :H7 are available. However there is no EU-wide accepted method for the identification of VTEC. The AGES Institute for veterinary disease control in Graz analysed 237 samples of cattle and sheep to make a contribution to a consistent method.

MATERIAL AND METHODS

Samples to compare were swabs of rectal mucosa from both animal species versus colon contents from cattle and fleece from sheep. Intestinal contents, swabs of rectal mucosa and fleece were put into 9 ml of mTSB in a 100ml flask. The flasks were incubated at 37 ± 1° C on a shaker for 5 hours. 1 ml of this pre-enrichment was transfered in a tube with 4ml EHEC- Directmedium with Mitomycin C and incubated for 18-20 hours at 37 ± 1° C on a shaker. The detection of toxin from supernatant was done with ELISA test kits from Novitec®Verotoxin Veterinary ELISA, Premier™ EHEC and Ridascreen®Verotoxin according instruction sheets. From samples with a positive result the sediment was cultured on CT-SMAC- and MacConkey-agar plates. Out of these cultures Verotoxin genes 1 and 2 were examined using rt- Fluorescence PCR.

RESULTS AND DISCUSSION

The first comparison affected the Verotoxin detection with three commercial ELISA test kits. At the first test (n=57) Novitec®Verotoxin Veterinary ELISA was checked against Premier™ EHEC and in the second test (n=21) Ridascreen®Verotoxin against Premier™ EHEC.

	Test 1 (n = 57)		Test 2 (n = 21)	
	n	%	n	%
Premier positive	25	44%	12	57%
Novitec positive	13	23%	-	-
Ridascreen positive	-	-	5	24%

Table 1: Comparison Verotoxin detection with three commercial ELISA test kits

Novitec®Verotoxin Veterinary ELISA and Ridascreen®Verotoxin found less Verotoxin positive enrichments than Premier™ EHEC. To check the specifity of the ELISA tests the sediment of the first 21 positive samples were also analyzed with PCR and all results were confirmed. So Premier™ EHEC proofed to be the most suitable of the three ELISA test kits.

From the second comparison of 70 cattle the results of swabs of rectal mucosa against colon contents are presented here:

Table 2: Comparison of Verotoxin detection and *VTEC* –Isolates from swabs of rectal mucosa or colon contents of cattle (n =70)

	Rectal swabs positive	Colon contents positive
Verotoxin ELISA positive	57,0%	21,4%
Isolates PCR positive	27,0%	14,3%
n = 70		

In the third comparison of rectal mucosa swabs against fleece of 89 sheep these results were obtained:

Table 3: Comparison of Verotoxin detection and *VTEC*-Isolates from swabs of rectal mucosa and fleece from sheep (n=89)

	Swabs positive	Fleece positive
Verotoxin ELISA positive	76,4%	36,0%
Isolates PCR positive	68,5%	4,5%
n = 89		

CONCLUSIONS

As the results showed clear differences in the rate of positive samples the method in our lab was adapted to using rectal mucosa swabs from cattle and sheep and PREMIER™ EHEC ELISA.

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"MEAT JUICE MULTISEROLOGY" FOR OPTIMIZING THE SO-CALLED FOOD CHAIN INFORMATION

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SUMMARY

The new EU food safety legislation is not any longer prescribing exactly the inspection procedure, but defines the common food safety goals: improving food safety, animal health and animal welfare. One way to reach these goals is to adapt the meat inspection intensity to recognisable risks stemming from the pre-harvest production phase and to inform animal producers about food safety, animal health and welfare deficiencies recorded during slaughter. This so called "food chain information" still lacks reliable data on the infectious status of the supplying pig herds, especially on the on the occurrence of latent zoonoses and production diseases. The paper describes the general concept and first results of a meat juice based "multi-serological" monitoring system by continuously testing random samples of meat juice from the supplying pig herds for antibodies against pathogens relevant for food safety and for the health and welfare of the animals. The basic approach of collecting meat juice samples at slaughter and testing them manifold makes the multi-serology highly informative and cost-efficient and has the opportunity to include anytime other tests to detect e.g. Hepatitis E and even notifiable diseases as support of state early warning systems.

INTRODUCTION

The new European food safety concept for products of animal origin contains three main principles for optimizing food safety, animal health and animal welfare:

a) the major responsibility for food safety lies on the food producers themselves including the primary production,
b) knowledge about the animal health and production procedures obtained through the "food chain information" is used for risk-based decisions during meat inspection (2), and,
c) continuous improvement of the production processes along the food chain complements the so far sole end product inspection.

The EU-Regulation No. 853/2004 defines for the pork production chain as minimum nine criteria for the food chain information. One of them is the demand for taking into consideration existing bacteriological and serological laboratory results. So far, except of various Salmonella monitoring programmes in some European countries, there is no systematic serological monitoring for any other pathogen occurring in pig herds. The paper presents first results of a "multi-serological" monitoring system using meat juice as valuable part of the food chain information and describes its usability for improving herd health and food safety.

MATERIAL AND METHODS

Based on the recently finalized validation of using meat juice instead of blood serum for identifying antibodies against a set of bacterial and viral pathogens in pigs relevant for pig health and food safety (1), random samples of meat juice from selected carcasses from six pig herds located in the same region of Germany with similar husbandry systems were tested repeatedly in 2009 and 2010. Taking into consideration the needs for a meaningful serological monitoring system in the pork production chain, a set of ELISA tests was selected, which provides results with relevance for human health (measuring antibodies of zoonotic pathogens) as well as for pig herd health (measuring antibodies of infectious pathogens for pigs). For the study, it was decided to start with the ELISA tests for the indirect detection of following pathogens:

a) *Salmonellla spp.*, Trichinella spiralis, Toxoplasma gondii and *Yersinia enterocolitica* as representatives of relevant food (pork) borne zoonoses, and
b) *Mycoplasma hyopneumoniae*, Influenza A subtype H1N1 and Influenza A subtype H3N2 as representatives of relevant production diseases of pig herds.

In autumn of 2009 meat juice samples from 291 slaughter pigs out of six conventional pig herds (A-F) located in the Northwest of Germany were collected and tested with the seven selected ELISA-tests. The testing procedure followed accurately the test producers' instructions, and, for those tests that are not licensed for meat juice as specimen (i.e. all ELISAs for the production diseases), a ten times lower dilution for the meat juice samples compared to the prescribed blood serum dilution was

chosen. In the pretesting study, this dilution ratio turned out to produce the most comparable results (1).
After twelve months, 160 meat juice random samples from pigs of the same six herds were tested again with the same tests under the same laboratory conditions to look into potential changes of the serological herd profiles over time.

RESULTS

Table 1 shows the positive test results of the meat juice samples per pig herd. The test results from 2009 and 2010 are coupled in the same row per herd. On the one hand this table structure is to demonstrate the "multi-serological" herd profiles (seven antibody frequencies per herd) in a certain time window for each of the two years, on the other hand the changes within the herd profiles over time, by contrasting the 2009 to the 2010 results.

In herds A and B, the serological profiles of 2009 and 2010 show quite little differences, whereas the herds C – F underwent more pronounced changes in their serological profiles. In contrast to the cumulative comparison of the serological results of all 291 pigs/carcasses (Salmonella: 12% - 18%; Yersinia: 52% - 72%; Toxoplasma: 2% - 6%; Mycoplasma: 36% - 48%; H1N1: 20% - 21%; H3N2: 6% - 7%), the serological results of the individual serological parameter show, except for Trichinella, a remarkable inter-herd variation (e.g. Salmonella: 0% - 80%; Yersinia: 0% - 100%; Toxoplasma 0% - 20%; Mycoplasma: 0% - 90%).

The repeated testing showed that several serological herd profiles change over time, which proves that the monitoring needs to be permanent, and that such a serological monitoring allows for an early detection of changes in the bacterial and viral burden of pig herds.

Tab. 1: Comparison of the proportion of positive meat juices per herd in 2009 and 2010 (changes in the herd profiles over time)

Herd	Year	Salmonella (n/N) [0-80%]	Yersinia (n/N) [0-100%]	Toxopl. (n/N) [0-20%]	Trichinella (n/N) [0%]	M. hyo (n/N) [0-90%]	SIV H1N1 (n/N) [0-37%]	SIV H3N2 (n/N) [0-90%]
A	2009	11 % (12/108)	69% (75/108)	3% (3/108)	0% (0/108)	45% (49/108)	26% (29/108)	10% (11/108)
	2010	10% (8/80)	61% (49/80)	9% (7/80)	0% (0/80)	45% (36/80)	24% (19/80)	0% (0/80)
B	2009	6% (2/31)	58% (18/31)	3% (1/31)	0% (0/31)	39% (12/31)	26% (8/31)	3% (1/31)
	2010	0% (0/10)	100% (10/10)	0% (0/10)	0% (0/10)	30% (3/10)	30% (3/10)	0% (0/10)
C	2009	10% (2/20)	20% (4/20)	10% (2/20)	0% (0/20)	90% (18/20)	0% (0/20)	0% (0/20)
	2010	80% (8/10)	0% (0/10)	20% (2/10)	0% (0/10)	60% (6/10)	10% (1/10)	90% (9/10)
D	2009	0% (0/28)	86% (24/28)	0% (0/28)	0% (0/28)	32% (9/28)	25% (7/28)	25% (7/28)
	2010	5% (1/20)	35% (7/20)	0% (0/20)	0% (0/20)	0% (0/20)	25% (5/20)	5% (1/20)
E	2009	14% (9/63)	82% (52/63)	0% (0/63)	0% (0/63)	51% (32/63)	0% (0/63)	0% (0/63)
	2010	5% (1/20)	25% (5/20)	0% (0/20)	0% (0/20)	0% (0/20)	10% (2/20)	0% (0/20)
F	2009	27% (11/41)	90% (37/41)	0% (0/41)	0% (0/41)	51% (21/41)	37% (15/41)	0% (0/41)
	2010	50% (10/20)	60% (12/20)	5% (1/20)	0% (0/20)	60% (12/20)	20% (4/20)	0% (0/20)

Total	2009	12% (36/291)	72% (210/291)	2% (6/291)	0% (0/291)	48% (141/291)	20% (59/291)	7% (19/291)
	2010	18% (28/160)	52% (83/160)	6% (10/160)	0% (0/160)	36% (57/160)	21% (34/160)	6% (10/160)

DISCUSSION

Creating a flexible system of serological herd profiles for important zoonotic and porcine infections in pigs provides the opportunity for introducing benchmarking systems. Such systems are the basis for targeted decisions on
a) risk-based meat inspection procedures,
b) improvement measures for herd health and animal welfare in the pre-harvest phase, and
c) the food safety oriented logistics of the food production chain.

Apart from these opportunities, a major advantage of the suggested approach is that this kind of multi-diagnostic monitoring addresses three groups of stakeholders: the veterinary authorities, the pig producers, and the food business operators.

Offering all three groups continuous information that serves their specific interests will provide the benefit to share the costs of such monitoring systems.

CONCLUSIONS

The tested serological meat juice monitoring of multiple pig health and zoonotic pathogens has proven to be feasible without any disturbance of the regular slaughter process, with high degree of accuracy in terms of assigning results to pig supplying herds, and very cost effective, if added to an existing salmonella monitoring programme by the multiple use of the same specimen. As for the test results, the tested herds had highly heterogeneous serological profiles. The proposed meat juice multi-serology provides a useful tool for improving the meaningfulness of the food chain information, especially for the risk-based meat inspection.

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SPECIFIC HUMAN PATHOGEN FREE (SHPF) PIG HERDS – DREAM OR REALITY?

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SUMMARY

This paper describes that Norwegian Specific Pathogen Free (SPF) herds defined and kept free from important animal diseases such as sarcoptic mange, swine dysentery and enzootic pneumonia, are also mostly free from *Salmonella*, *Trichinella*, *Toxoplasma gondii*, and *Yersinia enterocolitica.* In this respect the SPF herds could also be defined as Specific Human Pathogen Free (SHPF) herds since *Campylobacter* is decimated during blast chilling post-harvest and does not need to be a part of a SHPF concept. Since a successful elimination of an important human pathogen such as *Y. enterocolitica* is achieved in the SPF herds, a reduction of the prevalence of the zoonotic human pathogens in the general pig health and breeding pyramids is possible if some of the management practises and preventive measures in the SPF herds are exploited in these pyramids.

INTRODUCTION

In 1996 the first Specific Pathogen Free (SPF) nucleus herd was established by hysterectomy in Norway. Since 1997 more than thirty new SPF herds have been established with gilts recruited from the first SPF nucleus herd or from the two SPF nucleus herds established from the first one and maintained as closed herds. This closed SPF breeding pyramid is defined and kept free from important animal diseases such as sarcoptic mange, swine dysentery and enzootic pneumonia [1]. Since Norwegian SPF herds are managed to prevent specific pig diseases, it might be feasible to expand the list of microorganisms to include human pathogens and thus create Norwegian Specific Human Pathogen Free (SHPF) production pig herds.

MATERIAL AND METHODS

Materials and methods are described in the papers: 2, 5, 6, 7, 8, 9 and 10. In addition the "Annual Report from the Surveillance and Control Programmes for Terrestrial and Aquatic Animals in Norway" [3] and the national "Zoonosis Report 2008" [4] constitute a basis for the results and the discussion.

RESULTS AND DISCUSSION

The results and the discussion are based mainly on own epidemiological studies in the SPF herds [2, 5, 8] and in the general health and breeding pyramid in Norway [5, 6, 9, 10] also including studies of blast chilling of carcasses in the abattoir [7] and surveillance programmes regarding *Salmonella* [3] and reported data from the compulsory control of *Trichinella* [4].

SPF herds have many advantages for continued and further developments. These herds have been shown to have cost-benefits for farmers by lowering the costs of veterinary services and medicines as well as supplying animals which have a greater feed to weight ratio [1]. In the future, SPF herds might benefit food safety by becoming SHPF herds.

Some comments for the specific zoonotic agents in the SPF herds and in the general health and breeding pyramid in Norway:

Y. enterocolitica

Fifteen of the 16 SPF herds studied were free from *Y. enterocolitica* in the Norwegian SPF breeding pyramid, and the nucleus herd at the top of this SPF pyramid has been free from this agent since the establishment in 1996 [8].

Campylobacter

Five of the ten SPF herds tested were free from this agent [2]. However, the results from a study by Weijtens et al. [11] in The Netherlands suggested that a *Campylobacter*-free pig population might be established in breeding farms by combining a top-down approach with *Campylobacter*-free top-breeding farms with a strict regime of hygiene management. Anyway, *Campylobacter* is decimated during blast chilling and does not need to be a part of a SHPF concept [7].

For the other zoonotic agents we do not have specific data from the SPF herds, but based on surveillance programmes [3, 4] and research studies in the general

health and breeding pyramids in Norway [6, 10] the following conclusions might be presented:

Salmonella

A prevalence of less than 0.1 % at herd and animal level based on the level in the general health and breeding pyramids in Norway [3].

Trichinella spiralis

All pigs are investigated by compulsory analyses connected to the post-mortem inspection in Norway. *Trichinella spiralis* has not been detected since 1994 [4].

Toxoplasma gondii

Probably a prevalence of less than 1% in the SPF herds since the general protection level (management and pest control etc.) in SPF herds is higher compared to the general health and breeding pyramids [10]:

- 5.2% of the specialized slaughter pig herds,
- 2.0% of the combined herds,
- 1.2% of the multiplying herds.

CONCLUSIONS

Since *Campylobacter* is already controlled under current processing techniques such as blast chilling, attention should be focused on establishing and maintaining SHPF herds free from *Salmonella*, *Trichinella*, *T. gondii*, and *Y. enterocolitica*. Since Norwegian SPF herds are already mostly free from these agents, they might even be defined as SHPF herds in Norway today.

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RECENT TRIALS FOR DIAGNOSIS OF BOVINE EPHEMERAL FEVER IN EGYPT

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SUMMARY

Bovine ephemeral fever is a non contiguous epizootic arthropod viral disease infecting cattle and water buffaloes. The present work aimed to isolate virus particle and identify the causative agent by molecular techniques then serological characterize the virus antigens.

Infections with bovine ephemeral fever virus were noticed in 7 governorates; during summer seasons in 2006 and 2009. Collected samples were 115 serum, 95 blood with anticoagulant and 206 nasal swabs. All samples were collected from small stalk holders having small number of animals and from dairy farm in Damietta. The virus was isolated in Vero cell line reported 17.8 % from Buffy coat. While isolation in 2 days old baby mice intracerebrally inoculated were 23.1% from Buffy coat not nasal swabs. Viral identification by indirect immunoflourcent technique showed positive records of 36.6% from buffy coat. Reverse transcriptase polymerases chain reaction done on positives from buffy coat samples for molecular identification of the virus showed 500 bp clear single band in agarose gel stained with ethidium bromide revealing 45.4% positives. Serum neutralization test was used for antibodies screening developed against the virus recording 36.5 % positive result, and the titers ranged 4 to 64. Comparison between the sensitivity of the utilized techniques in identification and diagnosis of infections revealed 23.1%, 36.3%, and 45.4% for virus isolation, IFAT, and RT-PCR, respectively. Despite that virus isolation is the gold standard technique but IFAT and RT-PCR proved to be rapid, sensitive and specific for BEFV identification. In addition, SNT is valuable for mass screening.

Key words: Bovine, Ephemeral fever, Epizooti, Arthropod, Viral disease.

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INTRODUCTION

The BEFV classified as the type species of the genus Ephemerovirus in the family Rhabdoviridae and is known to cause an acute febrile disease in cattle and water buffalo manifesting in anorexia, depression, ocular and nasal discharge, salivation, muscle stiffness, lameness, rumenal stasis, sternal recumbency and other inflammatory responses [23]. The disease has major economic significance, as there are major economic losses due to drop in milk production in diary herds and reduction in condition of prime animals or disruption of stock movements and disruption of markets [27]. The BEF virus life-cycle is maintained through a vector-host system [15]. BEF is not transmitted by close contact, bodily secretions, or aerosol droplets, and carriers are not known to occur [16, 23]. The virus agent has been isolated from various species of midges and mosquitoes, which are probably the main vectors [14]. BEF was first described in 1924 in Egypt (Rabagliati 1924, cited by Sen [22]) and in the Jordan Valley in Palestine in 1931 [20] The BEF virus was probably carried in vectors transported by air streams across the Rift Valley and the Red Sea. In the 2004 outbreak, the primary focus of the disease was the southern Mediterranean coastal plain and the disease agent was apparently brought by infected mosquitoes carried from their breeding site in the Nile Delta by the south-western winds. The disease broke out under optimal ecological conditions, among a vulnerable cattle population and spread rapidly; it showed essentially a spring-summer herd incidence and terminated soon after the night average ambient temperature fell below 16◦C in late autumn [30]. Recent outbreaks of the disease were characterized by increase in morbidity and morality rates as in Saudi Arabia during 1990 and 1996 as well as in Egypt 2000 and 2001. The sever clinical manifestations and economic losses during the last time created substantial awareness both in individual and industry owners about the epidemiology, transmission, prevention and control of the disease to avoid the enormous economic losses [32]. There for the present work aims to isolate and identify the causative agent of suspected cases of BEFV appeared in Egypt in 2006 and 2009.

MATERIAL AND METHODS

All samples were collected during feverish time of the infected animals. suspected samples were collected during summer season in 2006 and 2009 from 7 Egyptian governorates. 95 blood samples on EDTA were collected from cattle for separation of buffy coat for virus isolation according to Van Der Westhuzen [26]. 206 nasal swabs were collected as a trial for virus isolation according to Payment & Trudle [18]. 115 Coagulated blood samples were collected for separation Serum samples for serological diagnosis according to Lannette, [13].

Isolation of BEF virus by intracerebral inoculation of baby mice

The intracerebral inoculation of leucocytic fraction of feverish cattle in baby mice (48 hours age) by injection of 0.01ml of 10% leucocytic fraction or nasal swab extraction of feverish cattle in normal salin. Baby mice were kept under observation until nervous symptoms appeared or slaughtered at 21 days old.

Isolation of BEF virus in cell culture

African green monkey kidney cell line (VERO) established by Yasummara and Kawatika [29]. They were kindly supplied by Animal Health Research Institute (AHRI) Egypt. A confluent monolayer of VERO cell cultures 50 ml were inoculated with o.2ml/ of 10% leucocytic fraction or nasal swab extraction and left for one hour to allow virus adsorption. Subsequently infected cells were washed with HBSS then supplemented with maintenance media and incubated at 37°c and subjected to daily microscopical examination to detect the induced cytopathic effects.

Identification of the viral isolates

To identify BEF virus we used two methods. 1) IFAT according to Payment and Trudle [18]. 2) RT-PCR by using QIAamp Viral RNA Mini Kit (QIAGEN, Germany). Cat. no. 52904. The using primer was bef19T7-TTAATACGACTCACTATAGGGAGATTTACAATGTTCCGGTGA A at position 19 of the G gene, the reverse primer GGTATCCATGTTCCGGTTAT bef523R at position 523 according to Stram et al. [24]. All steps of the techniques had been applied in (AHRI). Primers were manufactured by (METABION) and delivered in a lyophilized form. Reconstitution of the primers was attempted in nuclease free water buffer to prepare concentrated stocks. Working solutions of 20 pm were prepared by individual dilution of the primer stocks in nuclease free water (according to their concentrations). The reaction conditions were: 95 ◦C for 1 min; 35 cycles of 94 ◦C for 45 s; 56 ◦C for 45 s; and 72 ◦C for 50 s.

Serological survey

42 naturally infected cattle out of 115 samples in the main survey served as an indicator of the prevalence of BEF virus activity. BEF infection elicits neutralizing antibodies in sera of infected cattle. The antibody titers were assessed by a serum neutralization test (SNT) in a microassay system of Vero cells cultured against 100 TCID50 of the BEF virus. All sera were titrated in duplicates in a 2-fold dilution series, with an initial dilution of 1/4 – 1/64. The plates were read for CPE under the microscope after 96 h or stained with 0.2% Amidoblack in methanol. The SN titer was expressed as the negative log of the highest serum dilution.

Biological materials

Anti-bovine immunoglobulins conjugated with FITC are commercially available (ICN, U.S.A.). The conjugate was used for IFAT. Anti sera of BEFV supplied by AHRI. Positive controlled virus strain is kindly supplied from (AHRI) locally identified isolates with titre 100 $TCID_{50}$(is field isolate was previously isolated and identified by electron microscope, indirect IFAT and RT_PCR). New born calf serum was purchased from Sigma USA. It was used to supplement cell culture media and tested to be free from viruses and mycoplasma.

RESULTS

Virus isolation: The isolation of the virus on vero cell line gave CPE in the form of cell vacculation progressed gradually into appearance of granulation of cytoplasm, rounded cell , elongation and tapering of infected cell ended with cell free area floating in the medium 2 &3 days post inoculation if compared of normal cell. Nervous manifestations like convulsions, paralysis of hind limb, abnormal gait, twesting and turning around axis were observed on baby mice infected of BEF virus intracerebrally. BEF isolation from tissue culture & baby mice resulted 17 positive samples out of 95 samples (17.8 %) and 22 positive samples out of 95 samples (23.1 %) respectively.

virus identification :The application of indirect Fluorescent antibody technique (IFAT) on smears from brain of positive mice appeared the form of greenish yellow flourcent intracytoplasmic granules in the cytoplasm of the infected cell (Fig 1-A). Moreover, in the comparison study between IFAT and RT-PCR techniques for BEFV identification, it was obviously that PCR technique is more accurate than IFAT technique. It produced a clear single band on agarose gel stained with ethidium bromide. corresponded to 500 base pairs (bp) in length (Fig 1-B). It gave 10 positive samples out of 22 Buffy coat samples with percentage 45.4%, where IFAT gave 8 of positive samples out of 22 samples with percentage 36.3% .

serological diagnosis : Screening of antibodies against BEFV in sera of infected animals by SNT test in the 7 governorates revealed that, the highest positive samples percentage was 45%, 40,6%, 40% and 33.3% in Damietta, Al Fayoum, Suez and Alexandria respectively (Table 1). Wile, the lowest percentages were in behira (20%), sharqiua (28%) and menoufia (28.5%). The titer of infected cattle serum ranged between 4- 64 by using SNT test (Table 1).

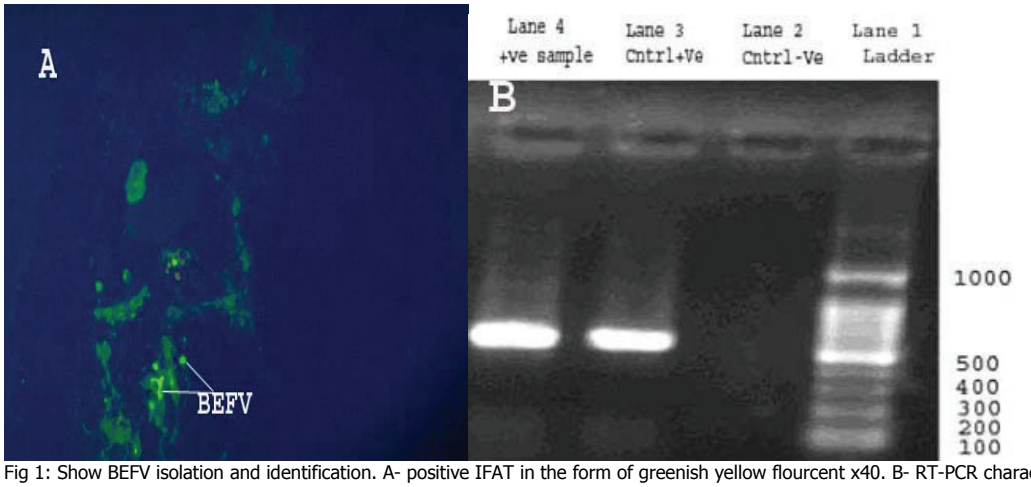

Fig 1: Show BEFV isolation and identification. A- positive IFAT in the form of greenish yellow flourcent x40. B- RT-PCR characteristic band at 500 bp.

Table 1: SNT test for serological diagnosis of BEFV with its titration of infected cattle sera in the 7 governorates.

Governorates	No. of serum samples	% of +v samples	Titration by SNT 1/4	1/8	1/16	1/32	1/64
Damietta	20	45.0	1	3	2	2	1
Al-Fayoum	32	40.6			2	3	2
Suez	20	40.0			2	2	1
Alexandria	6	33.3			1	1	
El-Menoufia	7	28.5			0	1	1
Al- Sharqiua	25	28.0			1	2	1
Al-Behira	5	20.0			0	1	
Total	115	36.5					

DISCUSSION

The two outbreaks of BEF virus occurred in summer months in 2006 and 2009 in Egypt had been studied in 7 governorates. The highest positive serum samples was (45%). It recorded from dairy herd in Damietta governorate north of the Nile Delta. Similar results were reported by Hamoda et al. [10] and Hsieh et al. 2005 [11]. It may be related to insect activity during these months. The primary source of BEF infection in the 2004 outbreak in Israel was the southern coastal plain, which is located 180–200 km from the Nile Delta. The Nile Delta offers favorable conditions for breeding large population of biting flies, mainly mosquitoes, and it is known to be a permanent focus of BEF infection. Such a distance could easily be crossed by infected mosquitoes carried with the wind [4]. The carriage of flies over long distance by air currents has also been reported by Farag et al. [8], Braverman and Chechik [6], Polydorou [19], Ward [28], and A1ba et al. [3]. It was reported that *Anopheles pharoensis*, a known malaria vector, was blown 180 km from the Nile delta in Egypt [9] to Tantura at the northern coastal plain of Israel [21]. BEF virus isolation on tissue culture from nasal swabs gave negative results where the buffy coats isolation gave positive results17.8% out of 95 samples. In the serological diagnosis, our results recorded the highest positive samples percentage was 45%, in Damietta wile, the lowest percentage was in behira (20%). Our results found to be in agreements with the findings recorded by Nawal et al. [17] during isolation of the BEFV in Egypt in outbreak 2000 as 9%-16% with average13.4% of the collected samples.

In this investigation, it was very clearly that isolation of BEFV on baby mice (23.1 %) was higher than isolation on tissue culture (17.8 %). The same results were recorded previously by Bastaweesy et al. [5], this may be attributed to the inability of all strains of BEFV to be adapted to vero cell line or due to presence of interfering viral particle [25]. It might be worth to note that BEFV does not replicate in tissue culture of bovine origin or does so only poorly and not all BEFv strains produce cytopathogenic effect and the presence of virus is generally demonstrated by immunofluorescence [23]. The application of IFAT on the positive isolates from the baby mice infected with the suspected material of BEFV revealed that 8 out of 22 buffy coat samples giving positive result (36.3%). In contrast to

the major outbreaks of the disease. The cases suspected of BEFV in the present study were sporadic in occurrence in the difference governerorates. RT-PCR application technique introduces a fast and specific method for BEFV diagnosis. Our experiment revealed that 10 out of 22 Buffy coat samples gave positive result (45.4 %). This results agree with Khalil et al. [12] and Zaghawa et al. [32]. Serological investigation of serum samples is one of a good diagnostic method for antibody detection of infected cattle. Our result showed that positive percentage ranged between 20 % & 45 % with average 36.5 %. These result agreed with that recorded in Israel in summer 2000 by Yeruham et al. [31] who recorded that the positive serological reaction rate was 20%. While differ completely than the result recorded in Saudi Arabia in 1997 as no titer (0%) of antibodies against BEFV was detected in 910 surveyed animals by Abu-Elzein et al. [2]. On the other hand in Egypt during outbreak of summer 2000 the antibody against BEFV were detected in 75% of the collected samples by The highest percentage of animals having neutralizing antibodies were recorded in Damietta and Al-Fayoum 45% and 40.6% respectively. Although many outbreaks recorded in El-menoufia 28.5% of the collected samples have neutralizing antibodies, But it may be attributed to the low number of the collected samples. The titer of sera were ranged between 4 to 64 by SNT. These results were previously recorded by Abd El Rahman et al. [1] and Nawal et al. [17]. The highest titers were recorded in Damietta during 2006 , while in 2009 these high titers were recorded in Al-Fayoum and Suez. Although the rest of governerorates have low titer of antibodies but this may be due to the time of collection of samples as the titer was low in the early stages of the disease. This result was confermed with Burgesss [7] and Abd El Rahman et al. [1].

CONCLUSION

Our assuming that the governerorates that have high titers may be due to the occurrence of unnoticeable interepizootic outbreak lead to elevation of the titer. In this situation it is worth to note that animals which had high tier of antibodies against BEFV in Damietta governerorate were vaccinated before the onset of the outbreak of 2006, a matter that lead us to suggest that the recurrence of BEFV infection among these vaccinated animals might denote to the presence of variant Egyptian strains which had emerged during that time and caused infection among vaccinated animals.

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PREVALENCE AND MOLECULAR CHARACTERIZATION OF BOVINE COENUROSIS FROM TURKEY* (Abstract)

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INTRODUCTION

Coenurosis is a central nervous system disease of ruminants, horses, pigs and human beings, caused by Coenurus cerebralis (Taenia multiceps metacestode), a bladder worm stage of Taenia multiceps (Leske, 1780), which inhabit the small intestine of dogs and wild carnivores, the definitive hosts. Following the ingestion of grass contaminated with eggs by the intermediate hosts, embryos (oncosphere) burrow their way through the intestinal wall and reach the brain via the bloodstream. Once in the central nervous system, a cyst develops taking after 3 months of age to grow to a size which will result in the onset of clinical signs. Nervous lesions, due to presence of cysts, lead to neurological symptoms which results in ataxia, hypermetria, blindness, head deviation, headache, stumbling and paralysis. However, the animals in most cases remain normal without clinical symptoms and condition was diagnosed only after the death of the animal. The specific identification of tapeworms in the Taenidae is usually based on a combination of ecological, biological and morphological criteria, including the morphology of the adult stage and the morphology and type of asexual reproduction of the larval stage, and the level of host specificity in different geographical regions. However, unequivocal identification based on these criteria is often difficult. Recently, techniques, such as partial or whole mitochondrial genome sequencing, based on the use of the PCR have been found broader applicability, mainly because their sensitivity permits the analysis of particular genes from tiny amounts of DNA from fresh, frozen or even ethanol fixed parasite material. While there is significant DNA sequence information for taeniids of socioeconomic importance in GenBank, there are limited data for the T. multiceps, particularly those from intermediate hosts and no sequence information has been reported from cattle. This study was conducted to determine the prevalence and molecular characteristics of *Coenurus cerebralis*, the metacestode of *Taenia multiceps* in cattle from Erzurum province located in eastern region of Turkey.

MATERIALS AND METHODS

The study was conducted on 1045 cattle brains from November 2009 to April 2010 in Erzurum province located in eastern part of Turkey. One visit per week was done to the abattoir during the 6-month period. Every week, 30–50 heads were examined in the abattoir. During each visit, after the slaughtering process skulls of each cattle were dissected by butchers working in the abattoir. Brain of each cattle was systematically inspected by visual inspection, palpation and incisions against *Coenurus* cysts. The gross and microscopic morphological appearance of the cysts were evaluated and identified morphologically, according to a combination of characters. Genomic DNA's were extracted from the cysts and were examined by PCR using specific primers that amplified the mitochondrial NAD1 and COX1 gene regions. PCR amplicons were sequenced; multiple sequence alignments and phylogenetic analyses were performed.

RESULTS

Five of 1045 brains of cattle (0.47%) were found to be infected with *Coenurus* cysts. The characteristics and morphology of *C. cerebralis* were seen in all the cysts. The cyts from three infected cattle were genetically analyzed and confirmed to be *T. multiceps* metacestodes by NAD1 and COX1 mitochondrial gene sequence analysis. Pairwise comparison between the NAD1 sequences of the *T. multiceps* isolates from Erzurum and other *T. multiceps* isolates available in GenBank showed differences ranging from 0.6 to 2.9%, while COX1 sequences showed differences ranging from 0.2 to 2.6%. Considering the two genes, it was seen that all of the three isolates from Erzurum province were in the same group according to phylogenetic analyses.

CONCLUSİONS

The present findings could provide a stimulus for future studies on the systematic relationships and epidemiology of lesser-known taeniid cestodes in the region, employing mitochondrial sequence data sets.

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SEROBIOCHEMICAL ALTERATIONS IN DROMEDARY CAMELS NATURALLY INFECTED WITH *THEILERIA* SPP. IN IRAN

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SUMMARY

Pathogenic protozoa belonging to the order piroplasmida, which include *Theileria* spp. are common pathogens transmitted by ticks and are of significant importance in many domestic and wild animals. The aim of this study was to evaluate the effects of natural infection with *Theileria* spp. on selected serum biochemical, enzymatic and hormonal parameters.

Blood samples were obtained from 114 apparently healthy dromedary camels aged 3 months to 18 years, held in husbandries of Yazd, Iran, during summer 2008. The 19 measured serum parameters included glucose, urea, cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatin kinase, calcium, magnesium, sodium, potassium, phosphorus, albumin, total protein, tri-iodothyronine, thyroxine, and cortisol. A Giemsa-stained peripheral blood smear from each camel was also examined.

Theileria organisms were found in blood films of 18 camels (15.79%), all of them being mature and over 5 years old. There was no significant abnormality in those parameters (p-value>0.05).

The frequency of theileriosis in camels is considerable. *Theileria* do not seem to induce significant alteration in routine serum laboratory tests in naturally-infected dromedary camels.

Keywords: *Theileria*, Camel, Iran, Laboratory test

INTRODUCTION

Theileria spp. are obligate intracellular tick-borne protozoan parasites belonging to the order piroplasmida, which cause world-wide infections of many domestic and wild animals. Their classification and nomenclature, though still controversial, are being gradually elucidated by molecular characterization. Theileriosis in ruminants is manifested by fever and lymphoproliferative disorders, associated with varying degrees of cytopenia [1]. One-humped camel (*Camelus dromedarius*) is an important multipurpose animal of arid and semi-arid parts of the world, including Iran. According to the last enumerationin 2010, there have been 154000 camels in Iran, 21830 of them counted in the Yazd province [2]. *T. camelensis*, also referred to as *T. dromedarii* has been reported from most of the regions which camels are raised in [3-8]. It is transmitted by common camel tick *Hyalomma dromedarii* [9] provided that the erythrocytic piroplasm stage of the parasites present. No microschizont stages have been yet described and thus, the taxonomic status of these parasites remains unclear. *T. camelensis* is generally thought to be non-pathogenic, and its economic impact appears to be small [6]. Although many investigators working on *Theileria* have shown alterations in blood constituents and tissue lesions in naturally and experimentally-infected domestic animals in Iran [10-12], little is known about the consequences of camel theileriosis worldwide. Nevertheless, Rao and coworkers studied five biochemical parameters on sera of naturally-infected camels [13].

The aim of this research was to investigate the effect of subclinical camel theileriosis on routine biochemical, enzymatic and hormonal parameters of dromedaries in Iran.

MATERIAL AND METHODS

The study was carried out during summer 2008 in the Yazd province, a semi-arid region in center of Iran having minimum and maximum summer temperature 14.50^{oc} and 45.50^{oc}, respectively. Camels were kept by local farmers and were fed low quality. Blood samples were obtained from jugular vein of 114 apparently healthy dromedary camels aged 3 months to 18 years. Serum was separated and stored at –20^{oc} till analysis. Since all of the positive cases were above 5 years old, we omitted 4 camels under the age of 5. The 19 measured serum parameters are mentioned in table 1. A Giemsa-stained peripheral blood smear from each camel was also examined by 2 parasitologists and a pathologist.

A spectrophotometer (Shimadzu, model AA200, Japan) was used for biochemical colorimetric assays, flame photometry method (digital flame analyzer, model 2655–00, Cole–Pulmer Instrument, USA) for determination of sodium and potassium concentrations, and ELISA plate reader (Awareness, USA) for hormone assays. Data were analyzed by SPSS 16 software, using independent

Student's t–test. Non infected animals were considered as control for comparison of results.

RESULTS

Theileria were found in blood films of 18 camels (15.79%), all of them being mature and over 5 years old. There was no significant abnormality in parasitized camels (as compared with non-parasitized camels) in any of measured parameters (p-value >0.05). The main data are summarized in table 1.

Table 1: The mean (±SE) concentration of serum constituents of *Theileria* spp. infected and non–infected dromedary camels

Parameter	Method	Units	Infected camels (n=18)	Non-infected camels (n=92)
Glucose	enzymatic (GOD-PAP)	mmol/L	3.31±0.38	3.40±0.14
Urea	enzymatic (urease)	mmol/L	10.25±0.96	9.56±0.34
Cholesterol	enzymatic (CHOD-PAP)	mmol/L	0.86±0.97	0.87±0.04
Triglyceride	enzymatic (GPO-PAP)	mmol/L	0.75±0.24	0.49±0.05
ALT	enzymatic (IFCC)	IU/L	20.72±3.34	16.41±1.01
AST	enzymatic (IFCC)	IU/L	93.55±4.87	114.55±8.62
ALP	enzymatic (DGKC)	IU/L	104.22±8.67	116.48±8.96
LDH	enzymatic (DGKC)	IU/L	438.78±40.72	513.79±40.61
CK	enzymatic (IFCC/DGKC)	IU/L	102.72±21.15	100.99±17.99
Calcium	cresol phethalein complex	mmol/L	2.55±0.06	2.52±0.03
Magnesium	xylidyl blue	mmol/L	1.13±0.03	1.07±0.02
Sodium	flame photometry	mEq/L	167.11±3.09	167.48±1.31
Potassium	flame photometry	mEq/L	6.69±0.26	6.25±0.11
Phosphorus	ammonium molybdate	mmol/L	2.11±0.12	1.97±0.05
Albumin	bromcresol green	g/dL	3.71±0.13	3.73±0.05
Total	biuret	g/dL	7.59±0.16	7.40±0.08
protein	ELISA	nmol/L	4.90±0.81	4.87±0.37
T3	ELISA	nmol/L	172.30±23.68	152.57±8.25
T4	ELISA	nmol/L	9.13±1.95	10.57±0.98
Cortisol				

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase; CK: creatin kinase; T3: tri-iodothyronine; T4: thyroxine.

DISCUSSION

According to this study, the frequency of theileriosis in camels is considerable (15.79%). Scientists from all around the world have reported different infection rates of camels to *Theileria* spp., from those of Nassar who reported *Theileria* organisms in blood films of 60 (30%) of the 200 examined camels in Egypt [8], to Borji et al. who did not find *Theileria* spp. organisms in their epidemiologic study on 262 camels in eastern Iran [14], and also Sloboda et al. who found no positive animal in their survey on 70 Mongolian Bactrian camels [15]. Salimabadi et al. noticed that *Hyalomma dromedarii* (vector of camel theileriosis) was the predominant tick species and accounted for 55.92% of the 583 collected hard ticks in their study on domestic animals in the same area as ours [16].

In our study there was no significant abnormality in the measured parameters in the infected subjects except a mild reduction in AST levels (p-value>0.05). The only well-known study on serum parameters in camel theileriosis is that of Rao et al. who measured serum total protein, glucose, cholesterol, acid phosphatase and ALP of 14 asymptomatic camels infected with *T. dromedarii*. The serum ALP activity was lower and the serum glucose higher than in uninfected controls. The values for total proteins, cholesterol and acid phosphatase were not significantly different in infected animals vs. uninfected individuals [13].

The rest of our knowledge about metabolic changes induced by theileriosis comes from studies on other animals (mostly cattle), which is accessible in the published literature.

CONCLUSION

Regarding the considerable frequency of camel theileriosis in Yazd province, control programs seem necessary, although *Theileria* do not appear to induce significant change in routine serum laboratory tests in naturally-infected dromedary camels.

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HEALTH PERFORMANCE AND SOME BLOOD SERUM BIOCHEMICAL STUDIES OF THYROID DISORDERS IN SHEEP AT ASSIUT GOVERNORATE, EGYPT (Abstract)

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INTRODUCTION

Sheep are very important for animal production activity in tropical countries (Baker and Gray, 2003 and Kosgey et al, 2006). For productivity study special attention must be conducted to thyroid gland function as one of the main regulator of metabolic activity. It is already known that thyroid hormones affect health performance, which is reflected on some biochemical blood serum constituents. The aim of the present investigation is to evaluate the thyroid activity in sheep and the occurring changes in health performance and reflection of thyroid disorders namely hypothyrodism on some blood serum biochemical constituents.

ANIMALS, MATERIALS AND METHODS

In this study a total number of 180 adult sheep (2-6 years old) selected from sheep flocks at different villages of Assiut governorate, Egypt. Blood serum samples of examined sheep were used for determination of thyroid indices (T3 and T4), total protein, total cholesterol, glucose and iodine levels were also estimated.

RESULTS

Correlations and regression analysis of the normal distribution of serum iodine, T3 and T4 levels revealed that the thyroid hormones were positively correlated with the values of iodine levels. Furthermore, levels of T3 are positively correlated with T4 values.

The metabolic profile of sheep with hypothyroidism was characterized by hypoproteinemia, hyper-cholesteraemia and hypoglycemia.

Iodine levels in blood serum of sheep were not significantly correlated with serum total protein concentration, whereas thyroid hormones (T3 and T4) were positively correlated with protein values. On the contrast, blood serum iodine thyroid hormones (T3 and T4) were negatively correlated with the concentrations of cholesterol in blood serum. On the other hand, the values of blood serum iodine and T3 were not significantly correlated with the glucose values, whereas the levels of T4 were positively correlated with serum glucose concentrations.

The incidence of hypothyroidism in Assiut governorate is quietly high, and suggests that these animals are likely at the risk of iodine deficiency and thyroid dysfunction especially in the newly reclaimed areas of Assiut governorate.

CONCLUSION

The thyroid dysfunction affects the metabolic function of the affected sheep as denoted by the disturbances in total protein, cholesterol and glucose levels in blood serum.

Block 6

PREVENTION OF NECROTIC ENTERITIS OF POULTRY WITH HERBAL PREPARATIONS

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SUMMARY

Acute outbreaks and subclinical manifestation of necrotic enteritis (NE) caused by *Clostridium perfringens* in broiler flocks result in significant production losses due to increased mortality. The strain's wide-ranged adaptive qualities makes it a significant helath hazard in the food chain also. After the AGP ban in the EU, the use of ionophore coccidiostats, targeting both *Eimeria* spp. and certain Gram-positive bacteria, including *C. perfringens*, has increased in broiler chicken production and seems at present the only reliable disease control measure. The long-term use of ionophores as feed additives is questioned within the EU, and hence there is an urgent need for identifying alternative ways for suppressing adverse effects of Clostridium infections. A small-group and a field study were conducted to test the effectiveness of a herbal preparation against *C. perfringens*. In both of our experiments the Herbanoplex CP showed a good tendency of its effectiveness, confirmed by lower number of bacteria in the intestines, lower mortality, improved weight gain and feed conversion parameters.

INTRODUCTION

Clostridium perfringens is a Gram-positive, rod-shaped, anaerobic, spore forming bacterium, the most important clostridial pathogen of poultry, causing necrotic enteritis (NE) [1].

Acute outbreaks of NE in broiler flocks result in significant production losses due to increased mortality. The sub-clinical form of the disease is characterized by reduced broiler growth, poor feed utilization [2] and wet litter causing skin burns and food pad lesions representing a serious welfare problem in broiler production [3].

Due to its ubiquity, spore-forming ability, short generation times, the wide temperature range in which the organism grows, its ability to adapt and grow in different food environments, and the diseases caused, beside the above mentioned problems *C. perfringens* makes a significant health hazard in the food chain resulting some 250,000 cases of human food poisonings annually in the US [4].

Production problems related to NE were formerly controlled by the use of antibiotic growth promoters (AGP). After the AGP ban in the EU, the use of ionophore coccidiostats, targeting both *Eimeria* spp. and certain Gram-positive bacteria, including *C. perfringens*, has increased in broiler chicken production and seems at present the only reliable disease control measure. The long-term use of ionophores as feed additives is questioned within the EU, and hence there is an urgent need for identifying alternative ways for suppressing growth and toxin production by *C. perfringens* [5]. With this object, two experiments were conducted to prove the effectiveness of a new herbal preparation developed against *C. perfringens*.

MATERIAL AND METHODS

Experiment 1. Two groups (experimental and control; 10-10 animals) were formed with laying hens. The animals were fed ad libitum with the same layer feed. The feed of the experimental group was supplemented with the herbal additive (Herbanoplex CP) at 1kg/t concentration. Both groups were artificially infected orally with *C. perfringens* ($5x10^8$ cell/dose) at day 3, 4 and 5. Cloaca tampon-samples were collected daily, and they were washed with 1.5 ml tap-water. The solution was cultured on TSC agar with Cycloserine and it was incubated at 42 °C. After 24h the colonies were counted.

Experiment 2. The second experiment was carried out in a broiler farm where clostridial diseases have been recorded for long. Two groups of 18,000 broilers were formed. All animals received the same, commercially available broiler starter and finisher feed. The diet of the experimental group was supplemented with the herbal additive as described above. The occurrence and the severity of the disease and production parameters in both groups were recorded.

Data were statistically analysed with Statistica 9.0 software by using General Linear Models.

RESULTS

In the **first Experiment** the tampon-samples in the control group contained 10^5-10^6 cfu/ml with severe symptoms of NE and 10^4-10^5 cfu/ml with mild symptoms, while in the experimental group the result was significantly lower, 10^2-10^3 cfu/ml ($p<0.05$), and there were no symptoms of NE.

In the **second Experiment** 3 weeks after the start of the trial there were clear signs of Necrotic Enteritis in both groups, however the occurrence of the symptoms was higher (15 vs 65 %) in the Control group. Lower mortality was detected in the Experimental group (3.4 vs. 4.1%), although this difference was not significant. The Experimental group had higher averaged end body weight at day 42 (2201.3 vs 2112.2 g, $p<0,01$). Despite the initial body weight was also significantly higher in the Experimetal group (33.6 vs 32.6 g, $p<0,01$), the overall weight gain was significantly higher in the Experimental group ($p=0.037$). Average feed consumption in the experimental group was lower (102.5 vs. 113.5 g/day). Additionally, litter was less wet in the experimental group (no data measured, only organoleptic examination).

DISCUSSION

Despite there are several NE inducing models, there is no generally accepted disease model for the experimental induction of necrotic enteritis in broiler chickens because consistent replication of the disease is difficult to achieve. In our first experiment the chickens were orally challenged in three consecutive days by 1 ml solution containing 10^8 cfu virulent *C. perfringens* similarly to Long an Truscott [6]. Normally the number of *C. perfringens* in the intestine is low (about 10^4 cfu/g of digesta) but disorders of normal intestinal microflora may cause rapid proliferation of *C. perfringens*, increasing bacterial numbers to 10^7-10^9 cfu/g of digesta resulting in the development of clinical NE [7]. This intestinal proliferation of *C. perfringens* was succesfully reproduced in our first experiment, with no mortality, however morbidity rate was 100% in the Control group. Similarly to others using direct-fed microbials [8, 9], yeast extract [10] or essential oils [11], our herbal preparation significantly reduced the number of *C. perfringens* in the intestine of the animals in the Experimental group in our first Experiment.

In the second Experiment lower mortality was detected in the Experimental group. However this difference was not significant, the results are similar to the findings by Hofacre et al. [12] using lactic acid producing bacterial culture alone or combined with mannan-oligosaccharides. In their experiment, neither addition of FOS or MOS alone to the diet had a significant effect on mortality. Mortality was higher in their experiment, than in ours, as the animals were artificially infected with *C. perfringens* after an oral inoculation of coccidial strains. Thanissery et al. [10] found similar mortality rate in their experiment, however the feed supplementation with NuPro had no effect on the mortality rate. McReynolds et al. [9] also showed a lower mortality rate in the group supplemented with direct-fed microbials.

In our second experiment a higher weight gain was measured contrary to others [5, 10, 12, 13]. Hofacre et al. [12], similarly to our results, measured a better feed conversion rate in the groups supplemented against C. perfringens using lactic acid producing bacterial culture alone or combined with mannan-oligosacharides, while others [5, 13] did not confirm their finding.

CONCLUSIONS

In both of our experiments the Herbanoplex CP showed a good tendency of its effectiveness, however further research and development is needed to improve the product. The lack of residues and resistance makes these substances a good option to supplement or replace the use of antibiotics.

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A MULTI-STRAIN PROBIOTIC TO REDUCE NECROTIC ENTERITIS IN CHICKEN

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SUMMARY

Intestinal diseases, which are related to the overgrowth of enteric pathogenic bacteria, are an important concern of the modern poultry industry. Certain members of the intestinal microflora are by nature capable of resisting the establishment of pathogens. In course of a multinational project (C-EX QLK-CT-2002-71662), a total of 477 bacterial isolates was obtained from various chicken intestinal compartments by using selective conditions, characterized and representatives (n = 121) screened for their antagonistic properties. By using a co-cultivation agar spot assay, a reduced number of 15 well-characterized strains, belonging to *Lactobacillus*, *Enterococcus*, *Bifidobacterium* and *Pediococcus*, showed growth suppression of a broad range of pathogenic indicator strains, including two *Escherichia coli* serotypes, *S. enterica* serovar Enteritidis and Choleraesuis, *Campylobacter jejuni* and *Clostridium perfringens*. Several strains exhibited the ability to inhibit toxin-producing *C. perfringens*, the aetiological agent of necrotic enteritis (NE), with inhibition indexes ranging from 1.50 to 2.13. A cross-section of five strains affiliated to *Bifidobacterium animalis*, *Enterococcus faecium, Pediococcus acidilactici, Lactobacillus reuteri* and *L. salivarius* were combined to a multi-strain feed additive and examined *in vivo* for their effects on the development of NE in broilers. By using a subclinical NE model with ninety commercial broilers, the disease could be reproduced with lesion scores ≥ 2, only in the infected groups. The study revealed that feed supplementation with the multi-strain probiotic (0.1 %) resulted in a significant ($P<0.05$) lower amount of birds displaying necrotic lesions compared to the positive control group.

INTRODUCTION

Enteric diseases like necrotic enteritis (NE) in chickens are notably rising, causing increased mortality, poor growth and additional medical costs. In the past, antibiotic growth promoters (AGPs) have been the gold standard by which performance and disease resistance were measured. A natural alternative to AGPs might benefit from the competitive nature of probiotic gut bacteria, with the advantage of acting naturally in their habitat without leaving any residues. In an attempt to counteract possible problems in poultry production like increased occurrence of infectious disease, performance losses during production, food borne disease in humans and the increased use of therapeutic antibiotics, a multi-national project (C-EX QLK-CT-2002-71662) funded by the European Union was initiated in order to generate a mixture of host-adapted, effective probiotic strains for protecting young chickens against enteric infections.

MATERIAL AND METHODS

In order to evaluate a combination of host-adapted, probiotic strains for its use as multi-strain feed additive, a great variety of bacteria was isolated out of different gut compartments of healthy chickens (n = 477), characterized and representatives (n = 121) screened for their antagonistic properties [2]. By using a co-cultivation agar spot assay, a first pre-screening was done in order to obtain chicken-derived strains with the ability to reduce the growth of *Salmonella enterica* serovar Enteritidis. A reduced number of well-characterized, antagonistic strains were further studied for inhibition of a broader set of chicken pathogens including *Clostridium perfringens* type A, *Campylobacter jejuni*, as well as various serovars of *Escherichia coli* (*E. coli* O157:H7, O147:H19) and *Salmonella enterica* serovar Choleraesuis. The diameter of the inhibition zone and the growth zone of the test strain was measured and from these data an inhibition index (diameter inhibition zone [cm]/diameter test strain [cm]) was calculated. The test for each isolate was carried out in triplicate. Strong antagonists were further analyzed for inhibition of two pathogenic *C. perfringens* strains (CCUG 47895, # 56) by studying their cell-free supernatants with and without pH neutralization, proteinase K and catalase treatment. Strains were examined for hydrogen peroxide production by using a semiquantitative assay [3], and H_2O_2 producers were classified as low or high producers.

A mixture of chicken strains was examined *in vivo* for its ability to inhibit a *C. perfringens* strain (code # *56*) producing the α-toxin and the recently identified *net*B-toxin [3] by using a subclinical NE broiler model. This strain was isolated from the gut of a broiler with severe necrotic gut lesions from a flock with weight-gain problems. The strain was classified as a type A strain, carrying the *net*B toxin gene (no beta-2 or enterotoxin genes) and produces moderate amounts of α-toxin *in*

vitro. In this model a high percentage of the treated animals develop grossly visible necrosis of the intestinal mucosa, but without induction of mortality [1]. Ninety commercial broilers were divided in three floor pens, and randomly assigned to an uninfected, an infected and an infected group which received additionally 0.1 % of the multispecies combination (PoultryStar®, Biomin GmbH) via the feed. Drinking water and feed were given *ad libitum*. From day 1 to 8 birds received a starter diet, from day 9 to 16 a grower diet and from day 17 onwards a grower diet with a different protein source. The starter and grower diets were wheat/rye and soybean based. The second grower diet replaced soy with fishmeal as the protein source. A Gumboro vaccine Nobilis Gumboro D78 (Intervet, Mechelen, Belgium) was given in the drinking water at day 16 in all groups. All groups, except the negative control group were challenged orally three times a day with approximately 4.10^8 cfu *C. perfringens 56* strain at days 17, 18, 19 and 20. At day 18 all birds were orally gavaged with a ten-fold dose of vaccinal *Eimeria* oocysts (Paracox-5, Schering-Plough Animal Health, Brussels, Belgium). At days 22, 23, and 24, ten birds from each group were euthanized and intestinal lesions in the small intestine (duodenum to ileum) were scored using the following criteria: 0: no gross lesions, 1: congested intestinal mucosa, 2: small focal necrosis or ulceration (1-5 foci), 3: focal necrosis or ulceration (5-16 foci), 4: focal necrosis or ulceration (16 or more foci), 5: patches of necrosis 2-3 cm long, 6: diffuse necrosis typical of field cases. Lesion scores of 2 or more were classified as necrotic enteritis positive. The statistical analysis was carried out for the 3 sampling days (total) by the means of multivariable logistic regression.

RESULTS

A first *in vitro* pre-screening revealed that a reduced number of 90 strains exhibited the ability to suppress the growth of *Salmonella enterica* serovar Enteritidis (data not shown). The 15 most effective strains belonging to *Lactobacillus*, *Enterococcus*, *Bifidobacterium* and *Pediococcus* were able to inhibit a broader range of pathogenic indicator strains, including two *E. coli* serotypes, *S. enterica* serovar Choleraesuis, *C. jejuni* and *C. perfringens*. Finally, five effective chicken strains of distinct species affiliation (*Bifidobacterium animalis*, *Enterococcus faecium*, *Pediococcus acidilactici*, *Lactobacillus salivarius, L. reuteri*) were combined to a multi-strain feed additive on basis of their functional, technological and safety features [2]. The five strains produced an antagonistic metabolite against the α-toxin producing *C. perfringens* strain (CCUG 47895), as indicated by clear inhibition indexes ranging from 1.5 to 2.13 (Figure 1). The *P. acidilactici* strain was found to produce an anti-clostridial metabolite of proteinaceous nature. Strains affiliated to *E. faecium* and *L. salivarius* turned out to be strong organic acid and hydrogen peroxide producers (H_2O_2>3 µl/ml), with the *E. faecium* strain producing the highest concentrations (H_2O_2≥10µl/ml), respectively. Sensitivity assays could not assign the antimicrobial agent of the two lactobacilli (strong organic acid and/or H_2O_2 producer), the organic acid producing *B. animalis* strain and the strong H_2O_2 producing *E. faecium* strain, since the inhibitory effect of the pH-adjusted filtrates was not eliminated neither by proteinase K, nor by catalase. For the *in vivo* examination of the multi-strain probiotic, a subclinical necrotic enteritis model was used to reproduce the disease. Table 2 shows the number of birds with necrotic lesions scores ≥ 2 in the jejunum for each group at day 22, day 23 and day 24, as well as the total number of birds with lesion scores ≥ 2 per group. The negative control group did not have animals suffering from necrotic enteritis, whereas the positive control group displayed the highest percentage of birds with necrotic lesions. Accordingly, after the damage induced by the *C. perfringens* challenge in the positive control group more than half of the birds had intestinal lesions scored ≥ 2. Birds treated with the multispecies probiotic showed the lowest percentage of birds with necrotic lesions. The statistical analysis was carried out for the 3 sampling days (total). By the means of multivariable logistic regression, the number of birds with macroscopic necrotic lesions in the multispecies probiotic group was compared to the positive control group. In the multispecies probiotic group a significant ($p<0.05$) lower amount of birds showed necrotic lesions in comparison to the positive control group.

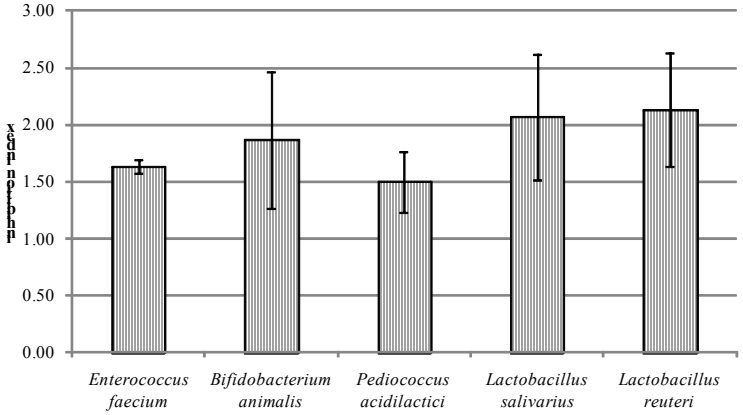

Figure 1: Ability of potential probiotic bacterial strains derived from the gastrointestinal tract of chicken to inhibit the growth of *Clostridium perfringens* based on inhibition zones obtained after co-cultivation on agar plate expressed as inhibition index (diameter inhibition zone [cm]/diameter test strain [cm])

Table 2: Number of birds with macroscopic necrotic enteritis lesions per sampling day

	negative control	positive control	multi species probiotic
day 22	0/6	3/9	5/10
day 23	0/5	9/10	2/10
day 24	0/5	5/10	1/9
Total	0/16	17/29	8/29
%	0	58.6	27.6

DISCUSSION

A large and diverse pool of bacteria was isolated out of the gastrointestinal tract of healthy chickens and subjected to microbiological studies in order to obtain optimal strains for a competitive exclusion (CE) product [2]. The *in vitro* screening demonstrated antagonistic properties of several gut-derived strains against various chicken pathogens. Mixed cultures may contain bacteria that complement each others' effect. Five probiotic strains of distinct bacterial species affiliation were carefully selected based on their broad range of competitive features, but also because of their good technological properties and their origin, deriving from different niches of the chickens gut. The *in vivo* results from the *C. perfringens* challenge study supported the significance of bacterial antagonism by the ability of the gut-derived bacteria to compete with the pathogen. However, further investigations are needed to explain the nature of pathogen inhibitory properties of the antagonistic strains of this study. Current knowledge of synergism of strains within CE cultures as well as the effective species of the various bacterial genera is still inadequate. Future work will first involve monitoring of survival of the introduced bacteria along their journey through the chickens GI tract and examination of changes in the gut microbiota and immune response of the host after feeding the multi-strain probiotic by using molecular methods, with later work focussing on the ability of the probiotic feed additive to inhibit other important pathogenic organisms (e.g. *Salmonella*) under these circumstances.

CONCLUSIONS

Data of this study revealed that the multi-strain probiotic may be capable to protect chickens against NE. These results encourage us to proceed with a more detailed NE study to get insights into the pathogen-exclusion mechanisms of these probiotic strains.

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EFFECT OF DIETARY GARLIC SUPPLEMENTATION ON PERFORMANCE, CARCASS TRAITS, AND MEAT QUALITY IN BROILER CHICKENS

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SUMMARY

A study was conducted to assess the effect of garlic (G-NUTRA®) supplementation in diets of broiler as a replacement antibiotic on productive performance, dressing percentage, weight of heart, gizzard and liver, and meat quality of the broilers. Three hundred and sixty, one-day-old Cobb chicks were randomly allocated to 3 groups and each group consists of 4 replicates, with 30 chicks in each over a period of 6 weeks. The groups were assigned to receive the treatment diet as follows: Group A served as control and was fed ration without any supplementation (basal diet); whereas group B and C were fed diet 2 and diet 3, respectively. Diets 2 and 3 contained supplementary raw garlic powder at 0.5 and 1.0 Kg/ton diet respectively. The birds (in group B) using ration supplemented with 0.5kg/ton garlic gained the highest live weight (g) among the treated groups and the best-feed conversion ratio although they consumed the same food ($p < 0.05$). There was no significant difference in mortality rate of the broilers due to treatment. There was a significant difference between the average dressing percentages, while this difference was not significant for giblet weight (heart, gizzard, and liver) of the broilers fed rations with or without supplementation of garlic. Meat cholesterol concentration in thigh and breast muscles decreased significantly ($p < 0.05$) with garlic powder supplementation. There was a slight decrease in APC and coliforms for garlic supplemented groups. It is therefore concluded that dietary inclusion of garlic in the rations may be used for economical and efficient production of broilers.

Keywords: garlic, broiler, performance, meat quality.

INTRODUCTION

The fast growing nature of broilers and their short generation interval has been associated over the years with the use of antibiotic growth promoters in animal feeds in order to improve the quality of the product. Although birds raised with these feed additives achieved good performance, their potential side effects became a real public health problem worldwide [8]. In pursuit of improved chicken healthiness and in order to fulfil consumer expectations in relation to food quality, poultry producers more and more commonly apply natural feeding supplements, mainly herbs. The positive effects of herbal supplements on broiler performance [14], carcass quality and quality traits of meat [3 and 14] have been demonstrated.

Garlic (Allium sativum)) is well known as a spice and herbal medicine for the prevention and treatment of a variety of diseases . The major active ingredients of garlic are allicin, ajoene, S-allyl cysteine. Garlic has been found to demonstrate antimicrobial activity [1], lower serum and liver cholesterol [16] and improve productive performance of broiler chicks [19]. Some studies, however, suggested that commercial garlic oil, garlic powder and commercially available garlic extract may be hypocholesterolemic [3]. In addition to its antimicrobial activities, garlic has been shown to increase feed palatability and thus feed intake [5]. The objective of this study was to investigate the effect of garlic supplementation in diets of broiler on productive performance, carcass traits; meat cholesterol and meat quality.

MATERIALS AND METHODS

Bird and Management

This study was conducted at the Faculty of Veterinary Medicine, Cairo University, Egypt. One-day old 360 broiler (Cobb) chicks were reared in a group for one week (adaptation period). A starter diet (23 % CP and 3029.84 kcal/kg ME) were supplied from 0–21 days while finisher diet (20 % CP and 2949.69 kcal/kg ME) were fed from 22–42 days. Feed and water were provided *ad-libitum.* All the birds were provided the same management conditions (floor space, temperature, relative humidity, ventilation ,light and vaccination programme). Commercial garlic-yeast extract compound (G-NUTRA 2S DAT) as feed additive produced by AMECO-Bios & Co., ARCADIA, CA 91006, USA was used. The product composed of: inactive dry yeast (Saccharomyces *cerevisiae*); silicon dioxide and garlic aroma .

Experimental design

At day eight all the birds were weighed and were randomly divided into twelve experimental units (replicates) having 30 chicks each. These experimental units were further allotted to three treatment groups, A, B, and C. The birds were fed *ad-libitum* an experimental ration with or without supplementation of garlic. Group A served as control and was fed ration without any supplementation (Diet 1). Whereas group B and C were fed ration supplemented with 0.5 kg/ ton (500 mg/kg) (Diet 2) and 1.0 kg/ton (1000 mg/kg) garlic (Diet 3), respectively.

Parameters measured

The data collected were utilized to calculate the initial body weight, final live body weight and weight gain, total feed consumption and mortality were recorded at the end of the experimental period (6 weeks). At the end of experiment, five birds from each replicate were picked up randomly and slaughtered for their dressing percentage and organ weight (heart, liver, and gizzard). At the end of the experiment (42 d) three birds per pen were randomly chosen and were killed , thigh (*Biceps femoris*), and breast (*Pectoralis major*) muscle samples were taken, to determine the total cholesterol content of theses tissues .

Microbiological examination

The thigh and breast muscle samples were prepared according to technique recommended by [10].Determination of aerobic plate count was done according to [11]**.** Total Enterobacteriaceae count was applied using Violet red bile glucose agar medium (OXOID). Count of Fecal Coliforms using EC broth at45.5°C for 48±2 hours. Estimation of total mould and yeast count was done by the technique described by [4]. Also Determination of anaerobic count by the technique described by [12] was adopted using Reinfoced Clostridia Agar medium (RCM) .

Statistical analysis

The data thus collected were subjected to the analysis of variance (ANOVA) technique in completely randomized design. The differences in the means were compared by the Duncan's Multiple Range following [18].

RESULTS

Table 1: Performance of broiler chickens fed diets containing supplementary garlic

Parameters	Treatments		
Supplementary garlic (mg/kg diet)	A (control)	B (500 mg)	C (1000 mg)
Initial live weight (g/bird)	115.92±3.17^{a}	116.13±3.77^{a}	115.12±3.28^{a}
Final live weight (g/bird)	1960.24±22.6^{a}	2093.51±16.53^{b}	1997.01±27.70^{c}
Total weight gain (g/bird)	1844.32±28.9^{a}	1977.38±22.20^{b}	1882.89±19.80^{c}
Total feed intake (g/bird)	4179.14±148.0^{a}	3912.12±220.0^{a}	4020.33±202.8^{a}
Feed conversion ratio (FCR)	2.3 ± 0.14^{a}	1.9 ± 0.15^{b}	2.1 ± 0.15^{c}
Mortality %	2.50 (3/120)^{a}	4.17 (5/120)^{a}	3.33 (4/120)^{a}

Table 2: Carcass characteristics of broiler chickens fed diets supplemented with garlic

Parameters	Treatments		
Supplementary garlic (mg/kg diet)	A (control)	B (500)	C (1000)
Final live weight (g/bird)	1960.24±22.6^{a}	2093.51±16.53^{b}	1997.01±27.70^{c}
Weight after slaughtering (g/bird)	1357.37±18.2^{a}	1588.14±16.3^{b}	1408.69±21.4^{c}
Dressing %	69.24±3.66^{a}	75.86±4.83^{b}	70.54±3.76^{a}
Heart weight	13.06 ± 0.63^{a}	14.19 ± 0.93a	12.88 ± 1.06^{a}
Liver weight	44.24± 1.93^{a}	46.35± 1.95^{a}	44.85± 2.65^{a}
Gizzard weight	32.35± 2.34^{a}	34.87± 1.72^{a}	33.67± 1.95^{a}

Abc = means within rows with different superscripts are significantly differ at $p \leq 0,05$.

Table 3: Influence of dietary garlic on thigh and breast muscle cholesterol

Variables	Treatments		
Supplementary garlic(mg/kg diet)	A (control)	B (500)	C (1000)
Thigh muscle cholesterol (mg/100 g wet tissue)	145.3±17.2^{a}	112.1±8.7^{b}	121.4±12.1^{c}
Breast muscle cholesterol (mg/100 g wet tissue)	43.24±3.4^{a}	32.15±3.7^{b}	35.11±7.3^{c}

Table 4: Effect of garlic powder on bacteriological Examination of chicken meat (Thigh and breast)

		Aerobic Plate Count			Coliforms			Fecal Coliforms			Enterobacteriaceae			Anaerobic Count		
	mg	con	500	1000	con	500	1000	con	500	1000	con	500	1000	con	500	1000
Thigh	Mean	4.2 $x10^4$	4.2 $x10^4$	6.8 $x10^4$	1.56 $x10^2$	3.1 $x10^2$	62.45	<3	<3	<3	1.1 $x10^2$	1.92 $x10^2$	4.98 $x10^2$	2.03 $x10^4$	2.6 $x10^4$	2.7 $x10^4$
	SE	7.1 $x10^3$	5.9 $x10^3$	2.0 $x10^4$	66.5	60.42	14.27	<3	<3	<3	23.4	60	1.1 $x10^2$	1.8 $x10^4$	2.4 $x10^3$	2.9 $x10^3$
Breast	Mean	3.7 $x10^4$	2.3 $x10^4$	3.8 $x10^4$	51.4	7.3 $x10^2$	9.72 $x10^2$	<3	<3	<3	5.3 $x10^2$	2.06 $x10^2$	1.4 $x10^2$	1.93 $x10^4$	2.1 $x10^4$	1.5 $x10^4$
	SE	6.4 $x10^3$	6.3 $x10^3$	5.7 $x10^4$	9.75	1.5 $x10^2$	85.33	<3	<3	<3	26.12	3.28 $x10^2$	3.05 $x10^2$	9.78 $x10^2$	1.6 $x10^3$	9.4 $x10^2$

Table 5. Effect of garlic-yeast extract compound on meat quality of broiler meat

Samples		Mould count			Yeast count		
		Cont.	0.5kg/ton	1.0 kg/ ton	Cont.	0.5kg/ton	1.0 kg/t on
Thigh	Mean	-	1.09 $x10^2$	72.73	2.5$x10^2$	2.64$x10^2$	9.2x 10^2
	±SE	-	24.94	29.05	90.73	74.24	1.72 $x10^2$
Breast	Mean	2.7$x10^2$	5.8$x10^2$	10^2	4$x10^3$	2.4$x10^3$	2.08 $x10^3$
	±SE	39.58	2.17$x10^2$	29.81	1.2$x10^3$	5.29$x10^2$	2.73 $x10^2$

DISCUSSION

Performance

The use of garlic 500mg/kg showed more increase in live weight of the birds as compared to non-supplemented and 1000 mg/kg level in this study, which is also in agreement with the findings of [14], who concluded that powdered garlic at 0.5% level may be incorporated as a growth promoter in the ration of Japanese quails. Addition of garlic improved the weight gain of the broilers in this study. These results are in line with those reported by [2] who reported higher weight gain in broilers fed rations supplemented with garlic. The improvement in weight gain of the birds using garlic in their rations may probably be due to the fact that allicin (an antibiotic substance found in garlic), inhibits growth of intestinal bacteria such as *S. aureus* and *E. coli* and inhibit aflatoxins producing fungi [13]. Resultantly, when the load of these bacteria in the intestine is low, birds may absorb more nutrients, thus leading to the improvement in weight gain of the birds using rations supplemented with *alum sativum.* This study clarified that, the birds fed rations supplemented with garlic utilized their feed more efficiently than those feed ration without addition of garlic.These findings were in agreement with [9]. Better feed conversion ratio of the broilers may be attributed to the antibacterial properties of this supplement, which resulted in better absorption of the nutrients present in the gut and finely leading to improvement in feed conversion ratio .

Carcass and organ characteristics

All organ weights and carcass characteristics were not affected by the treatments, except for a slight increase in dressing percentage of birds fed on low level of garlic (500 mg/kg diet) compared with other treated groups. These findings conciede with those of [7 and 2], who reported a non-significant effect on broiler dressing percentage values due to the inclusion of garlic in the diet of broilers.

Meat quality

Tissue cholesterol concentration

Results in table-3 showed that, garlic supplementation lowered thigh muscle cholesterol by 23 and 17% and lowered breast muscle cholesterol by 26 and 19%, in group B (500mg/kg) and C(1000 mg/kg), respectively than control non supplemented group. Reduction in cholesterol with garlic has also been reported previously [13;15 and 16]. This reduction may be attributed to that organic tellurium compounds are found in high concentration in garlic buds, which may inhibiting squalene epoxidase, the penultimate enzyme in the synthetic pathway of cholesterol [16]. Cholesterol concentrations were found to be much higher in the thigh than in breast muscle (Tables 3). A possible explanation is that cholesterol is usually associated with adipose tissue, which is more abundant in thigh than in breast muscle.

Bacteriological examination

From the results in tables 4 and 5, it can be concluded that, there are only observable decreases for APC and coliforms counts in breast muscles and also for yeast in both groups B and C (fed 500mg and 1000mg/kg diet, respectively), in breast and thigh muscles. While no valuable changes were noticed for other bacteriological examination (Enterobacteriaceae, and anaerobic counts); so addition of garlic powder in meat products is more useful than its dietary supplementation and this agree with [20] who stated that, exogenous addition of garlic-derived organosulfur compounds significantly delayed both oxymyoglobin and lipid oxidations ($P<0.05$). and presence of diallyl sulfide (DAS), diallyl disulfide (DAD) in ground beef significantly reduced total aerobes and inhibited the growth of five inoculated pathogenic bacteria, these results suggested the application of these organosulfur compounds in meat or other food systems could enhance color, lipid and microbial safety.

CONCLUSION

Garlic supplementation of broiler chicken diets improved weight gain and it was better at low level of supplementation (500 mg/kg diet) which may be useful for economical and efficient production of broilers

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INFLUENCE OF BAMBOO VINEGAR SUPPLEMENTATION ON GROWTH PERFORMANCE, APPARENT TOTAL TRACT DIGESTIBILITY, BLOOD CHARACTERISTICS, MEAT QUALITY, FECAL NOXIOUS GAS CONTENT AND MICROBIAL CONCENTRATION IN FINISHING PIGS

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SUMMARY

A 42-day trial with 60 pigs (79.7 ± 1.42 kg) was conducted to evaluate the effects of bamboo vinegar (BV) supplementation in finishing pigs. Dietary BV increased ADG ($p < 0.05$) than CON group during 0 to 3 weeks and 0 to 6 weeks. Pigs fed BV2 treatment had a greater G:F ratio than other treatments. The inclusion of BV led increased ATTD of dry matter and nitrogen ($p < 0.05$) than the pigs fed CON diet at 6 week. Pig fed BV supplemental diet had a greater lymphocyte count than those fed CON diet ($p < 0.05$). The fecal E. coli numbers were reduced with increasing BV supplementation ($p < 0.05$). Pigs fed BV2 diet evidenced better meat color and firmness ($p < 0.05$) than other groups. Pig fed BV2 increased redness values ($p < 0.05$) compared with CON group, whereas the lightness value was higher ($p < 0.05$) in pigs fed CON diet than BV2 treatment. Dietary BV2 group reduced NH3 emissions ($p < 0.05$) compared with CON and BV1 groups on day 0 and 5. At day 10, pigs fed CON treatment evidenced greater NH3 emission ($p < 0.05$) than that in BV1 treatment. Pigs fed CON diet had greater ($p < 0.05$) H2S concentrations than those fed BV2 diet. In conclusion, dietary BV supplementation increased growth performance and apparent total tract digestibility, along with its beneficial effect on the intestinal microbial population, meat quality and fecal noxious gas concentrations.

INTRODUCTION

Bamboo vinegar (BV) is a naturally derived liquid obtained from the condensation occurring during the production of bamboo charcoal. It is composed principally of more than 200 chemical components, with acetic acid (about 60%) being the main one [11]. Akakabe et al. [1] have confirmed that BV, with a pH of 2.5-2.8, can function as insecticide, bactericide and deodorant for treating malodor from pets. Baimark and Niamsa [2] also proposed that BV has a higher antifungal efficiency than both acetic acid and formic acid in vitro, and suggested that the phenolic compound found in BV may evidence some antifungal effects. However, the applications of BV in animals have not been vigorously investigated, and only Kook et al [10,11] suggested that supplementation with BV and BV liquids can improve the growth performance of ducks and pigs, respectively. Our study was conducted to investigate a bamboo vinegar powder product, which is supposed to contain 27.745 g/kg acetic acid and 14.63 g/kg total phenolic compound. We hypothesized that BV could be used in finishing pig, exerting beneficial effect of both organic acid and phenolic compound to the pigs.

MATERIAL AND METHODS

Animals and Facilities

60 pigs were allotted to 1 of 3 dietary treatments in a randomized complete block design according to their sex and BW. Each treatment was consisted of 5 replications, with 4 pigs (2 barrows and 2 gilts) per pen. The experimental treatments were: 1) CON, basal diet; 2) BV1, (CON + 0.1% BV supplementation); 3) BV2, (CON + 0.2% BV supplementation). The pigs were housed in an environmentally controlled, slatted-floor facility in 12 adjacent pens (1.80 × 1.80 m) and were permitted ad libitum access to feed and water throughout the experimental period.

Sampling and Measurements

Individual pig BW and feed consumption were monitored daily to determine the ADG, ADFI and G:F. The coefficient of apparent total tract digestibility (CATTD) of DM and N were determined using chromic oxide (0.20%) as an inert indicator.

The WBC, RBC counts and lymphocyte concentration were determined with an automatic blood analyzer (ADVIA 120, Bayer, NY). The blood urea nitrogen (BUN) and creatinine was analyzed using the Abbott Spectrum urea nitrogen test (Series II, Abbot Laboratories, Dallas, TX).

One gram of the composite fecal sample from each pen (1 gilt and 1 barrow) was diluted with 9 mL of 1% peptone broth, and homogenized. Viable counts of bacteria were then conducted by plating serial 10-fold dilutions (in 1%

peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate E. coli and lactobacillus, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 39°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. The E. coli and Lactobacillus colonies were counted after incubation.

Samples of the right loin from 60 pigs were removed between the 10th and 11th ribs to evaluate the meat color, marbling, and firmness scores according to NPPC (1991) standards at 26°C. The lightness, redness, and yellowness values were measured at 3 locations on the surface of each sample (Model CR-410 Chromameter, Konica Minolta Sensing Inc., Osaka, Japan). The longissimus muscle area (LMA) was measured by tracing the LM surface at the 10th rib with digitizing area-line sensor (MT-10S, M.T. Precision Co. Ltd., Tokyo, Japan). The 2-thiobarbituric acid-reactive substances (TBARS) were measured using the method described by Witte et al [13]

Fresh fecal samples were collected from at least two pigs in each pen to evaluate the fecal noxious gas emission content. The NH3 concentration was determined using the method described by Chaney and Marbach [3]. In order to determine the fecal H2S concentration, 300 g of fresh fecal samples were transferred to a sealed box and fermented in an incubator for 30 h (35°C). The fermented samples were then analyzed with a gas search probe (Gastec Corp., Kanagawa, Japan).

Statistical analysis

All data were subjected to statistical analysis via a randomized complete block design using the GLM procedures of SAS (SAS, 2001, Inst. Inc., Cary, NC), with the pen serving as the experimental unit. The initial BW and blood values were employed as a covariate. A logarithmic conversion of the data was carried out prior to statistical analysis of the microbial counts. Differences among the treatment means were determined by Duncan's multiple range test, with a $p < 0.05$ indicating significance. Orthogonal polynomials were employed to evaluate the effect of the dosage of BV.

RESULTS

Pigs fed diets with BV supplementation evidenced a greater ADG ($p < 0.05$) than those fed CON diet during 0 to 3 weeks and the overall period. The inclusion of BV linearly increased G:F ($p < 0.05$) when the entire experiment was evaluated. No difference was observed on ADFI ($p > 0.05$) in this study. The CATTD of DM in BV diets was greater ($p < 0.05$) than that in the CON diet at the end of the experiment. Pigs fed BV diets linearly increased N digestibility ($p < 0.05$) in the current study.

No significant differences were noted on RBC, BUN and creatinine concentrations among the dietary treatments. The lymphocyte concentration was greater in BV diets than that in CON diet ($p < 0.05$), while a tendency was observed on WBC concentration with increasing BV levels.

The numbers of the lactobacillus were not influenced by BV supplementation. The E. coli numbers were linearly reduced with increasing doses of BV supplementation ($p < 0.001$).

Pigs fed diets supplemented with BV2 evidenced greater meat color and firmness ($p < 0.05$) than the other groups. Dietary BV2 increased the redness value ($p < 0.05$) relative to CON group, whereas the lightness value was improved ($p < 0.05$) when pigs received CON diet compared with BV2 treatment. No difference was observed ($p > 0.05$) on WHC, LMA and TBARS values in this study.

NH3 emission was linearly reduced ($p < 0.05$) with dietary BV supplementation on the first and 5 day. The inclusion of BV supplementation reduced ($p < 0.05$) NH3 emissions compared with CON group. Additionally, BV1 group evidenced greater ($p < 0.05$) NH3 emissions than BV2 group. Pigs fed CON diet evidenced greater ($p < 0.05$) H2S concentration than those fed BV2 diet.

DISCUSSION

Kook et al [10, 11] had reported that ADG and G:F were greater in finishing pigs when 2% bamboo vinegar liquids was supplemented. Kishi et al [8] suggested that acetic acid influence nutrient digestibility by modulating the balance of intestinal microflora and pathogens. Shiomi et al [12] also demonstrated that phenolic compound exhibit antimicrobial activity and could be incorporated in various food products as natural antimicrobial additives. Therefore, the greater growth performance may be attributed to the improved circumstance of intestinal lumen induced by bamboo vinegar, which is also augmented by the results of apparent total tract digestibility. Besides, our study suggested that E. coli is decreased by the administration of BV, which again confirmed that the maturation and optimal development of intestinal could be considered as a reason for the boost nutrient digestibility and growth performance in this study. Moreover, Kishi et al [7] have suggested that acetic acid can affect immunity system of ovariectomized rats by controlling the balance of intestinal microflora and pathogens. Knekt et al [6] also suggested that polyphenolic compound derived from plant evidenced great importance antitumor and anti-disease properties. Therefore, the acetic acid and phenolic compound in the BV may be responsible for the improved immune related-blood characteristics observed in this study.

It has been suggested that dietary phenolic compound could react with lipid and hydroxyl radicals and convert them into stable product [14]. Previous study also reported that the inclusion of phenolic compound in animal diets led to a greater oxidative stability [5].

Therefore, a possible mode of action for the effect on the meat quality could be its antioxidant properties. Moreover, several studies have suggested that acidifier may improve the utilization of minerals, such as Ca or Fe [10]. Thus, supplemental BV may be involved in this process by influencing Fe status and microorganisms in meat, as the chemical state of the Fe present in the heme ring may affect the meat color.

Chen et al [4] have suggested that bamboo vinegar could induce a reduction in total Kjeldahl nitrogen losses occurring during the composting of porcine manure; Our previous study suggested that intestine microbial and nutrient digestibility are related to the fecal noxious gas content in pigs [15]. Therefore, we hypothesized the effects may be attributed to the following factors: 1) Improved nutrient digestibility induced by BV. 2) Positive effect of BV on the intestinal microbial.

CONCLUSIONS

In conclusion, dietary BV supplementation exerted beneficial effects on growth performance and nutrient digestibility via its beneficial effects on intestinal microbes, and positively affected meat quality and fecal noxious gas (NH3 and H2S) concentrations.

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GRASS/RED CLOVER SILAGE TO GROWING/FINISHING PIGS – INFLUENCE ON BEHAVIOUR AND GROWTH

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SUMMARY

Grass/red clover silage has potential to improve pig welfare by increasing possibilities for foraging and exploration behaviour, supply the pigs with nutrients and contribute to a sustainable use of arable land. This study investigates the effects of grass/red clover silage inclusion in pig diets on pig behaviour and growth. Inclusion of grass/red clover silage in pig diets resulted in pigs spending a lower proportion of time interacting with other pigs and manipulating pen fittings compared to pigs fed solely commercial feed. Moreover, when silage was fed in pelleted form, pigs reached the same average daily gain as pigs fed solely commercial feed. Thus, grass/red clover silage has the potential to improve pig welfare and contributes with a substantial supply of nutrients for pigs.

INTRODUCTION

Access to roughage increase pigs' time spent eating and gives the pigs increased possibility's to perform species specific behaviours such as foraging and exploration (Roberts et al., 1993; Vestergaard, 1996; Olsen, 2001). Traditionally, provision of straw as substrate for foraging and exploration is considered to be one of the most efficient ways to reduce the occurrence of injurious and potentially harmful behaviours in pigs (EFSA, 2007). However, straw does not contribute significantly to the nutrient supply of the pigs. Grass/red clover silage, on the other hand, has potential to improve pig welfare, supply pigs with nutrients and contribute to a sustainable use of arable land.

Ley crop with legumes (e.g. clover and alfalfa) in combination with grass have an important role in agriculture due to the ability to fixate nitrogen from the atmosphere. Moreover, when ley crop is included in the crop rotation it contributes to increased biologic diversity and weed, pest and disease control. Many crop production systems are relying on ley crops for nitrogen supply, and when ley crop is available on the farm it can be used as a locally produced feed resource to pigs.

The ley crop consumption reported from previous studies have been relatively low (0.1-0.5 kg/day and pigs) (Kelly et al., 2007; Høøk Presto et al., 2009) and there is a large variation in nutrient and energy utilisation between different ley crops and different inclusion ratios (Andersson & Lindberg, 1997a,b). There is a need for more knowledge about effects of different roughage feeding strategies on pig behaviour and production.

The aim of the present study was to assess how grass/red clover silage, fed separately, as a complete feed or in pelleted form to growing/finishing pigs, affected pig behaviour and growth.

MATERIAL AND METHODS

In total 64 growing/finishing pigs (Yorkshire x Hampshire) were included in the study. At 12 weeks of age, pigs from 10 birth litters were allocated to 8 pens with 8 pigs per pen. The distribution of pigs was balances with regard to birth litter and sex, e.g. never litter mates in the same pen and 4 gilts and 4 castrates per pen. Pigs were fed either commercial feed + chopped silage mixed and fed together (SM), commercial feed + intact silage fed separately (SS), commercial feed + grinded silage, mixed and pelleted (SP) or commercial feed alone (C), with two pens per treatment. The pigs were fed twice daily according to the Swedish energy recommendations (Simonsson et al., 2006). In the silage treatments, silage (9.04 MJ ME and 129 g crude protein per kg dry matter) constituted 20% of the diets on energy-basis (ME). All pens were provided 1 kg of straw per day. Representative samples of feeds was collected and analysed from DM, CP, ash, EG-fat, NDF and gross energy.

Pig behaviour (table 1) was registered from video recordings (4 occasions distributed over the growing/finishing period x 48 hours) with instantaneous scans every 30 minutes. For each scan, body posture and activity was registered for each pig.

Table 1: Ethogram of behaviours registered from the video recordings

Category	Variable	Definition
Body posture	Lie	Lying on the side or sternum, strait or bended legs
	Sit	Front feet on the ground, back legs in lying position
	Stand	Stands on all four feet or walks
Activity	Eat	Snout in feed through or silage hedge
	Drink	Snout touch water nipple
	Nose/bite other pig	Snout touch other pig
	Nose/bite pen fittings	Snout touch pen fitting
	Nose/bite floor	Snout touch floor
	Nothing	Snout in air

Pigs were weighed every second week from the start of the study (when pigs weighed on average 30 kg) until slaughter (when pigs weighed on average 115 kg live weight).

Statistical analyses were performed using SAS software, version 9.2 (SAS institute, Inc. Cory, NC). Behaviours were analysed with generalised linear models with binomial distribution and logit link and the models included the fixed effects of treatment (SM, SS, SP and C), pen nested within treatment (8 pens), video recording occasion (4 occasions distributed over the growing/finishing period) and the interaction between treatment and recording occasion. Growth was analysed with a mixed general linear models. The model included the fixed effects of treatment (SM, SS, SP and C), pen nested within treatment (8 pens), sex (gilt or castrate) and the random effect of birth litter. Weight at the beginning of the growth period was included as a continuous covariate.

The study was performed in accordance with Swedish regulations governing animal use in experiments.

RESULTS

SM pigs spent most time and SP pigs spent least time active, i.e. not lying down (p $p \leq 0.001$, Figure 1). SS pigs spent most and SP and C pigs spent least time eating ($p \leq 0.001$, Figure 1) and inclusion of silage in the diet reduced the time spent interacting with other pigs ($p \leq 0.001$, Figure 1). Pigs in the SM treatment spent most time manipulating floor and pen fittings while not eating and among the other three treatment groups, C pigs spent most and SS pigs least time manipulating floor and pen fittings ($p \leq 0.001$, Figure 1). Pigs in SM and SS treatments had lower average daily weight gain compared with pigs in the SP and C treatments (763^{a} and 783^{a} vs. 850^{b} and 856^{b} g/day in the SM, SS, SP and C treatment, respectively, $p \leq 0.001$).

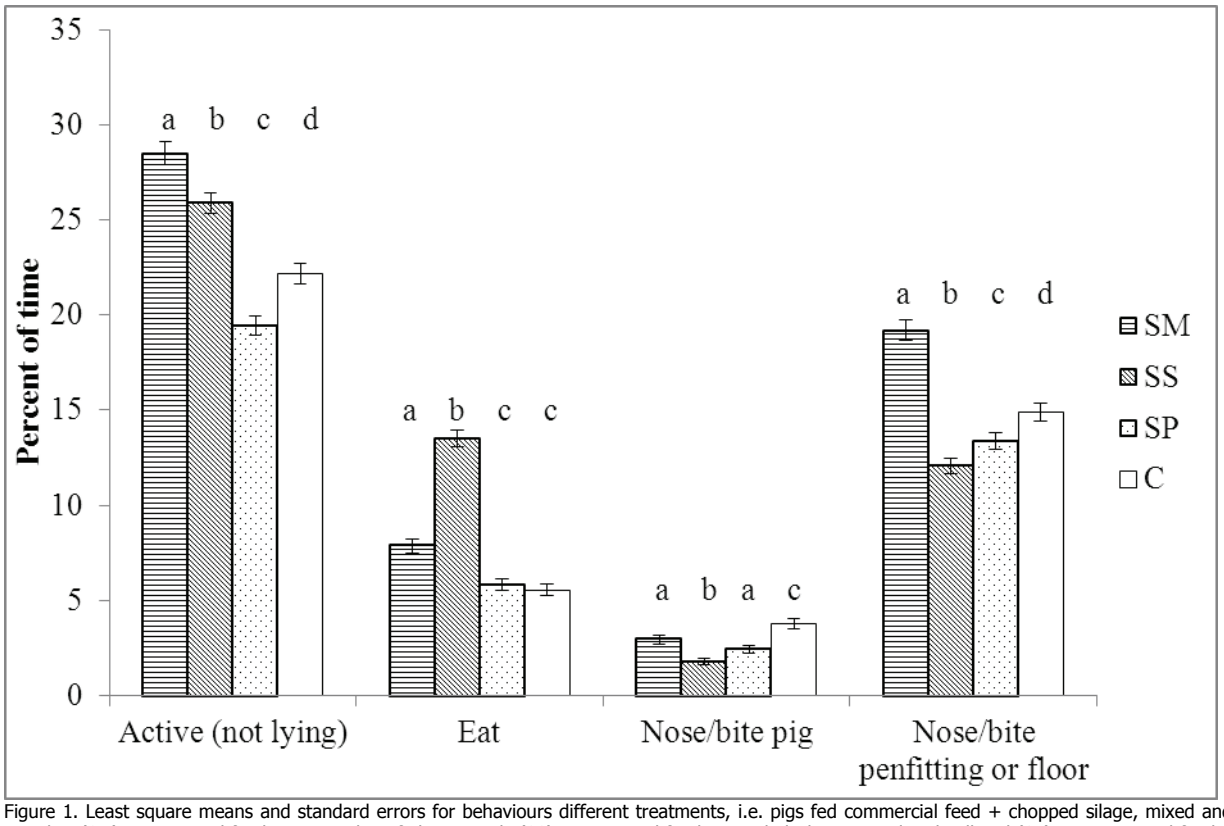

Figure 1. Least square means and standard errors for behaviours different treatments, i.e. pigs fed commercial feed + chopped silage, mixed and fed together (SM), commercial feed + intact silage fed separately (SS), commercial feed + grinded silage, mixed and pelleted (SP) or commercial feed alone (C). Different letters indicate significant ($p<0.05$) differences between treatments.

DISCUSSION

Inclusion of chopped or intact silage to pigs made them more active and increased time spent on time eating compared with pigs fed pelleted feed. The reason that pigs in the SM treatment spent most time manipulating floor and pen fittings, while not eating, might be that they separated silage and commercial feed on the floor outside the feed trough. The results of the present study confirms previous reports (Roberts et al., 1993; Vestergaard, 1996; Olsen, 2001), that provision of chopped or intact silage activate the pigs due to increased time spent on foraging. Silage fed in a pelleted form, on the other hand, resulted in pigs spending more time lying down resting, possibly due to a higher fibre intake resulting in more time needed for digestion.

Pigs fed silage spent less time interacting with other pigs and manipulating pen fittings compared with control pigs. This finding suggests that, even though pigs are provided straw, inclusion of silage in pig diets can reduce the occurrence of stereotypic, injurious and other potentially harmful behaviours even further.

Interestingly, pigs fed silage in pelleted form grew as fast as pigs fed commercial feed, even at this relatively high silage inclusion level (20 %). This suggests that grass/clover silage have substantial potential as a nutrient source for pigs.

CONCLUSIONS

Inclusion of grass/red clover silage in pig diets results in pigs spending a lower proportion of time interacting with other pigs and a lower proportion of time manipulating pen fittings, indicating improved pig welfare, compared to pigs fed diets with solely commercial feed. Moreover, if silage is fed in pelleted form pigs can reach the same average daily gain as pigs fed commercial feed alone. In conclusion, inclusion of grass/red clover silage in pig diets has a positive impact on pig welfare and contributes with a substantial supply of nutrients for pigs.

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Improvement of Palm Oil Fronds Digestibility by Fermentation Using Fibrolytic Microbial Inoculum Isolated from Buffalo Rumen Liquid

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SUMMARY

Experiments were conducted into two steps. First experiment was isolation of fibrolytic microbial of ruminal buffalo using agricultural and estate by-product (palm oil fronds, rice straw, bagasses and sawdust) as carbon source, in arrangement of completely randomized design with four (4) treatments and four (4) replication. Result of this experiment then was used to increase the quality of palm oil fronds through fermentation in arrangement of factorial 3x2 based on completely randomized design. First factor were percentation level of using inoculum (0,1 and 3 ml inoculum/g dray matter of palm oil fronds). Second factor were time of fermentation of 14 and 28 days.
The utilization of sawdust as carbon source produced the highest specific activity of endoglucanases, eksoglucanases and xylanases such as follow : 75.91 U / g protein / h; 103.83 U / g protein / h and 39.52 U / g protein / h. Interaction effect were reflected from the utilization of time of fermentation and level percentation of using microbial on neutral detergent fiber, acid detergent fiber, lignin, cellulose, hemicellulose, dry matter and organic matter digestibility (P<0,05) of palm oil fronds.
Conclution of these experiment showing that agricultural and estate by-product (sawdust) were had very potentioal to be used as carbon source in fibrolytic microbial isolation and the utilization of 1-2% inoculum with 14 days of fermentation effective to increase nutritional value of palm oil fronds.

INTRODUCTION

Indonesia has a lot of agroindustrial and estate by product which is rich of crude fiber. Palm oil fronds is the most one (10.500.996 ton/year). The production of enzyme from agroindustrial by-product by microbial treatment taking advantage of presence sellulose in agroindustrial by-product. Limited research have been undertaken to provide better utilization of agroindustrial by product for nutritional value added. Based on these trend treating agroindustrial by-product, Therefore, this study inspired to find out fibrolytic microbial cultures that can degrade the complexity of fiber caracter in palm oil fronds.

MATERIALS AND METHODS

Experiment step one

Sample collection : substrat as a carbon source used for these work was palm oil fronds, rice straw, bagasses and sawdust is cheap and hight content of fiber. All material milled and sieved through a filter size of 0.7 to 1.4 mm. Microbial source comes from buffalo rumen liquid fed rice straw.

Fibrolitic microbial screening: Fibrolitic microbial screening was conducted by using each 100 ml of selective liquid medium placed in erlenmeyer. Within each added 2% (weight : vol) of agroindustrial by product as carbon sources. 5% fresh rumen liquid inoculated at each liquid medium riched with carbon source treatment. The samples incubated at 40^{o}C for 7 days in anaerobic condition (Tampoebolon, 1997).

Analysis of Sellulases and Xylanases Activity: After 7 days of incubation, samples was analyzed for cellulases and xylanases of specific activity, caried out by the method sugested by Mendel *et al (1976)*.

Experiment step two

Sample preparation : Palm oil fronds was grinded at 1-2 cm^{3}. Fermentation was done by adding 0,5% of urea and 0%; 1% or 3% inoculan (based on dray matter of palm oil fronds). Sample made in 60-70% of moist, than kept in anaerobical boxes and placed in incubator (40^{o}C). Fermentation was kept for 14 and 28 day.

Statistical Analysis: All data analyzed in varians where the significant effect (P<0,05) then analysed in LSD (first steps experiment), Duncan multiple range test (second steps experiment) to detect differences among the treatments and polinomial ortogonal to analys the optimum response.

RESULTS

Specific Activity of cellulases and xylanases: Each carbon source had sicnificant effect (P<0,001) for specific activity of endoglucanases, exoglucanases and xylanases. Tabel 1 shows specific activity of endoglucanases, exoglucanases and xylanases.

Tabel 1. Specific Activity of Endoglucanases, Exoglucanases and Xylanases in Agroindustrial By-product as a Carbon Source

Carbon Source	Endoglucanases	Exsoglucanases	Xylanases
	---------- U/g protein/jam ----------		
Palm oil fronds	57,58[b]	68,16[b]	30,85[b]
Rice Straw	51,66[c]	70,77[b]	21,36[c]
Bagasses	48,69[d]	53,11[c]	38,72[a]
Sawdust	75,91[a]	103,83[a]	39,52[a]

Interaction between percentage of inoculum with day of fermentation sicnificanly (P<0,05) affecting NDF, ADF, hemicellulose, Cellulose and lignin content Fiber contents of fermented palm oil fronds showed at tabel 2.

Tabel 2. Fiber content of fermented palm oil fronds

Treatment Factor	day	ADF	NDF	Lignin	cellulose	Hemicellulose
		----------%----------				
FPS + 0% inoculant	14	67,49[a]	88,36[a]	22,14[a]	43,71[d]	20,87[d]
	28	67,20[a]	85,76[b]	22,15[a]	43,11[d]	18,56[c]
FPS + 1% inoculant	14	66,19[b]	79,70[c]	38,89[bc]	25,36[b]	13,51[b]
	28	68,39[a]	79,91[c]	36,03[bc]	31,31[c]	11,52[a]
FPS + 3% inoculant	14	65,72[c]	79,81[c]	33,50[b]	24,47[a]	14,09[b]
	28	66,75[b]	79,85[c]	41,47[c]	24,10[a]	13,10[b]

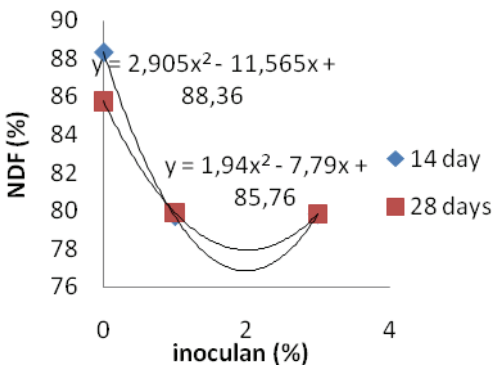
Tabel 3. Dry mater and organic digestibility of palm oil fronds fermented with selected inoculum from the first steps of experiment.

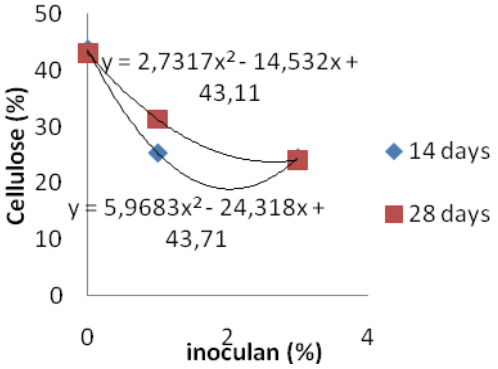
Treatment	DM digestibility			Organic mater digestibility		
	Fermentation time		Avg	Fermentation time		Avg
	14 day	28 day		14 day	28 day	
	----------%----------					
FPS + 0% inokulum	27,59[a]	27,60[a]	27,59[y]	14,05[k]	14,46[k]	14,26[q]
FPS + 1% inokulum	39,84[b]	41,58[c]	40,71[x]	41,76[m]	41,2[m]	41,50[s]
FPS + 3% inokulum	37,68[b]	41,22[c]	39,45[x]	41,27[m]	40,18[l]	40,73[r]
Rata-rata	35,04[q]	36,80[p]		32,50[v]	31,8[w]	

DISCUSSION

Differenties of specific activity of fibrolitic enzymes indicate that each carbon source had a different content of micro cristalin, amofr cellulose and hemicellulose. Lowest specific activiy of endoglucanases reached by rice straw (51,66 U/g protein/h). Lowest specific activiy of exoglucanases reached by bagasses (53,11 U/g protein/h). Sawdust had the Highest specific activity of endoglucanases, exoglucanases and xylanases (75.91 U/g protein/h; 103.83 U/g protein/h and 39.52 U/g protein/h). The different result of xylanases activity happen because of xylanases was induced enzym (Ryu and Mandels, 1980; Kubicek, 1992; Biely, 1993; Kubicek *et al*., 1993; Saloheimo *et al*., 1998). Higher xylanases activity was happened when the microbial had phisical contact with xylan. Xylan will stimulate gen translation produced a xylanases (Ryu and Mendels, 1980; Biely, 1993). High reduced of hemicellulose give a highest chance for cellulolitic microbial to degrade cellulose because hemicellulose bounds a layer of cellulose formed a microfibril to increase the stability of cell wall (Perez *et al.,* 2002).

Canges of fiber content in fermented palm oil fronds (experiment steps two) show the efectivity of both factor treatment. Polinomial ortogonal analysis used to determin the best treatment combination. Result of these analysis can see below.





NDF content is a component from cell wall aranged by ADF and hemicellulose (Cullison, 1979). Decreased of hemicellulose followed by ADF content will decrease NDF content directly. Kuadaratic curve of hemicellulose content show the optimum level of inoculant adding at 1,85% with 14th days of fermentation can decreas 9,6% of hemicellulose contetnt in palm oil fronds. Cellulose, ADF and NDF content also show the same curve with optimum level in 1,8 - 2% of inoculant adding wih 14th days of fermentation. Curve of all of fiber content also show a feedback mechanism in every enzym reaction. Increaed of lignin content show that there is a lignohemicellulases and a lignocellulases. These enzyme hidrolized a lignohemicellulose and lignocellulose bonds so hemicellulose and cellulose microbial can degrade much hemicellulose and cellulose (Abdullah, 1992).

Increasement of dry mater digestibility indicate the effectivity of fibrolitic microorganism degrade the complexity of fiber content. Highest organic mater digestibility reached by 1% inoculant adding in 14th days of fermentation not in 28 day of fermentation it because after 14 days of fermentation, the fibrolytic microbia have a trouble in degrading organic mater that still exist because it's high of lignin and soluble ash content.

CONCLUSIONS

Agricultural and estate by-product (sawdust) were had very potentioal to be used as carbon source in fibrolytic microbial isolation and the utilization of 1-2% inoculum with 14 days of fermentation effective to increase nutritional value of palm oil fronds.

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STUDY ON THE UTILISATION OF GLYCINATE- AND INORGANIC BOUND TRACE ELEMENTS IN CALVES

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SUMMARY

The goal of the laboratory model experiment was to compare inorganic and organically-bound (chelated) microelements (Cu-, Zn-, Fe- and Mn-glycinate) with respect to their effects on health and integrity, immune responsiveness and weight gain of growing calves.
Two experimental and two control groups (4 calves in each) were formed with Holstein Friesian bull calves. Average age and weight at assembling were 68 days and 80 kgs respectively. Groups: Control-A (Co-A): no trace element supplementation; Control-B (Co-B): supplemented with inorganic trace elements; Experimental-A (Ex-A-100%): supplemented with glycinate-bound trace elements at concentration that was identical with Co-B; Experimental-B (Ex-B-50%): supplemented at 50% of glycinate-bound trace elements of the Ex-A-100% group. Duration of the experiment was 56 days. Weight gain, solid feed consumption, feed conversion efficiency, hematological status (packed cell volume, qualitative and quantitative blood picture), metabolic status (blood glucose, BHB, NEFA, urea, total-protein, albumin, AST, Ca, inorganic-P, Mg, Cu, Zn, Fe, pigmented hair and feces Cu, Zn, Fe, Mn) were examined. Cellular immune response was studied by lymphocyte stimulation tests.
Supplementation with glycinate-bound Fe, Cu, Mn and Zn increased the daily weight gain, improved the feed conversion efficiency, had beneficial effect on the biological weight gain, decreased the amount of solid feed consumed for unit of biological weight gain, provided good trace element supplementation at 50% of the suggested concentration and the fecal excretion of Mn and Fe decreased. The glycinate-bound trace element supplementation had effect on the erythrocyte count, hemoglobin concentration and packed cell volume and had positive effect on the mean corpuscular volume of erythrocytes that indicates positive influence on erythropoiesis. Improved cellular immune parameters were detected.

INTRODUCTION

Trace elements are miniscule metal and non-metal components of the plant and animal organism, of which only few (e.g. iron) forms structural parts of tissues.
Minimum trace element requirement of livestock can not be characterised by a single figure because this might be relevant only for one, individual feed formulation. This is because biological availability of dietary trace elements is function of multitudes of environmental and other influences.
Even the most carefully designed and implemented experiments can show variable figures of utilisation or absorption; the differences might reach one order. This variability might be attributed frequently to the different chemical and physical status and various concentrations of the element in question. In other instances the absorption is influenced by inorganic (e.g., sulphide, thiomolibdenate etc.) or organic (e.g. chelate) components of the feed or by substances synthesised in the intestines. There are antagonistic and synergistic connections among trace elements. In special cases these connections might have decisive importance (e.g. cadmium – copper/zinc; molybdenum – copper etc.).
In the light of the foregoing considerations reference values of textbooks for trace elements, expressed as unit mass per dry matter kg, in practical conditions should be regarded as values that contain certain safety plusses, therefore these values in most of the cases meet the trace element requirement of livestock an. It follows, when formulating daily feed rations, these reference values might be relied on unless strong antagonist effects prevail.

MATERIAL AND METHODS

Two experimental and two control groups (4 calves in each) were formed with Holstein Friesian bull calves. Average age and weight at assembling were 68 days and 80 kgs respectively. Groups: Control-A (Co-A): no trace element (Cu-, Zn-, Fe- and Mn) supplementation; Control-B (Co-B): supplemented with inorganic trace elements; Experimental-A (Ex-A-100%): supplemented with glycinate-bound trace elements at concentration that was identical with Co-B; Experimental-B (Ex-B-50%): supplemented at 50% of glycinate-bound trace elements of the Ex-A-100% group. Duration of the experiment was 56 days.

Examined parameters

Weight gain, solid feed consumption, feed conversion efficiency:
Weight of the calves was taken immediately upon their arrival to the Environmental Laboratory than measurements were repeated by two week intervals always on the same week-day and same hour. Feed consumption of the calves was measured daily and calculated for the relevant two week periods between consecutive weight scales. Feeds (prestarter, and milk replacer) and faeces were sampled by calves on days when the weight of the calves was measured for determination of the trace element concentrations.
Hematological status: packed cell volume, qualitative and quantitative blood picture were examined.
Metabolic status: blood glucose, BHB, NEFA, urea, total-protein, albumin, AST, Ca, inorganic-P, Mg, Cu, Zn, Fe, pigmented hair and feces Cu, Zn, Fe, Mn) were examined.
Cellular immune response was studied by lymphocyte stimulation tests. These tests measure the blastogenic response of lymphocytes to effects of non-specific mitogens (concanavalin-A /Con-A/, phytohaemagglutinin /PHA/ and pokeweed mitogen /Pwm/). All mitogens were tested in 4 parallel repetitions with $1.5x10^5$ lymphocytes separated with standard procedures from the same blood sample. Degree of stimulation was measured by the colour change of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromid (MTT) reagent.
Blood samples were obtained from *v. jugularis* in the period between 9-10 a.m. on days indicated in the table above. Samples were released into test tubes containing heparin or EDTA.

RESULTS AND DISCUSSION

Supplementation with glycinate-bound Fe, Cu, Mn and Zn increased the daily weight gain. In spite of the fact that no differences were found among the average daily weight gains of calves the biological weight gain (weight gain calculated for the metabolic body weight /MBW: body weight $kg^{0.75}$/) was better in both Experimental groups. The overall difference between Co-A calves and the Experimental (Ex-A-100% and Ex-B-50%) calves was considerable: 8.4 and 11.7%, respectively.
The glicinate-bound supplementation improved *the feed conversion efficiency*. The amount of solid feed consumed for unit of biological weight gain decreased. The difference between Co-A calves and the calves in groups of Ex-A-100% and Ex-B-50% was 24.8 and 21.4%, respectively.
According to the *metabolic status* of the calves, neither considerable, nor significant differences were found among the metabolic parameters (blood glucose, BHB, NEFA, urea, total-protein, albumin, AST, Ca, inorganic-P, Mg) were measured. All figures fell within the physiological range.
The *plasma concentrations of Fe* showed fluctuations within the physiological range in all groups but relevant values of the experimental calves were below of those found in calves of the Co-A group. The difference ranged 3.2 and 45.6%, the differences were statistically not significant. The *plasma Cu concentration* showed tendentious decline towards the end of the experiment. Although Ex-B-50% calves had the lowest plasma copper concentration, the values measured were still above the lower level of the physiological range. This may conclude that in spite of the 50% less copper supplementation of this group the copper supplementation of Ex-B-50% calves was still satisfactory. *Zinc concentrations of the plasma* suggested good Zn supplementation. The Zn satus in Ex-B-50% calves was satisfactoty in spite of the 50% less supplementation as well. Data of the groups were similar (differences were ≤ 2%) with slight declining tendency toward the end of the experiment.
We may conclude that the actual trace element supplementation of calves in all groups was satisfactory.
Long term trace element supplementation was studied by analysis of pigmented hair samples. *Fe-content* of the samples indicated good supplementation. However, we need to mention that Fe content of hair samples is not the best indicative of supplementation. Fe deficiency might lead to anaemia with decreased Fe concentration in the blood plasma with parallel increase of iron binding capacity. However, it was worth to notice that iron content of the Ex-A-100% calves' hair samples was considerable, 21% higher than that of the control calves and close correlation (R^2=0.848) was found. *Copper content* of the pigmented hair was higher in all groups throughout the experiment than the lower limit of the physiological range (6 µg/g). Best supplementation was found in Ex-A-100% calves; the difference to the two control groups was 4.6% at the end of the experiment (R^2=0.981). *Zinc content* of the pigmented hair was higher in all groups throughout the experiment than the lower limit of the physiological range (100 µg/g). Supplementation was best in Ex-A-100% and Ex-B-50% calves. At conclusion of the experiment the difference to values of the controls was 4.7 and 3.3%, respectively with medium correlation (R^2=0.655). *Manganese content* of the pigmented hair was lower in all groups throughout the experiment than the lower limit of the physiological range (6 µg/g). Best supplementation was found in the Co-A and Ex-B-50% groups with medium correlation (R^2=0.789). We need to mention, however, that checking the manganese status by laboratory examinations gives no reliable results.
Trace element excretion of calves was studied by determination of the trace element concentrations of the faecal samples.
The glicinate-bound trace element supplementation at 50% of the suggested concentration provided a decrease in the fecal excretion of Mn and Fe. At conclusion of the experiment faecal samples of Co-B calves had the highest Mn concentration and tendentious decrease was found for the advantage of Ex-A-100% (12.1%) and Ex-B-50% (8.5%) calves. Between group differences in excretion was characterised by medium correlation (R^2=0.624). Iron concentrations of the faces samples showed tendentious decline towards the end of the trial in all groups. The

decline was most expressed in the experimental groups and the difference of the Ex-A-100% and Ex-B-50% calves to Co-A calves were 12.2 and 10.3%, respectively.

The glycinate-bound trace element supplementation had effect on the *erythrocyte count*. At the beginning of the experiment the least count was found in the two experimental groups and it is also seen that these groups had the highest number of erythrocytes at conclusion. Erythrocyte count increased with measure of supplementation with glycinated trace elements (R^2=0.894), that is it was bigger in the Ex-A-100% calves than in calves of the Ex-B-50% group. In summary we may conclude that the experimental feed additive had favourable effects on the erythropoiesis with a resultant higher erythrocyte count. This is especially important because increased number of red blood cells improves the oxygen supplementation of tissues.

The hemoglobin concentration, the packed cell volume and the mean corpuscular volume of erythrocytes indicates positive influence on erythropoiesis. On day 56 of the experiment Ex-A-100% calves had the highest haematocrit value (36.6%). This value was followed by the Co-B and Ex-B-50% calves (34.5 and 33,8%, respectively). This is another indication that changing the 100% dose of the experimental feed additive is not feasible.

No treatment associated deviations from the physiological data were found in case of examined parameters of the blood leucocites.

Improved *cellular immune parameters* were detected. *Lymphocyte stimulation with PHA* data suggest that cellular immune responsiveness of the calves improves with progressing age. Least response was found at day 14. This finding might be attributed to the inadequate adaptation to the new climatic and housing conditions. From day 28 definite cellular reactions were measured in all groups; noteworthy, the best results were found in Ex-A-100% calves. Calves in the group of Ex-B-50% have shown good cellular reaction from day 28 onwards, and this group produced the best reaction at the of the trial. *Lymphocyte stimulation with ConA and Lymphocyte stimulation with PwM* data are comparable to the lymphocyte responsiveness evoked by PHA. An improved cellular immune reaction detected after day 14 of the trial. In summary we may state that cellular immune reaction of young calves develops with the age which might be assisted with feeding glycinated trace element supplementation.

CONCLUSIONS

Supplementation with glycinate-bound trace element (Fe, Cu, Mn and Zn), increased the daily weight gain of calves, improved the feed conversion efficiency, had beneficial effect on the biological weight gain, decreased the amount of solid feed consumed for unit of biological weight gain, provided good trace element supplementation at half of the suggested concentration, faecal excretion of manganese and iron decreased, had favourable effect on the erythrocyte count, haemoglobin concentration and packed cell volume, had positive effect on the mean corpuscular volume of erythrocytes that indicates positive influence on erythropoiesis, and improved the cellular immune parameters.

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FMD VACCINATION RESPONSE ON CALVES WITH COLOSTRAL ANTIBODIES

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SUMMARY

This paper describes the effect of colostral antibodies (CAb) in the response to Foot-and-Mouth Disease (FMD) vaccine The aim of this study was to evaluate the effect of the CAb on the induction of the antibody (Ab) response to the vaccination of calves with a commercial vaccine. Three groups of 20 calves of 30, 60 and 90 days of age (G30, G60 and G90), born to regularly vaccinated cows, received a first dose of the oil-adjuvant-tetravalent vaccine (O1/Campos, A24/Cruzeiro, A/Argentina/01, C3/Indaial) on day 0 and a second dose on day 180 of the trial. Serum samples were collected on days 0, 60, 180 post vaccination (dpv) and 60 days post revaccination (dpRv). The proportion of calves with Ab titers post vaccination compatible with protection was estimated for the three groups. Calves with Ab titer ≥ 2.2 were considered "protected". Liquid phase ELISA was used to determine the Ab titer against A/Argentina/01 strain contained in the vaccine. Serum samples collected at day 0 indicated, as expected, that CAb titers decreased significantly ($p<0.05$) with increasing age, with G30 titers being the highest and G90 the lowest (95%, 60% and 20% of calves from G30, G60 and G90 were protected, respectively). Although at 60 dpv, Ab levels did not differ significantly among groups ($p=0.56$).,At 180 dpv, Ab titers were inversely related to age at vaccination and the proportion of calves protected were of 0.61, 0.25 and 0.20 for G90, G60 and G30, respectively. At 60 dpRv, Ab titers increased in calves of the three groups with no significant differences among them ($p<0.05$), being the proportion of protected animals greater than 0.93. Calves born to vaccinated cows should be revaccinated to reach adequate and persistent levels of immunity.

INTRODUCTION

Argentina has two different sanitary status regarding Foot-and-Mouth Disease (FMD): the South of the country is recognized by OIE as "FMD Free Zone where vaccination is not practised" and the North, which concentrates more than 95% of the national bovine herd, is recognized as "FMD free zone where vaccination is practised" [3]. In this latter zone, a compulsory vaccination campaign is performed to the bovine and bubalines. The cows and bulls are vaccinated once a year and the heifers, steers and calves, twice [6]. The oil-adjuvant vaccine used is tetravalent (contains the strains O1/Campos, A24/Cruzeiro, A/Argentina/01, C3/Indaial) and is inactivated with binary ethylenimine. The National Service for Agrifood Health and Quality (SENASA) performs every year national population immunity surveys to estimate the immunity in the vaccinated herds to assess the effectiveness of the vaccination campaigns.

The aim of this study was to evaluate the effect of the colostral antibodies (CAb) on the induction of the antibody (Ab) response to the vaccination and revaccination with a commercial vaccine in calves so as to generate information that contributes to the population immunity surveys understanding.

MATERIAL AND METHODS

Three groups of 20 calves of 30, 60 and 90 days of age (G30, G60 and G90), born to regularly vaccinated cows received a first dose of the oil-adjuvant-tetravalent vaccine (O1/Campos, A24/Cruzeiro, A/Argentina/01, C3/Indaial) on day 0 and a second dose on day 180 of the trial. Serum samples were collected on days 0, 60, 180 post vaccination (dpv) and 60 days post revaccination (dpRv). The proportion of calves with Ab titers post vaccination compatible with protection was estimated for the three groups. Calves with Ab titer ≥ 2.2 were considered "protected" [5]. Liquid phase ELISA was used to determine the Ab titer against A/Argentina/01 strain contained in the vaccine.

RESULTS

Serum samples collected at day 0 indicated that CAb titers decreased significantly ($p<0.05$) with increasing age, with G30 titers being the highest and G90 the lowest. At that day, 95%, 60% and 20% of calves from G30, G60 and G90 have Cab titers compatible with protection, respectively (Fig. 1 and Fig. 2).

Although at 60 dpv, Ab levels did not differ significantly among groups (p=0.56), at 180 dpv, Ab titers varied significantly among groups (p<0.05) with a decreasing trend in the animals from G90, G60 and G30, respectively, being the proportion of protected 0.61, 0.25 and 0.20.

At 60 dpRv, Ab titers notably increased in calves of the three groups and the proportion of protected animals was greater than 0.93 in all three groups.

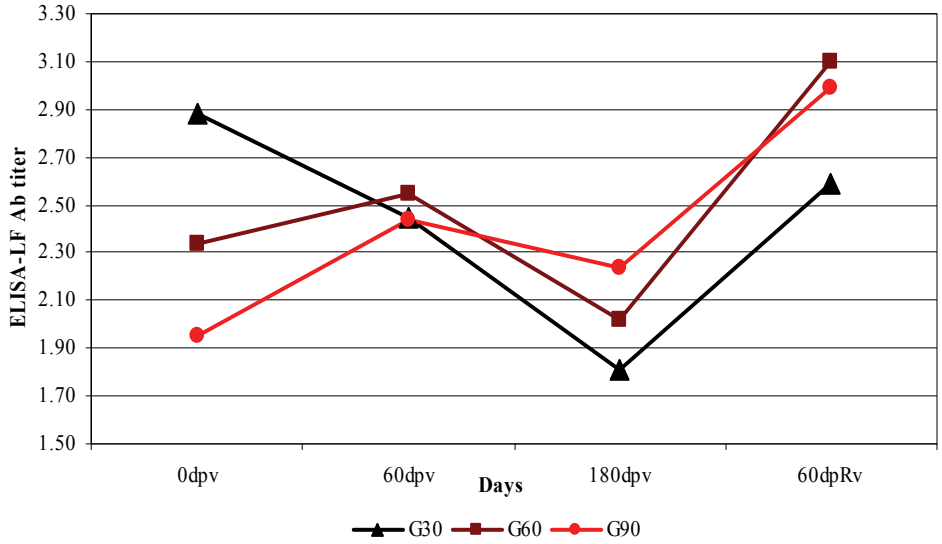

Fig. 1: Ab titers against FMD virus (strain A2001) in the days post vaccination and post revaccination

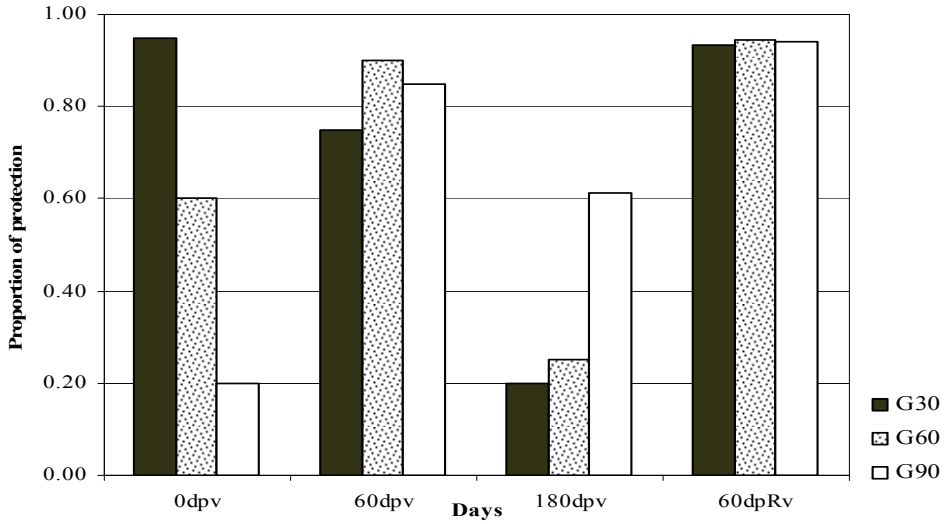

Fig. 2: proportion of protected calves to the FMD virus (strain A2001) in the days post vaccination and post revaccination

DISCUSSION

Serum samples collected at day 0 indicated, as expected, that CAb titers decreased significantly with increasing age. As described by [1], at 60 dpv, more than 75% of calves were protected. Results obtained show that Ab level reached at 180 dpv was inversely related to the level of CAb at the moment of vaccination, suggesting an interference between the CAb and the response to the vaccination, similar to that described by different auhtors [1], [2], and [4]. Sixty dpRv, animals from the three groups showed high levels of protection confirming the need to revaccinate the calves.

CONCLUSIONS

The CAb interfere in the response to the vaccination. Calves born to vaccinated cows should be vaccinated twice so as to reach adequate and persistent levels of immunity, especially if the first vaccination was performed in the presence of high level of CAb.

Interpretation of serological surveys to estimate the efectiveness of vaccination campaigns in young cattle needs to take into account the age at first vaccination and the number of vaccinations.

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CLINICAL SIGNS AND BACTERIOLOGICAL BACKGROUND OF CLINICAL MASTITIS CASES IN DAIRY COWS

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SUMMARY

This paper shows the first results of our long term investigation. 442 milk samples were taken from clinically sick udder quarters and microbiological examination were carried out. Besides in 186 cases the macroscopic view of the mastitic milk and the sings of inflammation of the udder were also recorded. Our results showed that *Streptococci* and bacteriums from the *Enterobacteriaceae* family are the most common causative agents of clinical mastitis. In almost half of the cases only minor changes were identified in the milk and no signs of clinical mastitis were seen on the udder, which shows the importance of the proper investigation of the fore strips before milking.

INTRODUCTION

Mastitis is one of the most important herd health problems in dairy cows. While clinical cases cause only one third of the total economical loss, they are the most seen and better known problem, not the subclinical form which is harder to identify in the everyday milking routine. The lack of early identification of the problem lowers the quality of the milk while it raises the SCC, and also makes the treatment more expensive and less effective. The knowledge of the causing pathogens can help to find the most important and effective preventive measures.

MATERIALS AND METHODS

In our survey we examined 442 milk samples taken from clinical mastitis cases collected in large scale Holstein Friesian dairy herds. The samples were collected during the morning milking only from previously not treated quarters in an aseptic way. The teat end was disinfected with alcohol and a few ml. of milk was collected in sterile tubes. In 186 cases the macroscopic view of the milk and the signs of inflammation of the udder (pain, heat, redness, swelling etc.) were also recorded. After the sampling, the milk samples were transported to the microbiology laboratory in a coolbox. In the laboratory the samples were frozen. The next day the samples were cultured on four media, Columbia agar with aesculin, MacConkey agar, Edwards agar and Sabouraud agar. The cultures were incubated for 48 hours on 37°C. The colony morphology, the presence and type of haemolysis, the ability to split aeculin, the result of catalase test and latex tests (like Staphylase Test) were all considered during the identification.

RESULTS

In 127 (28.73%) cases no pathogen was cultured from the milk samples. The most common pathogens in the background of clinical mastitis were different kind of environmental *Streptococci* (without *Str. uberis* and *Str. dysgalactiae*), we found these in 67 samples (15.16%). Bacterium from the *Enterobacteriaceae* family (mostly *Escherichia coli* and *Klebsiella spp.*) was found in 52 samples (11.99%). *Str. uberis* alone was in 60 samples (13.57%). We could confirm the presence of *Staphylococcus aureus* in 43 samples (9.73%), coagulase-negative Staphylococci in 32 samples (7.24%), *Prototheca zopfii* in 25 samples (5.66%), *Streptococcus dysgalactiae* in 23 samples (5.20%), and *Corynebacterium bovis* in 5 samples (1.13%).

In 87 cases we found only little clots in the milk, and the identification of these cases was the hardest for the milkers. Middle sized clots were found in 30 cases, large clots 36 cases while pus in 21 cases. The milk was watery in 21 cases, bloody in 7 cases. In 87 cases there wasn't any visible sign of the inflammation. We could see redness 27 cases, the udder was swollen in 32 cases, smaller in 14 cases. It was painful in 37 cases, firm in 49 cases and warmer in 48 cases.

CONCLUSIONS

In our survey environmental pathogens (mostly *Streptococcus spp.*) caused most of the clinical mastitis cases, while the occurrence rate of *S. aureus* was much higher in the sub-clinical cases.

While in 99 cases there were visible signs of the inflammation of the udder, in 87 cases the only change was the quality of the milk which shows the importance of the examination of the fore strips in test cups otherwise many clinical cases are not identified in time. Without the recognition of the sickness the milk of the inflamed quarter is milked in the bulk tank. It elevates the SCC of the bulk tank and also raises food hygiene concerns. Without the early recognition the treatments are more expensive and the chance of total recovery is reduced.

ASSOCIATION BETWEEN HERD CHARACTERISTICS AND UDDER HEALTH IN SWEDISH DAIRY HERDS

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SUMMARY

This paper describes a study of association between herd characteristics and herd-level indicators of udder health. The study was based on information from the report "Welfare Signals" from the Swedish Dairy Association and included data from 4,722 herds during the time period December 2008 to November 2009. A high calculated bulk milk somatic cell count was associated with herds with Swedish Holstein as the predominant breed, having an automatic milking system, a herd size above 100 cows and low milk yield. A higher incidence of veterinary treated clinical mastitis was associated with a herd size below 50 cows and a high milk yield. A high culling rate due to udder disorders were associated with a small herd size, parlour- or rotary milking and a high milk yield.

INTRODUCTION

The number of dairy herds in Sweden has nearly halved during the last decade. At the same time the average herd size has increased from an average of 39 cows in 2000 to 64 cows in 2010 [11]. Also the proportions of herds with free stalls and organic management have increased as well as the number of herds with an automatic milking system (AMS). In the beginning of 2011, over 50% of the Swedish dairy cows were kept in free stalls and about 20% were milked in an AMS. During this time period there has also been a steady increase in the calculated bulk milk somatic cell count (BMSCC) and a decrease in the incidence of veterinary treated clinical mastitis (VTCM) [10].

In Sweden, a web based report "Welfare Signals" is provided to dairy farmers and advisory workers as a tool to identify weaknesses and strengths in the production. The report includes 24 welfare indicators based on data in the Swedish cattle database. The report is a result from a research project with the aim to use register-data to identify herds with poor and good welfare [6, 7].

With the structural changes currently occurring in Swedish milk production it is important to evaluate whether the systems that gradually become more common are associated with impaired animal welfare under Swedish conditions. The association between each welfare indicator in the report and herd characteristics has been evaluated and here we present the result for the udder health indicators, including calculated BMSCC, incidence of VTCM, and culling due to udder disorders.

MATERIAL AND METHODS

Data from 4,722 dairy herds during December 2008 to November 2009 was retrieved from the Cattle Database at the Swedish Dairy Association in January 2010. Three different outcomes were used; calculated BMSSC (log transformed arithmetic mean), incidence of VTCM and culling due to udder disorders. The associations between herd characteristics (predominant breed, organic vs. conventional management, herd size, milking system and average milk yield) and calculated BMSCC, incidence of CM and culling due to udder disorders and were evaluated using linear regression model and negative binomial regression models, respectively. Bulk milk somatic cell count was calculated using the arithmetic mean of cow composite SCC at 12 monthly test-milkings and log-transformed in order to improve the linearity. For incidence of VTCM and culling due to udder disorders the outcome was number of events and total animal-days at risk was used as exposure. The association between the outcomes and each herd characteristic was first tested in a univariable analysis and characteristics with a p-value <0.2 (likelihood ratio test) was included in a multivariable model. The herds were categorized as Swedish Red (SR) or Swedish Holstein (SH) if at least 80% of the cows were pure-bred and mixed or other breeds otherwise. Herd size and milk yield was included as continuous variables and categorized if not linearly related to the outcome. The model was then reduced by backward elimination until all variables had a p-value <0.5. Confounding was checked for during all steps and interaction between characteristics remaining in the final main effects model were tested, but none were significant. The linear regression model was evaluated by visual examination of the normality of residuals, Q-Q plots, and DFITS were used to identify influential observations. The fit of the negative binomial regression models were assessed with Goodness-of-fit test and visual examination of the plot of Ancombe residual versus predicted values. Cook's distance and deviance

residuals were used to identify influential observations and outliers.

RESULTS

During December 2008 to November 2009 the 25th, 50th and 75th percentiles for herd-level incidence of VTCM were 2.9, 9.7 and 19.2 and for culling rate due to udder disorders they were 4.1, 8.2 and 13.4. For calculated BMSCC the 25th, 50th and 75th percentiles were 175, 222 and 280 (1000 cells per ml). The results for the statistical analyses of the association between herd-level characteristics and incidence of clinical mastitis, BMSCC and culling due to udder disorders are presented in table 1, 2 and 3, respectively.

Table 1. Negative binomial regression analysis of the association between herd-level characteristics and incidence of veterinary treated clinical mastitis.

Herd-level indicators	Category	n	IR	95 % KI	
Herd size	<50	2444	1		
	50-99	1552	0.90	0.84 -	0.96
	100-199	598	0.91	0.83 -	1.00
	≥200	128	0.95	0.79 -	1.13
Milk yield (kg ECM)	<8 500	1141	1		
	8 500- 9 399	1199	1.38	1.26 -	1.51
	9 400- 10 200	1254	1.47	1.34 -	1.60
	>10 200	1128	1.60	1.47 -	1.75

Table 2. Linear regression analysis of the association between herd-level characteristics and bulk milk somatic cell count (log transformed arithmetic mean, 1,000 cells per ml).

Herd-level indicators	Category	n	Estimate	SE	95 % KI	
Constant			5.38	0.01	5.36 -	5.41
Predominant breed	SR	1283	0			
	SH	1324	0.13	0.01	0.10 -	0.16
	Mixed SR*SH	1878	0.05	0.01	0.02 -	0.07
	Other breeds	237	0.02	0.02	-0.03 -	0.06
Herd size	<50	2444	0			
	50-99	1552	0.11	0.01	0.08 -	0.13
	100-199	598	0.17	0.02	0.13 -	0.21
	≥200	128	0.20	0.03	0.13 -	0.27
Milking system	AMS	514	0.07	0.02	0.04 -	0.11
	Parlour Rotary	650	-0.01	0.02	-0.04 -	0.03
	Rotary	26	0.08	0.07	-0.05 -	0.22
	Pipeline	3532	0			
Milk yield (kg ECM)	<8 500	1141	0			
	8 500- 9 399	1199	-0.07	0.01	-0.10 -	-0.04
	9 400- 10 200	1254	-0.17	0.01	-0.20 -	-0.14
	>10 200	1128	-0.25	0.02	-0.28 -	-0.22

Table 4. Negative binomial regression analysis of the association between herd-level characteristics and culling due to udder disorders.

Herd-level indicators	Category	n	IR	95 % KI	
Herd size	<50	2444	1		
	50-99	1552	0.93	0.88 -	0.98
	100-199	598	0.80	0.74 -	0.87
	≥200	128	0.74	0.65 -	0.84
Milking system	AMS	514	1.07	0.99 -	1.15
	Parlour	650	1.11	1.03 -	1.19
	Carousel	26	1.61	1.24 -	2.09
	Rotary	3532	1		
Milk yield (1,000 kg ECM)			1.09	1.07 -	1.11

DISCUSSION

Having SH as the predominant breed, high milk yield, free stalls and large herd size were all associated with impaired udder health, in at least one of the udder health indicators investigated. However, most of the significant differences are rather small. The higher calculated BMSCC in herds with AMS are consistent with previous result [3] whereas we did not find differences between breed or housing type which have previously been shown under Swedish conditions [1,4,8,12]. The association between calculated BMCSS and milk yield could probably be explained by a dilution effect [2]. It could further be speculated whether the lower culling rate due to udder disorders in larger herds could be an effect of problems with higher calf mortality rates and higher cow mortality, resulting in reduced ability of planned removal of cows. Management system was the only herd characteristic investigated that was not statistically associated with any of the udder health indicators which is in accordance with recent Swedish studies of differences in udder health between organic and conventionally managed herds but other studies have shown result in favor of organic or conventionally managed herds (5, 9, 13).

CONCLUSIONS

In Sweden organically managed dairy farms, automatic milking systems, proportion of Holstein cows and herd size are increasing. There is a concern if these trends will have a negative impact on animal welfare in the Swedish dairy production. The associations between udder health measures and herd characteristics found needs to be considered to maintain good animal health in the Swedish dairy herds. Efforts need to be focused on preventive measures to stop the increasing BMSCC.

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LUNG ALTERATIONS AND THEIR AETIOLOGY IN ORGANIC KEPT LAMB

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SUMMARY

This paper describes path morphological, bacteriological, and electron microscopically investigations of lungs from organic kept lambs. Aim of the study was to get information about the aetiology, antibiotic resistance and where the alterations developed. About 90% of the lungs showed purulent pneumonia, which arose in more than 50% within a gathering stable, where the animals were kept before slaughtering. Primary pathogen mycoplasma and viral infection seemed not to be important for the genesis of the acute pneumonia, but transport and crowding.

INTRODUCTION

Mannheimia haemolytica, Pasteurella multocida [2], *Mycoplasma ovipneumoniae* as well as Parainfluenza3- and Retrovirus are known as infectious agents causing pneumonia in sheep [5, 7]. Some of them are normal mucosal residents. After some viral infections appear mild acute respiratory symptoms, chronically, mainly lymphocytic inflammations arise because of mycoplasma infection [3]. Severe purulent pneumonia develop from bacterial infections, retro virus infection causes chronically alterations as adenocarcinoma [9] or ovine progressive pneumonia.

In this study organic produced Styrian lambs were brought from the farms to a gathering stable and kept there until a weight of at least 40kg to be submitted to slaughter. During meat inspection health problems, especially of lungs, were found. The study was conducted to find out the causative agents of the lung alterations, whether these developed on the farm or in the gathering stable and the resistance of relevant bacteria against antibiotics.

MATERIAL AND METHODS

From June 2008 to June 2009 1458 lambs were slaughtered and inspected. 212 out of 273 altered lungs have been sent to the IVET Graz, out of them samples were taken for histological examination, bacteriology and electron microscopy. Localization, qualifying and quantifying of alterations [4] was done by macroscopic and histological examination. Bacteriological examination took place by culture and biochemical differentiation (API sytemes, Biomerieux); antibiotic resistance testing was performed for relevant bacteria by agar diffusion test. Mycoplasma were isolated on specific agar plates under micro aerophil conditions and differentiated by mycoplasma-specific PCR of the 16S-23S Intergenic Spacer (IGS)-Region and subsequent restriction analysis [8]. Electron microscopy was done with the method of "negative staining" after suspension and ultracentrifugation of the lung material. Age determination of the alterations was performed on H.E stained slices by evaluation of characteristic inflammatory changes [1, 10].

RESULTS

90 % of the lungs presented a purulent, partially apostematous bronchopneumonia with desquamation of alveolar macrophages (alveolar histiocytosis) (Fig.1,2 and 3). Hyperplasia of bronchial associated lymphoid tissue (BALT) occurred in 57 % (Fig. 2), interstitial pneumonia in 35 % and bronchio-alveolar hyperplasia in 25 %. In 20 % of the cases fibrosis of lung tissue was diagnosed. Two lungs showed early stages of pulmonary adenocarcinoma Fig. 3 and 4). Bacteria were isolated from 157 out of 212 organs, of which 51 % were *Mannheimia haemolytica,* 21 % *Pasteurella multocida,* 7 % *Moraxella sp.,* 3% *Pasteurella sp.*. *M. haemolytica* and *P. multocida* were highly sensitive to most of conventionally used antibiotics as amoxicillin, ampicillin cephalolexin, florfenicol and varying degrees resistant to neomycin, kanamycin, streptomycin and gentamyin. In 28 % of the lungs mycoplasma were found; in 24% *M. arginini*, in 14% *M. bovigenitalium* and in 1% *M. ovipneumoniae.* 17 % of the lungs contained viruses; 10% Retrovirus, 4% Herpesvirus, 2% Mammal Orthoreovirus and 1% Adenovirus. The length of stay in the gathering stable differed between 1 and 131 days. 57% of the alterations developed in the

gathering stable, 27% of the animals had pneumonic lesions that were older than this period, there fore they already arose on the farm.

DISCUSSION

In meat inspection purulent pneumonia is a quality problem. 90% of the lungs in this study showed purulent alterations; in 70% pathogen bacteria were isolated. Mucosa residents *(M. haemolytica, P. multocida)* become pathogen in stress situation like transport, crowding or contact with chronically ill animals. Existing mycoplasma and viral infection favour bacterially induced inflammation. About 60% of the purulent pneumonia developed in the collecting stable, which supports this thesis. Ovine Lung adenocarcinoma is caused by a lenti-(retro)virus; clinically symptoms appear normally up from 1,5 to 2 years [9]. The 2 histological diagnosed cases prove that incubation periods can be much shorter. Bronchiolo-alveolar hyperplasia is discussed to be a praecancerogen stage of lung adenocarcinoma in human [6].

CONCLUSIONS

Visible alterations of the lungs result mainly from bacterial infection. Although purulent pneumonia was seen in nearly all altered lungs, none of the sheep showed clinically symptoms that prohibited shlaughtering. To minimize lung alteration and improve the quality of carcasses farming and transport should be improved, "all in-all out" system has to be established in the gathering stable to reduce the length of stay there and chronically ill animals must be eliminated.

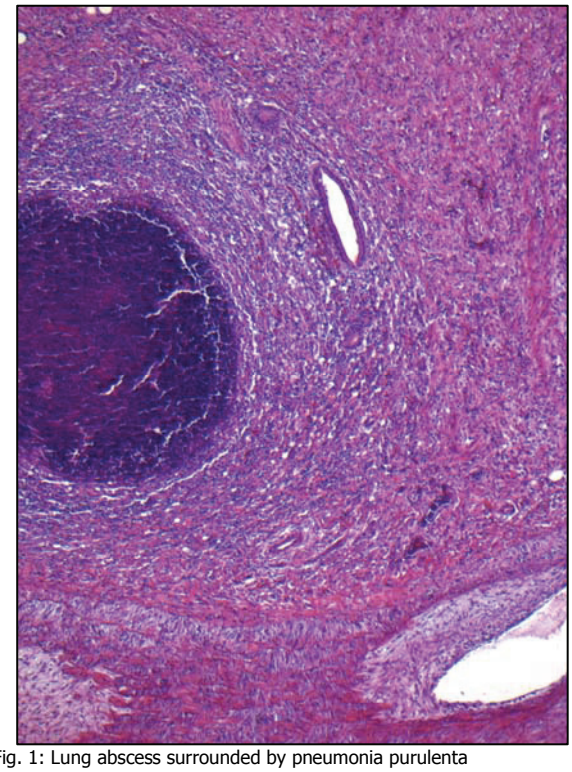

Fig. 1: Lung abscess surrounded by pneumonia purulenta

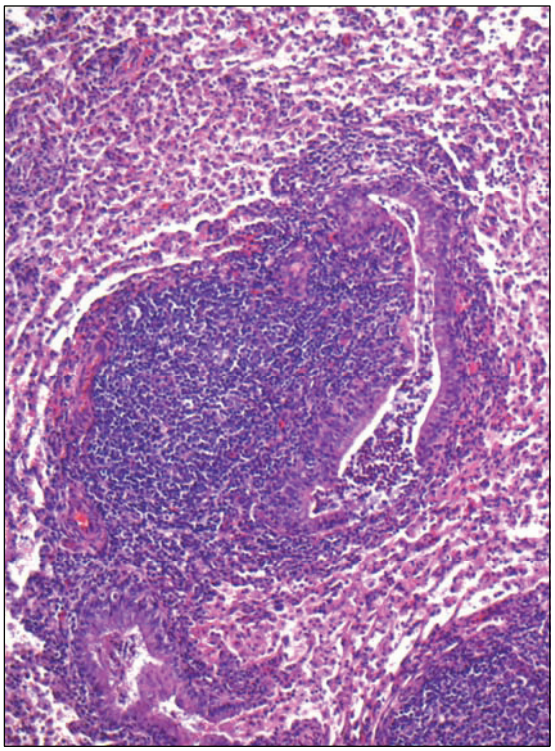

Fig. 2: BALT hyperplasia. Acute broncho- granulation tissue

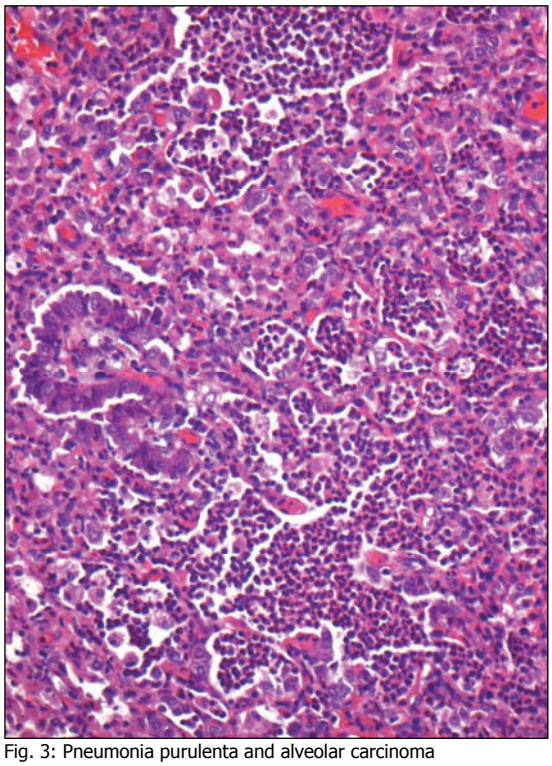

Fig. 3: Pneumonia purulenta and alveolar carcinoma

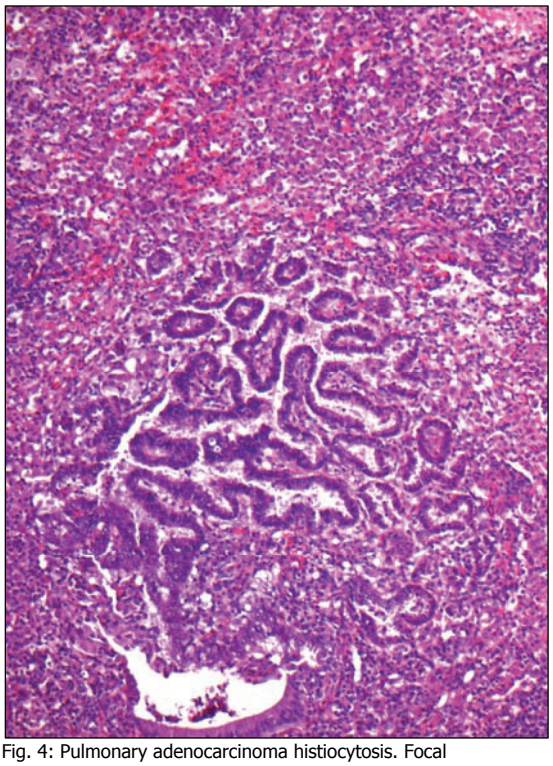

Fig. 4: Pulmonary adenocarcinoma histiocytosis. Focal

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ULTRASONIC EVALUATION OF HEAT SRESS ON OVARIAN ACTIVITY IN BUFFALOES

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Key words: Heat stress- ultrasonic- ovarion activity mineral mixture

INTRODUCTION

High ambient temperature, relative humidity and feed supply are identified as causes of summer anoestrus in buffaloes. Thermal stress during summer causes lack of LH surge which contribute to ovarian inactivity. In heat stressed dairy cows, there a reduction in dry matter intake (rock, et al., 2001) which prolongs the period of negative energy balance then by turn lead to an-ovulatory condition in dairy cows during the early post-pastum period (buttler, 2001, Rajani and Nobukazu 2008). Previous studies of Adams and Robinsan (1994) showed that addition of Amino acids as glutamine indirectly increase GnRH and LH secretion and directly on ovarian functions. Moreover smith and steworts (1990) and Nattle et al., (1997) showed that addition of amino acids alone or with glucose (Downing et al, 1995) induced dynamic effects on ovulation. The probability of gonadorophin in combination with prostaglandin F $2\propto$ as treatment results in 50-100% ovulation (troxiel et al., 1983). Besides the hormonal treatments, application of douching were done (Abou-Zina et al., 2009) who applied douching with antioxidants to improve fertility. In several studies. Ultra sonography has been used to describe changes accwing in the reproductive tracts of cows and heifers throughout the estras cycle (Texzano et al., 2007). Previous knowledge on changes in the reproductive organs are mainly confined to rectal examination. There for the objective of the present work is to study some managerial treatment as mineral & amino acids mixture and hormonal treatment with douching application to experimental group on ovarian morphological characteristics and functions.

MATERIALS AND METHODS

Seven anoestrus multiparious buffaloes and heifers raised at National Research Center farm were used. In addition to farm ration a commercial preparation (vigest) ® composed of amino acids and mineral mixture besides dextrose and electrolytes were given to animals by rate of 80 ml / animal daily in drinking water for a period of one month.

Another managerial treatment (hormonal) by injection of gonadotrophic releasing hormone (GnRH) . Receptal® (5ml/animal i.M) and synthetic prostaglandin (Estromate ® 2ml /animal 1.M) was applied for all animals. Moreover, application of water douches two times daily at mid day with one hour interval were done from the beginning of hormonal treatment till the appearance of oestrus. The ovarian activity and follicular dynamics in the experimental group were detected and followed every 5 days by rectal examination and ultrasonography (M. 1000 linear scan probe 3.5 M ZH).

RESULTS

First exper.

A- Morphological characters

Table, 1. showed wide variation in dimensions of the left ovary of both heifers No. 509 and 511.

Also increased ovarian size in both heifers is the direct response to reproductive hormones at that age (18 month – age of puberty).

B- ovarian activity

Table, 2 and Fig. (1) category A2 revealed improvement in ovarian activity of animals number 492 but without developmental growth.

On the other hand animal number 511, 476 category B and number 512 category C, showed ovarian response (follicular development) after addition of minerals.

Table, 1. Morphology: Effect of adding minerals and amino acids mixture on morphological characters of the ovaries.

Animal number	Dimensions			
	Before addition		After addition	
	length (cm)	width (cm	length (cm)	width (cm)
509	2.00	1.70	1.40	3.40
511	1.90	0.70	2.30	1.00
491	1.8	1.7	2.0	1.4
492	Small follicle not measured		0.6	0.4
476	Not measured		1.5	0.6
512	0.6		1.1	1.6

Table, 2 Follicular activity: Effect of adding mineral and animo acid mixture (vigest) on follicular activity

Animal number	Category	Pretreatment	Post treatment
491, 509, 497	A	Smooth	Smooth
492	A	Follicular	The same follicular without growth
511	B	No follicles	Two follicles
476	B	One follicle	Two follicles
512	C	One follicle	Three follicles

Second experiment

(A) Morphological characters

Table (1) showed changes in dimensions of ovaries of both groups as hormonal treatment for estrous induction.

B. Follicular activity:

Table (2) Fig (1,A), ultrasonographic (U.S) examination revealed para ovarian cyst in animal no 497 with appearance of estrous signs but not standing for male while in animal no 491 U.S examination revealed follicle formation. on the other hand only enlargement in size of ovaries in animal No 512. This means that ovarian activity were improved as a result of douching.

In case of experimental group, the condition of the ovaries are some white better than that of the control ones as shown from table (2) and sonography images (Fig, (1, B) specially animal numbers 476 and 492.

Table (1) Effect of hormonal treatment and douching on ovarian dimensions (cm)

Animal Number / Exper. Ovary	Ovary	Before estrous	After estous & douching
511	R.0	<0.5	Larger ovarian size 2.2 x 1.4
509	L.O R.O	< 0.5	1.3 X 1.1 large ovarian size
492	R.O (follicle)	0.9X 0.6	1.1 x 1.1
476	R.O Follicle	0.5 X 0.5	Large ovarian size with follicle 1.4 x 2.2
491 control	R.O	C.L. 1.5 x 2.7	Small follicle
497		Paraovarian cyst 1.1 x 1.7	Para Paraovarian cyst 4.0 x 50 cm 2.1x 0.6
512	R.O.	C.L. 1.4 x 1.7	1.3 x 2.4

2. Follicular activity

Table (2): Effect of douching on ovarian activity in buffaloes during summer season.

Group Treatment	Control group			Experimental group			
	491	497	512	476	492	509	511
Finding before 1st induction of estrous	C.L.	Para ovarian cyst	R.O C.L	Smooth	L.O Small follicle	L.O Large Smooth	Smooth
Finding before 2nd induction of estrous	L.O Follicle	Para ovarian cyst	R.O Smooth	Small Follicle	Follicle	Large And Smooth	Large And smooth

R.O= Right ovary

L.O = Left ovary

DISCUSSION

The results of the first experiment as shown (table , 1) indicated a wide variation in the dimensions. of left ovary of both heifers numbers 509 and 511. similar observation were noticed at the age of puberty (Terzano et al., 2007) as a direct response to reperoductive hormones. With regard to ovarian activity animal numbers (512,511 and 476) showed follicular developments afte addition of minerals & amino acids mixture. These results agree with the findings of smith & Stwart (1990), Nottle (1997) and Newar et al., (2000) who noticed 80% of animals exhibited estrus within 120-132 days of mineral supplementation compared to 20% in non-supplemented group which occur within 168-178 day of experimental period.

The results of the 2nd experiment denotes also changes in morphological characters as dimensions of ovaries of both groups after hormonal treatment (table,1). This findings coincides with that reported (De Rensis et al., 2002).

Table (2), figure (1,B) showed ovarian activity (presence of follicles) which indicate improvement as a result of hormonal treatment and douching application in animal numbers (476, 491 and 512 respectively). These results agree with that reported (Abou-Zina et al., 2009) who stated that the application of douching with zinc methionine improved fertility but with lower rate (20% compared to 50%).

CONCLUSIONS

Results of morphological characters and imaging of sonographic follicular development reached up to 43% in mineral mixture addition and 50% in dushing management.

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ANNEX

Ultrasonic Images

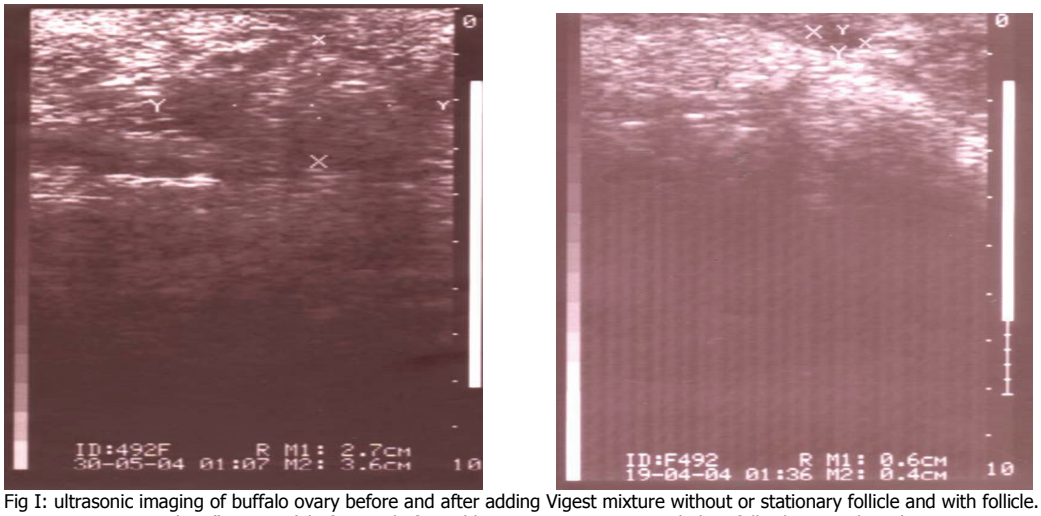


Fig I: ultrasonic imaging of buffalo ovary before and after adding Vigest mixture without or stationary follicle and with follicle. Category A-2: Animal No "497, 492) before and after adding Vigest mixture revealed no follicular growth in their ovaries.

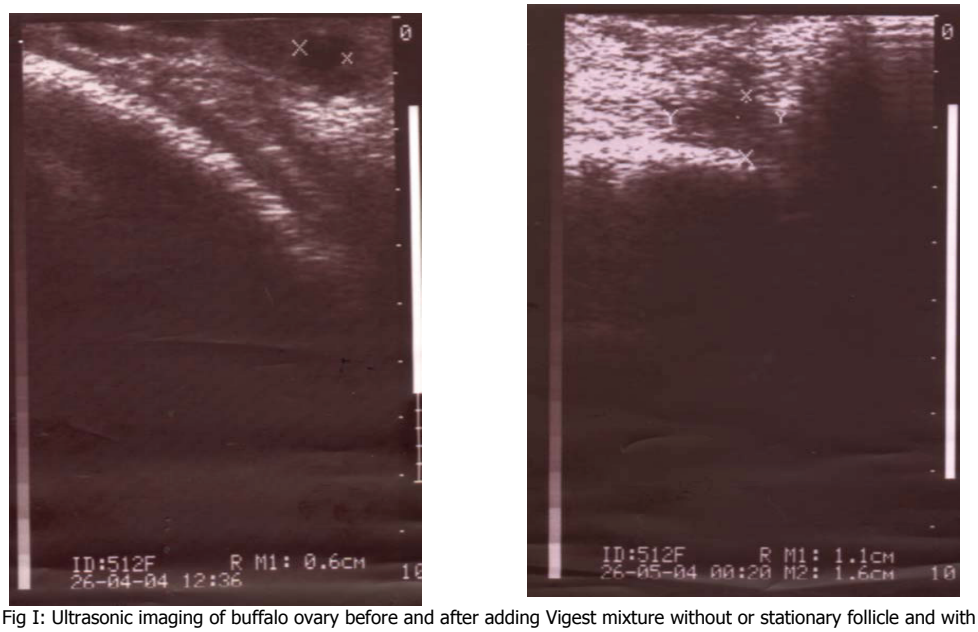
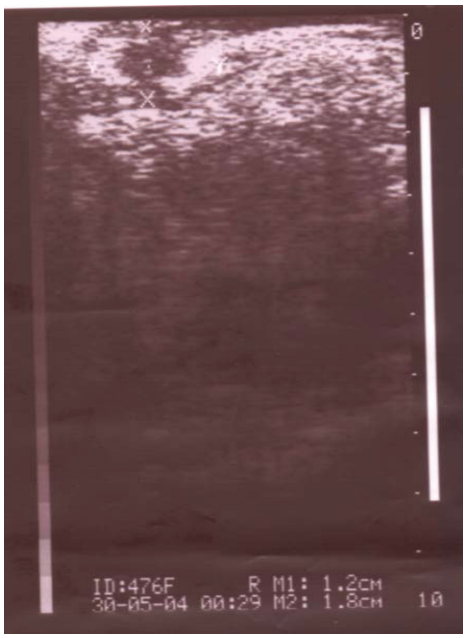
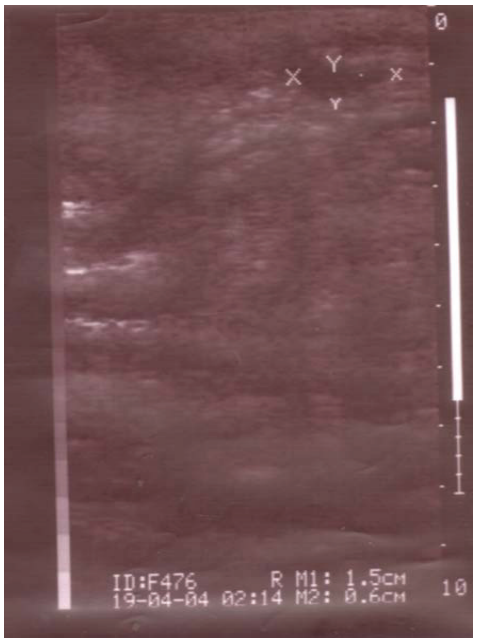
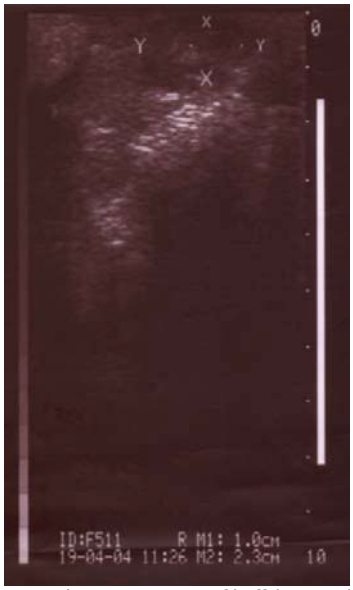
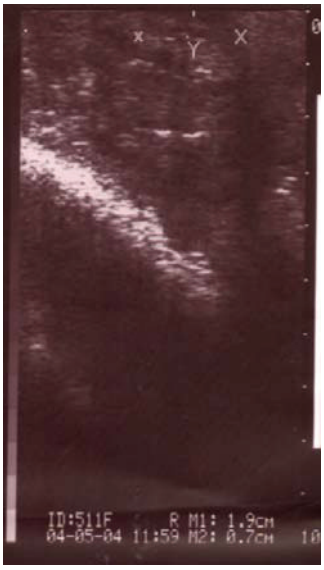


Fig I: Ultrasonic imaging of buffalo ovary before and after adding Vigest mixture without or stationary follicle and with follicle. Category C: Animal No. (512) after adding mixture rapid enhancement.









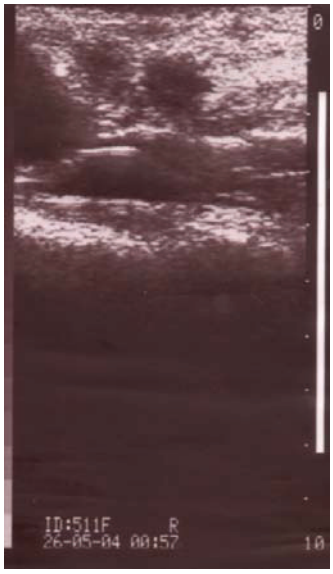


Fig I: Ultrasonic imaging of buffalo ovary before and after adding Vigest mixture without or stationary follicle and with follicle.
Category B: Animal No " 511,476"
In animal No. 511 before adding of Vigest mixture, ovary appears smooth followed by activity after adding mixture; in animal 476 enhancement of activity.

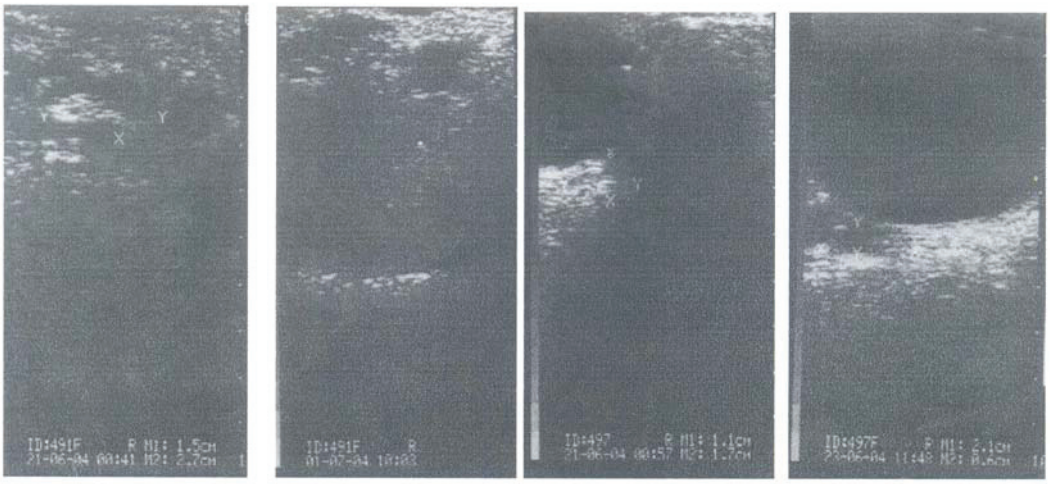
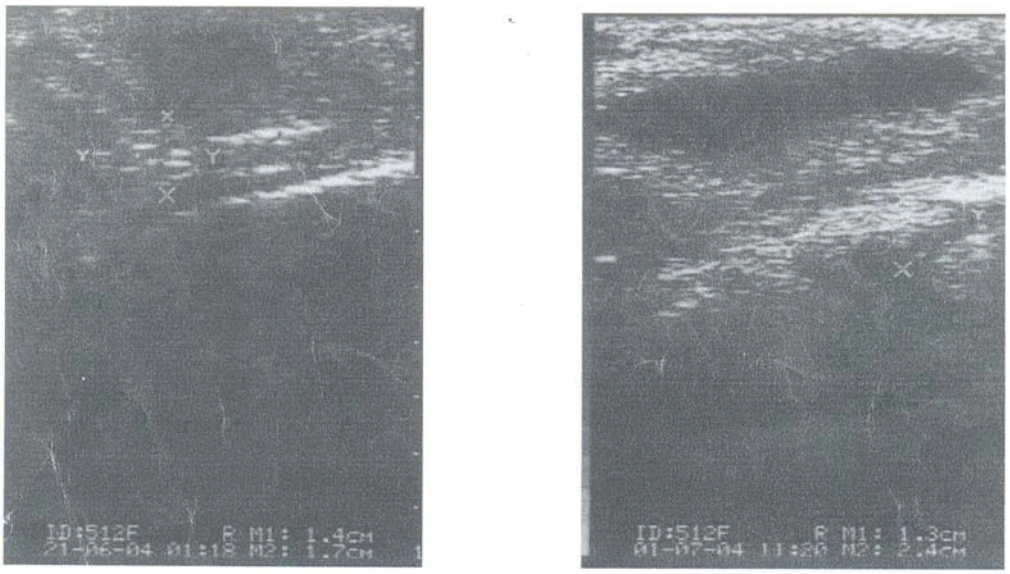


Fig.(١-A): Ultrasonic imaging of buffalo ovaries for control group.

Control animal (١): Animal No."٤٩١, ٤٩٧" only injected for estrous induction Animal (٤٩١), showed CL.; Animal (٤٩٧), showed paraovarian cyst.



Control animal (٢): Animal no."٥١٢" Right ovary showed CL.

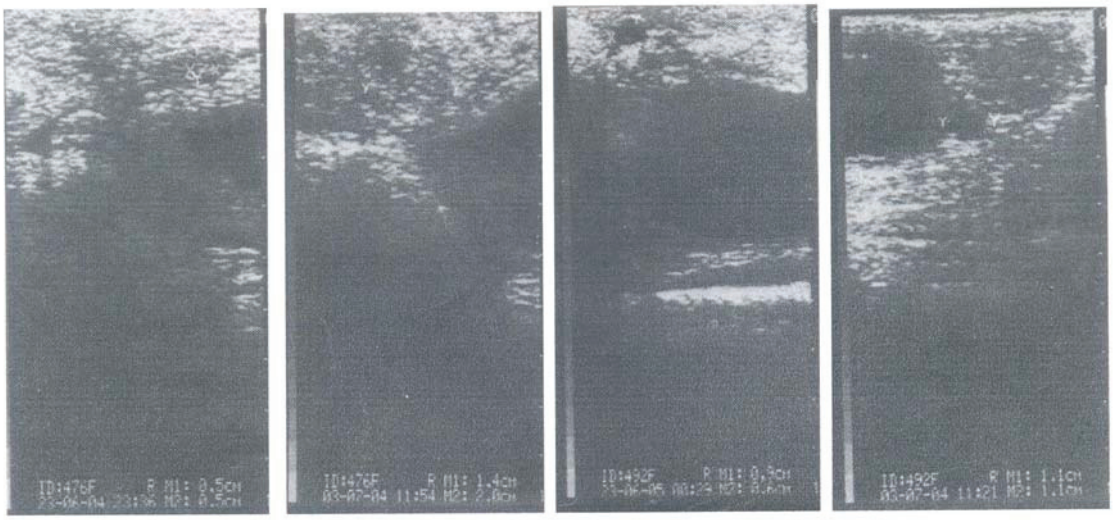
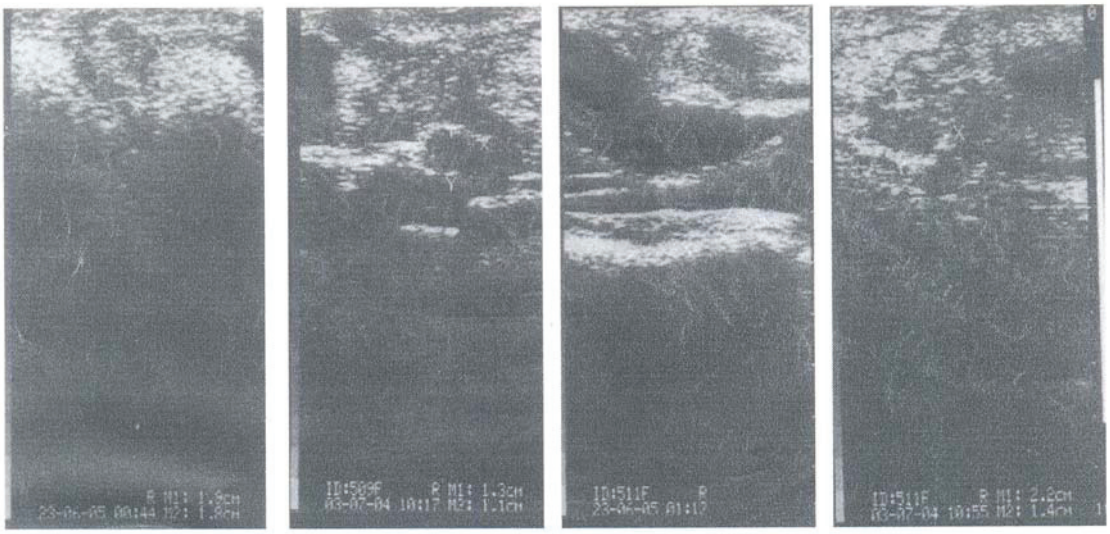


Fig.(١-B): Ultrasonic imaging of buffalo ovaries for experimental group (Douching)

Experimental animal No.(١): Animal No."٤٧٦, ٤٩٢" induction of estrous and douching.

Animal no. ٤٧٦, sonographic image showed smooth ovary before estrus

Animal no. ٤٩٢, from sonographic image showed small follicle after ١st and ٢nd injection.



Experimental animal (٢): Animal no."٥٠٩, ٥١١" both heifers showed large and smooth ovaries without any change.

INFECTION KINETICS AND HOST SPECIFICITY OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN PIGS

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SUMMARY

In this study, we investigated the colonisation kinetics and host specificity of three different clonal lines of MRSA (ST8, ST9 and ST398) in pigs.

MRSA prevalence on skin, nasal mucosa, conjunctiva, feacal shedding and distribution patterns of MRSA in internal organs in weaning piglets are studied.

MRSA strains of the clones ST8, ST9 and ST398 were able to colonize all pigs and to spread within the body of the inoculated animals. Because of the regularly observed colonization of mandibular and ileocolic lymphnodes it must be assumed, that MRSA can really "infect" susceptible pigs.

INTRODUCTION

The transmission of MRSA and its different clonal lines in practice, as well as the influencing factors of a transmission are largely unclear in the field of animal husbandry (2). It is known that MRSA can pass from animals to humans. Moreover people with direct daily contact to animals such as farmers, holders of livestock and pets, veterinarians and the staff in slaughterhouses show a higher colonization with MRSA than those without frequented contact to animals (1).

With regard to MRSA the number of studies in larger domestic animals is increasing. However these are mainly field studies dealing with the prevalence of MRSA (3) carried out in practice, with naturally contaminated animals in farms, barns and slaughterhouses. There are no previous reports about the rate of recovery of MRSA in internal organs with regard to animal experiments with large domestic animals.

MATERIAL AND METHODS

A pool of 58 piglets were randomly divided into four test groups and one control group. Three test groups were infected with MRSA ST8 (isolated from pig), MRSA ST9 (poultry) and MRSA ST398 (pig), respectively. The fourth group was a fusion of MRSA ST398 infected and non infected "sentinel" animals. The infectious dose of 5,0 x 10^8 cfu/animal was determined in preliminary animal studies.

Clinical symptoms, the nasal, conjunctival and skin colonisation of MRSA, faecal excretion and organ distribution of MRSA, as well as different environmental samples were examined.

RESULTS

After nasal application with MRSA piglets of all four test groups showed no clinical signs of an MRSA infection. MRSA was present on the nasal mucosa, skin and conjunctiva in all four test groups, including sentinel animals. Likewise, faecal excretion and internal colonization of MRSA ST8, ST9 and ST398 could be shown in each group. Colonization was less efficient with the MRSA ST9 strain (originated from poultry) as indicated by a lower proportion of positive nasal swabs and a numerically reduced colonization of internal organs, feces and skin, in comparison to the ST8 and ST398 groups.

DISCUSSION

MRSA strains of the clones ST8, ST9 and ST398 were able to colonize all pigs, to spread within the body of the inoculated animals and to contaminate the environment throughout the whole study period. Results of our study suggest existing strain specific colonization mechanisms of the different MRSA types that might be associated with a certain degree of host specificity. Furthermore, because of the regularly observed colonization of mandibular and ileocolic lymphnodes it must be assumed, that MRSA can really "infect" susceptible pigs.

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MRSA IN AIR OF GERMAN BREEDING AND FATTENING PIG FARMS

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[2] *University of Veterinary Medicine Hannover, Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour, Hannover, Germany;*

SUMMARY

This paper describes the occurrence of MRSA in pig house air as well as in samples from pigs and their housing environment. 27 pig barns of different sizes and management schemes have been extensively studied. The animals themselves but also other environmental samples like dust, boot socks or feaces showed a high rate of MRSA colonization or contamination, respectively. The MRSA prevalence was significantly higher in fattening farms than in breeding farms. MRSA was regularly found in the air of the investigated pig houses. This indicates that aerial emissions from piggeries seem to be an important transmission way of LA-MRSA within pig herds but also between neighbouring farms.

INTRODUCTION

So called LA-MRSA (livestock associated methicillin resistant *Staphylococcus aureus*) can be present in various farm animal species especially in pigs (4). They belong predominately to a specific sequence type which usually does not appear in humans but can be transmitted from animals to humans (1, 6). However, the transmission routes of MRSA in livestock production are still scarce. Therefore studies, funded by the German Federal Ministry of Food, Agriculture and Consumer Protection, were carried out about the occurrence of MRSA in pigs, their keeping their environment and in animal house air. This paper reports about results from breeding and fattening pig farms.

MATERIAL AND METHODS

Selection of farms

27 MRSA positive pig barns (14 fattening farms, 11 breeding farms and 2 farrow-to-finish-farms) were investigated. The farm size varied between 700 und 12.000 animals (mean about 3500) in fattening farms, 90 to 1600 sows (mean about 540) in breeding farms and 2 farrowing-to-fattening farms with 960 and 2700 weaners.

Sampling at pig farms

Nasal and skin swabs were collected from 60 pigs within each barn. Animal house air was sampled by two different methods in parallel, impingement and filtration. For impingement the All-Glas-Impinger (AGI-30) filled with 30 ml phosphate buffered saline (PBS) was used. For filtration a personal air sampler pump (GilAir-5) with an I.O.M. dust sampler and a polycarbonate filter was applied. Air samples were collected at three different spots in the barn in 1.50m height above the floor. Furthermore environmental samples like boot swabs, pooled dust samples, pooled faecal samples and samples from feed were taken.

Laboratory analyses

All samples were investigated qualitatively by enrichment. Air samples, pooled dust samples and nasal swabs were analyzed also quantitatively. Nasal and skin swabs were investigated pooled and individually. Five swabs in general formed one pool resulting in 12 pools per farm in which the first swab of each pool was analyzed individually. For enrichment Mueller Hinton Broth with 6.5% NaCl was used and for selective detection of MRSA a commercial chromogenic MRSA screen agar.
Confirmation of MRSA suspected isolates was done by coagulase reaction and PCR for a *S. aureus* specific DNA-fragment as well as the presence of the *mec*A gene.

Statistical analyses

Statistical analysis was conducted using the software SPSS 16.0 (SPSS, Inc., Chicago, IL). To calculate the differences between detection rates of different sample types the χ^2 test was performed. Differences were considered significant if p was <0.05. Cohen´s kappa was calculated to compare the two air sampling techniques.

RESULTS

Main results are shown in Figure 1. MRSA was found in 85.2% of the investigated barns in the stable air (at least one out of all six air samples was MRSA positive) with a higher detection rate using impingement. Also in other environmental samples like dust, boot socks, faeces and feed MRSA could be found regularly. Furthermore, there was a high occurrence of MRSA in pooled as well as individually analyzed animal samples (78.8% - 88.3%). Thereby, the MRSA detection rate was higher than in breeding farms in nearly all samples.

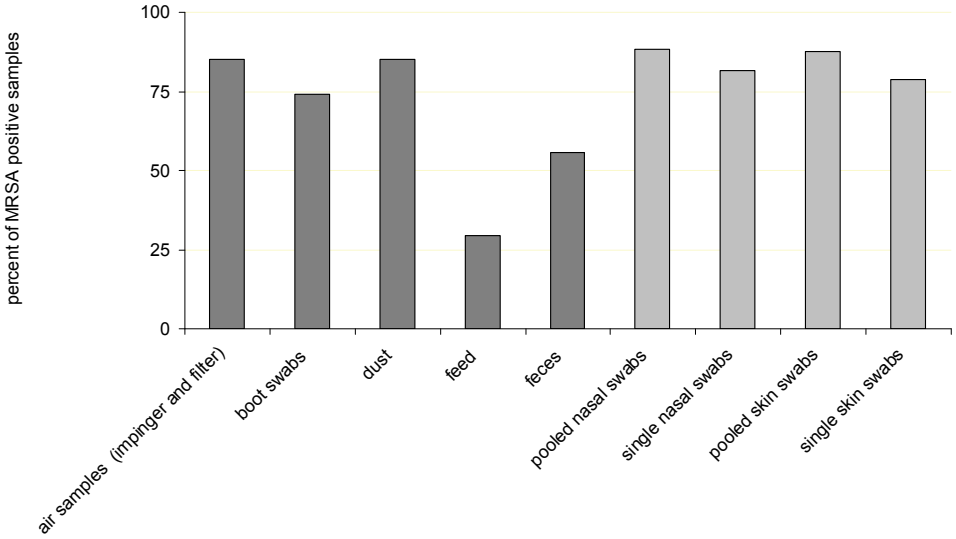

Figure 1: MRSA detection rates in all investigated stables (n = 27). MRSA was detected in air samples (at least one out of six air samples collected in one stable was MRSA positive), boot swabs (n = 27), dust (n = 27), feed (n = 17), faeces samples (n = 27), pooled nasal swabs (n = 325), single nasal swabs (n = 325), pooled skin swabs (n = 325) and single skin swabs (n = 325).

DISCUSSION

MRSA was detected in the air of most stables (85.2 %). Impingement turned out to be significantly more sensitive than filtration. This is probably caused by the more gentle collection in the impinger liquid where microorganisms are less exposed to mechanical stress and exsiccation during air sampling than during filtration. The results indicate that dust is a main carrier of MRSA in air since dust was always MRSA positive whenever the microorganisms were found in the air. The high prevalence of MRSA in air samples as well as in dust samples reveals the difficulties to reduce a spread of the bacteria within the animal house. In other environmental samples like boot socks, faeces or feed MRSA could be regularly found, however, the prevalence in pooled faeces samples and feed samples was lower. It can be assumed that there is no initial contamination but a secondary contamination due to dust and animals themselves.

Besides the spread of MRSA within the animal house the microorganisms are also emitted in the ambient air outside the barns. In terms of prevalence among the animal samples MRSA was widespread (78.8% - 88.3%). However, other studies resulted in lower MRSA prevalences by sampling nasal swabs (3) (5). The reason for higher prevalences in the present study might be the selective choice of sampled farms by the initial screening of dust samples. Consequently only farms classified MRSA positive were investigated and a higher prevalence among the animals could be expected. When comparing fattening and breeding farms, the prevalence of MRSA in animals and in samples of air and environment (except samples of feed) of fattening farms was significantly higher than in breeding farms. This may be related to various factors like stable size, transportation or contact to many other pigs (2).

CONCLUSIONS

This study was the first survey with extensive investigations about MRSA in air and housing environment of fattening and breeding pigs. There was a high prevalence of MRSA in samples taken from animals and in the environment of the animals. Especially dust and air turned out to be a possibly important transmission way of MRSA within pig herds but assumingly also between individual farms.

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ANTIMICROBIAL RESISTANCE OF STAPHYLOCOCCI ISOLATED FROM MASTITIS MILK SAMPLES FROM CATTLE IN BRAZIL

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SUMMARY

Bovine mastitis is the main cause of economic loss in milk production worldwide and coagulase positive staphylococci are the agent most frequently associated with the disease. The aim of this study was to assess the prevalence of coagulase positive staphylococci in milk samples from cows with clinical or sub clinical mastitis and to assess the sensitivity of these pathogens against some antibiotics used for treating of mastitis. From the 50 milk samples obtained from cows with clinical or sub clinical mastitis from different farms in Bahia state, Brazil, were isolated 103 morphologically distinct bacterial colonies, which 59 strains was *Staphylococcus* spp. and 54 of those strains (92%) were producing the enzyme coagulase. The genus *Staphylococcus* spp. was isolated in 40 (80%) samples, proving to be the major agent of bovine mastitis in the farms studied. The agar disk diffusion was used for susceptibility testing of mastitis pathogens and all isolates were subjected to the following antimicrobials: gentamox (35μg), ampicillin (10μg), cephalothin (30μg), tetracycline (30μg), erythromycin (15μg), oxacillin (1μg); ciprofloxacin (5μg), neomycin (10μg), norfloxacin (10μg), penicillin G (10μg) and sulfametoxazol + trimethoprim (25μg). The interpretation of the results followed the recommendations of the National Committee for Clinical Laboratory Standards. The results showed that the most effective drug in vitro against coagulase positive staphylococci were ciprofloxacin (90%), norfloxacin (78%), sulfa+ trimethoprim (70%), and cephalothin (68%). Penicillin G (40%) and ampicillin (44%) showed the lower indices of sensitivity for the treatment of clinical or sub clinical mastitis caused by these microorganisms in Bahia's farms. The present study confirmed the *Staphylococcus* spp. as the main role in the etiology of bovine mastitis in Brazil, which reinforces the concern about the possibility of pathogens being served by food of animal origin like milk and dairy products to humans can cause serious damage to public health.

Keywords: 1. Bovine mastitis; 2. *Staphylococcus*; 3. Antimicrobial resistance.

INTRODUCTION

Brazil has the largest commercial herd of cattle in the world, with about 215 million head, and is the sixth largest producer of milk, representing a key role in the development of agriculture in the country. However, it is known that many problems in the sanitary conditions or management of livestock may result in loss of milk quality and cause serious harm to public health. Given that reality, mastitis in cattle is considered the main disease that affects dairy cattle, not only in Brazil but worldwide, causing large economic losses to producers of milk and dairy industry (6). Among the bacterial species involved in the etiology of infectious mastitis, the genus *Staphylococcus* occupies a prominent role in cattle, and some strains are resistant to several antibiotics used routinely in the treatment of mastitis. The increasing presence of multidrug-resistant strains further hindering treatment (5). Thus, whereas *Staphylococcus* stand out as the most important microorganisms in contagious mastitis, with difficult to treat because of high antimicrobial resistance, the aim of this study was to investigate the presence of *Staphylococcus* strains as agents of bovine mastitis and verify the antimicrobial susceptibility of them to better understanding of the etiology and therapy of bovine mastitis in Bahia state, Brazil.

MATERIAL AND METHODS

Were evaluated 190 lactating cows from farms in Bahia state, Brazil. Cows were subjected to clinical examination and then carried out the screening for presumptive diagnosis of mastitis (California mastitis test) to detect sub clinical mastitis. From of all animals with clinical or sub clinical mastitis were collected, in order entirely random, 50 milk samples were seeded in defibrinated sheep blood 5% for microbiological evaluation. The samples were incubated at 35°C for a period of 24 to 48 hours, when the readings were taken. The colonies were analyzed for morphology, color and production of hemolysis. Then, the colonies of different characteristics were submitted to

Gram staining for microscopic observation. All bacteria with characteristics of *Staphylococcus* genus were tested for coagulase production. Finally, to determine the pattern of antimicrobial susceptibility testing was performed by agar diffusion (2) and tested the following antibiotics: gentamox (35μg), ampicillin (10μg), cephalothin (30μg), tetracycline (30μg), erythromycin (15μg), oxacillin (1μg), ciprofloxacin (5μg), neomycin (10μg), norfloxacin (10μg), penicillin G (10μg) and sulfametoxazol + trimethoprim (25μg). The interpretation to the sensitivity profile was based on Clinical and Laboratory Standards Institute.

RESULTS

Of the 50 milk samples from clinical or sub clinical mastitis analyzed in this study, *Staphylococcus* spp. was isolated in 40 (80%), proving to be the major causative agent of bovine mastitis in the farms studied. Of this total, 26 (66.7%) strains were producers of hemolysin. Although *Staphylococcus* spp. has been the most frequently isolated agent, other groups of microorganisms were identified, which were often responsible for the mastitis or were associated with other etiological agents involved in cases of mastitis in this study (Table 1).

Table 1: Frequency of microorganisms isolated from 50 samples of milk obtained from cows with clinical mastitis or sub clinical infections with single or mixed dairy farms in Bahia state, Brazil.

Microorganisms	Absolute Frequency	Relative Frequency
Staphylococcus spp.	18	36%
Staphylococcus spp. + Gram positive rods	14	28%
Staphylococcus spp. + Gram negative rods	5	10%
Staphylococcus spp + Gram positive + Gram negative rods	2	4%
Staphylococcus spp. + Yeasts	1	2%
Gram positive rods	4	8%
Gram negative rods	1	2%
Gram positive + Gram negative rods	2	4%
Gram positive cocci + Gram negative rods	1	2%

Were isolated and stored 59 strains of *Staphylococcus* spp., all brought to the coagulase test, and was possible verify that 54 (92%) of the colonies were producing the enzyme coagulase and 5 (8%) were coagulase negative. After that, four strains not grew in BHI broth, and only 50 strains identified as coagulase positive were subjected to antimicrobial susceptibility testing (Table 2).

Table 2: Antimicrobial resistance of 50 strains of coagulase-positive staphylococci isolated from cows with clinical or sub clinical mastitis of dairy farms in Bahia state, Brazil.

Antimicrobial	Sensitive	Intermediate	Resistent
Gentamox	34 (68%)	12 (24%)	4 (8%)
Ampicillin	22 (44%)	0 (0%)	28 (56%)
Norfloxacin	39 (78%)	7 (14%)	4 (8%)
Tetracycline	29 (58%)	4 (8%)	17 (34%)
Cephalothin	34 (68%)	1 (2%)	15 (30%)
Ciprofloxacin	45 (90%)	3 (6%)	2 (4%)
Erythromycin	33 (66%)	1 (2%)	16 (32%)
Neomycin	28 (56%)	18 (36%)	4 (8%)
Oxacillin	32 (64%)	1 (2%)	17 (34%)
Penicillin	20 (40%)	0 (0%)	30 (60%)
Sulfa+Trimethoprim	35 (70%)	2 (4%)	13 (26%)

As shown in Table 2, antimicrobial that showed greater action *in vitro* against *Staphylococcus* coagulase positive were ciprofloxacin (90%), norfloxacin (78%), sulfa **+**trimethoprim (70%), cephalothin and gentamox (68%). Since penicillin (40%) and ampicillin (44%) were the antimicrobials had lower effect on the strains of coagulase-positive staphylococci examined in this study. According to the profile of antimicrobial susceptibility, it was possible to verify the presence of 21 strains (42%) multidrug resistant, being observed also strains resistant to up to nine of the 11 drugs tested (data not shown).

DISCUSSION

In another study conducted in the state of Minas Gerais, Brazil, were analyzed 50 milk samples from cows with clinical and sub clinical mastitis, it was possible to determine the prevalence of 60% of *Staphylococcus* spp. (7). The present study revealed that was found other Gram positive cocci, Gram positive and Gram negative rods and yeasts, thus demonstrating the variety of microorganisms that can cause infectious mastitis. Other studies also report a wide variety of microorganisms as etiologic agents of bovine mastitis (1, 3). Regarding the antimicrobial susceptibility test, similar results were observed by Andrade et al. (1), where only 23.7% of strains of *S. aureus* showed sensitivity to penicillin. Even more worrying data were reported in Brazil (4), were *S. aureus* isolated from cows with mastitis showed very low rates of susceptibility to penicillin (2.8%) and ampicillin (4.2%). It is believed that this high resistance to β-lactamic antibiotics is mainly because the production of the enzyme-mediated plasmids from bacteria. In addition, β-lactamic antibiotics are widely used for treatment of intramammary infections, contributing further to the possibility of bacterial resistance against these antimicrobials. The large number of multiresistant *Staphylococcus* strains isolated, demonstrating the need to perform microbiological tests and antimicrobial susceptibility testing for the mastitis treatment is done correctly and effectively.

CONCLUSIONS

The genus *Staphylococcus* was the main isolated from clinical or sub clinical infections mastitis in milk cows of Bahia's farms in Brazil. The number of resistant strains suggesting the inappropriate use of antimicrobial agents in the treatment of clinical or sub clinical mastitis and probably other infectious processes in livestock, and serves as a warning to microbial resistance to drugs used inappropriately. Finally, it is known that the contaminated milk and milk products can pose serious risks to public health.

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DETECTION OF AIRBORNE MRSA IN AND AROUND PIG FARMS

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SUMMARY

The objective of this study was to investigate LA-MRSA emissions from MRSA positive pig holdings. Six MRSA positive pig barns (two breeding, four fattening) from six different farms were included in the study. Each barn was investigated four times in about three month intervals. Inside the animal houses air samples and samples from animals were taken. Outside air samples were taken at different distances downwind (50m, 150m) and upwind (100) the barns by impingement. In 21 out of 24 indoor air samples MRSA was found. Airborne MRSA was detected in low concentrations (1 to14 cfu/m³) only on the downwind side of three barns. There seems to be a permanent entry of airborne LA-MRSA into the vicinity of positive pig holdings which may contribute to the uncontrolled spread and farer transmission of these bacteria. Further studies are necessary to investigate the consequences of this entry.

INTRODUCTION

Livestock associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) is reported widespread in conventional fattening and breeding pig farms in Germany. In a baseline study of EFSA 43.5% of breeding holdings and 41.3% of production holdings were tested MRSA positive (1). Culturable MRSA can be isolated from colonized pigs and from environmental samples of MRSA positive herds such as dust, sedimentation dust and air (2). In clusters or attached to dust particles these bacteria may emit via the exhaust air into the vicinity of contaminated animal houses. However, very little is known about the concentrations of MRSA outside the barns in the air. Therefore air samples were taken at the downwind side and the upwind side (as a control) of six MRSA positive pig holdings.

MATERIAL AND METHODS

Samples were taken from two breeding farms with 500 sows each and from four pig-fattening farms with herd sizes between 1500 and 6300 pigs. The animal houses were sampled four times in about three month intervals. Nasal swabs from 60 randomly selected pigs and three impinger (AGI-30, Ace Glass Inc., Vineland, N.J.) air samples (sampling time 20 min) were taken inside the animal houses at each visit. Simultaneously, impinger air samples (sampling time 90 min) were taken downwind and upwind the barns according to the prevailing wind direction during the visit. Air samplings outside were performed at 50 m and 150 m downwind and at 100 m upwind the barns. The wind direction was measured and monitored by a compass and 3-axis ultrasonic anemometer (Gill Instruments, Hampshire, England).
MRSA was isolated from pooled nasal swabs (5 swabs per pool) and impinger samples by using selective media (CHROMagar MRSA, MAST DIAGNOSTICS) and selective broths (Müller-Hinton-Bouillon with 6.5% NaCl and Trypton-Soya-Bouillon with 3.5 mg/l cefoxitin and 75 mg/l aztreonam). Suspected MRSA colonies were confirmed by testing catalase, oxidase and koagulase reaction and by triplex PCR (3).

RESULTS

The results of the study are summarized in Table 1. Pooled nasal swabs inside the barns were always positive. Only in three cases (12.5% of all samplings) MRSA was not detected in the animal house air. Airborne concentrations of MRSA inside the barns varied between 1 x 10^1 and 7 x 10^3 cfu/m³. Outside the animal houses airborne MRSA was found in five air samples downwind from three different farms. The concentrations were very low, only 1 cfu/m³ was found two times in 150 m distance and 11 to 14 cfu/m³ were measured in distances of 50 m.

Table 1. MRSA detection inside and in the ambient air of six pig farms. Qualitative results from four samplings.

farm no.	downwind from the barn		inside the barn		upwind from the barn
	150m (air)	50m (air)	air	pigs	100m (air)
1	- - - -	- - - -	+ - + +	+ + + +	- - - -
2	- - - -	- - + -	+ + + +	+ + + +	- - - -
3	- - - -	- - - -	+ + + +	+ + + +	- - - -
4	- - - -	o - - -	+ + + +	+ + + +	- - - -
5	- + + -	- + - -	+ + + +	+ + + +	- - - -
6	- - - -	+ - - -	+ + - -	+ + + +	- - - -

+ = MRSA positive sample; - = MRSA negative sample; o = no sample was taken in this interval

DISCUSSION

The results show that in piggeries with a high prevalence of MRSA positive animals also the air can be regularly contaminated. With the exhaust air these LA-MRSA are emitted in the vicinity of the contaminated holding where they are present in air. The relatively low detection frequency in the air (five times out of 47 samplings) on the downwind side of three out of five MRSA positive barns in low concentrations is probably caused by two reasons. Firstly, the concentrations of MRSA in the animal house air are not very high. They present only a small part of the airborne flora in the investigated pig houses (4). Secondly, in the ambient air there is a fast decline of concentration due to dilution with the unpolluted air.

However, the tenacity of LA-MRSA seems to be high enough to enable the bacteria to survive for longer periods in an airborne state, even in outdoor air conditions. This is probably supported considerably by the fact that most airborne bacteria, incl. MRSA, are attached to dust particles and they are emitted together from the positive barns into the environment. No MRSA were found on the upwind side of the farms which shows the most important influence of the wind and the wind direction on the spread of MRSA through the air. A more detailed characterisation of the isolates from indoor and outdoor air samples could give proof whether the strains origin from the respective pig barn or from other sources.

CONCLUSIONS

Airborne LA-MRSA can contaminate not only the indoor air of piggeries but also the ambient air in the vicinity of colonized pig herds. The bacteria are spreading with the prevailing wind direction. However, because wind directions are changing regularly and frequently the surrounding on all sides of the barns is affected by the spread of MRSA. This route of transmission should be considered for various reasons including protection of neighbouring pig farms, residential dwellings or hygienic sensitive areas such as food processing or hospitals. Future studies are necessary to verify aerial transmission distances of LA-MRSA in the outdoor environment of animal houses.

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TRACEABILITY OF ENTEROTOXIGENIC *STAPHYLOCOCCUS AUREUS* IN THE PROCESSING OF SEMI-HARD CHEESE USING GENOTYPICAL METHODS (Abstract)

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The entrance of *S. aureus* in the dairy chain is multifactorial: Particularly, contamination of raw milk is possible during milking (shed by cow, milking equipment, farmer) and storage of milk in the farm tank. Each further processing step facilitates the entrance/growth of *S. aureus* associated with bovine, human and environmental origin. The following research was based on a total chain approach: semi- hard cheese made from raw milk was investigated in a small-scale dairy from each individual cow to the end product at retail level. This study was focused on the determination of the possible entrance and pathways of *S. aureus.* Suspicious colonies were tested for various phenotypic properties according to ISO 6888-1. Furthermore, presumptive *S. aureus* colonies were confirmed by PCR method targeting the *nuc* gen and enterotoxin genes *sea* to *sej*. In addition, the expression of enterotoxins (A-E) was determined. Subtyping of selected isolates was performed by Pulsed-field Gelelectrophoresis (PFGE), *spa* and MLST typing. Preliminary results reveal a high prevalence of the enterotoxin genes *sea, sed, sej* and *seg, sei* within a subset of 164 confirmed *S. aureus* isolates (83%). This study provides a comprehensive characterization of *S. aureus* isolates originating from quarter milk samples, bulk-tank milk samples and the whole dairy chain process. The high prevalence of potential toxin-producing *S. aureus* is an issue requiring consideration as it is relevant to food hygiene.

MONITORING OF *STAPHYLOCOCCUS AUREUS* BY MEANS OF FTIR-SPECTROSCOPY ALONG THE DAIRY PRODUCTION CHAIN– FROM COW TO PRODUCT (Abstract)

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One of the most important contagious pathogens connected to mastitis in dairy cattle is *Staphylococcus aureus*, accounting for approx. 30-40% of all cases. *S. aureus* causes chronic, clinical, or subclinical bovine mastitis and is associated with great economic losses in dairy herds. Knowledge of the epidemiological pattern and the potential sources of infections is important to control *S. aureus* in dairy herds. *S. aureus* can easily be shed into the milk during collection and enter the milk processing chain. Each further processing step facilitates the entrance/growth of *S. aureus* associated with bovine, human and environmental origin. The objective of the study was to determine the occurrence of *S. aureus* in a small-scale dairy in western of Austria, enclosing the levels of farm, production and retail. The production chain from quarter milk samples (n= 1176), via bulk tank milk (n=18) to different cheese production stages were investigated for lecithinase positive and –negative *Staphylococci*. *S. aureus* suspicious colonies grown on Sheep blood agar and/or Baird Parker agar were investigated with traditional microbiological methods and FTIR-spectroscopy. The heterogeneity of *S. aureus* isolates was analysed by hierarchical cluster analysis (HCA) using Ward algorithm. In total 185 isolates were identified by FTIR spectroscopy and traditional microbiology as *S. aureus*. The major outcome of the screening was that most *S. aureus* isolates were spread into the dairy chain from one dairy farm into the processing. The *S. aureus* FTIR-spectra from quarter-milk were similar to the spectra from bulk tank milk and ripened cheese. This study reveals that a transmission of udder pathogens from dairy farms as primary producers is possible. Nevertheless, there were also *S. aureus* isolates which were only found in samples taken during the processing and possibly related to biofilms in the dairy equipment.

MOLECULAR TYPING AND TOXIN PROFILES OF *S. AUREUS* STRAINS ISOLATED FROM BOVINE MILK SAMPLES

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SUMMARY

The purpose of this study was to apply pulsed-field gel electrophoresis (PFGE) method of *Staphylococcus aureus* (*S. aureus*) isolated from 39 bovine milk samples collected in dairy farms from Sao Paulo State, Brazil. It was also aimed to characterize isolates and to verify the presence of enterotoxins genes A, B, C and D, and also *mec*A gene in these strains and to contribute the comprehension of *S. aureus* genetic population in Brazilian dairy herds. PFGE analysis revealed three major clonal types and three subtypes at a similarity level of 80%, and two polyclonal types. These results showed a higher prevalence of enterotoxins genes (toxin A, C and D) and mecA gene in two clonal types. These findings are high alarming, mainly because of the high dissemination of these strains in the studied dairy farms.

INTRODUCTION

S. aureus is an important human and animal pathogen and the most prevalent and economically significant agent causing intramammaray infections in dairy ruminants worldwide [4]. Infected cows' udders are the main reservoir from which *S. aureus* is transmitted to other cows in the herd, and prevention of pathogen transmission from cow to cow reduces mastitis incidence. Enterotoxigenic *S. aureus* strains in dairy products have been reported to cause food-borne diseases [7]. Although a limited number of dominant clones are responsible for the majority of infections, there has been a rise in the number of subtypes with elevated virulence and epidemicity [6]. Molecular studies of *S. aureus* isolated from dairy farms in Brazil are still limited. Thus, the objective of this study was to characterize isolates by Pulsed-field gel electrophoresis (PFGE) and to verify the presence of enterotoxins genes A, B, C and D, and *mec*A gene in *Staphylococcus aureus* isolated from 39 bovine milk samples collected in dairy farms from Sao Paulo State, Brazil.

MATERIAL AND METHODS

This study included 39 bovine *S. aureus* isolates collected from ten different dairy herds in Sao Paulo State, Brazil, between December 2008 and August 2010. Pulsed-field gel electrophoresis (PFGE) after *Sma*I digestion (Fast Digest SmaI, Fermentas Life Science, Canada) was used in order to investigate the persistence of specific genotypes of bovine mammary gland isolates of strains of *S. aureus* from cows with subclinical mastitis. PFGE was performed after SmaI digestion. The resulting band patterns were analized by visual inspection, followed by analysis with BioNumerics software (version 6.1; Applied Maths, Belgium) for relatedness evaluation. Dendrograms was generated from similarity matrixes calculated with the Dice coefficient and patterns was clustered by the unweighted-pair group method with arithmetic averages, using an optimization and a tolerance of 1%. Profiles with more than 80% similarity were considered closely related.

RESULTS

Among the thirty-nine (39) *S. aureus* isolates, it was identified three major clonal types (A, B and E) with seven (17.94%), ten (25.64%) and six (15.39%) bacterial strains each one respectively, and three subtypes (D, F and H) with two (5.13%) strains in each one. They were identified through visual interpretations of gels. A and B types were present in three different dairy farms. As for E strains, they were all from the same farm. Polyclonal types were represented by C and G letters and did not show high similarity to the other types. The results also showed a higher prevalence of enterotoxins genes (toxin A, C and D) and *mec*A gene in clonal types A and B **(Figure 1).**

DISCUSSION

Strains of isolated *S. aureus* belonging to pulsotype A, B, D and E are from five different visited farms, located in Nova Odessa, São Pedro, São Manuel, Botucatu and Itatinga; Nova Odessa and Sao Pedro are next to each other, as well Sao Manuel, Botucatu and Itatinga, suggesting that the strains are probably circulating among

these farms. Pulsotype F is represented for strains of farms located far away from each other (Sao Pedro and Botucatu). That is interesting due to the possibility of detecting the circulation of these strains among the visited farms. These findings are high alarming, mainly because of the high dissemination of these strains in these dairy farms.

S. aureus is an important human and animal pathogen. This is the most common microorganism isolated from subclinical mastitis, which is a considerably costly disease for dairy cattle industry all over the world [5,1]. The finding of enterotoxigenic genes represent the main cause of staphylococcal food poisoning and are potential virulence factors that contribute toward mastitis pathogenesis [2].

Pulsed-field gel electrophoresis (PFGE) for genetic typing of *S. aureus* has been described in a large number of reports and shown to be a well-suited method regarding its discriminatory power [3]. Our results demonstrated the highest detection of enterotoxins genes A, C and D and also mecA gene in PFGE types A and B, with disseminated strains in several farms.

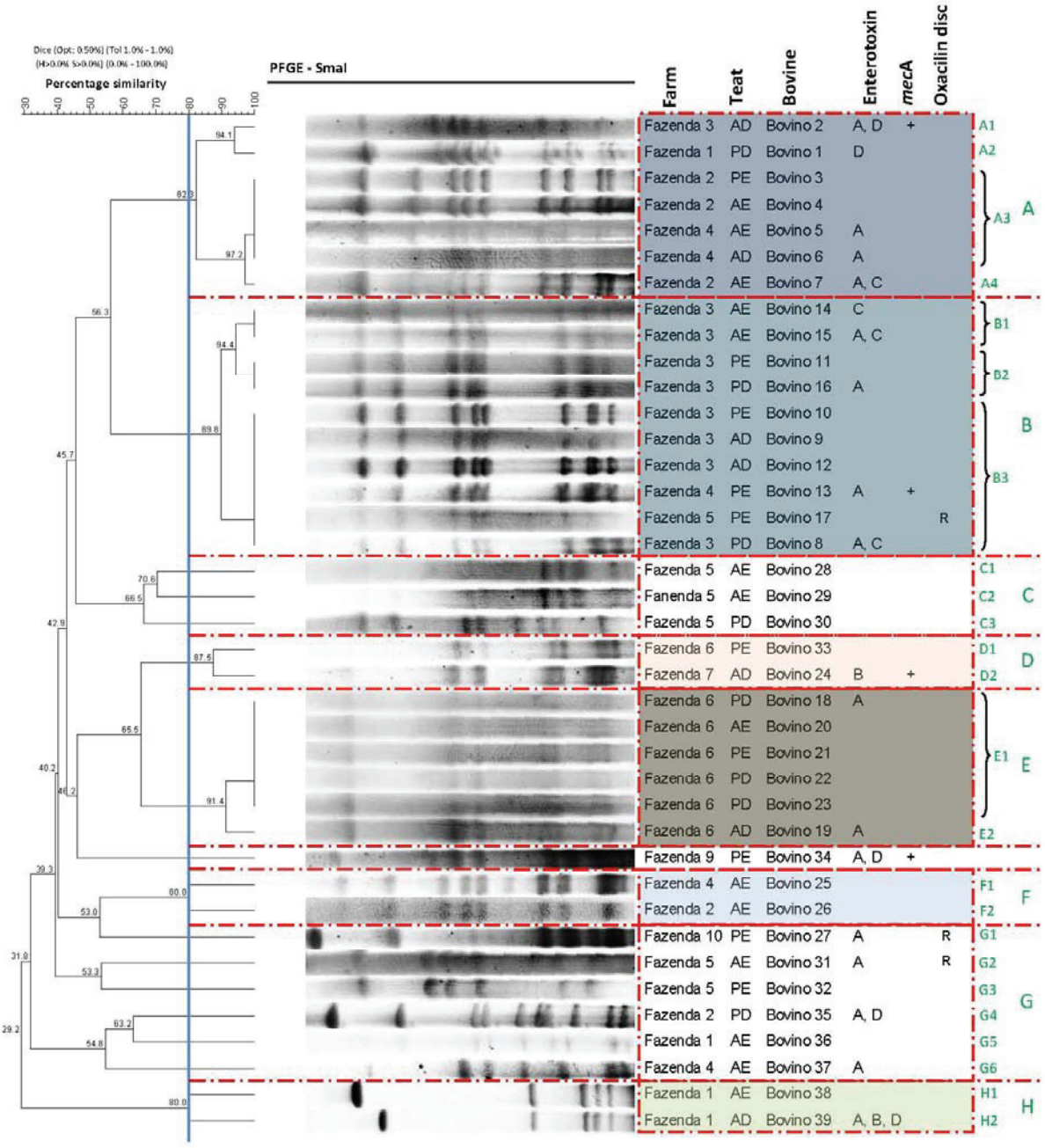

Figure 1. Dendrogram of PFGE patterns and the relatedness of 39 bovine *S. aureus* strains examined in this study. The cluster cutoff was set at 80% similarity. Columns to the right of the dendrogram show the identification of the farm and the respective udder quarter milk from the animals, the presence of enterotoxins A, B, C or D and the presence of oxaciline resistance and mecA genes. Capital letters indicate pulsotypes and subtypes based on visual interpretation of PFGE results.

CONCLUSIONS

Genetic diversity and toxin profiles of *S. aureus* isolates recovered from cases of bovine mastitis were studied. The results showed the importance of the investigation and identification of potential virulence factors that are circulating in clonal and polyclonal types among dairy farms. These findings contributed with the mastitis epidemiology study, which will, in a short-term, help to develop a more effective mastitis control program. Finally, our findings highlighted the presence of enterotoxins genes (toxin A, C and D) and the mecA gene in two clonal types. More studies and a nationwide surveillance program to control *S. aureus* transmission are deeply needed among dairy farms in Brazil. This study shows that PFGE can be successfully applied to characterize *S. aureus* isolates of bovine mammary origin and may contribute to farm-specific recommendations for the management of problems with this agent in dairy farms.

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SMALLHOLDER DAIRY PRODUCTION IN NORTHERN MALAWI: PRODUCTION AND HEALTH CONSTRAINTS

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SUMMARY

Animal production contributes more than 11% of total Gross Domestic Product of Malawi. Smallholder dairy production is becoming increasingly important as source of food supply and income. Milk production is still unsatisfactory despite efforts by different stakeholders. A survey to investigate production and health constraints on smallholder dairy farming was conducted in 282 farms around Mzuzu, Malawi. Average herd size and milk yield were 2.3 ± 1.9 cattle per farm and 8.7 ± 5.8 litres per cow per day, respectively. Poor animal health and inadequate extension services, inadequate artificial insemination (AI) service, low milk prices and feed shortage were the most important constraints to smallholder dairy farming. Further, clinical examination of animals and study of recorded health data of 249 animals in 91 farms showed that mastitis and East Coast Fever were the main animal health constraints. Daily milk production was higher in farmers with above primary school education (10.3 ± 7.4 vs. 7.8 ± 5.1 litres/cow/day), farmer whose main activity was dairy farming (9.1 ± 5.7 vs. 7.4 ± 5.9 litres/cow/day) and those with at least two years of dairy farming experience (9.6 ± 5.3 vs. 7.7 ± 6.2 litres/cow/day). Future dairy programs intended to increase milk production in Malawi should also focus on improvement on farmers' education, experience and knowledge about health problems.

INTRODUCTION

Agriculture contributes to about 39% of Malawi's Gross Domestic Product (GDP) and employs about 85% of the Malawian labour force. Taken alone, the livestock sector accounts for 36% of total agricultural domestic products. The growth of population and the rapid urbanisation around the major cities of the country has led to an increased demand for milk as food source as well as source of income for farmers. To satisfy this need, the Malawian Government initiated new strategies such as facilitating farmers' access to improved dairy breeds. Farmers in Malawi are organised into Milk Bulking Groups (MBGs) located around the three major cities of the country: Blantyre (Southern Region), Lilongwe (Central Region) and Mzuzu (Northern Region). Farmers within an MBG transport their milk to a central cooling unit from where milk is collected by processors and other buyers. Milk consumption in Malawi is estimated to be only 4 to 6 kg per capita and year [1, 3]. This is far below the estimated 30.8 kg per capita for sub-Saharan Africa. Moreover, over 20% of the estimated 73,295 tonnes of milk and milk products consumed in the country are imported [1]. To satisfy the increasing demand of dairy products, the productivity of Malawian dairy animals has to improve by alleviation of the various constraints that affect these animals. This study was initiated to identify production and health constraints faced by smallholder dairy farming, to establish the association between farm characteristic and milk yield and to propose sustainable interventions to alleviate constraints identified.

MATERIAL AND METHODS

This study was carried out in smallholder dairy farms around Mzuzu, Malawi (11° 27' 0" South, 33° 55' 0" East). The area has a subtropical climate with an altitude of 1,200 metres. The rainy season lasts from November to April with a mean annual rainfall of 1,750 mm. The dry season lasts from March to October. A questionnaire survey was conducted in April 2009 and based on face-to-face interviews with 40% of randomly selected dairy farmers registered at Mzuzu Agriculture Development Division [6] (N = 282 farms). The structured questionnaire included information on personal details, number and breed of animals, average milk production and sales, feeding method, constraints faced as well as information on disease occurrence. A follow-up study on animal health was carried out from July to August 2010 and included clinical examination of affected animals, clinical signs described by farmers and secondary data from farm records of the previous year (N = 91 farms). Diseases or animal health condition were categorized into eight groups based on clinical signs.

RESULTS

The questionnaire revealed that average herd size and milk yield were 2.3 ± 1.9 cattle per farm and 8.7 ± 5.8 litres per cow per day respectively. Farmers had been involved in dairy production for an average of 5.9 ± 4.5 years. Milk production was significantly higher in farmers owning more than 3 cattle, farmers with above primary education, farmers whose main activity was dairying and those with more than 5 year of dairy farming experience (Table 1).

Table 1: Association between selected smallholder farm characteristic and milk yield

Factor and category	Number of farms	Milk yield/cow/day (l)		
		Mean	SD	P
Herd size				
<3 cattle	208	8.1	5.9	0.01
>3 cattle	74	10.1	5.3	
Education				
Primary education	211	7.8	5.1	0.00
Above primary education	71	10.3	7.4	
Main activity				
Only dairy production	186	9.1	5.7	0.01
Dairy production and others	96	7.1	5.9	
Duration in farming				
< 5 years	162	7.7	6.2	0.01
>5 years	120	9.6	5.3	

Further, the survey showed that poor animal health and extension service was the most important constraint faced by a majority of smallholder dairy farmers. Numerous farmers also complained about inadequate AI services, poor milk prices and the limited availability of feed for the animals (Table 2).

The supplementing health data obtained by clinical examination of animals and by analysing the recordings of farmers revealed that almost 50% of all animals had mastitis during the previous year. Next to numerous other diseases, East Coast Fever (ECF), worm infestation and retained placenta were found to be of high incidence (Table 3).

Table 2: Summary of constraints faced by farmers in the northern region of Malawi

Constraint	Number of farmers facing constraint (%)
Poor animal health and inadequate extension services	158 (56)
Inadequate artificial insemination services	130 (45)
Poor market for milk	111(39)
Feed shortage	105 (37)
Poor farm management	45 (16)
Poor farmer group management	20 (7)

Table 3: Mean annual incidence of clinically manifested dairy health constraints *

Disease and condition	Composition (number of cases)	Total number of cases	Mean annual incidence per 100 susceptible animals
Mastitis	Clinical mastitis	63	46
Specific diseases	East coast fever (18), Blackquarter (7), Pinkeye (8), Heartwater (2),	35	14
Reproductive disorders	Abortion(4), cystic ovaries (1) dystocia (2), retained placenta (11)	18	13
Respiratory disorders	Coughing (4), dyspnoea (3)	7	3
Metabolic diseases	Milk fever(2), ketosis (3)	5	4
Locomotor disorders	Sprains associated with arthritis (1), limping (7)	8	3
Gastrointestinal disorders	Diarrhoea(1), indigestion of plastics(2), worms (13)	16	6
Miscellaneous	Inappetence of unknown cause and others	8	3

*Average number of study animals was 249 and included 137 cows, 37 heifers, 55 young stock, and 20 bulls in 91 smallholder farms between July 2009 and July 2010

DISCUSSION

Smallholder dairy farming is an emerging business in the Northern Region of Malawi. It is a significant source of income, food and employment. Constraints identified in this study are similar to those reported in smallholder dairy farming in other East African countries [4, 7]. Inadequate veterinary and production services as well as irregular prophylactic programs were the most cited reasons for poor animal health observed in this survey. Clinically manifested diseases diagnosed were comparable to those observed in smallholder dairy farms near Addis Ababa, Ethiopia [5]**.** Mastitis, specific diseases including ECF and reproductive disorders were the main clinically manifested health constraints to smallholder dairy development in the region. On the other hand, metabolic diseases did not occur regularly, probably due to low milk production. Farmers attribute poor animal health observed to irregular extension services, high cost of veterinary inputs and inadequate knowledge of disease prevention. Farmers use imported semen through the national AI scheme. However, the breakdown of the liquid nitrogen machine made the AI services ineffective at the time of the survey. The region has only one dairy processing plant giving farmers little or no choice on where to sell their milk. Introducing value addition and encouraging processors from other regions to collect milk could give farmers more market opportunity.

Dairy farming in the region is being promoted by different stake holders who import exotic dairy cattle breeds (mainly Holstein-Friesian and Jersey) and distribute them to farmers on loan basis. This could account for the higher average milk production observed compared to 5.5 ± 3.8 litres recorded in the centre region of the country were most dairy cattle are cross bred animals [2]. However, the relatively high standard deviation observed in this study suggests that milk production could be improved. The association of some farm characteristic indicate that farmers' education and main activity are some of the aspect to be taken into consideration in a dairy development program in the region. Moreover, significantly higher milk yield was recorded in more experienced farmers. This suggests an increase in average daily milk yield per cow in the region with time.

CONCLUSIONS

In the northern region of Malawi, farmers with above primary education, whose main activity was dairy farming and those with more than 5 years of dairy farming had higher milk yield. Because most smallholder dairy farms started recently, a rise in milk production in future years is likely. Poor animal health and extension services, inadequate AI services, low milk prices and feed shortage limit smallholder dairy development and should be considered in future dairy programs. Mastitis and East Coast fever are the most encountered animal health constraints and farmers should acquire knowledge about prevention.

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NEW TECHNOLOGIES & SUSTAINABLE LIVESTOCK PRODUCTION IN PAKISTAN

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The contribution of science and technology to the growth of modern livestock sector in Pakistan since the mid-1960s is self-evident. Developments in research and production infrastructure and encouraging government policies acted as catalysts for the technology-driven growth in livestock sector, helping the country achieve self-sufficiency in food grains and many other commodities. In other areas such as horticulture, fisheries and livestock considerable technical progress has taken place, but impacts have been slow and sporadic and its analysis in an economic framework has largely remained undocumented. The contribution of livestock to income and employment generation is second only to that of crops. However, its productivity is low computed to the world average. Animals are fed on crop by-products and grasses from roadsides and other marginal lands. Feeding of grains and other concentrates is inadequate and the competition for grains will intensify with increasing human and livestock populations. Thus technology will be a key factor in improving the sustainable productivity of Pakistan's livestock sub sector.

Key word: Pakistan, sustainable livestock production, modern technology

INTRODUCTION

The economical production of food for human consumption is a continuing and increasing problem. It has been estimated that by the year 2025 the world population will have grown by at least 3.5 billion to reach a total of 8 billion. Sustainable livestock production includes financial, environmental, ethical, social and product quality issues. Growth of livestock is especially strong in the developing world and livestock production is the second biggest economic sector (Contribute app. 11.3 percent to national GDP, GOP: 2009-10) after crop production in Pakistan. The development of this sector as whole needs new husbandry innovations for farm animals with the cost effective technologies options, which are effective, efficient, practicable and most appropriate to local conditions. In this article, different technological options and their possible applications are presented with special reference to the sustainable livestock production.

Animal Breeding & Genetics

The eventual application of new technologies like molecular genetics in breeding programs depends on developments in the following four key areas:

- Molecular genetics: identification and mapping of genes and genetic polymorphisms
- QTL detection: detection and estimation of associations of identified genes and genetic markers with economic traits
- Genetic evaluation: integration of phenotypic and genotypic data in statistical methods to estimate breeding values of individual animals in a breeding Population
- Marker-assisted selection: development of breeding strategies and programs for the use of molecular genetic information in selection and mating programs.

Most animal characteristics of interest to food and agriculture are determined by the combined interaction of many genes with the environment. The genetic improvement of locally adapted breeds will be important to realizing sustainable production systems. The molecular genetic technologies provide a major opportunity to advance sustainable animal production systems of higher productivity, through: characterizing and better understanding animal genetic variation; manipulating the variation within and between breeds to realize more rapid and better-targeted gains in breeding value and in conserving genetic material.

I) Characterizing and better understanding of animal genetic variation: The increasing knowledge of mammalian genetic structure and the development of convenient ways of measuring that structure have opened up a range of new possibilities in the areas of animal and product identification.

ii) Increasing the speed of genetic improvement of locally adapted breeds: There are many links in the chain to realizing rapid genetic progress in the desired goals, with the objective being to rapidly transmit from selected breeding parents to offspring those alleles which contribute to enhanced expression of the traits of interest. There is rapid progress in the preparation of sufficiently dense micro satellite linkage maps to assist in the search for genetic traits of economic importance.

iii) Molecular conservation: The first step in considering the sustainable management or conservation of a particular population of animals is genetic characterization. The development of efficient methods of reading the molecular structure of populations has added a totally new range of instruments which can be used for the development of rational and balanced genetic management strategies.

The compelling need for conserving domestic species is to prevent the loss of the many differentiated populations that, because of geographic or reproductive isolation, have evolved distinct characteristics and now occupy different environmental niches.

Methods of preserving animal germplasm: Three basic approaches can be identified for preserving genetic diversity: maintaining living herds or flocks, cryo-preserving gametes or embryos and establishing genomic libraries. A major advantage of preserving live herds and flocks is the opportunity for selection, thereby allowing the breed to adapt to shifting environmental conditions. Frozen storage of gametes and embryos offers a cost-effective method to preserve the genetic material of a breed for an indefinite period of time. Embryo storage has not been used for conservation, in part because of the cost of sampling, but research on collecting and handling oocytes and embryos is advancing rapidly. Cryo-preservation can complement efforts to preserve live populations and it should be used as a safeguard when population numbers are dangerously low or when certain breeds or lines are likely to be replaced with other populations. Genomic libraries are of little use for breed preservation. As technologies develop, however, they may provide an important mechanism not only for conserving diversity, but also for accessing particular genes. Their value will be enhanced with continuing advances in molecular genetics research.

Animal Health

Animal diseases are a major and increasingly important factor reducing livestock productivity in developing countries. Use of DNA biotechnology in animal health may contribute significantly to improved animal disease control, thereby stimulating both food production and livestock trade.

1) Diagnostics and epidemiology: Advanced biotechnology-based diagnostic tests make it possible to identify the disease-causing agent(s) and to monitor the impact of disease control programmes, to a degree of diagnostic precision (sub-species, strain, and bio-type level) not previously possible. Molecular epidemiology is a fast growing discipline that enables characterization of pathogen isolates (virus, bacteria, parasites) by nucleotide sequencing for the tracing of their origin. This is particularly important for epidemic diseases, where the possibility of pinpointing the source of infection can significantly contribute to improved disease control.

ii) Vaccine development: Although vaccines developed using traditional approaches have had a major impact on the control of foot-and-mouth disease, rinderpest and other epidemic and endemic viral, mycoplasmal and bacterial diseases affecting livestock, recombinant vaccines offer various advantages over conventional vaccines. These are safety (no risk of reversion to virulent form, reduced potential for contamination with other pathogens, etc.) and specificity, better stability and importantly, such vaccines, coupled with the appropriate diagnostic test, allow the distinction between vaccinated and naturally infected animals. Recombinant DNA technology also provides new opportunities for the development of vaccines against parasites (e.g. ticks, helminthes, etc.).

Animal Nutrition

i) Nutritional physiology: Applications are being developed to improve the performance of animals through better nutrition. Enzymes can improve the nutrient availability from feedstuffs, lower feed costs and reduce output of waste into the environment. Prebiotics and probiotics or immune supplements can inhibit pathogenic gut micro-organisms or make the animal more resistant to them. Administration of recombinant somatotropin results in accelerated growth and leaner carcasses in meat animals and increased milk production in dairy cows. Immuno-modulation can be used for enhancing the activity of endogenous anabolic hormones. In poultry nutrition, possibilities include the use of feed enzymes, probiotics, single cell protein and antibiotic feed additives. The production of tailor-made plant products for use as feeds and free from antinutritional factors through recombinant DNA technology is also a possibility. Plant biotechnology may produce forages with improved nutritional value or incorporate vaccines or antibodies into feeds that may protect the animals against diseases.

ii) Rumen biology: Rumen biotechnology has the potential to improve the nutritive value of ruminant feedstuffs that are fibrous, low in nitrogen and of limited nutritional value for other animal species. The potential applications of biotechnology to rumenmicro-organismsare many but technical difficulties limit its progress. Methods for improving rumen digestion in ruminants include the use of probiotics, supplementation with chelated minerals and the transfer of rumen microorganisms from other species.

CONCLUSION

There is a serious need for capacity- building, in the country, because, new technologies may be successfully applied to improve the management of animal genetic resources, for the benefit of farmers and consumers and needs to be at all levels. There is a need to strengthen competence in the areas of science and technology, but also for regulatory issues and policy analysis. Governments should inform the public as to the benefits and risks of new biotechnologies and their potential role in the management of animal genetic resources. The new biotechnologies have targeted improvement in livestock production in industrial countries and have increasingly been developed in the private sector. Such research will necessarily concentrate on species and breeds that can generate a near-term profit, to the exclusion of research on less profitable species or

traits. A key role for governments is to ensure that an open, transparent and effective regulatory system in place, that permits the harmonious development of animal production, particularly in the light of the on-going livestock revolution, so as to maximize production while minimizing ecological risks. The use of new and frequently proprietary, new technologies in the management of animal genetic resources will require country more systematically to consider their relevant intellectual property policies and legislation, in order to provide enabling environments for the conservation and utilization of animal genetic resources and for associated public and private sector research and product development.

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KUDU HARVESTING: DAY OR NIGHT?

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SUMMARY

This paper describes the effects of day and night cropping on kudu (*Tragelaphus strepsiceros*) meat quality. Eight animals were cropped during the day and eight at night. Day-cropped animals had higher mean stress scores and cortisol levels (stress score = 3.0 ± 0.641; cortisol = 68 ± 1.28 nmol/L) than night-cropped animals (stress score = 1.8 ± 0.955; cortisol = 14 ± 2.15 nmol/L). The muscle ultimate pH (pHu) values differed significantly between the two treatments (day-cropped animals = 5.40 ± 0.030; night-cropped = 5.48 ± 0.041). Significant differences were also found in drip loss (day-cropped = 2.76 ± 0.261%; night-cropped = 1.36 ± 0.361%) and in shear force between treatments (day-cropped = 3.45 ± 0.171; night-cropped = 4.06 ± 0.237 kg/1.27 cm diameter). No differences were found between the treatments for any of the colour ordinates, except L* values (day-cropped: 33.45 ± 0.435; night-cropped = 32.13 ± 0.601). The results of this study are inconclusive in that although day-cropped animals experienced more ante-mortem stress and, as a result produced meat with higher drip loss, they had a lower shear force and a lighter colour, positive meat quality attributes associated with less stress.

INTRODUCTION

The production and especially the export of game meat from South African is steadily increasing and with this growth, it is inevitable that more emphasis is being placed on the quality of game meat. Kudu has become a popular ungulate species for game meat production; in 2008, 3 542 animals were harvested producing 280 tonnes of meat; equivalent to 12.5% of the total game meat exported. In 2009, 1 370 animals produced 108 tonnes of meat that was exported and in 2010, the values were 1 254 animals and 96 tonnes exported, respectively. For export purposes, kudu are harvested at night using spotlights [2]. A problem with night harvesting of kudu is that this animal is known to close its eyes thus making it difficult to see during moonless nights. It would thus be more efficient to crop this species in the day time.

Research regarding the effect of different cropping periods on *ante-mortem* stress, and as a result, on meat quality in wild ungulates, is lacking and thus the purpose of this study was to investigate the effect of some of the commonly used cropping methods on the meat quality of kudu

MATERIAL AND METHODS

Eight animals were cropped during the day and eight at night. *Ante-mortem* stress was measured using serum cortisol levels (nmol/L) immediately after death, a subjective stress score (Table 1) allocated to each animal as well as the rate and extent of pH decline in the *M. longissimus dorsi* [3]. Special emphasis was also placed on the meat quality parameters drip loss, cooking loss, colour and Warner-Bratzler shear force (kg/1.27 cm diameter) [3].

Analysis of variance (ANOVA) was used to test for differences in meat quality, as well as pH_u with treatment (shooting method) as main effect, using SAS version 8.2 [5]. Pearson correlation coefficients were calculated where applicable. The differences between cropping methods were, where appropriate, tested separately by means of the null hypothesis (H_o), with H_o:$\mu_1 = \mu_2$ and the alternate hypothesis (H_a) being H_a:$\mu_1 \neq \mu_2$. Differences within the main effects were accepted as being significant if the probability of rejection of H_o was less than 5 % ($P < 0.05$).

RESULTS

Day-cropped animals had higher mean behavioural scores and cortisol levels (behavioural score = 3.0 ± 0.641; cortisol = 68 ± 1.28 nmol/L) than night-cropped animals (behavioural score = 1.8 ± 0.955; cortisol = 14 ± 2.15 nmol/L). The muscle ultimate pH (pH_u) values differed significantly between the two treatments (day-cropped animals = 5.40 ± 0.030; night-cropped = 5.48 ± 0.041). Significant differences were also found in drip loss (day-cropped = 2.76 ± 0.261%; night-cropped = 1.36 ± 0.361%) and in shear force between treatments (day-cropped = 3.45 ± 0.171; night-cropped = 4.06 ± 0.237 kg/1.27 cm diameter). No differences were found between the treatments for any of the colour ordinates, except L* values (day-cropped: 33.45 ± 0.435; night-cropped = 32.13 ± 0.601).

DISCUSSION

The higher stress scores of the day-cropped animals was also measured in their blood cortisol levels. This indicated that the day-cropped kudu may have experienced more *ante-mortem* stress during the cropping operation. The stress scores were strongly correlated with the blood cortisol levels ($P < 0.0001$; $r = 0.823$), indicating that the subjective stress score was a good indication of the stress experienced by the animals. It must be noted, however, that the cortisol levels of both the day and night-cropped animals are within the mean cortisol ranges that were reported for other African ungulates such as impala (*Aepyceros melampus*) (19.4 – 148 nmol/L) and roan antelope (*Hyppotragus equinus*) (23.0 – 135.0)[1].

The major influence that pre-slaughter stress has on meat quality is its effect on muscle glycogen content which can cause a higher than normal ultimate pH (pH_u) or rate of pH decline. The differences in pH_u measured in this investigation may be attributable to the differences in serum cortisol levels in that the higher serum cortisol levels of the day-cropped animals caused the mobilisation of the animals' glycogen stores shortly before death which resulted in an increased rate and extent of lactic acid formation and muscle acidification *post-mortem.* It must also be noted that although the mean pH_u differed statistically between treatments, biologically, these values may not necessarily differ significantly with regard to their effects on meat quality. It could therefore be argued that the statistical difference may have become insignificant had the sample size been larger.

Drip loss and cooking loss are both functions of the water-holding capacity (WHC) of meat. *Ante-mortem* stress can affect the WHC of meat by affecting the pH of the meat since pH affects the affinity of meat to bind and hold water. When a high rate of pH decline occurs because of acute *ante-mortem* stress, low pH values are usually reached while muscle temperatures are high so that denaturation of the muscle proteins occurs that causes a decrease in their ability to bind water and thus decreases their WHC. On the other hand, chronic stress, particularly when experienced over a long period, can lead to a higher than normal pH_u that increases the WHC of the meat such that little or no exudate is formed. In the current investigation there was a difference ($P = 0.012$) in drip loss between the treatments such that day-cropped animals produced higher drip loss values than night-cropped animals. This is consistent with the finding that day-cropped animals may have experienced more *ante-mortem* stress, which may have caused a more rapid pH decline whilst the carcass temperatures were still relatively high causing a denaturation of muscle proteins and a loss of WHC.

Tenderness is often considered to be a decisive factor in determining meat quality by consumers. Differences ($P = 0.003$) were found between treatments for shear force, with night-cropped animals producing tougher meat than day-cropped animals. This differs from day-cropped impala that produced tougher meat than night-cropped impala[4]. The correlation between pH_u and tenderness ($P < 0.0003$; $r = 0.506$) may partly explain the difference between treatments, since night-cropped animals had higher pH_u values than day-cropped animals. Furthermore, the differences in ambient temperatures between day and night (daytime ambient temperatures ranged between 12°C and 15°C, while night-time ambient temperatures ranged between 2°C and 3°C), and the fact that six of the animals cropped during the day were not loaded into the cooling truck until at least 6 hours *post-mortem* may have resulted in night-cropped carcasses cooling at a much faster rate and, since temperature is known to affect tenderness, this increased cooling rate may account for some of the differences in shear force between the treatments.

Meat colour is an important selection criterion for consumers and can often give an indication of the effect of pre-slaughter treatment on the meat quality. Although game meat is generally accepted as being a dark-red colour, exceptionally dark meat may be indicative of DFD which is caused by chronic *ante-mortem* stress [2]. The only difference for colour ordinates between treatments was for L* values ($P = 0.015$), with day-cropped animals producing lighter meat than night-cropped animals. On the other hand, no differences in any of the colour ordinates between day- and night-cropped impala were found [4]. Differences in L* may be attributable to the differences in pH_u since the myofilament lattice shrinks as the pH decreases, causing an increase in the myofibrillar refractive index and an increase in the light scattering of the meat. This would cause the meat to be more pale or lighter.

CONCLUSIONS

The results of this study are inconclusive in that although day-cropped animals experienced more *ante-mortem* stress and, as a result produced meat with higher drip loss, they had a lower shear force and a lighter colour, positive meat quality attributes associated with less stress. Neither cropping methods produced pH values high enough or low enough to be considered detrimental to the meat quality[2], even though the serum cortisol levels and the subjective stress scores indicated that day-cropped animals experienced more *ante-mortem* stress than night-cropped animals. The biological significance of the small difference in pH_u is also debatable. Although the meat quality values were not extreme enough for it to be considered as PSE, it is likely that the stress experienced by the day-cropped animals in the current investigation had an effect on the pH of the muscle *post-mortem* and that this, coupled with the differences in rates of temperature decline, was enough to cause differences in the meat quality between the treatments. As a result, day-cropped animals produced meat that had a higher mean drip loss percentage and lower mean shear force, as well as being lighter in colour. Although differences in drip loss, shear force and lightness of the meat, does suggest that night cropping caused less *ante-mortem* stress than day cropping, it is argued that the differences are slight and would not negate the advantages of day cropping.

Some of these advantages include: easier visibility; better off-take rate as more animals are visible at longer distances – an experienced shooter has no difficulty in shooting an animal in the head at distances over 200m; the better visibility in the day-time allows for a more rapid loading of carcasses – not only because the carcasses are more visible but also the route to the carcasses is more visible and it is easier to avoid holes, trees stumps, etc.; selective cropping is possible because of the visibility of the animals/herd; a longer cropping period is possible as night cropping is restricted to dark moonless nights.
However, the higher day time ambient temperatures and the presence of flies would require that the carcasses be processed and placed into a cooler as soon as possible.

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Table 1. Stress scale for the kudu (*Tragelaphus strepsiceros*) as pertaining to perceived *ante-mortem* stress experienced [x]

Value	Stress experienced
1	No stress: died immediately after being shot
2	*Ante-mortem* exercise: was chased for up to 15 minutes prior to being shot and died immediately after being shot
3	Stressed: shot only once although death was not instantaneous - moved a short distance (up to 50 m) after being shot before falling and dying
4	Stressed: fatally wounded – shot a second time after moving a short distance
5	Severely stressed: wounded although not fatally - ran for a long time before being killed by a second or third shot

THE EFFECT OF BREED AND THE BODY CONDITION SCORE IN ECONOMIC TRAITS OF SHEEP

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SUMMARY

This study aimed to estimate the effect of breed and Body Condition Score (BCS) on the production economic traits of our sheep in Kurdistan region ,Kurdi, Awassi and Mamesh sheep. The sheep were consisting of (53 Kurdi, 44 Awassi and 47Mamesh) in Erbil / 2009. Results showed a significant effect of breed ($p < 0.01$) on milk production, were highest in Mamesh. Moreover, wool, birth and weaning weights having already over taken Mamesh on Kurdi and Awassi. The study also showed that the (BCS) have significantly affected ($p < 0.01$) on milk production, birth weights and wool. As the ewes (BCS) 3 and 4, has excellent in all the traits.

INTRODUCTION

Livestock are an important factor in agricultural production as well as in securing sources of animal protein, which is a measure of the evolution of per capita food from the strategic of the development of livestock and the achievement of food security and self-sufficiency in livestock products. The body weight and growth rate are important economic traits, which are considered one of the basic components to increase the productivity of sheep including meat as well as to increase the flock sheep outcome [1]. Moreover, the wool of the three types of sheep is characterized as rough quality wool and with long fiber which covers almost the entire body. The study of economic traits for various animals in farm including sheep is very essential of serious education and plans for improvement. Furthermore, those economic traits are known as the clan members and they are varying in one different clans in the rate of production performance due to different combinations of genetic and environmental conditions. The objective of this study was to investigate the effect of different breeds between Kurdi, Awassi and Mamesh sheep and BCS in milk production, birth and weaning weights and weight of wool.

MATERIAL AND METHODS

The experimental of this study carried out with: (53) Kurdi sheep (44) Awassi and (47) Mamesh in 2009. The data were analyzed using the statistical method of General Linear Model (SAS) [2] .The ewes fed with residues of wheat and barley with concentrated feed (600-750) g / day / head and hay freely and water templates and salt freely. Furthermore, all sheep were vaccinated and the used vaccinations were followed according to the preventive health program in each field.

RESULTS

Milk production

The mean of milk production 94.657 kg (table 1), results showed that the mean of breed (Kurdi, Awassi and Mamesh) were (88.254, 92.684 and 102.269) kg, respectively. Ewes with the 4 recorded of (BCS) was the highest average of milk production and below the 102.883 kg milk production of ewes (BCS) 2, accounted to 86.391 kg. Correlation between the (BCS) and average of milk production was highly significant ($p<0.01$) accounted to 0.295. Ewes had a significant effect of age ($p<0.05$) in the mean of milk production as excelled ewes aged 3.5 and 4.5 years in the mean of milk production (100.727 and 103 906) kg, respectively on the other age groups (table 1).

Birth and Weaning weight

The total weight of lambs at birth of the sheep Kurdi , Awassi and Mamesh 4.403, 3.968 and 4.885 kg, respectively, (table 1). Moreover, the age of the sheep had significant effect ($p < 0.05$) in birth weight that reached peak at the age of (3.5, 4.5) years, accounted to (4.980 and 4.736) kg respectively [3]. In addition, these differences were significant, as characterized by (BCS) 3 and 4 and it gave the highest weight of infants at birth (4.805,4.695) kg respectively ,compared with ewes (BCS) (2) 3.976 kg.

Wool production

Results showed that the mean weights of wool shearing were 2.220 kg , 1.829 kg and 2.585 kg for breeds of sheep Kurdi, Awassi and Mamesh, respectively (table 2). Sheep weights have significant effect ($p < 0.05$) on shearing weights of wool reached a maximum at the age of 3.5 and 4.5 years, accounted from (2.735 to 2.660) kg respectively. Whereas produced ewes which weighed 60 kg more weight shearing heavier by 2.911 kg wool. Research found that the weight of the fleece begins with the increase of the ewes with the (BCS) (2) as the total weight fleece 1.387 kg up to 2.612 kg in ewes (BCS)(3) the state of the body and reaches a maximum (2.710) kg in the ewes with the (BCS) (4). It was found that the correlation coefficient between the (BCS) and wool shearing weights has reached 0.430, ($p < 0.01$).

DISSCUTION

The study of variation and genetic traits between breeds of the status of milk production.

Table 1: least squares means ± Stander Error of the impact of breed, (BCS) and age of sheep on the milk production , birth and weaning weights (kg). * Significant at .05 ** Significant at .01

Classification	No.	Milk Mean±S.E	Birth wt. ± S.E	Weaning wt.±S.E
Overall Mean	144	94.657± 0.125	4.467±0.022	24.625±0.046
Breed		**	**	**
Kurdi	53	88.254±0.386b	4.403±0.020b	23.937±0.115b
Awassi	44	92.684±0.417b	3.968±0.023c	21.556±0.136b
Mamesh	47	102.269±0.302a	4.885±0.016a	27.612±0.116a
BCS		**	**	
2	48	86.391±0.387c	3.976±0.017b	22.940±0.121a
3	56	95.083±0.344b	4.805±0.019a	25.649±0.137a
4	40	102.883±0.341a	4.695±0.021a	25.533±0.160a
Age of dam(year)		*	*	*
2.5	43	83.256±0.525b	4.021±0.019b	23.018±0.145c
3.5	35	100.727±0.386a	4.980±0.024a	26.286±0.187ab
4.5	33	103.906±0.376a	4.736±0.035a	27.385±0.251a
5.5	33	84.242±0.650b	4.208±0.027b	22.128±0.185c

Under any environmental conditions is important to determine the most appropriate breed that could be adapted to the circumstances of that particular environment. The results indicate that the moral influence ($p < 0.01$) for the breed in the mean of milk production is evident that the highest rate of milk production was in sheep Mamaesh 102.269 kg and the lowest in sheep Kurdish 88.254 kg . However, the results did not show significant differences between the Kurdi and Awassi sheep. Furthermore, The results of this study were agreed with [4,5] . The reason for increasing the productivity of milk ewes aged 3.5 and 4.5 years for 2.5 and 5.5 years traced to the development and integration of the Milk system which is responsible for milk production with increasing age, in addition to increase the weight of the mother as a result of increasing the size of the gastrointestinal tract and utilization of feedstuffs [5]. The results showed significant effect ($p < 0.01$) of breed and (BCS) on birth weight, as characterized by the (BCS) of ewes 3and 4 it gave the highest weight of infants at birth. The results of this study are identical with many of the studies on improving the productive performance of ewes to improve the (BCS) (4). The effect of breed had significant ($P < 0.01$) in weight at weaning with (BCS), Kurdi, Awassi and Mamesh that weighted an average of 23.937,21.556 and 27.612 kg,respectively [3].The moral influence of the breed of this type is attributed variation in weaning weight to the variance in genotypes and weight at birth and the amount of milk lactation period [6]. The results indicated there were significant differences ($p<0.01$) in weight of wool shearing, resulting from the impact of race, overtook sheep Mamesh weigh in shearing, wool on the Kurdi and Awassi due to the differences in weight of wool between the breed to the variance in each of the size of the animal's body and the ability of animals efficiency of feed conversion to wool [1] .The body weight as the economic traits is an important to breeders and that their importance in the production of wool because there is a positive relationship between body weight and weight of fleece raw because the increased size of the animal increases the surface area covered with wool. While the correlation coefficient between the weight of sheep when fleecing and weight moral ($p<0.01$) were accounted to (0.433), whereas the

(BCS) had a significant effect ($p < 0.01$) in weight shearing wool. This may be due to the increased size of the uterus, accompanied by the advancement of age of the sheep so that they can create an environment and conditions of the uterine best for the growth of the fetus. As the small age ewes are in the process of growth and development and thus affect the amount of food available for fetal growth and development .The findings come in line with [1].

Table 2: least squares mean ± Stander Error of the impact of factors on the wool production (kg). * Significant at .05 ** Significant at .01

Classification	No.	**wool** Mean±S.E
Overall Mean	144	2.238±0.007
Breed		**
Kurdi	53	2.220±0.020b
Awassi	44	1.829±0.022b
Mamesh	47	2.585±0.018a
BCS		**
2	54	1.387±0.020b
3	45	2.612±0.019a
4	42	2.710±0.015a
Weight of ewes at shearing(kg)		**
40 - 50	60	1.630±0.016c
50 - 60	41	2.245±0.023b
60 ≥	43	2.911±0.018a
Age of dam(year)		*
2.5	44	1.661±0.020b
3.5	38	2.735±0.024a
4.5	37	2.660±0.023a
5.5 ≥	25	1.977±0.050b

CONCLSIONS

Effect of breed and Body Condition Score in economic traits was highest in Mamesh than Kurdi and Awassi sheep, (BCS) of ewes 3and 4 it gave the highest weight of infants at birth. Correlation between the (BCS) and milk production was highly significant.

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ASSESMENT OF BACTERIOLOGICAL QUALITY OF RAW CAMELS' MILK IN AB-'ALA, NORTH EASTERN ETHIOPIA

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SUMMARY

In most pastoralists, camel milk management is poor and always consumed either fresh or in varying degrees of sourness in the raw state without heat treatment thus, can pose a health hazard to the consumer. This study was there fore, aimed to determine the bacteriological quality of raw camels' milk from udder and milking vessels in Ab-'Ala through the assessment of standard plate count (SPC) and identification of bacteria genera/species. The bacterial isolates from the milking vessels were *Escherichia coli* (25 %), *Rhodococcus equi* (25 %), *Alcaligens spps (*12.5 %), *Pasteurella haemolytica* (10.71 %), *Acinetobacter spps* (10.71 %), pathogenic *Staphylococcus aureus* (7.14 %), *Pseudomonas spps* (5.35 %), non pathogenic *Staphylococcus spps (1.79 %)* and *Bacillus spps (*1.79 %). The isolates from udder were pathogenic *Staphylococcus aureus* (31.82%), *Rhodococcus equi* (22.73%), *Acinetobacter spps* (18.18%), non pathogenic S*taphylococcus spp* (9.09%), *Pasteurella haemolytica (*9.09%), *Escherichia coli* (4.54%) and *Alcaligens spps* (4.54%). Species of *Pseudomonas* and *Bacillus* were isolated from the milking vessels only. Among 34 samples collected directly from the udder, 15 samples (44.12 %) were free of growth of mesophilic bacteria. According to the comparisons made with bacteriological standards of raw milk as prescribed by Bureau of Indian Standards (BIS), the bacteriological quality of the 35 samples collected from the milking vessels was 2 (5.7%) very good, 9 (25.71%) good, 11 (31.43%) fair, 11 (31.43%) poor and 2(5.7%) very poor. On the other hand, out of 34 milk samples collected from the udder, 32 (94.12 %) were graded as very good and 2 (5.88%) fair. The difference in bacterial load among the two types of specimens was statistically significant (R^2= 0.02, F= 0.49, P-value=0.002). The results of this research had indicated that milk is getting contaminated by the handling of pastoralists.

INTRODUCTION

Ethiopia's camel population is estimated to be one million head. This number ranks the country third in Africa after Somalia and the Sudan and fourth in the world (India included) [12]. Because of its outstanding performance in the arid and semi-arid areas of eastern lowlands of Ethiopia where browse and water are limited, pastoralists rely mainly on camels for their livelihood. In these areas, camels are mainly kept for milk production and produce milk for a longer period of time even during the dry season when milk from cattle is scarce [4]. The annual camel milk production in Ethiopia was estimated to be 75, 000 tones [5]. In most pastoralists, camel milk is always consumed either fresh or in varying degrees of sourness in the raw state without heat treatment thus, can pose a health hazard to the consumer. Poor management and unhygienic milking practices prevalent in the traditional husbandry systems, which include tying the teats with soft barks to prevent the calf from suckling, tick infestations and cauterization of the udder and skin, are few of the factors responsible for contamination of milk [1, 2, 7, 13]. This study was therefore, aimed at determining the bacteriological quality of raw camels' milk in Ab-'Ala, Afar regional state, North eastern Ethiopia.

MATERIALS AND METHODS

About 15-20 ml of milk sample was collected either from the milking vessels and or the last streak directly from the udder of lactating camel using sterile test tubes. During sampling, observation was made about the condition of the udder for the presence of lesions. A total of 69 samples; 35 from the milking vessels and 34 directly from the udder were collected. Each specimen was labeled and placed in ice box till transported to the laboratory. These were placed in a refrigerator at +4 ^{O}C and culturing was conducted with in 24 hours [6]. For bacteriological culturing and subculturing, about 0.01 ml of milk from each specimen was streaked on nutrient agar and incubated for 48 hours at 37 ^{O}C [9]. Isolated colonies were selected, transferred to nutrient Broth and incubated at 37 ^{O}C for 24 hours. Cultures were then grown on nutrient agar plates for further studies. For subsequent biochemical tests, individual colonies were picked up and cell morphology, Gram staining, catalase test, oxidase test, oxidation fermentation (O-F) test, presence or absence of growth on mackonkay agar were conducted [10]. Further more, haemolysis on blood agar plate, citrate utilization, growth on manitol salt agar and fermentative activity of sugars on kligler Iron agar (KIA) were checked [6]. For standard plate count (SPC), serial ten fold dilutions using sterile 0.85% saline solution up to 10 X 10 $^{-6}$ dilutions were prepared for each specimen.

Pour plate method was used to make viable count. In this method, one ml inoculum was mixed thoroughly with molten plate count agar, previously held in water bath at 47°C. Four plates were inoculated with each dilution. The agar was allowed to set and then incubated at 37°C for 48 hours. Plates inoculated with a simple dilution that yielded between 30 and 300 colonies per plate were counted [6]. For each dilution, the viable counts in the four plates were counted and the mean was calculated. The result was compared with Indian bacteriological standards of raw milk stated as very good (< $2x10^5$ SPC/ml), good (2-$10x10^5$ SPC/ml), fair (10-$50x10^5$ SPC/ml), poor (> $50x10^5$ SPC/ml) and very poor (not specified) [11]. For data entry and analysis SPSS version 12 was used. Percentages were used to express the proportion of bacterial isolates. The difference in bacterial load between milk samples from udder and milking vessels was analyzed using Regression analysis. The result was reported as significant if P-value was less than 0.05.

RESULTS

Major bacterial isolate from udder was pathogenic *S. aureus* where as from milking vessels were *Escherichia coli* and *Rhodococcus equi* (Table 1).

Table 1. Different bacterial isolates from raw milk of milking camels

Bacterial isolate	Frequency, %	
	Milking vessels	Udder
Pathogenic Staphylococcus aureus	4 (7.14 %)	7 (31.82%)
Non pathogenic *Staphylococcus spps*	1 (1.79 %)	2 (9.09%)
Escherichia coli	14(25 %)	1 (4.54%)
Rhodococcus equi	14 (25 %)	5 (22.73%)
Alcaligens spps	7(12.5 %)	1 (4.54%)
Pasteurella haemolytica	6 (10.71 %)	2 (9.09%)
Acinetobacter spps	6 (10.71 %)	4 (18.18 %)
Pseudomonas spps	3 (5.35 %)	0 (0%)
Bacillus spps	1 (1.79 %)	0 (0%)
No bacterial growth	nil	15 (44.12%)
Total bacterial isolates	56	22

The bacterial load (SPC/ml) and hygienic standard of the two types of samples is indicated in Table 2. The difference in bacterial load was statistically significant (R^2= 0.02, F= 0.49, P-value=0.002). Majority of specimens from the milking vessels were more contaminated having grade of fair and poor while 94.12 % of the udder samples were having very good grade. The traditional handlings of camels' milk and milking vessels in Ab-'Ala is indicated in Table 3.

Table 2. Bacteriological quality of raw camels' milk in Ab-'Ala

Sample type	Milk Quality grade				
	Very good (<$2X10^5$)	Good (2-10 $X10^5$)	Fair (10-50 $X10^5$)	Poor (>50 $X10^5$)	Very poor (not specified)
Udder (34)	32 (94.12%)	0 (0%)	2 (5.88%)	0 (0%)	0 (0%)
Milking vessel (35)	2 (5.7%)	9 (25.71%)	11 (31.43%)	11(31.43%)	2 (5.7%)

Table 3. Traditional camel milk hygiene practices by pastoralists in Ab-'Ala (n = 30)

Traditional camel milk hygiene control methods	Time interval	Number and proportion (%) of responses
Washing hands before milking (by water and ground soil)	Before milking	30 (100%)
Washing and smoking milk vessels	Every 3 days	10 (33.33)
	Every week	20 (66.67%)
Keeping milk in a cold place	Till consumption	5 (16.67%)
Boiling		0 (0%)

DISCUSSION

Staphylococcus aureus, Escherichia coli, Pseudomonas spps, Bacillus spps, and *Alcaligens spps* were reported as common microflora of raw milk [11]. *Staphylococcus aureus*, *Pasteurella haemolytica* and *Escherichia coli* have been reported to be responsible for mastitis in camel [3, 8]. The isolation of species of *Pseudomonas* and *Bacillus* only from milking vessels samples may indicate that these were the common environmental contaminants. The absence of growth of mesophilic bacteria in 15 (44.1%) cases was almost in agreement with previous reports [8]. As indicated in Table 3,in the study area pastoralists wash and smoke the milking vessels only once or twice per week and the personal hygiene of the milker is down graded by lack of awareness or inaccessibility to use soap and insufficient clean water supply; all can contaminate the milk after milking. On the other hand, the lesser bacterial load from the udder of the camel could be attributed to observation of few udder lesions and less tick infestation on camels' udder as a result of the undergoing ecto-parasite control through the assistance of USAID.

CONCLUSIONS

The results of this research had indicated that milk is getting contaminated by the handling of pastoralists. The isolated bacteria can inflict public health concerns especially in immune compromised individuals and pose animal diseases such as mastitis. The high ambient temperature prevailing in the area coupled with lack of cooling facilities can also reduces the shelf life of the milk and it is an undeniable fact that camels are kept in infrastructure and resource poor marginal areas. Early problem recognition and improved hygienic action can reduce the health ailments in animals and humans. Thus, pastoralists should keep regular hygiene of personal, milking vessels and boil or sub boil the milk. Further more; they should avoid traditional beliefs such as branding of udder and professionals should work a lot on further study in identification and virulence of bacterial isolates and perform systematic regular ecto-parasite control of livestock.

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EVALUATION OF SOME BIOCIDES AVAILABLE IN LOCAL IRAQI MARKETS

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SUMMARY

Seventeen types of disinfectants and antiseptics (antimicrobial biocides) that were used in different fields of public health and animal production were obtained from Iraqi local markets. Three types of bacteria were used, *Salmonella*, *Escherichia coli* and Pseudomonas. The test was performed by agar well diffusion technique by making wells in the agar using Pasteur pipette and then filled with disinfectants and antiseptic materials as instructed by the manufacturer, and were incubated for 24 hours. The results were noticed by the inhibition diameter measurement for bacterial growth. Also results of the experiment showed the existence or development of microbial resistance to some antimicrobial agent and for some types of alcohols and iodine especially *Salmonella* and *E.coli*.

INTRODUCTION

Sterilization and disinfection are regarded as basic work associated with our public lives and since there are rules which organize every type of these works and despite the increase in the number of commercial formulations used in the sterilization and disinfection in recent years, yet they are not considered as new effective materials.

The methods of mixing and processing techniques enabled the business of production of the best and inspite of that most disinfectants and detergents remained of chemical origin, and these materials classified into inorganic materials such as elements of iodine, chlorine, and organic elements which most of them are disinfectant chemical liquids which needed to be dissolved before using them [1].

The significant progress has helped a lot in understanding the mechanisms of antiseptics and disinfectants action [2], and comparitive studies that point to the way they work against fungi [3], viruses and protozoa[4], which different from one type to another. In addition to the little knowledge about how the impact of these compounds was to disrupt the work of prions [5]. Whatever the type of microbial cell is, it is likely that there will be a series of join events, among them where the concept of these events interpenetrate the sterilizer or cleanser with cell surface and then it will be followed by penetrating the cell and work from the target location. This is in addition to the disparity in the nature of the surface and the composition of the types of microbial cells from one to another, in addition to the changes that occur in the environment [6].

The aim of this study is to evaluate some of these types of antiseptics and disinfectants (Biocides) in the local markets that were used for long time, in public health and livestock production.

MATERIALS AND METHODS:

Salmonella, E. coli And *Pseudomonas* bacteria isolated from cases of disease and identified biochemically in Department of biology in the Faculty of Science at the University of Sulaimaniya were used to test 17 types of disinfectants and antiseptics (antimicrobial biocides) that are common in different sectors of public health and animal production, and from different origins like Syria, Egypt, Turkey, India, Iraq, Jordan and Iran were collected from local Iraqi markets (table.1). Nutrient agar, Petri dishes, Benzen burner, Pasteur pipette, micropipette were used and the bacteria were tested according to [7],[8], by work of the well in agar record in Petri dishes planted germs by the Pasteur pipete then filled the well with materials sterile and antiseptic after diluting it down as instructed by the manufacturer, and then incubated for 24 hours. The results were read by measuring the diameter of the inhibition zone of bacterial growth.

RESULTS:

The results showed microbial resistance to some of the antimicrobial agents such as alcohols, potassium permanganate and iodine. Table 1 shows the presence of resistant bacterial species to the three Alcohols, Potassium Permanganate, Iodine, Hydrogen Peroxide and Timzielen. On the other hand, Savelon, Hygen and Berfytol show high area of inhibition zone to Salmonella E. coli and Pseudomonas as illustrated in Table (1).

Table (1): Results of examination of inhibition of certain types of antimicrobial

No.	Biocide	Result by zone(mm) *		
		E-coli	*Salmonella*	*Pseudomonas*
1	Berfytol	30	35	40
2	Hygen	38	33	40
3	Savlon	33	37	40
4	Medical alcohol	15	20	40
5	Snow alcohol 68%	-v	-v	10
6	Alcohol 90%	-v	-v	30
7	Chloroxylenol	25	25	23
8	Potassium Permanganate	-v	-v	20
9	Heptan	25	25	32
10	Ribas (menthol)	10	10	18
11	Oxygen(Hydr. Peroxide)	15	11	17
12	Imaj (Temizleme)	-v	-v	18
13	Podovidone	-v	-v	14
14	Bidalkin (Sod. Bycarbo.)	17	10	24
15	Alcohol 96%	-v	-v	-v
16	Alcohol 60%	-v	-v	-v
17	Formaldehyde	10	14	30

* Sensitive >13 > resistance [8]

DISCUSSION

The great interest of the microbial adaptation and resistance to antiseptics and disinfectants was associated with an increased bacterial resistance to antibiotics [9]. Therefore it is very important to assess the types of some of these biocides that were used for long periods of time, in addition to other modern types which are used for clinical purpose and animal production in all its forms [10]. The group (phenolics and hypochlorites) of the most disinfectants have been used since the early nineteenth century and the beginning of the twentieth century and then used the quaternary ammonium compounds and formaldehyde and finally Glauterdhayd and Chlorhexadine and hydrogen peroxide and Chlorhexidine, Organic mercurials, silversalts, peroxygens, hydrogen peroxide, peraceticacid and ozone. Several reports and studies that were based on laboratory tests, indicated a lower impact of some of these compounds on germs. Bacteria acquired complete resistance to some chemicals when increasing dose was given such as boric acid and mercuric chloride, although the inclusion of spores germ in these studies was not clear, in addition to the studies that indicate adaptation of bacteria to antiseptics. The linking of disinfectants and mode of action with the theory of sterilization closely related to the microbial resistance [11]. The evolution of Pharmaceutical stability to any kind of bacteria in the environment associated with the decrease in vulnerability of germs to other compounds in the event of a continuous exposure to it. E. coli falls within these types of bacteria that have developed stability to silver nitrate, mercurochrome, formaldehyde, acriflavine, hexylresorcinol and phenol [12].

That the resistance of different types of bacteria can be normal for the organism or acquired through change or possessing plasmids (self-reproduction, extrachromosomal DNA) or converged plasma [13].

Generally bacteria Gram-negative bacteria are more resistant to antiseptics and disinfectants, and there are differences in the sensitivity of E. coli to quaternary ammonium compounds QAC. There are also a few differences in the susceptibility of Pseudomonas to chlorhexidine, which is largely resistant to most of these compounds [6], where the outer membrane of Gram negative bacteria acts as inhibitor determines the entry of many chemically unlinked types of anti-germ compound in addition to the ongoing mutation in the outer membrane of *E. Coli*, *S.typhimurium,* and *P.aeruginosa* that referred to by [14], and this support the present study especially in types of alcohols and iodine, which had not affect on the growth of *E. coli* and *Salmonella*.

As it is the case with antibiotics and other drugs, the resistance to antiseptics and disinfectants can be gained through a change in the genetic material and possessing plasmids [6]. In order to reach its target the sterilizing must be to cross the outer layers, and depend on the nature and composition of these layers on the type of organism where it works as a contraceptive or attempt to enter the permeability of the sterilizing material or the antimicrobial action of some disinfectants and antiseptics [2].

Results of present study indicated the importance of re-consideration of the chosen type of sanitizer or disinfectant to be used in various fields to be sure of the concentration of active substance and test its effectiveness in eliminating pathogens. This is considered key to the success of the sterilization and cleansing and the better use of all forms and public health agents.

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HORSE'S MEDICINE IN ANCIENT ARABIC HERITAGE

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SUMMARY

The ancient Arabs loved their horses as themselves and their families, may be spent their nights without foods but horses drink and eat some milk and some foods. The horse was very important for Arabs especially resident in desert (Sahara), because it was a suitable for fast transport, carrying and fighting against enemy who attack the trip, other sporting and social purposes as marriage as a gift for woman and her fami-ly, for prodding, horses were resemble a highly economical values and the horse was a standard for economic state. The ancient Arabs wrote many books which have valuable knowledge and skills about horses, information about pure and good horses descried horse body from lips to hooves, main colors and secondary colors, marks, gross and featured anatomy of horse body and cleared a good and bad of this organs, colors, marks and other charac-ters which related to horse ships and sporting. They wrote about importance of characters of Arabian horse , especially genetics and differentiated between congenital and acquired disadvantages, study animal sexology and reproduction, characters of reproductive performance, libido, ejaculation, quali-ty of sperms, fertility, infertility, normal parturition and dystocia and other gyneco-logical and obstetrical problems, uterine and vaginal prolapse, embryotomy, cesa-rean. They differentiated between infectious and non infectious diseases, studied infectious diseases of horse as tetanus, rabies tuberculosis and internal medicine and classified as regiona , diseases of head, neck, respiratory, digestive, wrote about general states as fever , vomiting , diarrhea, constipation and describe the clinical signs and treatments of many diseases as hepatitis, rhinitis, pneumonia, blepharitis, myositis, eczema, diseases of heart, diseases of kidney , and so on . They used many drugs as purgatives, laxatives, astringents, diuretics, and pharma-ceutical preparation as eye drops, powders, ointment, lotions, syrups, injections and used many materials for manufacture of drugs especially medical plants. They practiced a surgery in some surgical affection, ophthalmology, wounds, abscess, hernia, castration and other things.

Key words: Arabic hippiatry , Horse, Islamic hippology, Horse's medicine, Veterinary history

INTRODUCTION

Medicine of horse, it is a science and art of diagnosis, treatment and prevention of diseases of horse. Which was found in many old civilizations .In Arabic civilization it developed before advent of Islam, where recorded in Arabic poetry. In golden time, Arabian veterinarians depended on their experiences, they learned from their teachers usually fathers and families ,and classical works of Persian, Indian, Greek and Byzan-tine works , lots of Arabic veterinary manuscripts scattered in many libraries in all over the world, the study of horse's medicine in ancient Arabic heritage, especially the most importance books of the greatest veterinarians in Arabic civilization as, Ibn Ibn Ahi Hizam Al-Huttuli, Abu Beker Ibn Al Badr Al Mundir Albytar,, Ahmed ibn al –Hasan Al-Ahnf and Al Ashraf.

The aim of this study is clarifying and evaluation of horse's medicine depend on an-cient Arabic heritage. 2

Horse

The earliest reliable evidence for the domestication of the horse comes from Ukraine, where people lived by herding horses and cattle on the grass steppes 6,000 years ago. At the same time, the African wild ass was being domesticated in ancient Egypt and Arabia. At first horses and asses were not usually ridden, but were harnessed in a pair to a cart, or chariot. (Juliet and Brock, 2008)

Arabian horse

Arabian horse, One of the oldest of all the breeds of horse. Arabians originate from the Arabian Peninsula, where they were bred by the Bedouin people around 3,000 years ago. (Juliet and Brock, 2008) Arabian horse had special characters than other horses, Hordepaigt mentioned, 310 All breeds of horses compete in endurance, but serious competitors prefer the Arabian horse and Arabian crosses. It is said that the shorter back, very dense bone, and natu-ral ability of Arabians to go for long periods of time with little food or water are what make them a favorite choice for endurance riding. (Hordepaigt, 2007), and Brown added, hoof horn seems to be of better quality on hardier native types and Arabs. (Brown etal, 2003), Arabian horse breed is considered the

oldest purebred horse in the world and many other breeds are derived from it. The Arabian horse was developed in the deserts of the Middle East. It is an extremely hardy breed with a distinctive appearance and ex-ceptionally friendly disposition. The head is characterized by a dished profile, promi-nent eye, large nostrils, and small muzzle. The neck is arched and the back is shorter than most breeds, and it has a high-set tail, as a learned Arabian once wrote, "paradise on earth is to be found on the back of a Horse" (Bickel. 1965)

Medicine of horses in ancient civilization

Veterinary sciences before their birth. In particular, Horse medicine is discussed. Each riding maestro, each stud farm leader had, at that time, to know how to maintain horses in good conditions and healthy, and had to treat ill horses too! This kind of knowledge, normally, was transmitted orally, from the Maestro to the pupil, and each one had special secrete recipes well established. The manuscript, recently published, is a testimony of the very early Renaissance veterinary science, and for this reason is here presented.(Pignatelli , 2004).

The specialized genre of hippiatric literature, which makes its first appearance in cuneiform tablets of the fourteenth century BC found at ras Shamra-ugait in Syria.

Simon of Athena, the first known Greek writer on horses (fifth century BC),. He own treatise on the art of horsemanship is not concerned with diseases or their treatment, though refers in passing to three conditions: surfeit of blood, exhaustion, and lamini-tis.(McCABE , 2007).

The first elements of Greek veterinary science started to "hatch" in the context of the very ancient civilizations of Egyptian, Minoan and Mycenae periods. .(Seimens.and Charissis ,2004). Semenis and Charissis reported that Veterinary medicine as an independent medical branch was based on the Alexandria Greek medical studies centre. It became self-dependent, in first place as medicine of horses, during period of letter decline in Greek and Rome, reaching its higher development with the Greek "Hippators"and "Hippiatrika" during the Byzantine period.(Seimens.and Charissis ,2004)

and Charisis mentioned that Absyrtos (Klazomenes, Asia minor,300 A.D.) educated in Alexandria, he served in the army under the emperor Constantine the great during his excursion against the Goths. He left many letters addressed to fellow veterinarians 3 and Horse breeders, concerning the pathology of Horses. He is supposed to be one of the most well known veterinarians in the empire.(Seimens.and Charissis ,2004)

In China in ancient time,Chi min yao shu , wrote an official treatise (nug shu) which included, a section on veterinary medicine for horses, asses, cattle , sheep and goats according to Chinese methods, most probably as attempt to exclude any outside (nomadic influence. (Meserve, 1996), also, Profuse information on elephants and Horses, their diseases and control, is available in ancient Indian literature on veteri-nary sciences.(Garg,1987).

Medicine of horses before advent of Islam

The medicine of Horse before advent of Islam by Arabian Bedouin was developed, Mcdonald mentioned , The most basic strand comes from pre-Islamic Arabia and draws its material from the ideas of the Bedouin about the Animals which formed such an important part of the world about them and which were so vital for own sur-vival, whether wild or domesticated..) Mcdonald ,1988), and Sarton (1931) stated that the word baitar was used by the pre-Islamic poets in the sense of leech, and that the baitar was as itinerant doctor of men, as well as of animals, who attended the great fairs or assemblies of the Bedouin. (Hare, 1984). Mohamed Adel, explained the probability of origin word of veterinary, in ancient Arab the veterinarian is –Bitar(surgeon of animals in Arabic language) – in Arabic language, betar modified to vetar, and by time became Veterinary, then the suitable origin of veterinary word is Arabic language (Mohamed Adel,1983).

Vocabulary of Arabic language before Islam proved that ancient Arab practiced Horse's medicine, (Maswani, 1938) and Kutasi reported that, the earliest descriptions about the horses can be considered the descriptive parts of the pre Islamic (gahiliya) poetry. The pre-Islamic poetry was handed down orally and was only written down after the rise of Islam, in the 7th and 8th centuries.. .(Kutasi Z.2008), and in the clas-sical (pre-Islamic and Mukhadram) qasidah (poem) may have images, or (stories) of quite specific animals, conforming always to very formalized appearance and beha-vior. (Stetkevych, 2002).

Medicine of horses after advent of Islam

Islamic Veterinary Medicine in its true context, can thus be defined as a body of knowledge of Veterinary Medicine that was inherited by the Muslims in the early phase of Islamic, from mostly Greek sources, but to which became added veteri-nary medical knowledge from, Persia, Syria, India and Byzantine. This knowledge was not only to become translated into Arabic, but was, assimilated, Islamic zed . The Veterinarians of the times both Muslim and non-Muslim were then to add to this, their own observations and experimentation and convert it into a flourishing and practical science, thus helping in not only in curing the ailments of the masses, but increasing their standards of health. The effects of its domineering in-fluence extending not only in the vast stretches of the Islamic lands, but also in all adjoining nations including Europe, Asia, China, and the Far East.

The Arab society of the classical and medieval periods was one which, on the whole, lived fairly close to nature, while the literate classes were heir to a Bedouin tradition in

which Animal love played a prominent part, and, in addition, were much given to country pursuits such as hunting and falconry. (Mcdonald, 1988)

It would appear safe to assume that by at least the twelfth century the title baitar was reserved for the veterinary surgeon and that some fro the Arab veterinary profession had been evolved. (Hare, 1984).

In the early period of Islam, the intelligential Muslim produced the great philologists and grammarians who helped evolution of Zoology and Natural history as special 4 sciences, by naming and giving the classifications of the large number of Animals known to the Arabs at that time, as also those imported from foreign languages. . (Maswani, 1938).

Medicine of horses in golden era of Arabic Islamic civalization the rapid development of Arab hippiatry is due to: The great role of the Horse during the Islam conquest; the rapid development of theoretical medical sciences and the high level of administration of medicines and preparation of instruments. Arabs had Horse Hospitals in which "baytars" (stablemen) treated sick Horses. Baytars were experts on breeding, riding, rising of foals and Horse-training as well and belonged to the head quarters as military physicians.Main mert: accepted and applied the practical teaching of former medical men: separated hippiatry from agriculture practice. In the therapy purgatives, washes, blood letting, burns, and administration of various medicines predominated. .(Seimens.and Charissis,2004) And many veterinary works were translated to Arabic language from Greek, Byzantine, Persian and Indian language. Weidenhofer men-tioned that since the late 9th century, scientific literature in Arabian language, based on the translation and compilation of works of the classical, Persian and Indian culture considerably increased. This also applies to the field of veterinary medicine, as is illu-strated by a number of hippological and hippiatric treatises. Affinities between texts on horse medicine in antiquity and in Arabian literature have been mentioned by phi-lologists, but the degree of dependence on classical texts could not be verified due to the lake of translations of the Arabian texts. (Weidenhofer, 2005and Garg, 1987).

There for, Arabian literature was attributed a prominent role in the conservation and transmission of the knowledge about ancient horse medicine. (Weidenhofer, 2005). **Arabic Veterinary Manuscripts**, at the beginning, knowledge about Horse's medi-cine was in poetry lexicography, books of language. In literature poetry lexicography have many descriptions of horses. It is because of the important role of the horse in the thinking of Arabs. There are countless descriptions of the horse with the titles ki-tab al- khayl (Book about the horses) and kitab al-faras (Book about the noble riding horse).and a lot – of fragments from poems as a kind of support to buttress the defini-tion of descriptions 0f the body – parts of the horse. The source of these works are the expressions used by the jahiliyh,s points and the Bedouins, who trained the horses on the desert fringes or in the oases. .(Kutasi ,2008).

lots of Arabic veterinary manuscripts scattered in many libraries in all over the world, very little of them were published, and only some in scientific form, where applied a rules of codecology and textile criticism, this need great project to study the very im-portant Arabic veterinary manuscripts.(Mohamed Adel, 1997).

Meri reported that, Hippology, numerous Arabic texts deal with Horse knowledge from either a theoretical or practical point of view. This knowledge " furusiyya " re-fers to hippological matters or to the nature of horses " Khalq al-khayl ", such as their different illnesses and cures " baytara " and equestrianism or horsemanship " siyasa al-khayl ".(Meri ,2005).

In Brockelmann,s encyclopedia about Arabic heritage (Brockelmann,s geschichte der Arabischen literature), a part of this literature is fairly technical, consisting of works on hunting, falconry, the care of Horses and veterinary medicine, but, as well as this, there is a large body of material which could best be described as " animal lore " it is this literature.

The veterinary manuscripts found in Turkey, were usually written about horses, and deal with the selection and breeding of horses, symptoms and treatment of diseases.(Dincer,1974).

Veterinary medicine 5

Greatest veterinarians in Arabic civilization as Ibn Ahi Hizam Al-Huttuli, Abu Beker Ibn Al Badr Al Mundir Albytar,,ibn Al Ahnaf and Al Ashraf. They wrote Encyclope-dias and important books in Horse's medicine, Weidenhofer, evaluated Ibn Ahi Hi-zam Al-Huttuli from his advice in one book and mentioned that this advise reflects the practical experience of Ibn Ahi Hizam Al-Huttuli, which is confirmed also by follow-ing prescription for some one traveling on horse :((if you are just on the way, and you have to fix the " intisar" without bandage, take old linseed and fill it in an iron dipper and mix it with hackled borax . boil it thoroughly and apply it on the tendon of the mount .)) and reported when he studied the affections of the extremities and their par-ticular treatment Ibn Ahi Hizam Al-Huttuli treatise appears unique. It, moreover, shows that he was an experienced horse doctor. (Weidenhofer,v 2005).

They made a good definitions, diagnosis, prognosis, control and treatment of horse's diseases, they used many tools for accurate diagnosis, case history, clinical examina-tion, by regional method, epidemiological parameter, effect of age ,sex, season and environment, they used a fire for sterilization of surgery instruments and for heat iron to cauterization.

Selen mentioned that, cauterization is one of the oldest means of treatment in the world:. The Arab Medicine has influenced the European Medicine in the Medieval times and cauterization became an important tool in the treatment of mankind and an-imals. (Selen, 2004). The ancient Arabic veterinarians had a valuable knowledge and

practical experience, in many branches medicine as, anatomy, physiology, therabeutic,internal and preven-tive medicine, infectious diseases, zoonosis, therogenology,and surgery. Seimens and Charisis reported that the ancient Arabian knew many diseases as colic, diarrhea, anthrax, rabies transmitted by bite, Dourine transmitted by coitus , grouping according the body regions , diseases of eye, nose, teeth, - diseases of head , neck, back, extremities. Contagious diseases, plague, equine lymphadenitis (Seimens and Charissis, 2004). Arabic treatises of a later date include first, the work of Ibn al-awwam, who wrote his agriculture compendium during the 12th century and second, the text of Ibn Al-Ahnaf, which was probably written around the year 605 AH (1209 AD), as well as the third and the most complete work on hippiatry named " an-Nasiri " by Ibn al-Mundir, vete-rinarian of the sultan Nasir ibn Qalawun during the first three decades of the 14th cen-tury . (Weidenhofer, 2006). **Anatomy,** philologist's works contain countless words about horse anatomy, Kutasi, mentioned that, In the middle ages the hippological books were written by philologists and they look like a register books of anatomy without illustrations. Later, in the 12th-15th century, appeared the illustrated anatomical books about horses..(Kuta-si,2008), and Dincer reported that, Kitab az-zardka ,in Arabic language, it can be said that the first known illustrated horse anatomy treatise in veterinary history.(Dincer 1974).Al-Masudi quotes a short story from the time of the Caliph Umar (634-644), who held examination for the horse to determine their nobility. They put a vessel full of water on the ground and led the horses one after another to drink from it. That horse which drank from the vessel with its forelegs upright, since its neck was long enough to reach the water, was declared a noble one, and that horse which bent its fo-relegs because of its short neck was recorded as a common horse. this test prove that ancient Arabian had the greatest experience in anatomy and applied anato-my(fig.1&2).

Therogenology 6

The history of AI is interesting. Old Arabian documents dated around 1322 A.D. indi-cate that an Arab chieftain wanted to mate his prize mare to an outstanding stallion owned by an enemy He introduced a wand of cotton into the mare's reproductive tract, then used it to sexually excite the stallion causing him to ejaculate. The semen was introduced into the mare resulting in conception. (Webb, 2003) There was a lack of detail regarding the c. 12th C. experimental breeding between Turkic mare and Persian stallion carried out by Ibn Ahi Hizam Al-Huttuli. (Me-serve,1996). **Cesarean**, Cesarean section was not performed as medical procedure in medieval Eu-rope the 14th century. Postmortem Cesarean section was also a doctrine in Islamic world until the modern ages, Cesarean section was accepted as a cultural event and it was not of medical importance (Lurie, 2005) although there is no evidence of Cesa-rean section in veterinary practice of medieval period, Kitab az Zardaka which is a book of Islamic period dated 1466 – 67 is the only manuscript consisting anatomical illustrations. In the second figure of this book a ten month pregnant mare and delivery of fetus are illustrated. This figure is the first gynecologic representation of the veteri-nary literature (dincer,1974 &Gultiken and Osmanagaoglu,2006). Mohamed Adel, eported the ancient Arabs before advent of Islam operated cesarean on sheep and goats (Mohamed,1988). **Fetotomy** , Al ashraf mentioned that, indication of this operation for sectioning of dead fetus in the abdomen of horse, this occur usually in first time of parturition, sec-tioning of fetus two or more parts within the uterus and vagina. Must be use a small It purpose it to reduce the size such that delivery through the birth canal become possi-ble. fetal parts will remove, fetal parts a symmetrically remove, if the obstetrician is certain that the fetus can be delivered by the employment of limited fetotomy, such as removal of the first forelimb until joint of shoulder, after that go out his hand and draw back the fore limb after that cut the head and neck, Noakes, Parkinson and Eng-land they that about same method, this will certainly be the method of choice.(Noakes et al, 2009) .if fetus posterior presentation begin cut with near part of fetus to vagina must use a small sharp held by palm in ventral side without appear any part will cut the tissue after that apply the mixture from specific type of oil, clear and clean ticked garlic and honey.(Fig.3). **Uterine prolapse:** Ibn Albetarand Ibn Ahi Hizam Al-Huttuli, they explained the problem of uterine prolapse, they mentioned\ the cause of uterine prolapse is abdo-minal straining during parturition, suitable position of mare, give mare a relaxant composed of babooning and king's in boiled water clean the inverted organs and wash with astringent, support the weight organs, replace the organs inside, suture the vagina with suitable thread not silk because cut the skin and recurrent prolapse, suture stay for seven days and injection with astringent as boiled crest of pomegranates.(Fig.4)

Surgery

Procedure : Cataract operation were performed, Abscesses were opened, osteal growth were resected, teeth were extracted , also polished to correct irregular abra-sion, bandages were used, suture were made, hammocks were used, blood let-ting.(Seimens and Charissis ,2004).

Arab practiced a good veterinary surgery, the word veterinary (Bitar) in ancient Arab-ic language mean a veterinary surgeon, they knew surgery utensils and a suitable one for every operation and for every step of operation and differ according species, ages and conditions, too they used a fire of sterilization and must be sharp. Different ma-terial for suture were used, cotton, types of silks and natural organic material for in-ternal suture from the large ants. 7

Abu Beker Ibn Al Badr Al Mundir Albytar the indication of castration are a – as treatment or for useful ( ) mentioned that 6 six methods for castration OF horses and may be fit for other animals : 1- first methods, the animal lay on dorsal position and lift two hind limbs, catch two tests by forceps, rope two tests from their organ by rope was made from cotton or Kanab, good legation, incision for

every tests alone, must be include all layers, the incision must be longitudinal by sharp scalpel (al mekwah al hada), the tests will appear without any discovered membrane, then catch tests from its origin by forceps of castration, then cut above forceps, put astringent on the end of vein until stop of bleeding, make the same in other tests, in the end remove the outside legation, applied on wound a mixture from oil, salt and garlic without left any place from wound from this mixture, and the animal must be walking, if bleeding conti-nuous after operation do not ignore this it is fatal, it is must be rope the vein. 2- collect two tests, remove membranes of scrotum, make incision, put castration for-ceps to held the origin of tests, cut with hot sharp iron, use astringent, apply a mixture prepared from oil, salt and garlic. 3- go to the origin of spermatic cord and vesicular, pressure on them, then use a bardi-zo, made from wood until the origin of two tests were pressed, let roped bardizo one or two days until remove two tests and fall. 4-This method is difficult because rope two tests and incision, after that torsion of the spermatic cord and vesicular part until remove from they origin, apply on wound a mixture from oil, salt and garlic, but this method is difficult for horse but suitable for cattle and kids.

5- Remove all tests, and use astringent, but astringent may be useless.

6- this method of castration suitable for horses, Equine, Cattle and kids, this by good and strong legation by rope the origin of testes, left rope until tests fall, this method not use because the animal will fell with pain. Al Ashraf, mention that, the scalpel and utensils from iron must be heated for sterili-zation. **Ophthalmology**, one of the most important achievements of medieval medical Arabic literature deals with of ophthalmology, which is passed down in comparatively large number of treatises focusing on the anatomy of the eye, its disease and their treatment. Arab invented, for example, a special technique for operation on cataracts. Inversion of the third eye lid (pterygium) : raise the membrane with a thread sewed with a needle, before the pterygium should be excised with a scalpel . (Weidenhofer, 2006). press on the large angle of eye with your finger until the third eye lid appear outside, needle with threat and gang it, pull outside of the eye, round cut the cartilage parallel to eye after that apply that. if blood vessel cut and bleeding do not ignore but use needle and yellow silk to close it, because I sea many horses dead from bleeding, use collyriumia powder for sterilization, may be formed from three types of salts Indian salt, yellow and gemme , ammonia and pepper one part from every one and half part from sugar candy grind and to sterilize.

CONCLUSION

Horse medicine began as a primitive in different ancient civilizations , Arab according to their life in desert and social, military among trips and defense on their selves , so-cial attitude where the Arabian Horse considered a Nobel horse with highly advan-tages than others until now this improve their importance and good caring with Ara-bian Horse, for this the medicine of Horse well developed , recorded orally before Is-lam in poetry especially and other orally Arabic heritage, the record of veterinary science was after Islam in different sources by philologists and books of history, geo-8 graphy, traveling, biology, in different literature, until specialists veterinarian prac-ticed and record horse medicine according to their individual experiences, from their professors, from neighbors country directly by traveling or veterinarians from this country came to Islamic State, or indirectly by translating their veterinary books, the ancient Arabic medicine for horse was a valuable in this era and in comparison with recent veterinary medicine , they knew, diagnosed and treatment many Horse diseases , made a differential diagnosis , zoonotic , infectious , internal , therogenology, sur-gery and most things deal with health of Horses and its development.

FOOTNOTES

Abu Beker Ibn Al Badr Al Mundir Albytar, (d.1340 AD), is greatest veterinarian of the medieval period. his famous book is "Kamil Al-Sinatain " .the work must have been written between 1310-1340 AD. The book was dedicated to The Mamluk Sultan Al-Nasir (1293-1294, 1299-1309, and 1310-1341 AD) Ahmed ibn al –Hasan Al-Ahnf was one of the writers of veterinary in the 13th century. His Arabic book is called Mukhtasar Al-bytarah .No records have been encountered about the author. Ibn Ahi Hizam Al-Huttuli on of the oldest available Arabian texts about hippology and hippiatry, which was written during the late 9^{th}

FIGURES

All figures from manuscript Mukhtasar Al-bytarah which wrote by Ahmed ibn al –Hasan Al-Ahnf (605Ad) 13th century Fig.1, Fig.2 clarify theoretical and practical a test for determine a noble Horse . Fig.3, clarifies fetotomy, Fig. 4, clarifies, a method for treatment uterine prolapse . Fig.5, clarifies, Examination of Horse before surgical operation. Fig.6, clarifies a surgical operation and the text explains a contraindication cut any nerve or blood vessels especially large one of them, and how deal and treat the bleed-ing.

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27. E mail, adelqena@lycos.com
28. Fig.1 Fig.2 Text in Arabic language explain Miniature show practical a test mine the nobility of horse to different between a noble and common horse 11

Fig.1
Text in Arabic language explain mine the nobility of horse

Fig.2
Miniature show practical a test to different between a noble and common horse

Fig.3 Fetotomy of dead fetus

Fig.4 Treatment of uterine prolapse

Fig.5 , Examination before surgical operation

Fig.6 , Surgery

VETERINARY PUBLIC HEALTH – HOW CAN ANIMAL HEALTH SERVICES CONTRIBUTE?*

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SUMMARY

Animal health services (AHS) are active in many countries around the globe with varying structures and diverse foci of activities. AHS are working on a range of livestock species, including cattle, pigs and poultry but also fish or bees. AHS typically focus on endemic or so-called production diseases and the optimization of productivity in general but some AHS also run or support programmes targeted at specific pathogens. Depending on the objective, both specific and unspecific interventions can be used. Historically, the direct contributions of AHS to public health appear to be limited. However, with increasingly constrained public budgets, some tasks may be passed on to AHS in the future. Examples of indirect contributions by AHS by controlling or eradicating economically relevant diseases are manifold and constitute a core activity. With an increasing awareness for food safety and zoonoses among consumers, new opportunities emerge that could lead, for example, to programmes for specific-zoonosis-free (SZF) production and expand the scope of AHS activities.

INTRODUCTION

Veterinary public health (VPH) is defined by the World Health Organisation (WHO) as a sub-area of public health which requires special knowledge, skills and understanding of veterinary medicine and contributes to the physical, mental and social wellbeing of humans. Traditionally, this included predominantly issues related to food safety and zoonoses, but more recently, the scope has been expanded to generally include the control of economically relevant animal diseases. Using this definition, it becomes clear that at least some activities of animal health services fall within the scope of VPH.

Animal health services (AHS) in this article are defined as organisations supporting and promoting animal health of food animals by managing infectious diseases and advising on good production practices. They are active in many countries around the globe with varying structures and diverse focus of activities. They are often industry-run organisations or cooperatives but may also be fully or partly government-funded. The source of funding of AHS is likely to impact on their area of activities and focus as the various funding bodies will set the priorities according to their own agendas. Clients of AHS can be individual farmers, but they also work for farmer groups, breeding organisations, cooperatives as well as local and national governments. Some AHS have grown into large, successful companies, such as the Animal Health Service Deventer (GD) with >500 employees, which is running its own laboratories and has an annual turnover of >50 million Euros (www.gddeventer.com).
The activities of AHS will vary between countries according to the economical significance of the different livestock industries and the health challenges relevant to them. AHS are therefore working on a range of livestock species, including cattle, pigs and poultry. In some countries, there are also AHS focusing on small ruminants, fish or bee health. AHS typically focus on endemic or multi-factorial, so-called production diseases and the optimization of productivity in general. The services provided can include consultations on specific cases or problems, administration and implementation of control programmes, diagnostic services (including laboratory diagnosis), information and education.

In this paper, I aim to provide an overview of current activities of AHS but also explore future opportunities for AHS' contributions to VPH. Examples used have a European focus, which does not mean to imply that similar examples do not exist in other parts of the world.

Direct and indirect contributions

VPH has a clear focus on the health and general wellbeing of humans. Contributions from AHS to VPH can be either by directly reducing the risk of hazards to humans related to animal-derived products, or contributions can be indirect, mainly by impacting on the economics of livestock production including competitiveness and international market access. VPH-relevant activities of AHS can also be grouped into either specific, i.e. targeted at an identified hazard, or unspecific activities. Some examples of these are provided in Table 1.

*This paper is dedicated to the memory of Prof. Hermann Keller, who was essential in developing the Swiss Pig Health Service. He passed away in April 2011.

Table 1: Examples of Animal Health Service activities with either a direct or indirect impact on Veterinary Public Health

	Impact on Veterinary Public Health	
	Direct	Indirect
Specific Animal Health Service activity	• Control of salmonella infection in pigs or poultry by vaccination	• Control of Bovine Virus Diarrhoea in cattle by vaccination • Control of foot-and-mouth disease by vaccination to achieve access to international markets
Unspecific Animal Health Service activity	• Reduced use of antimicrobials in food animal production by promoting good husbandry practice	• Disease prevention by increased biosecurity • Increased milk quality by promoting good milking hygiene

The requirements for animal-derived food increasingly include safety attributes particularly if the intention is to trade internationally. For example, some retailers expect evidence on the status of a farm regarding food safety hazards such as salmonella infections. In the absence of official control programmes targeted at such hazards, AHS can provide services related to monitoring and intervention for relevant hazards and thus directly contributing to VPH. Also, if official control programmes are introduced by governments, AHS can play an important role in their implementation. This can be achieved either by outsourcing such programmes to AHS or by contracting AHS for certain aspects of such programmes. AHS can also play a role in auditing specific public health safety standards, for example, in relation to antimicrobial usage or specific industry standards on welfare. One example of such involvement is the role of the Austrian Animal Health Service (www.tgd.at) in relation to monitoring of antimicrobial usage.

Historically, the direct contributions of AHS to VPH appear to be limited. This is likely due to the fact that the control and management of zoonoses is considered to be a statutory task and therefore within the scope of work of veterinary authorities. However, with increasingly constrained public budgets, some tasks may be passed on to AHS in the future. For example, in the United Kingdom there are continuing discussions about responsibility and cost sharing within the veterinary services. While this work is continuing and final directions are not yet known, there is currently no indication that zoonoses are likely to be a priority. However, disease control efforts that will benefit animal keepers and the industry may be discussed as candidates for responsibility and cost sharing in the near future. A recent study conducted among animal health experts in Ireland suggested a priority selection of diseases to be addressed by non-regulatory initiatives (More et al., 2010).

Indirect contributions to VPH are mainly related to the prevention and control of economically important diseases. Efforts to reduce diseases of animal also contribute to the reduced need for treatment and are therefore also impacting on public health. AHS have successfully developed and implemented disease control strategies at regional level. Such initiatives then sometimes evolve to become national or compulsory programmes. The strategies used by AHS can include both unspecific and specific interventions. Specific interventions often involve vaccination programmes, purchasing of animals with confirmed health status and specific-pathogen-free (SPF) production. Unspecific measures include increased biosecurity of farms, quarantine and purchase policies, farmer education and general quality assurance across all aspects of animal husbandry. The latter also covers all aspects of feed quality, housing aspects and veterinary care.

Examples of AHS programmes targeted at specific pathogens are the control of bovine virus diarrhoea (BVD) in cattle or the reduction of respiratory diseases in pigs. Such projects are advanced in many countries in Europe and elsewhere. Regarding respiratory diseases of pigs, many of these initiatives are run privately with the support of AHS with some governments providing at least partial funding (see for example Stärk et al., 2007). For BVD, some countries have regional or national programmes run privately and on a voluntary basis (Moennig et al., 2005; van Campen, 2010), while others have implemented national, compulsory programmes fully funded by the public.

AHS are also active in assuring the quality of animal-derived products, particularly milk. While these activities are often unspecific and impact mainly by assuring higher prices through improved quality, they can also include specific VPH-relevant aspects. For example, the Norwegian Cattle Health Services have been recording the incidence of diseases in dairy cattle for several decades and provide very detailed estimates on the frequency of diseases (Østerlås et al., 2007). Their data include information of public health relevance such as the incidence of mastitis which is likely to be linked with the use of antibiotics.

A further area where AHS contribute is by providing training and education. The Austrian Animal Health Service (TGD, www.tgd.at) mentions education of farmers and veterinarians as a key component to support implementation of farm assurance and general competitiveness. They mention usage and recording of drug usage and traceability as important concepts where training priorities are set.

DISCUSSION AND FUTURE OPORTUNITIES FOR AHS

While the examples of AHS successfully contributing to public health in an indirect way by controlling or eliminating economically relevant animal diseases, there is currently limited evidence of direct contribution. However, incidents involving non-statutory zoonoses may increase the debate of a possible contribution. For example, Q Fever, an infectious disease of cattle, sheep and goats caused by *Coxiella burnetti* has caused a large, continuing outbreak in the Netherlands which caused over 4,000 human cases and 11 deaths since 2007 (Schimmer et al., 2009). Due to the environmental persistence of the pathogen, control is difficult. Although Q fever is subject to statutory disease control in many countries, there is an opportunity for involving AHS in the monitoring and early warning of similar zoonoses.

There is also increasing public health concern world-wide regarding the use of antimicrobials in animal production. The WHO has chosen antimicrobial resistance as the topic of the World Health Day in 2011 (www.oie.int). In its policy package, WHO calls for a global, multi-sectorial approach to reduce antimicrobial usage, particularly also in food animal production. Topics such as this one offer ample opportunities for AHS to get involved. Current SPF programmes could evolve to become programmes for specific-zoonosis-free (SZF) production.

CONCLUSIONS

AHS have played a key role in a number of programmes relevant to VPH. With an increasing awareness for food safety, new opportunities emerge that could lead to an expanded scope of AHS activities. In order to play this role, AHS will have to be able to provide evidence-based, independent advice. They have the potential to be trusted, reliable partners of primary producers as well as retailers, and thus play a key role in the production chain.

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HACHAKLAIT ISRAEL - CLINICAL SERVICE, MONITORING AND SURVEILLANCE IN DAIRY HERDS

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INTRODUCTION

Israel has about 120,000 dairy cows kept in close to 1,000 herds. More than 95% of these are Israeli-Holstein cows. Herd size varies between smaller family-owned farms with 50 to 200 and larger Kibbutz farms with 250 to 1,100 milking cows. Dairy farms are scattered all over the country with a large variation in climatic conditions; from a short and wet winter and a long and humid summer in the Mediterranean coastal plain, to the arid and very hot Negev and Jordan valley regions, to the rainy and cooler Galilee and Golan. All dairy herds are kept in "zero-grazing" facilities, and the most common housing system consists of loose barns with a few free stalls, and no tie stalls barns. Cows in all the Kibbutz farms and some of the family farms are milked three times a day. Most milking parlors are herring-bone or parallel as well as some recently installed automated robotic milking systems. Almost all cows are artificially inseminated (AI), using mostly local semen produced by one bull station, and inseminated by professional regional inseminators. At the end of 2010 eighty percent of the herds and close to 90% of the cows were recorded monthly in the Israel cattle breeders' association (ICBA) Herd Book. All large herds as well as some of the smaller ones use automated identification systems of individual cows (mainly Afimilk® or SCR™). These systems automatically record the milk quantity, milk electrical conductivity and the amount of the cow's steps or neck movements at each milking. More than half of the farms and all of the large ones use comprehensive farm management software (NOA, Afifarm®) which enables easy and uniform data recording. Almost all farms feed Ad-lib total mixed ration (TMR) which is produced on site or purchased and delivered daily to the feed bunk. The typical feed ration of milking cows contains high concentrates (>60%) and low roughage with a variety of market available byproducts. The farm-gate milk price is affected by its fat and protein contents, somatic cell (SCC), bacterial counts and a summer-milk premium. In 2009, the average milk production per cow in 305d was 11,945 kg (ECM), with 3.51% fat and 3.13% protein, considered to be the highest in the world. The top herd produces just over 14,000 kg per cow per year. Bovine Somatotropin (bST) is not used in Israel. Each farm has a milk production quota adjusted by a semi-government Dairy Board according to market demands, and is divided into winter and summer quotas. Most farms rear their own replacement heifers - aiming for first calving at 24 months of age. The average annual culling rate is 30%. There are many disease and management risks inside and outside the farms. The objective of this paper is to describe and discuss the clinical veterinary service and the monitoring and surveillance activities performed by HaChaklait, that are aimed at optimizing production, and supporting the cows, the clients and the public in a harsh and challenging environment.

HaChaklait Organization

HaChaklait was established over ninety years ago in 1919 by a handful of enthusiastic pioneer farmers who had emigrated from Europe. Their vision was to establish an organization that combined a mutual insurance policy with a comprehensive veterinary service. The initial motivation was to protect the valuable and sensitive cattle that were imported from Europe and North America into a hot and arid land, burdened with diseases such as Rinderpest, Foot and Mouth, tick born diseases and many others. HaChaklait was founded as a cooperative, which was owned and managed by the farmers for their own benefit. The veterinarians were, and still are today, contracted as employees of this cooperative. From its small beginning, serving just few farms around the Sea of Galilee, HaChaklait grew hand in hand with the Israeli Food Animal Industry to encompass the entire country. Today, ninety two years later, HaChaklait is still a strong and thriving organization. Both in size and structure, it is unique in the veterinary world. About eighty percent of all dairy farms with approximately 100,000 milking cows are being served by HaChaklait Veterinary Services. HaChaklait is a non-government, not-for- profit farmers' cooperative. HaChaklait's basic goal is to give its clients the best veterinary service at a reasonable cost. Each farm is charged a monthly fixed rate per animal, calculated to cover all of its routine, emergency and consulting needs. No extra payment is charged per time spent or per task operated on a farm. Thus, HaChaklait has a long term and stable contract with the farmer, is committed to the well being of the animals and is dedicated to the sound economy of the farm. From a veterinary public health point of view, both food producing farmers and the vets are geared to supply wholesome safe and reasonably priced products. HaChaklait is also serving some beef, sheep and goats herds and feedlots which will not be discussed in this paper.

HaChaklait personnel include 50 vets and some management staff. Thirty-six of the vets serve as regional practitioners throughout Israel. Eight junior vets operate as replacements (locum) for the district vets and also work on various organizational tasks. Some of the vets operate

part time as consultants for epidemiology, applied nutrition, dermatology, parasitology, hoof-care, young stock, ultrasound, beef, feedlot and small ruminants. The mission of the consultants is to support the other vets and the farmers, and to represent HaChaklait in national and international interactions. The ability to become a company consultant gives the more ambitious vets a career path and extra stimulation beyond the routine chores of a farm vet. The Epidemiology (also called Herd Health) department, consist of 5 experienced vets of whom 3 also work as field practitioners. The department serves and supports the regional vets, the farmers and the HaChaklait management. It also assists the national veterinary services and other national bodies. Forty percent of the HaChaklait vets studied veterinary medicine in Israel, while the others studied in various schools around the world, bringing a healthy diversity of approaches and thinking.

Clinical Veterinary Services on Dairy Farms

The objectives of modern herd medicine today are geared at three different levels:

- The Cow - curing diseases and prevention of suffering.

- The Herd Owner - maintaining a sound, productive and efficient business.

- The Public- helping to supply abundant, healthy and hygienic food.

In order to achieve those objectives, HaChaklait practices intensive veterinary medicine at three levels: The individual cow level, the herd level and the multi-herds/ nationwide level. Its execution emphasizes frequent and dominant vet presence on farms. Vets initiate regular check-ups rather than just responding to calls, carry out part of the treatments and all of the vaccinations themselves and are involved in the farm management and economics.

High producing milking cows need high quality attention in order to maximize efficiency. Sub optimal production is a major loss potential on intensive dairy farms. Our vets visit each dairy herd between one to 3 times per week. Each cow is examined routinely several times per lactation: after calving to make sure she is free of clinical and subclinical post calving diseases, thereafter at the end of the voluntary waiting period for anestrous, and later on for pregnancy check. Early detection and rapid intervention are expected to maximize efficiency and minimize losses. Cows are scored for body condition (BCS) 3 times per lactation on most farms. All the data from each individual cow is recorded by the herdsmen onto the farm computer using nationwide uniform terminology and parameters. The data collected at the farm level is aggregated in a central database and is processed by the ICBA Herdbook and by HaChaklait. This high volume and high quality data set is also available for academia, researchers and government bodies.

Usage of Drugs, Vaccines and Medical Costs

Just about all of the veterinary drugs and vaccines in Israel are imported from a limited number of "recognized" countries (the EU, North America and Australia). HaChaklait handles purchase, registration, importation and distribution of a wide range of remedies, vaccinations and equipment. In order to perform these tasks, HaChaklait operates a large and modern central warehouse. Drugs are distributed only through the vets and are sold without profit to the farmers. The vets do not benefit financially from supplying the drugs and so are not motivated to distribute more than what they find necessary. HaChaklait advocates low and rational drug use both for food safety and for farm economics. HaChaklait strongly believes that intensive farm presence, regular checkups, early diagnosis and herd-monitoring activities reduce the farms drug use, drug costs and increase their product quality and safety. Treatments are recorded onto the management software, and are then aggregated and monitored. The total vet cost, service and drugs, was about 2% of production cost per liter of milk, or about 55 euro per cow per year in 2009. The composition was 66% for vet fees and 34% for pharmaceuticals. The pharmaceuticals cost are further divided into drugs (60%) vaccines and utilities (40%). Annual drug usage reports provide comparisons among vets and farms for specific years. Trends are identified, monitored and analyzed. This transparency promotes more rational drug use and the replacement of treatments by means of prevention and by management techniques. Figure I show the mean and the variation of pharmaceuticals costs among HaChaklait regional vets in 2009.

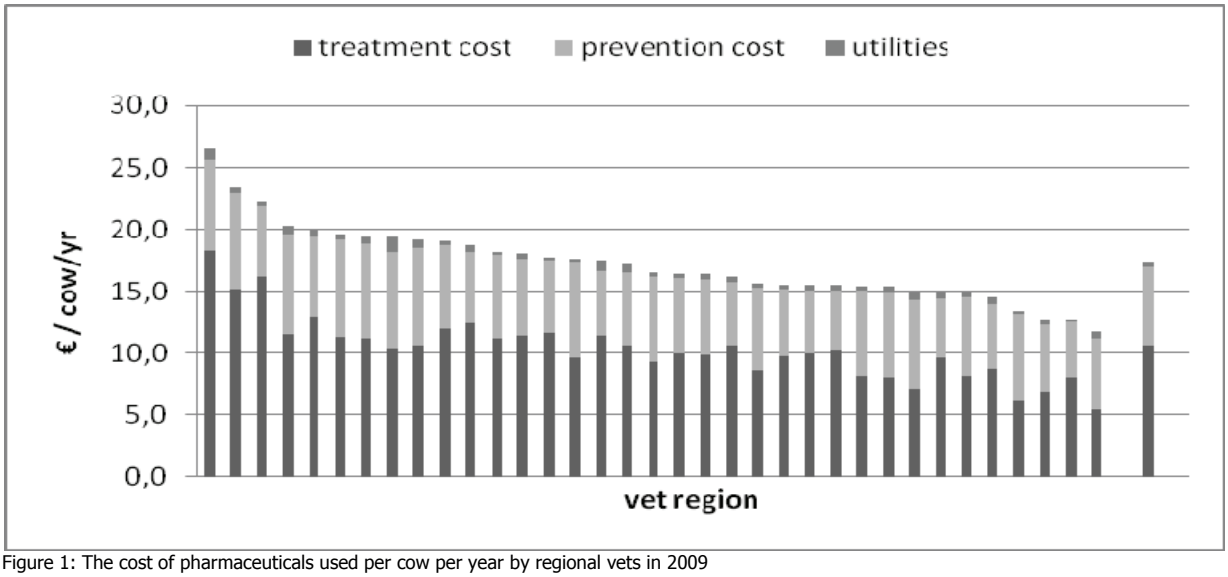

Figure 1: The cost of pharmaceuticals used per cow per year by regional vets in 2009

Monitoring of Laboratory Diagnosis and Findings

HaChaklait offers its client farms low cost comprehensive laboratory insurance for various diagnostic tests of sick animals. The diagnostic tests are done in one government and several private labs and are paid by HaChaklait. The herd vet is expected to lead and guide what, when and how to sample. The lab results are sent to the vet, who forwards and explains them to the farmer. Results are monitored at HaChaklait level by the chief vet and the epidemiologist who will intervene if results seem to deviate from the expected norm. Both the vets and the farms are monitored for their level of sampling of some important diseases, such as infectious causes of abortions (Figure II), causes of neonatal diarrhea, or changes in antimicrobial susceptibility. Vets who sample either much less or much more than anticipated thresholds are notified for epidemiological and economical reasons respectively. HaChaklait is running voluntary control programs for BVD and for Paratuberculosis. Farms are encouraged by their vet to join and get guidance and support to implement these programs successfully.

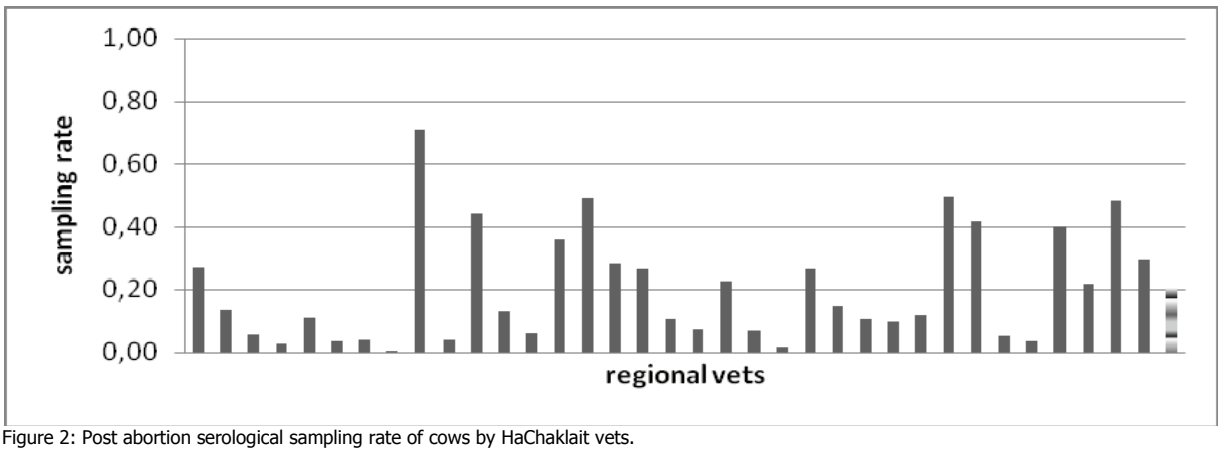

Figure 2: Post abortion serological sampling rate of cows by HaChaklait vets.

Surveillance of Production, Reproduction and Production Diseases

HaChaklait has an epidemiology unit (called "Herd-Health department"), established by Dr. O. Nir- Markusfeld some thirty years ago, which harvests, processes and provides valuable information to the vet and the farmer. The relevant data is collected from three sources: the farm, the AI cooperative (Sion Ltd.) and the ICBA herd book. After processing the data, HaChaklait produces a periodic (semi-annual or annual) report for each farm. The report includes a descriptive monitoring section and a multi-factorial causal analysis section. The report deals with major calving traits and diseases, milk production, reproduction and some of their economic implications. Parameters and goals are set separately for replacement heifers, first lactation (primiparous) cows and second or higher lactation (multiparous) cows. This vast data base enables HaChaklait to compare individual farm results nationwide, and to compare same farm results with its results in previous years. The benchmark values like mean, median, standard deviation, quartiles, minimum and maximum for each and every trait are available to all farmers, vets and researchers. New herd goals are set and updated annually based on the upper quartile figure of more about 150 large farms. (See sample from a farm annual report at the table below) During an annual farm visit by one of the herd-health consultants, the report is discussed with the farm owner, management team, the

farm vet and the nutritionist. At this meeting, the farm receives an analysis of its production problems and issues and is given recommendations and suggested remediation strategies. The execution of these changes will be monitored and assessed at the next meeting. The success of this process depends on cooperation, an understanding of the importance and an investment of the time required at all levels. Reliability of the recorded data is also crucial. Farmers need to be shown and convinced of the advantage and payback that this extra effort entails. The willingness of the farmers to expose and share their farm data is another major success factor.

Calving traits	Primiparous		Multiparous	
	Rate	Goal	Rate	Goal
a. Total calved	161		353	
b. % Twins	1.2	(0.0)	6.5	(5.1)
c. % Stillbirth	3.2	(4.7)	4.0	(4.3)
d. % Milk fever	0.0	(0.0)	5.7	(1.6)
e. % Prolapsed uteri	0.0	(0.9)	0.6	(0.5)
f. % Displaced abomasum	1.2	(1.0)	2.0	(1.5)
g. % Retained placenta	10.0	(5.9)	18.0	(10.2)
h. % Primary metritis	64.4	(34.1)	20.6	(17.8)
I. % Ketosis	13.1	(7.8)	28.1	(11.0)
j. % Calved with mastitis	3.1	(1.1)	0.3	(0.7)

Surveillance of Emerging and Re-emerging Diseases

Maintaining intensive farms in a harsh environment is not a simple task. The State of Israel is tiny in size yet it harbors many threats of epidemics - both emerging and re-emerging. Israel is situated in the tip of Asia, at the border of Africa and at the gate to Europe. Along with its intensive dairy herds, there are many extensive and nomadic sheep goat and cattle herds that are scattered all over. Some of these herds are unknown to the authorities, have no regular veterinary service and have low compliance to vaccinations and other controls. Large quantities of live sheep and cattle are imported each year from Europe and Australia for fattening and slaughter. Israel has long borders with countries with less intensive and partially vaccinated herds. There is also a significant wildlife population that plays a role in transmission of some of these diseases. The Rift Valley (stretched along the eastern border of Israel) is a migration route to hundreds of millions of birds twice a year. All these risk factors compose a complex epizootic environment to monitor and control, especially for high producing, sensitive and densely populated dairy cattle. Some Foot and Mouth cases are identified almost every year, despite mandatory vaccination, partially due to low vaccination compliance in some herds. Several ARBO viral diseases outbreak periodically, cause variable damage and require systematic monitoring and surveillance. Outbreaks of Bovine Ephemeral Fever (BEF) have occurred five times in the last decade, causing vast damage mainly by lowering milk production, abortions and salvage culling. Lumpy Skin Disease (LSD) likely arrived from Africa twice in the last decade (2006, 2007) and a major EHDV epidemic broke out in 2006. Several strains of BTV are regularly found in cattle and in sheep. The role of HaChaklait field clinicians is important as sentinels in identifying the first (index) cases of a new outbreak and reporting them to their colleagues and to the national veterinary services. Farm clinicians also help in vaccinating and in treating or salvaging sick animals during outbreaks, and assist in serological sampling and other monitoring activities. In the face of the low presence of state employed vets, the strong presence of the private sector vets is critical, and provides returns by early identification of known and unknown outbreaks, rapid intervention, thus in lowering the disease's economical damage as well as reducing animal suffering. The help of field vets in diagnostics, data recording and research help to prevent future epidemics.

Field Trials and Investigations

HaChaklait vets have been involved in field research long before the establishment of the local vet school in 1985. Several years ago, HaChaklait opened a small clinical research unit headed by a vet who is also a qualified epidemiologist. The aim of the unit is to initiate, manage and support various field surveys, investigations and prospective clinical trials. The company vast data sets are extremely valuable and useful for retrospective research. The regional vets are encouraged to participate in research activities, and to apply for research grants. They are given the time and resources required to execute applied research. We believe that through this extra work, the field vet is enriched professionally, stimulated to learn more and enabled to progress professionally and to contribute to their and to other client farms.

HaChaklait Affiliations and Education Programs

HaChaklait interacts with the following professional bodies in Israel: The Milk Board, the Israeli Cattle Breeders Association, the National Herd-Book, Sion (the Israeli A.I. cooperative), the Milk Quality & Udder Health Laboratory, The National Veterinary Services, the Kimron Veterinary Institute, the Koret Vet School of the Hebrew University, the Ministry of Agriculture Extension Service and various commercial companies. The state is trying to reduce government employed veterinary work force in the field and maintain adequate monitoring and surveillance at the same time. HaChaklait, due to its large size, extensive reach and high level of organization represents the farmers' needs while assisting the national vet services in executing various professional field tasks. Both the farmers and the national veterinary services trust HaChaklait vets to achieve optimal control with minimal harm to livestock, in a manner that is humane and facilitates animal health.

HaChaklait promotes continuing education for its vets through various channels such as monthly seminars, local and international conferences, courses and workshops. HaChaklait also offers short courses, day meetings and "wet labs" to its farmers, as it is appreciated that knowledgeable vets and farmers perform better. HaChaklait encourages vets with high potential for advancement to continue their professional education, to join the national specialization accreditation program and to teach in universities and other academic programs. It also encourages vets to take short consulting projects overseas. This is because Israel is isolated from other developed countries, and has only one veterinary school with limited opportunities for further study. HaChaklait hosts overseas students and vets from various countries and organizes an annual Herd Health work shop to teach its unique Herd Health program. These approaches keep HaChaklait’s vets up-to-date with the changes in veterinary knowledge, which in turn benefits both the vet and the company.

SUMMARY

Israeli climate, scarcity of land and water, high labor costs and numerous disease threats do not make it a natural or easy place to intensively raise dairy cows. Its success in doing so is attributed to the innovative, cooperative and untraditional spirit of its farmers, which also established and preserved the type of veterinary service practiced on their dairy farms, from the very first days until today. HaChaklait's unique structure was set and shaped many years ago, when a more cooperative way of thinking was popular in Israel. HaChaklait's survival through economic and political changes is probably due to its still appreciated benefits to both sides: the farmers who enjoy and are willing to pay for this intensive service, and the vets servicing them who enjoy financial and professional stability. Stability and continuity are important components of sustainability. As countries tend to privatize government services, the national veterinary service can benefit from intensive on-farm presence of professional vets who belong to a countrywide synchronized network. In today's heightened public awareness, increased legislative and regulatory scrutiny and demands related to the quality, health, welfare and environmental aspects of animal sourced food production, an organized structure like HaChaklait can successfully perform key control and surveillance functions.

However there are forces that threaten this pleasant picture. These include: economic pressure to further reduce milk production costs on dairy farms, reduced milk prices paid to the farmer, farmers' aspiration for more independence regarding if, when, how to use and how much to pay for their veterinary service, and the shortage of vets keen to work in food supply veterinary medicine. In HaChaklait’s vision the vet should play an important role in modern dairy farm management. Vets should learn to use advanced technologies and should be involved in epidemiology, nutrition, animal welfare and farm economics. They should do all of this for the benefit of the cows, the farmers, the public, and to ensure the sustainability of the veterinary profession.

SALMONELLA DUBLIN OUTBREAK IN CATTLE (Abstract)

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INTRODUCTION

Reproductive failure respectively abortion in dairy cows comprise a large number of different infectious and non-infectious causes. Enhancing reproductive efficiency is a main target in lifestock farming, therefore accurate diagnosis and eradication of venereal diseases are highly important for prevention of financial losses to the dairy industry. Due to the successful eradication of *Brucella abortus*, *Campylobacter fetus* subspecies *venerealis*, infectious bovine rhinotracheitis virus and the ongoing bovine viral diarrhoea virus control program, the number of infectious abortion outbreaks is relatively low in Austria. This case report describes a significant increase in abortions on two different mountain pastures in Upper Austria.

ANIMALS, MATERIALS AND METHODS

In late summer 2008 abortion material (fetus and placenta) from 5 cows in 5 different farms were sent to the AGES Institute for Veterinary Disease Control in Linz for examination. *Salmonella (S.) Dublin* antigen type 1,9,12:g,p was isolated from 5 fetuses and 2 placentas originating from 5 different farms. For detection of clinically inapparent *S. Dublin* infected cattle, 112 fecal samples were taken in 21 contact farms. Additionally, bulk milk samples from 26 farms were sent to the Danish National Veterinary Institute in Copenhagen for ELISA testing.

RESULTS

Totally 12 *S. Dublin* infected cattle kept on 6 farms were detected during this outbreak, whereas only one farm was tested positive by *S. Dublin* bulk milk ELISA.

CONCLUSIONS

As the annual reports of the National Reference Laboratory for *Salmonella* clearly demonstrates, *S. Dublin* infections are very rare in Austrian cattle and often restricted to single farms. Common grazing of cattle from different farms is a major risk factor for spreading diseases between herds and led to infection in 6 different farms. Bulk milk serology showed low sensitivity, only one of 6 affected farms could be detected by *S. Dublin* antibody ELISA, therefore feces sampling is still necessary for detection of *S. Dublin* infected cows respectively farms.

RISK BASED CLASSICAL SWINE FEVER SURVEILLANCE IN STYRIAN PIG HERDS

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SUMMARY

This paper describes the methodology of creating a risk based sampling scheme for classical swine fever surveillance in pig herds located in the Austrian province of Styria. By using information on various farm characteristics (herd size, husbandry system, movement of pigs, hunting activity of the farmer and gastronomical activity, biogas plants or compost works on the farm) and knowledge about the regional wild boar density all pig farms were classified according to their CSF-risk. Following this risk assessment 152 farms with the highest risk were selected for a serological CSF screening.

INTRODUCTION

Classical Swine Fever (CSF) is a highly contagious viral disease that affects both domestic pigs and wild boar [5]. Recent classical swine fever epidemics in the European Union (EU) resulted in enormous economic losses and social consequences [6]. Early detection of the introduction of a highly contagious diseases such as classical swine fever is well known as a crucial factor for confining the size of an outbreak. However, CSF is often associated with non-specific clinical signs and thus hard to detect in a short time period after introduction. Thus in 2010 the Austrian Federal Ministry of Health followed a recommendation of the CSF task force of the national expert group on animal disease control and established a nationwide classical swine fever surveillance program. This program is based on the Austrian Decree on Animal Health Surveillance Programs [1] and consists of the following elements: CSFV antigen testing of organ samples collected from suspicious carcasses in slaughterhouses, rendering plants and diagnostic laboratories, CSFV antibody testing of blood samples collected in the course of the surveillance scheme for Aujeszky`s disease and on farms with a high risk for an introduction of CSFV. The implementation of this program and the selection of high risk farms is the duty of the provincial veterinary authorities.

MATERIAL AND METHODS

According to the instructions of the Federal Ministry of Health the number of pig herds with a high CSF risk to be tested in the province of Styria is 152 per year and the number of blood samples to be taken is 4 per herd. In order to set up a risk based CSF sampling scheme for Styria we considered the major routes for CSFV introduction into free regions of the European Union: animal movements, feeding of improperly heated swill and direct or indirect contact with wild boar [4]. For assessing the risk of individual herds we tried to identify some specific risk factors and accessible relevant data. As the risk of illegal swill feeding increases with the amount of kitchen waste handled on a pig farm, gastronomical activities (e.g. restaurant, inn) and the operation of biogas plants or compost works on such farms were considered to be risk factors. The relevant data were extracted from the register of gastronomical business establishments and the register of animal by-product establishments. Since the probability of a CSF transmission from potentially infected wild boar to domestic pigs was considered to be higher in free range husbandry systems [2] district veterinary administrations were asked to provide a list of pig farms with free range husbandry systems. With regard to possible indirect contacts with wild boar, persons who are pig keepers and hunters at the same time could be a risk if they don´t respect biosecurity measures or if they even feed wild boar offal to their pigs. To identify pig farmers with a hunting license we matched the national pig holding database with the provincial register of hunting licenses. For an introduction of CSF into free regions transports of persistently infected live animals play a major role. Therefore we collected information from the TRACES-System of the European Commission on all international pig movements which had taken place to Styrian pig holdings within 2010.

After collecting the relevant data for all of the 3.578 Styrian pig farms with a minimum of 10 pigs a query of the established database showed the number of farms with no, one or more risk factors and the kind of different combinations of risk factors per farm. In order to establish a risk score and to prioritize the assumed risk factors we asked an expert panel of 18 veterinary officers to indicate their personal CSF risk assessment for each of the determined risk factors (A - free range husbandry system; B - gastronomical activity or biogas plants or compost works on the farm; C - introduction of pigs from abroad; D - hunting activity of the farmer) and for all observed combinations of these risk factors graphically on a developed scale. The assessments of the risk factors were

metricized and statistically evaluated in order to generate specific risk scores. For the actual selection of herds for the subsequent blood sampling those with the highest risk scores were prioritized. Giving the fact that an average pig density of 300 pigs/km² has been demonstrated to be a risk factor for CSF introduction [3] the average pig density per km² has been calculated. Additionally the local wild boar density was taken into account. Whereas the regional pig density was derived from data of the Austrian Veterinary Information System (VIS) the wild boar density in the different regions of Styria was calculated on the basis of the hunting bag data provided by the provincial hunters association. In order to visualize the regional differences in pig and wild boar densities we used a geographical information system (fig. 1).

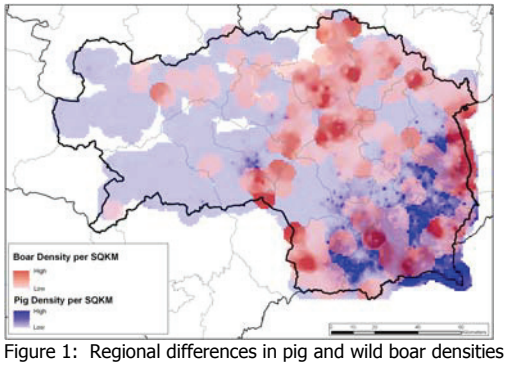

Figure 1: Regional differences in pig and wild boar densities

Table 1: Mean CSF-risk scores rescaled on a scale from 0 to 10

Category	Number of pig holdings	Median risk score
A+B+D	4	8.66
B+C+D	1	8.26
A+C	1	7.14
A+D	6	7.06
A+B	18	7.01
B+C	7	6.49
C+D	17	6.41
B+D	107	6.37
A	29	5.49
D	372	5.14
C	54	5.13
B	360	5.10

A - Free range husbandry system, **B** - Gastronomical activity or biogas plants or compost works on the farm, **C** - Introduction of pigs from abroad, **D** - Hunting activity of the farmer

RESULTS

At the reference date of 1st January 2011 pigs were kept in free range husbandry systems on 58 from a total of 3,535 pig farms with a minimum of 10 pigs. At the same time there was evidence of gastronomical activity on 493 of these farms and 4 pig farmers ran biogas plants or compost works. In total 507 of the mentioned pig farmers were holders of a hunting license and 39 had received consignments of pigs from EU member states or from third countries within the previous year. Additionally the analyses identified 1,142 of these farms (32.31 %) to have at least one CSF risk factor, 161 (4.55 %) at least two and 5 (0.14 %) three. The median CSF risk assessment of the members of the expert panel for the different single or combined risk factors are demonstrated in table 1. Due to higher pig and wild boar densities in the South of Styria the number of selected pig herds to be sampled was relatively higher in the southern districts of the province. After an introduction phase in 2010, the risk based classical swine fever surveillance program will be fully implemented in Styria in 2011.

DISCUSSION

As the sampling in high risk herds is carried out only once a year, this screening alone cannot guarantee, that possible introductions of CSFV are detected in time. On the other hand it should be kept in mind, that this program is only part of a broader surveillance scheme, which is conducted continuously throughout the year by testing suspicious organ samples and routinely collected blood samples. In future it is planned to improve the program by collecting information on mortality rates in pig herds. In order to calculate these rates we want to combine the herd size data extracted from the VIS with the number of dead pigs regularly collected on farms by the local rendering company.

CONCLUSIONS

By inclusion of the most important risk factors for an introduction of CSFV into domestic pig farms the established risk based surveillance should contribute to an early detection of this disease and thus help to prevent major economic consequences for the Styrian pig industry. The program may also add to the prevention of CSF, because the sampling in the high risk pig farms is carried out by veterinary officers, who make sure that the visited pig farmers follow legal provisions and adequate biosecurity measures.

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WILD GAME HEALTH MANAGEMENT AND IT'S INFLUENCE ON GAME MEAT SAFETY

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SUMMARY

This paper describes investigations into the farming practices of South African game farmers that may have an influence on the provision of safe game meat to the consumer. The South African game industry is expanding and game meat is readily available to both the national and international consumer. Food safety gaps in the farming practices were identified especially at the beginning of the supply chain of game meat entering the supply chain through game farmers. The results can contribute to the developmnet of policies by relevant authorities thereby ensuring that consumers receive game meat that is safe for human consumption.

INTRODUCTION

Africa has a high density and diversity of wild large herbivores, of which South Africa has some 300 different species of mammals [1]. With its initial focus on aspects such as ecotourism and breeding of game and rare/endangered game, South African game farmers realised the economic potential of game farming. As a result many domestic animal farmers changed over to game farming [5] and it is now widely recognized that game farming is the fastest growing agricultural industry in South Africa. When game farmers realised that hunting is another economic opportunity they started to charge hunters for hunting on their game farms [18].

Naturally, game meat is a residual product of hunting and therefore several game farmers also provide slaughter facilities where hunters can slaughter or process the meat [2]. The meat is finding its way to the consumer's table through the formal processes or structures as it is consumed by the immediate family, sold locally in order to provide cash income, or exported as a sought-after delicacy, especially in Western Europe [11; 12]. The consumption of meat from wild deer, antelope and other exotic animals as an alternative to beef is an age old practice worldwide, including Africa [16]. Current trends among consumers worldwide are to purchase lean meat cuts from animals [13]. Game meat has also been found to be very popular with South Africans and tourists to South Africa [11; 12].

Game farming and hunting is in the beginning of the game meat supply chain and practices followed on the farm impacts on veterinary public health / game meat safety. The occurrence of animal diseases such as *Bovine Spongiform Encephalopathy* (BSE) in beef in England and Europe and the recent outbreak of Rift Valley fever in South Africa highlight public awareness with respect to product origin, production methods and how safe it is to eat [15]. Although not specifically aimed at the production of game animals, the pre-farm-gate integrated farm assurance standard of GLOBALG.A.P can help with these concerns as the standard is designed to provide confidence regarding Good Agricultural Practices (GAP) [9]. Although the application of principles of GAP is not completely new to South African farmers, it was necessary to determine the level of agricultural and hygiene practices followed on game farms since this has not been determined before.

MATERIAL AND METHODS

We have investigated the health practices followed by game farmers based on the principles of GAP. Information regarding game animal health control practices that may impact on game meat safety was obtained through a desk top study of the relevant subject material and analysis of questionnaire responses from game farmers (N=139). The questionnaire responses were coded and all analyses were performed using SPSS (IBM® SPSS® Statistics) statistical software program.

RESULTS

When game farmers were asked to indicate whether they agree that game farming is part of the tradition of South Africa and is here to stay, 97.1% of them agreed with the statement.

Game sourcing, identification and traceability

Regarding sourcing of game, 24.4% and 25.2% of the respondents indicated that they purchase game from auctions and direct from other game farmers respectively for hunting purposes. Regarding identification of animals, 48% of the farmer respondents indicated that they do not have any method of identification of the animals. Altough the majority of the farmers during purchasing of game record the name of the seller / supplier (83.3%) and the origin (71.1%), only 37% obtain a record of the health status of the animals and 20.5% obtain a record of the medical treatment history and withdrawal periods. On selling game 71.8% of the farmers record the name of purchaser; 53.6% record of the destination of the game; 27.5% provide purchaser with a record of the health status of the animals and 27.9% provide the purchaser with a record of the medical treatment history and withdrawal periods.

The majority (85.7%) of the game farmer respondents (n=134) indicated that their game was free roaming and wild while the remaining 14.3% indicated that they farmed game semi-extensively. 71.4% of the game farmers (n=133) where game was free roaming and wild indicated that natural grazing was supplemented with feed. 49% of the farmer respondents indicated that they supplemented natural grazing during winter months, followed by farmers supplementing during drought periods (29%), all year (12.3%), spring (5.1%), autumn (4.3%) and summer (0.7%). Supplement feeds used to feed free roaming animals include compound manufactured feed, natural feeds, own mix feeds and licks (Mineral blocks). Farmers indicated that the compound feed that they used contain antibiotics (13.3%), internal parasite control agents (37.0%), hormones 4.3%), ionophores (6.5%), carcass meal (13%), bone meal (19.6%), blood meal (2.2%), fly control agents (2.2%) and poultry manure (8.7%). The majority of the water sources originate from rivers, streams and bore holes while 37% of the farmers indicated that the water sources are not protected against chemical contamination.

Forty-one percent of the farmers that are farming with domestic animals and game indicated that they have no control measures to prevent animal interaction in order to prevent disease spreading between game and domestic animals. Most (75.4%) of the farmers do not have a written Veterinary Health Plan in place and 71.6% have no quarantine area / camp for keeping ill animals. The treatment of sick animals is mostly done by veterinarians (59.5%) followed by trained farm staff (31.4%) and untrained farm staff (9.1%). Records such as name of product, date of purchase, batch number, expiry date, name of supplier, and the date of medicine administration are kept by only 41.5% of the farmers. Tick control is mostly done by Duncan applicator (59.5%) and Remote trigger spray (18.5%).

DISCUSSION

The sentiment of game farmers that game farming is part of the tradition of South Africa supports the opinions of other researchers [4; 18] who indicated that since people began to pay for hunting wildlife has been recognized as an economic force in South Africa.

Traceability of a product refers to the ability to track the inputs used to make food products backward to their source at different levels of the supply chain [14]. It is clear from the study that game farmer's source game meat from auctions as well as from other game farmers for hunting purposes. The fact that less than half of the farmers indicated that they do not have any method of identification of the animals as well as the low level of shating information regarding the animal's health status and medical treatment history during sourcing of game are major threads to the successfull implementation of a proper traceability system for game meat from farm to fork [9]. Traceability links producers and consumers in an effort to provide safe food supplies [17].

Animal feed is at the beginning of the food safety chain in the "farm-to-fork" model [6]. The fact that such a high number of the farmers indicated that their game is still free roaming supports the findings of other researchers who indicated that South African game meat can be considered to be an organic product [13]. Of concern however is that farmers supplement natural grazing with compound manufactured feeds. Additives such as antibiotics (antimicrobial agents) are commonly used in animal feed [3; 7]. Most food scandals started with contamination of animal feed, so feed has also gained interest for monitoring purposes [19]. A Hazard Analysis and Critical Control Point program should be instituted for the animal feed industry, and a *Salmonella*-negative policy for feed should be enforced [6].

The lack of (1) control measures by farmers to prevent animal interaction to prevent disease spreading between game and domestic animals and (2) the abscence of written Veterinary Health Plan is of public health importance as ±60% of emerging infectious diseases of humans are zoonotic of which 72% originate in wildlife [8]. In addition, the lack of of records of animal health treatment undermines the ability to track the inputs used during this animal production stage of the supply chain [14]. Regarding tick control, it seems that farmers prefer the use of agents that have a continual activity as it saves on the number of treatments and labour costs which as a result may lead to unacceptable residues in meat [10].

CONCLUSIONS

Conclusions drawn include: (1) Game farmers use the opportunity of a growing game meat demand; (2) certain health management practices do not support the principles of Food Safety Management (FSM); and (3) it is essential that veterinary public health authorities and game farming associations develop policies and strategies to train game farmers and their staff in the principles of GAP as well as other meat hygiene aspects that will support the principles of a FSM plan.

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ESTIMATING THE CONSUMPTION OF ANTIBIOTICS IN AUSTRIAN CATTLE, PIG AND POULTRY PRODUCTION

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SUMMARY

Records of 38,745 treatments and prescriptions from 18 veterinary practices were used as a sample to project the consumption of antimicrobial substances in the Austrian cattle, pig and poultry production. As a unit of measurement the number of prescribed daily doses (PDD) per livestock unit (LU) per year was estimated for each species by Monte Carlo simulation techniques. Antimicrobial substances were classified according to their ATCvet codes.

A median number of 6.62 PDD / LU was estimated for the consumption of antimicrobial substances per year in all species. Antibiotics were most frequently used in pig production (9.63 PDD /LU). The number of dosages prescribed per year in poultry production was 6.39 PDD / LU. Cattle were less often treated with antibiotics (2.82 PDD / LU). Polymyxins, Tetracyclines and Penicillins were the most frequently used substances. Antimicrobials classified as prioritized critically important by the WHO expert group (3rd and 4th generation Cephalosporines, Macrolides and Quinolones) added up to 12.8 % of all prescribed dosages.

INTRODUCTION

The usage of antibiotics is regularly followed by the resistance of bacteria against these substances. There are major concerns about the impact of antibiotics used in veterinary medicine on the spread of resistance genes to microorganisms because this resistance causes difficulties to treat human diseases. The prudent use of antibiotics is therefore crucial in animal production. In order to evaluate the effect of antibiotics on antimicrobial resistance, data about the usage of antimicrobial substances by veterinarians on food producing animals are a prerequisite. The Austrian Agency for Health and Food Safety was assigned by the ministry of health to develop methods with which the quantity of antimicrobials, applied or dispensed by veterinarians to livestock, can be determined and monitored. These methods are subject of the study at hand.

MATERIAL AND METHODS

Data recording and collection

In Austria veterinarians have to confirm the application and distribution of veterinary drugs to productive livestock owners. The diagnosis as well as the type and quantity of antimicrobial substances used have to be recorded. For the study at hand data on the treatment and prescription of antimicrobial substances was provided by 18 veterinary practices active in cattle, pig, and poultry populations. The data was submitted electronically or collected on-site and cover the use of antimicrobials from January 2008 to March 2010. Electronically submitted data refers to a period of 4 to 27 months. The data collected on-site refers to four months in the period from July 2008 and to June 2009.

For the storage of data a database was developed. An electronic interface was provided to enable an easy transfer of the collected data. Each data recording referred to a herd and included information of the treated species, the ID of the animal/population, the diagnosis, the marketing authorisation number as well as the amount of drug applied or prescribed.

List of antimicrobial drugs

Each antimicrobial veterinary drug registered in Austria was assigned a single, active component specific ATCvet-code [7]. The estimation of the consumption was conducted for ATCvet groups QJ (Antiinfectives for systemic use), QA (Alimentary tract and Metabolism), QD (Dermatologicals) as well as QP (Antiparasitic Products, Insecticides and Repellents).

Prescribed Daily Dose (PDD)

In Austria the legal basis for the dosing of veterinary drugs is the approved package information. Prescriptions often lack of the information of the exact number of animals being treated as well as of the information on the exact weight of the animals being treated. The prescribed dose per weight unit could therefore very often not be determined. Consequently, to derive a PDD value for each veterinary pharmaceutical product, the maximum dose recommended by the manufacturer was considered and adjusted by a factor of 0.8, correcting for the fact that not with every treatment the maximum dose indicated is used. The PDD for intramammary preparations was derived according to the manufacturers recommended dosage without any correction.

Number of Prescribed Daily Doses per Livestock Unit (n PDD/LU)

In order to be able to compare the use of antimicrobial substances among different species, the quantity of the substances applied with respect to the bodyweight was considered [4]. As a measure for the bodyweight the so called livestock unit (LU) was used. One LU is consistent with approximately 500 kilogram of bodyweight. To convert the number of animals into n LU, the criteria set by Agrarmarkt Austria Marketing (AMA) were used. A population at risk was defined to be the produced n LU within one year.

The produced n LU per herd was approximated on the basis of a reference date census for cattle and pigs (source: AMA for cattle, veterinary information system (VIS) for pigs). For poultry production data was provided by the Austrian poultry health service. Considering turnover ratios for each species, type of use as well as the different production levels of the participating farms, minimum values, most likely values as well as maximum values for n LU were calculated.

The population, in which at least one treatment occurred within the analysed period, could be determined. The population with no treatment needed to be approximated taking into account the n LU produced by member farms of the Austrian animal health service in which no treatment was documented by the contracted veterinarian. Consequently, factors for correcting the quantity of antimicrobials used in the total population at risk could be calculated.

Estimating n PDD / LU per year

(1) The data collection process in veterinary practices was done for different periods of time and included a various number of working days of the particular practice. Therefore the amounts of active substances were corrected by the assumed number of working days of an Austrian veterinarian dealing with cattle, pig or poultry.

(2) The amount of active ingredient was divided by the PDD of the used drug to estimate n PDD per year.

(3) After correction by the proportion of untreated herds, the total population at risk was used to estimate n PDD per year per LU by dividing n PDD by the sum of n LU produced.

The empiric variation of n PDD / LU was quantified by calculating 0,025 and 0,975 quantiles by Monte Carlo simulation techniques. To construct the probability model, a range of estimates (minimum, maximum and most likely value) for the working days, n LU produced and the proportion of untreated herds was set up.

RESULTS

Data of 38,745 treatments or prescriptions of antimicrobials in cattle, pig or poultry production could be analysed. 193,638 LU were produced by 2,593 herds with at least one treatment per year. The total weight of active substances amounted to more than 12 tons.

After correction by the proportion of untreated herds an annual amount of 44.0 grams (median) of active substances used per LU per year was estimated. A median number of 6.62 PDD / LU (quantiles = 4.91 – 8.46) was calculated for the consumption of antimicrobial substances per year in all species.

Results for ATCvet 1st level groups

Antibiotics for systemic use (ATCvet QJ) were in the largest amounts used (37.4 grams (median) / LU, 84.9 %). A smaller quantity was used of antibiotics of group QA (alimentary tract and metabolism, 6.0 grams (median) / LU, 13.7 %). 3.51 PDD / LU (quantiles = 2.64 – 4.44) of antibiotics belonging to ATCvet group QJ used per year were calculated (53.0 % of the total number of PDD used). Owing to the fact that antimicrobials belonging to ATCvet Group QA have lower recommended dosages the number of estimated doses used went up to 39.5 % of the total number of PDD (2.61 PDD / LU, quantiles = 1.91 – 3.39).

Results for ATCvet QJ 3rd level groups

The antimicrobials of which the largest amounts were used were Tetracyclines (16.20 grams / LU (median), 43.4 %), Macrolides, Lincosamides and Streptogramins (9.24 grams / LU (median), 24.8 %) and Beta-lactam antibiotics (4.36 grams / LU (median), 11.7 %).

The calculation of n PDD / LU used showed that besides Tetracyclines (1.22 PDD / LU (median), quantiles = 0.90 – 1.55), Beta-lactam antibiotics (0.61 PDD / LU (median), quantiles = 0.45 – 0.77) were most often applied or prescribed.

Results concerning cattle, pig and poultry production

Antibiotics were most frequently used in pig production (median = 9.63 PDD / LU, quantiles = 7.46 – 12.79). In poultry production the frequency of the use of antimicrobials was estimated at a median of 6.39 PDD / LU (quantiles = 4.58 – 8.00) per year. Cattle were less often treated with antibiotics (median = 2.82 PDD / LU, quantiles = 2.11 – 3.61).

Antimicrobials critically important

Polymyxins (median = 2.60 PDD / LU, quantiles = 1.91 – 3.39), Tetracyclines (median = 1.22 PDD / LU, quantiles = 0.90 – 1.55) and Penicillins (median = 0.61 PDD / LU, quantiles = 0.45 – 0.77) were the most frequently used substances. Antimicrobials classified as prioritized critically important by the WHO expert group (3rd and 4th generation Cephalosporines (median = 0.06 PDD / LU, quantiles = 0.04 – 0.07), Macrolides (median = 0.59 PDD / LU, quantiles = 0.44 – 0.74) and Quinolones (median = 0.20 PDD / LU, quantiles = 0.15 – 0.26)) added up to 12.8 % of all prescribed dosages.

DISCUSSION

Herds of which treatment and prescription data were recorded covered approximately 6.5 % of the total Austrian pig production per year, 12 % of the total poultry production and 3 % of the cattle production. Due to the voluntary participation in the project, data did not satisfy the criteria of an adequate random sample. Therefore, no estimation of the total consumption of antimicrobials in the Austrian cattle, pig and poultry production was carried out.

Dose measures like PDD are related to bodyweights of treated animals. The exact weight of the animals treated is commonly not recorded by the veterinarians. Similar to the procedure described in Denmark [5], the number of animals, their age and kind of use served as a basis for the calculation of the bodyweight. Livestock units (LU) were chosen as a comparison unit.

The ATCvet system was used to classify the antimicrobials used by the veterinarians [2]. Various active substances could be summed up to groups of antimicrobial classes. A divergent PDD could be defined depending on the systemic or intramammary use of an antibiotic [4].

Antibiotics ranked as „critically important antimicrobials" (3rd and 4th generation Cephalosporines, Macrolides and Quinolones, [1]) were used by the participating practises. Treatment intensity with these substances was low. Quinolones and 3rd and 4th generation Cephalosporines were rarely used (3.0 % and 0.9 % of overall prescribed n PDD respectively). Wether the prescriptions with critically important antimicrobials corresponded well to the guidelines on prudent use of antibiotics [6] cannot be answered based on the consumption data only.

To evaluate the influence of the consumption of antibiotics on the spread of resistant bacteria, the treatment intensity pertaining to the total population at risk must be known. The population treated has to be summed up with the population not treated [3].

CONCLUSIONS

A method to estimate the consumption of antimicrobials in the cattle, pig and poultry production was developed using treatment and prescription data of veterinary practices in Austria. The method used spot test data and calculated the empiric variation of quantities and dosages by Monte Carlo simulation techniques. Furthermore, this method should be mandatorily implemented into the Austrian resistance monitoring program.

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MONITORING PROGRAMMS FOR THE USE OF ANTIBIOTICS IN THE POULTRY PRODUCTION IN AUSTRIA

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SUMMARY

In europe a discussion about antimicrobial resistence and data on the use of anitmicrobial agents has started a few years ago. In 2008 EMEA puplished a document concerning this theme. New regulation for the use of antibiotics in case of Salmonella in poultry are layed down by in the EU. The national programs in Austria and the poultry hygiene regulation need a lot of data to be collected. So there is also a great need for validate data and a method how to collect these data.

INTRODUCTION

Antimicrobial resistence is one of the main topic in the Austrian federal Law on the control of zoonosis. Monitoring of antimicrobial resistence is done on indicator bacteria to follow trends. In Austria official Veterinarians use the Poultry Health Data to collect these data.

MATERIAL AND METHODS

All veterinarians specialised in poultry, work with a own database so called Poultry Health Data (PHD). This database is run by the Austrian Poultry Health Service. Within the poultry health regulation the users are forced to work in this system. If a flock is positive for Salmonella spp. database react by sending the positive results to a defined usergroup. In this database the Austrian Salmonella data are collected and every flock is registrated in the PHD. If positive findings occur in accordance to regulation 1273/ 2007 the flock is stopped for table egg production in the PHD. Authorised persons, authorities and companies receive a pin code allowing them to generate information relevant to them from the data base in a quick and simple way, including e.g. optimum dates for ante mortem inspections, clinical findings, results of salmonella control, vaccinations and diseases.

RESULTS

Since the PHD was introduced in 2002 a lot of work has been done. Today more then 100 veterinarians work with the PHD. In a project with the AGES, four veterinarians submitted their data from the PDH to database, 1056 records in 2008 and 1261 records in 2009.

DISCUSSION

Collecting data in poultry is a complex challenge. Nevertheless in Austria all professional poultry keepers are collected in the PHD. Data about the usage of antibiotics are of high sensibility. Data security therefore is the critical point of view in such a project.

CONCLUSIONS

Modern herd health management needs modern technologies. Data show that the PHD has following benefits:
The amount of drugs is a real figure and there is a clear connection to the animal species and to the medical indication.
Variations in the potency of active substance were taken into consideration and the data were comparable with results from other species like pigs and cows.

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VALIDITY OF MEAT INSPECTION DATA – A NOVEL APPROACH TO ASSESS THE QUALITY OF FEED BACK SYSTEMS IN THE PIG SLAUGHTER LINE

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SUMMARY

Meat inspection performance and meat inspection findings of 12 veterinarians were studied over the period September 2007 to December 2010 in 264 039 Austrian slaughter pigs in regard to quality of data, the validity and reliability of them and their comparability. Pulmonary lesions (32.1%) and visceral pleuritis (14.3%) ranked highest in organ findings and proved to be valid parameters of high significance for postmortem information feedback systems.

INTRODUCTION

The efficacy of traditional meat inspection within the EU has been highly debated, claiming that current procedures might not always fully safeguard public health [1, 2, 3, 4]. Traditional meat inspection procedures currently used in most European countries date back to the last century, when it became clear that meat could play a major role in the transmission of disease (e.g. trichinellosis, tuberculosis). Improvements in animal husbandry, the preventive and therapeutic use of veterinary drugs and programmes for disease control have led to improvements in the health of slaughter animals. Zoonotic diseases that lead to characteristic pathological – anatomical changes in slaughter animals, such as tuberculosis have become largely eradicated in the EU. Nowadays, potential threats to public health are caused by zoonotic agents such as *Salmonella*, *Yersinia* or *Campylobacter* species, carried by animals without clinical signs of disease and not detected by traditional meat inspection methods [2, 3]. In order to revise meat inspection towards the introduction of a risk-based approach, information feedback systems have been established throughout Europe as legally required by European Commission regulations (EC) 854/2004 [5] and (EC) 2074/2005 [6] to grant transparency along the production chain. It became mandatory for meat inspectors to provide information from the slaughterhouse to the pig producer and his/her veterinarian and thus assist in monitoring disease in national herds. To our knowledge, so far hardly anyone has questioned the quality of data recorded in such databanks, the validity and reliability of them and their comparability. Therefore, the aim of this study was to assess the potential of postmortem feedback systems to assure quality in the slaughter line and thus improve pig health from farm level on.

MATERIAL AND METHODS

Study Population

The population under study comprised all conventional pigs from 94 farms located in the province of Styria, Austria, that were slaughtered in the period September 2007 to December 2010. Farms included 28 (29.8%) fattening farms and 66 (70.2%) farms with "farrow to finish units". Herd size ranged from 70 to 2097 finishing pigs. All farms participating in the study had no "all in/all out" management, so each farm sent batches of finishing pigs to slaughter according to growth performance. Additionally, only farms which sent more than 250 finishing pigs per year to slaughter were included in the study. A total of 264 039 pigs were examined.

Meat inspection and Data recording

The study was conducted at one slaughter plant located in the province of Styria, Austria, slaughtering approximately 2300 pigs per week. Finishing pigs from the selected farms were sent to slaughter with approximately 96kg. The abattoir killed about 120 pigs per hour and used carbon dioxide stunning followed by conventional sticking with the animals lying on the side. At the slaughterhouse ante– and post mortem data were recorded by a total of 12 experienced official meat inspectors. Each veterinarian was registered under a certain "vetcode" in the system of the slaughterhouse. Along the pluck- and carcass line the veterinarian was able to select postmortem findings on a touch screen out of 60 defined parameters (code system), namely 17 carcass- and 15 organ criteria and approximately 26 criteria related to slaughter hygiene or –technology, as well as 2 codes for carcasses and organs without any pathological-anatomical abnormalities. Data implemented in the system were transferred to set up a model data bank and to assess the parameters for their validity and significance. In addition, the performances of meat inspectors were evaluated in regard to consistency in data recording over the full study period.

Statistical Analysis

The proportion of postmortem findings for each criterion was analyzed using a generalized linear mixed model (R 2.12.2). Farm type, animal type and quarter were included as fixed effects. This was done to assess possible differences between farm types and animal types. Additionally, the model should be flexible enough to follow nonlinear trends in time. The farms and official meat inspectors were included as random effects. It was assumed that these farms were a random sample out of the population under consideration. The same assumption was done for the official meat inspectors. Moreover, this approach allows quantifying the homogeneity of postmortem findings between official meat inspectors. The estimated variance components were used to assess this homogeneity and hence the reliability of the data. To get an impression of the variability of the assessment between the official meat inspectors the conditional modes of the random effects of the fitted model were illustrated in boxplots. The model selection was done using the Bayesian Information Criterion (BIC).

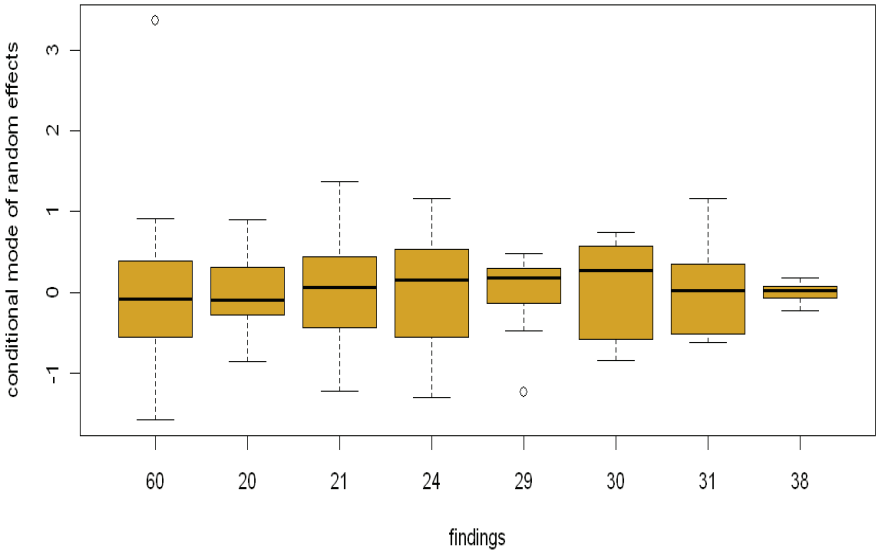
RESULTS

In the period September 2007 to December 2010 264 039 pigs were sent to slaughter and examined by 12 official meat inspectors. The percentage of positive and negative postmortem findings on an annual basis is listed in Table 1.

Tab. 1 Positive and negative postmortem findings (%) of 264 039 slaughter pigs in the period September 2007 to December 2010

		carcass findings (%)		organ findings (%)	
year	animals for slaughter	positive	negative	positive	negative
2007	23307	61.83	38.17	74.00	26.00
2008	72083	51.93	48.07	72.63	27.37
2009	82565	51.87	48.13	71.37	28.63
2010	86084	42.68	57.32	68.37	31.63

The percentage of positive and negative carcass and organ findings decreased in numbers from 2007 to 2010, namely 19.15% for carcass findings and 5.6% for organ findings. Out of the 60 criteria 17 were preselected according to prevalence and significance. Respiratory disorders such as pneumonia (32.1%) and visceral pleuritis (14.3%) ranked highest in organ findings, whereas bursitis (14.4%) ranked highest in carcass findings over the full study period. Eight criteria (Table 2) were further evaluated in regard to variability of the assessment between the 12 official meat inspectors (Figure 1). Variability was low for pericarditis (38) and pleuritis (29), increased for Milky spots of the liver (31, 30) and pneumonia (20, 21, 24) and highest for the criterion Scabies (60).



Tab. 2 Postmortem criteria for the assessment of variability in meat inspection among 12 official meat inspectors for the period Sept. 2007 to Dec. 2010

code	criteria (findings)
60	Scabies
20*	Pneumonia ++
21*	Pneumonia +++
24*	Pneumonia +
29	Pleuritis (visceralis)
30*	Milky spots liver <3
31*	Milky spots liver ≥ 3
38	Pericarditis

*according to the key of [8]

Fig.1 Variability of the assessment of 8 postmortem criteria between the 12 official meat inspectors in the period September 2007 to December 2010

DISCUSSION and CONCLUSIONS

The decrease in carcass and organ findings over the period September 2007 to December 2010 has to be considered with caution. Findings include pathological – anatomical abnormalities as well as parameters associated with slaughter technology and –hygiene. The number of organ findings was only reduced to 5.6% in comparison to 19.15% of carcass findings indicating that the lower prevalence rather reflect improved slaughterhouse technology and/or better trained personnel than better health state of slaughter pigs. That respiratory disorder ranked highest in organ findings came to no surprise, as respiratory disease is considered the most important health problem in pig production [7]. A distinction between acute and chronic forms of pleurits and pneumonia as described by [4] was not possible as not being included in the code system. The low variability in the assessment of pleurisy and the acceptable performance among meat inspectors to assess different forms of pneumonia indicate that these criteria can be used as valid parameters in postmortem feedback systems. Although some studies [8, 9] consider pericarditis and milky spots of the liver (results from migration of larval Ascaris suum worms) of lower significance for pig health than respiratory disease, raised prevalence of lesions might also be used as risk factors in postmortem feedback systems. The high variability in detecting scabies came as a surprise and has to be further investigated.

Inspection of pigs at slaughter has been widely used in epidemiological studies of risk factors associated with raised prevalence of lesions [10]. However, the prevalence of lesions recorded in databanks must be based on valid data and reflect a certain consistency in data recording of meat inspectors to establish a functional postmortem feedback system. This has been partially proven by this study. In future research the quantitative association between the occurrence of defined lesions in meat inspection and pig production parameters has to be assessed to implement a risk based approach in meat inspection.

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PUBLIC HEALTH POOL (PHP) – THE AUSTRIAN STUDENT INITIATIVE TO PROMOTE VETERINARY PUBLIC HEALTH (Abstract)

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Veterinary public health (VPH) essentially contributes to human health and well-being. Among others, adequate use of antibiotics, food safety, animal welfare and animal assisting therapy help maintain healthy environments, humans and animals.

In Austria, students and young professionals felt only poorly informed about educational and occupational possibilities in VPH. Thus, students from the Veterinary University in Vienna founded in 2007 an association called Public Health Pool (PHP) in order to facilitate the exchange of knowledge and information for people interested in VPH.

The specific objectives of the PHP are to encourage students and graduates with interest in public health, to offer access to specific education programmes by promoting networks and scholarships, to identify and help to establish working areas in the field of VPH, to strengthen the importance of veterinary medicine in the field of Public Health, to enhance interdisciplinary exchange with related disciplines of veterinary medicine and to identify educational demands within VPH in Austria.

The above mentioned aims are achieved by organizing lecture evenings with national and international experts as well as casual get-togethers, so called "Pool Runden", to exchange ideas and encourage teamwork of students. Through our newsletter (named "Pool Letter") we share job offers, upcoming conferences, traineeships and similar VPH-news with our registered members and supporters.

The PHP is a dynamic association and our members and supporters actively join us in creating new and improved opportunities within the field of VPH. Our association provides a neutral platform for the mutual exchange between students and professionals committed to VPH in order to ease the access into applied VPH.


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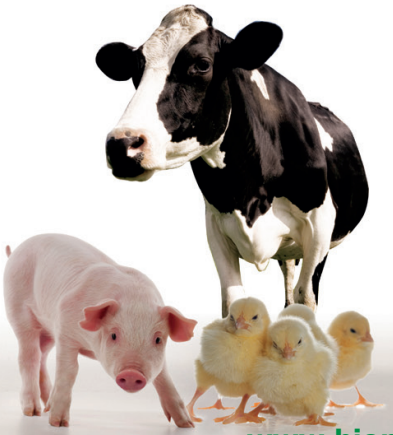

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