XVII INTERNATIONAL CONGRESS ON ANIMAL HYGIENE 2015

"Animal hygiene and welfare in livestock production – the first step to food hygiene"

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CONTENTS

FOREWORD

As we are writing this foreword about the XVII International Congress on Animal Hygiene, we are looking back in time to the year 1970 when the International Society for Animal Hygiene (ISAH) was founded on initiative of Prof. Dr. Ferenc Kovács from Budapest, in close collaboration with Prof. Dr.h.c. Johann Kalich from Munich and Prof. Dr. Jan Rosocha from Košice. They were supported by colleagues from other western and eastern European countries in their plan to create an association that would promote exchange of scientific information and practical knowledge, and organized collaboration among animal hygienists. Their efforts culminated in the statutory meeting of ISAH held in Budapest, and the first Congress of ISAH in 1973 also in Budapest, Hungary. After that the ISAH congresses took place every third year till the Warsaw congress in 2005, when the ISAH complied with the need for a more flexible and frequent exchange of information and decided to organize its congresses every second year. The XVI Congress of ISAH was held in Nanjing, China, with participants from 57 countries and areas all over the world.

Since 1970 the world has changed considerably but the XVII Congress of ISAH continues a tradition that had already been established by the previous congresses in that it provides a platform for the presentation of results from basic and applied research, new strategies and initiatives, and provides an opportunity to meet colleagues and friends and become acquainted with animal hygiene professionals from all over the world and also with the congress host country.

The present XVII International Congress of ISAH is the second one held in the Slovak territory as the IV Congress of ISAH was held in 1982 in the High Tatras when Slovakia was part of the former Czechoslovak Republic.

The opportunity to meet regularly with colleagues of the same professional orientation has gotten more important as we face new challenges related to changing climate, emerging infections, globalization of food supplies, increasing awareness of influence of animals on the environment, concern about safety of food of animal origin, economical implications and many other factors.

The Slovak Organizing Committee is greatly pleased and honoured to welcome delegates from 45 countries to the XVII ISAH 2015 Congress in Košice. The Congress is co-organized with the National Focal point for scientific and technical matter with EFSA.

The main theme of this Congress is “Animal hygiene and welfare in livestock production – the first step to food hygiene”. The invited lectures, scientific papers and posters deal with various aspects of product quality, resource efficiency, sustainability, animal welfare and agro-ecology. The programme covers various thematic topics, such as livestock farming systems, animal nutrition, animal management and health and also cattle, sheep and poultry, goat, pig and horse production.

We thank all participants, contributors, chairpersons, organisational and technical assistants for considerable efforts to make this Congress successful.

We wish you all an interesting, meaningful and pleasant ISAH Congress and enjoyable time in Košice.

Ass. Prof. Ján Venglovský

2nd Vice-president of ISAH 2015 Organising Committee

PLENARY LECTURES

SENTIENCE AND PAIN IN RELATION TO ANIMAL WELFARE

Donald M. Broom

Centre for Animal Welfare and Anthrozoology, Department of Veterinary Medicine, University of Cambridge, Cambridge CB3 0ES U.K.; dmb16@cam.ac.uk

Summary

The concept of sentience, which concerns the capacity to have feelings, involves cognitive concepts and awareness. The cognitive capacities of parrots, corvids, cows, pigs and sheep are substantial. All vertebrates, including fish, are shown to have pain systems. There has been rapid development in animal welfare science, including pain assessment. Examples of new pain indicators in sheep and other species are presented.

Sentience

The term sentience has generally been used to mean that the individual has the capacity to have feelings (DeGrazia 1996, Kirkwood 2006). This capacity involves awareness and cognitive ability so is principally in the brain. A major change in attitudes to the idea of awareness and feelings in humans and other animals has occurred as studies of behaviour have become more detailed. In addition, brain mechanisms have been studied using new methods such as brain-recording and scanning: e.g. electroencephalography (EEG), positron-emission tomography (PET-scanning), magneto-encephalography (MEG) and frequency-modulated magnetic resonance imaging (fMRI). Sentience implies a range of abilities, not just having feelings. A definition is: a sentient being is one that has some ability: to evaluate the actions of others in relation to itself and third parties, to remember some of its own actions and their consequences, to assess risks and benefits, to have some feelings and to have some degree of awareness (Broom 2006c, 2014). All of these concepts and abilities require definition and evidence.

The idea that animals used by people should not be treated like inanimate possessions but should be protected from actions that might cause suffering, is very old and widespread in human society. The term 'sentient' is now used in legislation about animals. The European Union Treaty of Lisbon, (European Union 2007), says in the course of a statement about animal protection and welfare (Article 6b), "since animals are sentient beings…". This wording had the intention to protect the animals commonly used by man, for example on farm, in the laboratory, or as companions. It came about because public concern about animal welfare has increased in many countries during the last thirty years and especially in the last ten years.

The development of animal welfare science

Scientists and legislators now use animal welfare as a term that is a scientific concept describing a potentially measurable quality of a living animal at a particular time. Such usage has rapidly become widespread during the last thirty years (Broom 2011). However, the use of the term animal welfare was not always as a scientific concept, and indeed there are still many people who are not aware of the modern approach to the subject.

The author's (Broom1986) definition of the welfare of an individual as its state as regards its attempts to cope with its environment, refers to all coping systems and so includes feelings and health. It is now used by most welfare scientists and is also, in modified form, by the O.I.E. (World Organization for Animal Health). As explained by Broom (2014) p.28, the O.I.E. text reads like a committee document so has some imprecise parts in it: (a) welfare is not "how an animal is coping" but is a state that reflects how well it is coping; (b) the animal has to cope with its whole environment and "the conditions in which it lives" might not mean that to all people; (c) the term "innate" would not be used by any modern animal behaviour scientist as it implies uninfluenced by the environment and no behaviour is uninfluenced by the environment.

Welfare can be assessed using a wide variety of behavioural, physiological, clinical, brain function and other measures. Welfare is always poor when animals are diseased but pain and other aspects of poor welfare vary with severity of pathological effects and can be measured (Corke 1997, Corke and Broom 1998, Corke et al 2014). It is impor-

tant to assess how good the welfare is as well as to evaluate poor welfare. The major changes in animal welfare science during the last 30 years have been the refinement in direct measures of animal welfare and the development of welfare outcome indicators that can be used by veterinary and other inspectors, as well as by those who use animals. Welfare outcome indicators have been developed by many scientists, including those involved in the E.U. Welfare Quality and Animal Welfare Indicators (AWIN) projects. Information on the subject is available at the Animal Welfare Science Hub www.animalwelfarehub.com.

Cognition, feelings, awareness

How clever are farm animals? Many people assume that cows sheep and pigs have very limited cognitive ability but those who work with farm animals know that they often work out ways to beat the system imposed on them. For example, sows with a transponder on their collar that operates a feeding station learn how to operate this rapidly. When a sow found a collar that had fallen off another animal, she picked it up and got a free meal for several days before the farmer noticed that she was doing so. In order to compare learning ability in different species, behavioural scientists started to use operants. These are actions, such as lever-pressing, carried out by an individual with consequent effects on its environment and are studied in a situation controlled by an experimenter. In the comparative studies, some of these operants depended upon motor abilities that were easy for some species but were very difficult or impossible for other species. For example, using a hoof to press a lever. Hence no unbiased comparison of learning ability was possible. A set of studies by Kilgour (1987) largely overcame this problem by the use of modified Hebb-Williams mazes for animals of different sizes. These mazes start with a decision point where there are two or more possible directions to take, one being towards a concealed target reached after two further turns. Such a maze still has some bias, as a comparison of learning ability, in that animals that often have to navigate around their surroundings would have had more experience of a sequence of decisions about which way to turn. This might favour animals that use discrete pathways. However, individuals of all of the species tested have to do this to some extent and the locomotion required to respond in a maze is common to all. When the numbers of errors were measured, cows, sheep, goats and pigs performed less well than 5-year-old children but better than dogs, cats, rats, horses and several other mammals and birds. When speed of learning was compared in the same study, the sequence was very similar but dogs performed as well as the farm ungulates.

In other studies, cows, sheep, pigs and fish learned rapidly to discriminate other individuals of their own species, or to discriminate between humans (Kendrick et al 1995, Swaney at al 2001, Mendl et al 2002, Hagen and Broom 2003). A variety of studies of learning by farm animals, fish and other animals are reviewed by Broom (2007a, 2010, 2014). It has sometimes been assumed that farm animals are not very intelligent but this has been shown to be untrue by many recent studies. Does a chicken have a concept of an object when it is not directly detectable? Studies by Vallortigara and colleagues showed that, not only could young domestic chicks go to objects hidden behind screens but that when two or three objects were hidden behind screens, the chicks went to the screen with the larger number of objects (Rugani et al 2009). Can farm animals remember and use a visual symbol for a resource? Langbein et al (2004) found that goats could respond by carrying out an action, or operant, in order to get water when they saw one particular picture rather than others.

A complex array of concepts in pigs were evident from studies by Held et al (2000). Pigs were put in a room and allowed to find hidden food. On the next day they were returned to the room and they went immediately to the place where they had found food. If another pig was watching, the pig waited and did not go to the food if that other pig was known from previous experience to be able to steal from it. If the other pig was known not to steal, the food was immediately approached. These pigs must have had a concept of an object in the absence of that object, a concept of a location, and an ability to predict that in the future it might have the food item stolen from it.

The ability to learn what is in a mirror is demonstrated for only a few species, pigs being one of these. Broom et al (2009) exposed 4-6-week-old pigs to a mirror for the first time in such a way that they could see a food bowl otherwise out of view behind a barrier. The young pigs went behind the mirror to the apparent position of the food bowl. However, when given five hours experience of a mirror, they responded initially to it as if to another pig but later by looking at it as they moved. After this experience with the mirror, seven out of eight pigs tested moved away from the mirror and around the barrier to the food bowl. Location by odour was prevented by fans and the naïve controls had exactly the same olfactory situation. To use information from a mirror and find a food bowl, each pig must have observed features of its surroundings, remembered these and its own actions, deduced relationships among observed and remembered features and acted accordingly.

In recent years there have been many studies of cognitive ability that lead to the conclusions that: (a) hardly any ability is uniquely human, (b) the best bird brains allow greater cognitive ability than any mammal except man, (c) learning by fish can be very complex, and (d) cognition in cephalopods, jumping spiders, ants and bees is much more sophisticated than we had previously thought. Communicating using symbols is possible for many animals so language is not just human. Using information from a mirror is now demonstrated for humans, chimpanzees, capuchin monkeys, pigs, elephants, dolphins, parrots and magpies (Gallup 1982, 2002, Reiss and Marino 2001, Plotnik et al 2006, Prior et al 2008), Broom et al (2009). A concept of future events is evident from work on many farm, companion and other animals (Mendl and Paul 2008).

Emotion, which has long been viewed as necessarily separate from intellectual activity, is now shown to be a facilitator of learning and a consequence of learning. An indication of the possible awareness of own actions and functioning comes from the studies of Hagen and Broom (2004) on young cattle. The heifers were put in a pen whose gate could be opened by pressing a panel with the nose, thus giving access to food 15m away. They learned to do this and at the time of learning showed an excitement response of increased heart rate and jumping or galloping. This "Eureka" effect was not shown by controls which just gained access to the reward or by heifers which had learned earlier how to open the gate. Evaluation of welfare can use the link between emotion and motivation or cognition, for example in studies of cognitive bias (Mendl and Paul 2008).

1. When investigating brain and behaviour in humans and other animals, academics should use precise scientific methodology to describe observations, to experiment, to analyse results and to write about these but should not be afraid to use concepts such as emotion, feeling, mood, pain, fear, happiness, aware, conscious, stress, need and welfare in presenting results. No concept should be avoided because there might be those who would criticise the use of complex concepts on the grounds that there must be parsimony in all description. If the subject is complex, some of the concepts must be complex.
2. Each concept used in cognition, awareness and animal welfare research should be properly defined in scientific writing rather than just being referred to in descriptive but imprecise ways.
6. High levels of cognitive ability may often help animals to cope with their environment. Hence a given level of a problem, such as pain, may be less in more complex animals than in simpler animals. There is a possibility that animals may have fear of possible future adversity. The relationships between negative feelings, such as fear and pain, and the role of cognition in the coping abilities of the animal should be investigated further and considered when evaluating the risk of poor welfare. Cognitive ability should also be considered when designing methods of enriching the environments of captive animals.

Pain

Pain is an aversive sensation and feeling associated with actual or potential tissue damage (Broom 2001 a,b). Its occurrence in animals is discussed by (Sneddon et al 2014). The management of pain in farm animals is often inadequate, resulting in poor welfare (Crook, 2014; Huxley and Whay, 2006). The reasons commonly cited by veterinarians for not administering analgesia to farm animals include difficulties in recognising and quantifying pain (Huxley and Whay, 2006; Ison and Rutherford, 2014; Lizarraga and Chambers, 2012). There is an evident need for an objective, reliable scoring tool that can be effectively used to recognise and assess pain severity.

Facial expression scoring systems for pain assessment have been recently developed for use in rodents, rabbits and horses (Dalla Costa et al., 2014; Langford et al., 2010; Leach et al., 2012, McLennan et al in prep). The procedure in Cambridge for onvestigating pain in sheep involved visiting eleven commercial farms when disease was reported, and evaluating the changes in clinical condition and facial expressions across recovery time. Of 111 sheep over one year of age, 73 were identified as having footrot by a veterinarian through lameness and lesion scoring. These sheep were matched with 38 control sheep identified as having no sign of footrot or other disease. All sheep were assessed for lameness using the five point gait scoring method devised by Ley et al. (1992). Photographic images of sheep faces were taken on the day of disease

identification after lameness and lesions were scored. Thermography was also used. All sheep were treated on the same day after images had been collected with antibiotic (tulathromycin subcutanerous and topical chlortetracycline) and with the non-steroidal anti-inflammatory meloxicam (subcutaneous). All sheep were revisited during their recovery period and facial images were recorded on day 90. Animals were reassessed for lesions and lameness to establish that they were fully recovered. A study of 17 sheep with mastitis and 12 control sheep was conducted in the same way but without topical antibiotic use.

The sheep pain facial expression scale involved scoring five facial areas; orbital tightness, cheek tightness, ear position, lip and jaw profile, and nostril and

philtrum position. These areas are scored as abnormal expression present (2), partially present (1), or not present (0). A total pain score of 1-10 was determined by adding the individual scores for each of the five areas for each set of photographs. On the first day, the total pain score was higher in the sheep with footrot than in controls ($p = 0.0005$) but at 90 days after treatment there was no difference. Sheep with mastitis also had a higher total pain score than controls ($p = 0.01$). Trained observers scored faces similarly.

Prompt recognition of pain through the use of the scale will enable farmers and veterinarians to treat and manage their flocks better, reducing the impact of pain on their sheep, thus improving welfare and production. The scale should be used together with other measures of pain.

In another study of actigraphy (McLennan et al 2015), ten ewes that were lame with footrot were chosen and carefully matched with ten non-lame healthy control ewes for age and weight. Their activity was monitored with an Actiwatch Mini in a small, waterproof box (350mm x 200mm x 350mm) attached to a standard collar fitted around the neck (Piccione et al. 2007, 2011). All ewes accepted the collars without apparent disturbance. The device contains an omnidirectional accelerometer to monitor the occurrence and intensity of movement producing an activity count.

Lameness affected the overall activity level with lame ewes being more active than control ewes at night ($p<0.05$. Lameness also reduced the activity level of ewes whilst walking ($p<0.001$), standing or standing ruminating ($p<0.001$). The results demonstrate the potential of using automatic monitoring devices to help identify lame sheep on farm as part of an automated husbandry system. The current limitation to actigraphy is the requirement to download data rather than observing in real time.

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DISEASE CONTROL IN A CHALLENGING GEOPOLITICAL REGION

Nadav Galon

Director of Veterinary Services and Animal Health, Ministry of Agriculture and Rural Development, Israel

Introduction

Israel is located in at a junction of continents. Although the small size of the country, 22,000 km^2, it has long international borders with several countries; Lebanon the north, Syria and Jordan in the east and Egypt in the south west. The Palestinian Authority (PA) is divided between the West Bank in the east and Gaza Strip in the west. Despite its small size Israel has a diversity of landscapes, climates and habitats; Mediterranean climate in the coastal plain, with a short and wet winter and a long hot and humid summer. A mountain range stretching from north to south in the middle of the country has a milder weather. The Jordan river valley along the eastern border is part of the Rift Valley, from the Hermon mountain in the north (2,300 m above sea level) through the Dead Sea (400 m bellow see level) to the Red Sea. The Negev desert which occupies the southern half of the country is very hot and extremely dry. The society of Israel is a colorful and vibrant mixture of ethnicities, religions and cultures. With nine million people, population density in the northern half of the country is among the highest in the world, leaving little land for agriculture. Trade relations with the PA are vast; raw materials, feed, livestock, food, workers and others cross continuously. Undefined international borders and an often tense political situation produce some major challenges for animal disease control.

The livestock population of Israel is relatively small; about half a million heads of cattle, one million heads of small ruminants, 200,000 pigs and 45 million poultry. These are kept mostly in four major types of husbandry systems; a. Kibbutz- a cooperative farm; modern, relatively large, isolated with proper biosecurity. b. Moshav- a village with up to one hundred family owned smaller farm units, with variable size and type of animal units, gradually urbanizing and cease farming due to changed life style and reduced profitability. c. Arab villages; with small to large traditional and extensive sheep and goats herds. With limited and diminishing pastures, elevated feed cost and more profitable professions available, the number of farms has been reducing fast in recent years. d. Bedouin shepherds in the Negev region- keeping some two thousands herds of small ruminants and camels, ranging from few to hundreds animals, some migrating to spring pastures in northern Israel and some keep them next to their homes. Legal and illegal trade of animals and commodities occur continuously between Israel and the PA. In the West Bank Jewish settlers' intensive farms are under the supervision of Israel veterinary services (VS), while neighboring Palestinian villagers and tent-living Bedouins herds and flocks are under the supervision of the PAT VS. Israel is importing annually about one quarter of a million heads of cattle and sheep, for fattening and slaughter, from Australia and the EU, to supply the local demand for fresh meat. The extreme diversity of animal farming systems between traditional and extensive sheep and goats herds to advanced technology zero grazing dairy cattle farms next to each other is a great control challenge. Israel is rich in endemic wildlife; wild boars, jackals and gazelles are the prominent ones. Half a billion migrating birds fly over Israel twice a year on route from Europe to Africa and back. Wild animals and birds are involved in disease epidemiology.

Major disease control challenges

Due to regional politics, crises and wars, there is very limited collaboration between the VS of countries in the region. Disease outbreak notifications to the OIE as well as to bordering countries are scarce and information transferring is minute. Due to limited recourses, monitoring and surveillance is almost non-existing or erratic and diseases often "surprise" the VS once they cross the border between neighboring countries. The amount of disease notifications by Israel to the OIE is one of the highest, certainly in proportion to the country size. Fig 1 shows the major ruminants' diseases incursions in the last decade, by route and year of the index case. The amount of disease events is also disproportionate to the VS size.

Foot and Mouth Disease (FMD) is a constant emerging and re-emerging challenge in Israel. It appears mostly in winter and spring through the northeastern borders of the country, irregularly, ranging from years without any events to years with multiple foci. Strain O is the most common type with rare outbreaks of strain A. Proper control is achieved by mandatory vaccination, performed by VS personnel only, using international commercial trivalent vaccines. Vaccine coverage ranges from 95% in intensive dairy herds to 50% in the Bedouin sheep herds. Economic losses are mostly due to the extra labor invested in vaccination, cost of control measures and the obstacles to ex-

porting animals and animal products, rather than to sick or dead livestock. Rare transboundary threat of other strains like SAT-2 strain, last diagnosed in 2012 in Egypt and in Gaza Strip, is another contingency control challenge.

Arthropod and Vector Borne Diseases outbreaks are on the rise in recent years, often suggested to be due to global warming and increased movements of animal, commodities and human. Bovine Ephemeral Fever (BEF) appears every several summers, spreading swiftly along the Rift Valley and the inner valleys, causing heavy losses in cattle by mortality, morbidity and drop in production, on both sides of the border. Vaccines are expensive and vaccination is not mandatory, leading to low compliance. Several Blue Tongue (BT) viral strains are endemic in sheep in Israel, while other strains are emerging, mostly in sheep and rarely (BTV 8) in cattle. Vaccine availability is limited and hardly used by farmers. Losses vary between years and regions, yet limiting export of animals and products. Lumpy Skin Disease (LSD) entered Israel from the Southwestern border three times in the past; in 1989, 2006 and 2007. The VS performed mandatory vaccination in cattle herds around Gaza Strip since 2006. In 2012, due to lack of reporting and communication, LSD unexpectedly invaded from the tip of the northeastern border, spread south and west, vastly infecting unvaccinated herds and was eradicated only by a massive mandatory vaccination campaign of the whole cattle population in Israel by both the VS and private vets. LSD had since spread through numerous Middle East countries and is now near EU countries. Rift Valley Fever is another vector borne threat, so far away from our borders, yet with very limited regional surveillance and collaboration; it forces the VS to maintain preparedness program, diagnostic capabilities and a costly vaccine reservoir.

Brucellosis; Israel is free of B. abortus, yet B. melitensis is endemic mostly among the Bedouin sheep, goat and camel herds in the Negev region, leading to numerous human cases. Cultural, traditional and trade practices make it difficult to control. The permeable border and the intense social and commercial ties with the West Bank is a source for reintroducing the disease in both directions, by illegal movements of animals and animal products. Alongside with Brucellosis, small outbreaks of Sheep Pox and PPR erupt occasionally in the less bio-secured herds due to uncontrolled animal movements within Israel and from the West Bank. Vaccination against the three diseases is mandatory in Israel.

Rabies; Israel is mostly free of the disease and all cases in Israel in recent years (none in human) were in the Golan Height and Upper Galilee, probably penetrated from the other side of the border (Fig 2). Paradoxically, the continuous crisis situation in Syria led Israel security forces to reinforce of the fenced barrier and this contributed to reduce the number of Rabies cases in the Golan height. Israel VS spread vaccine baits to wild carnivores from the air along the north and eastern borders, which proves as a successful preventative measure.

Avian Diseases; High Pathogenic Avian Influenza emerges every several years, mostly linked to the enormous annual spring migration of bird from Africa to Europe as an incursion route. Last outbreak in January 2013 involved eight large commercial farms and stretched several weeks. The VS are stamping-out sick flocks and farmers are compensated for their losses. Newcastle Disease (ND) is another heavy burden to the local poultry industry and to the VS. The VS monitor serologically all commercial flocks to enforce the mandatory vaccination in thousands of commercial farms and are stamping out every sick flock at high compensation costs, in order to maintain poultry products export to developed countries. In some villages poultry farms are extremely dense, bio-security is difficult to practice and vaccination compliance in some sectors is low. Many villagers in traditional living habitats keep unvaccinated backyard chickens, and trade ties with the West Bank are abundant. All these together challenge the VS control ability and cause occasional re-emerging ND events.

The role of the VS in control

Government and public resources for agriculture and animal health control are diminishing. Developed countries try to reduce the size and cost of the public sector by privatizing official control activities. The VS vets and inspectors numbers are constantly reduced while extra tasks are added in food safety and animal welfare. Therefor the VS are forced to shift from execution of vaccination and testing missions by government personnel to supervising and control, monitoring and surveillance, implementing a new strategy of Risk Analysis and Risk-Based Surveillance. Delegation of previously official tasks to private vets and to farmers; giving them the training, the tools, the responsibility, trusting them to act as reliable sentinels and to report new disease events to the VS, is a major change and challenge. Farmers do not like changes which they do not believe are in their favor, or which may impose extra costs. Therefore changes have to be well explained, slow and supported by the government funding. Farmers are also expected to comprehend their role-change from a traditional way of life to responsible food producers to the public. Consumer concerns have shifted from food-security to food-safety, animal welfare and the environment. These

changes are being engaged differently by advanced intensive farmers like modern dairy and integrated poultry farmers, compared with traditional agro-pastoral sheep and goats' shepherds. The media and animal activists are powering these change processes, by focusing on rare events, challenging the VS and other regulators in parliament and in courts, adding pressure and tension to government-farmers-consumers multifaceted relations.

Summary

Israel VS are challenged by many disease threats and outbreaks. While developed countries of similar size are often members of a region or a treaty like the EU, Israel has to diagnose, plan, monitor and manage a large verity of challenges by itself. The diversity and frequency of disease outbreaks and threats brings a great diagnostic workload to the national research institute (Kimron). Yet, every threat is a challenge and also an opportunity. Increased understanding of the benefits of collaboration by all stakeholders inside the country and on the other side of our long borders, despite and above political differences and tensions, may lead to a better control of transboundary diseases in the future. Since many diseases do not recognize man made borders as they spread, collaboration between neighboring VS should be separated, as much as possible, from other issues. Cross border collaboration in diagnosis, training, information sharing, epidemiology and surveillance can improve animal and human health in the region. This can be done directly between the neighboring countries, or by a third party moderation and support. International assistance and leadership can aid in disease control in this region, to contribute to reduce the risk of disease spreading to other regions of the world and to use the numerous disease threats as opportunities for joint research.

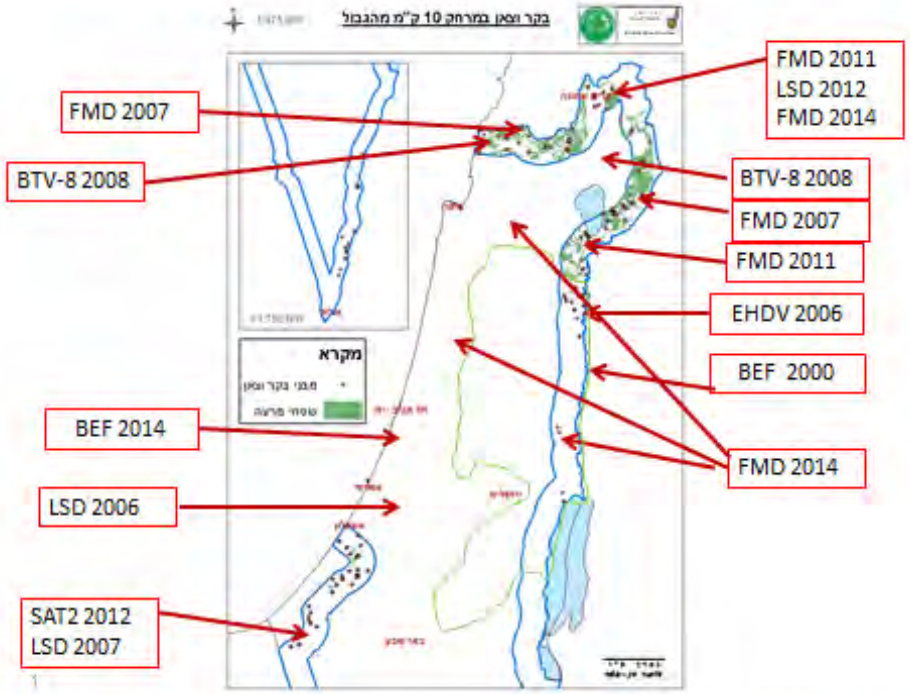

Fig. 1 Map showing index cases of major ruminants' emerging disease outbreaks in recent years. (Blue; a 10 km zone along international borders. Green; pasture zones. Black dots; ruminants' farms)

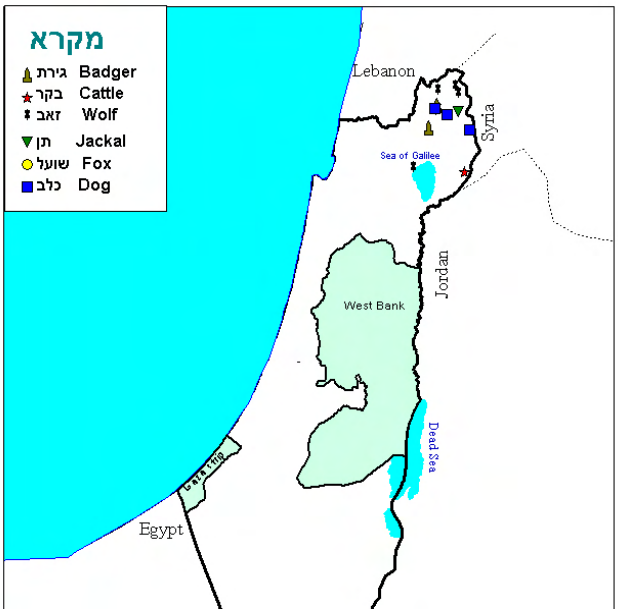

Fig. 2 Rabies cases in Israel in 2014 concentrated in the Golan Height and Upper Galilee.

FUTURES THINKING AND THE PERFECT STORM – BEING PREPARED FOR FUTURE ANIMAL HEALTH CHALLENGES

Alex Morrow

The Alexander Robertson Centre for Tropical Veterinary Medicine, Faculty of Veterinary Medicine, SCAR CWG Animal Health and Welfare Research / EMIDA, U.K.

Animal diseases cause serious social, economic and environmental damage and in some cases also threaten human health. It is estimated that 60% of human pathogens are of animal origin while 75% of emerging animal diseases can be transmitted to humans. Demand for animal protein is expected to increase significantly necessitating further intensification of production, which will bring with it its own challenges. Unanticipated and emerging threats are ever present. Changes in animal disease challenges are inevitable, but how these changes emerge and what the future outcomes could be are uncertain. The disease risks have increased over recent decades, especially as a result of the increased globalisation of trade and animal product movements, and the consequent transfer of associated fast evolving pathogens. These changes are exacerbated by interaction with environmental change, including changes to land use and the potential variabilities associated with climate change. Improved awareness of and preparedness for the animal disease threats are needed for their effective management. Identifying possible future threats is essential to improve preparedness and ensure the availability of the necessary research capacity and capability to address potential needs. With increasing disease challenges but declining public sector budgets for research, surveillance and disease control are we facing a "perfect storm" and how should we best prepare? Can new technology save us? Futures studies offer an approach to answering these questions by helping to understand uncertainty and complex interactions in order to anticipate and prepare for a range of plausible futures.

Traditionally the animal disease research community has focused very heavily on the pathogen, the host and their interactions, but increasing emphasis is now being placed on the context in which disease occurs, broadening host-pathogen interactions to host-pathogen-environment interactions. Disease challenges can arise through a number of sources including a) the emergence of new or previously unknown pathogens, b) extension of the geographic range of existing known pathogens, c) extension of the host range of known pathogens and d) evolution of existing pathogens allowing them to evade control methods. Factors contributing to the emergence or re-emergence of disease include biological factors, such as the rapid evolution of RNA viruses, to a range of human activity from production systems increasing evolutionary pressure on pathogens to encroachment on wildlife habitats resulting in transfer of pathogens from wildlife to livestock and humans. Woolhouse and colleagues have estimated the size of the as yet undiscovered pool of human viruses, suggesting that there is a pool of 38 – 562 of as yet undiscovered human virus species, with 10 – 40 to be discovered by 2020 (1). They also noted that a significant proportion of the viruses discovered in the last few decades is ecological spill-over from animal populations rather than newly evolved specialist human viruses. So far in the 21st Century we have seen the emergence of diseases such as SARS and MERS, but challenges are more likely to arise from extension of the geographic or host range of existing known pathogens. The past decade has also seen the emergence of Schmallenberg, a range of influenza viruses, and a major outbreak of ebola with dire warnings about the loss of antibiotic effectiveness returning infection control to the pre-antibiotic era.

A Strategic Research Agenda (SRA) with a 10-15 year horizon was developed in the EU-funded FP7 EMIDA ERA-Net on "Coordination of European Research on Emerging and Major Infectious Diseases of Livestock" in which it identified a list of pan-European research priorities; i)Surveillance systems and risk analysis, ii)Control measures and biosecurity**,** iii)Ecosystem change, vectorborne diseases and preparedness, iv) Host-pathogen interaction that serves the development of diagnostic tools and vaccination, v) Antimicrobial resistance, and vi) Zoonoses**.** Just after this SRA was published in 2011 Schmallenberg virus infection was identified in north-western Europe, initially affecting animals in Belgium, the Netherlands and the western part of Germany. The initial site of infection appeared to be similar to that of BTV8 when it arrived approximately 5 years earlier. It would not have been possible to predict the arrival of Schmallenberg virus in Europe, however, the need for research on improved surveillance, risk analysis, diagnostics, vector borne diseases and biosecurity were all highly relevant to the Schmallenberg situation that emerged subsequent to the publication of the SRA.

Animal diseases arising in one part of the world can rapidly spread to other regions and become global pandemics, as was seen with H1N1 Influenza in 2009. STAR-IDAZ (Global Strategic Alliances for the Coordination of Research

on the Major Infectious Diseases of Animals and Zoonoses) was a 48 month EU-funded (FP7) Coordination and Support Action (CSA) to improve coordination, at international level of research activities on the major infectious diseases of animals (including zoonoses) and hasten the delivery of improved control methods. Its scope included coordination of research relevant to emerging and major infectious diseases of production animals (livestock, including aquatic animals and bees) and those animal infections that may threaten human health. The development of a Strategic Research Agenda was considered a key component of building the global network into a forward-looking coherent structure of national animal health research funders capable of cross-programme collaboration that will better serve in terms of (human and financial) resources, the research needs of the Livestock industries and animal health policy makers. In developing the SRA foresight activities were undertaken to identify the scientific, technological and related needs to prevent, control or mitigate animal health and zoonotic challenges for the next 20 years which, together with the Criteria for Priority Setting, provide the partners with a framework for identifying targets for investment to improve preparedness and possible areas for collaboration. Regional foresight exercises were conducted in the Americas (using the Fore-CAN scenarios developed in Canada), Asia and Australasia (based on Seven questions to stimulate discussion), Africa and the Middle East (disease threats, impact and research) and Europe (Driver prioritisation and impact, Scenario building and Back-casting), with a separate focused study for the Mediterranean. The European exercise considered animal welfare challenges as well as health. Drivers identified in previous foresight exercises including in the development of the Strategic Research Agenda by the ERA-Net on Emerging and Major Infectious Diseases of Animals (EMIDA)(2), the Fore-CAN study(3), the APEC project on Road-mapping Converging Technologies To Combat Emerging Infectious Diseases (4) and the Foresight Infectious Diseases China Project (5) were prioritised in an online exercise involving experts from the four regions and their likely impact on a range of disease groups estimated. These regional exercises were developed further and integrated at a workshop in Moscow involving delegates from all four regions. The scientific, technological and related needs were presented in three separate lists, Specific Research Areas, Technology and Structural changes.

The results show that many of the important drivers were common to all four regions. Key drivers identified in all of the regions included i) Population Size, density and demographic change including movement of people and ii) climate change, including extreme weather events. Other key drivers identified by at least three of the four regions included i) Movement of animals and their products, ii) political leadership, including short-term thinking and loss of technical expertise and iii) intensification of livestock production.

Concerning the possible impact of the various key drivers the likely impact of demographic change combined with an increasing population size/density on the occurrence of most disease categories was considered to be high or very high with three of the regions rating it as very high in relation to the emergence of new diseases. Climate change including extreme weather events is likely to increase in importance and was considered to have a high impact on the occurrence of vector-borne diseases and endemic parasitic diseases. This supports comments in a World Bank report on climate change and disease risk that virtually any disease that is dependent on vectors or are waterborne could be included in the list of climate-sensitive livestock diseases (6). Disruption of ecosystems, with invasion of exotic species/pests is likely to intensify, increasing the risk of new diseases, vector-borne diseases and endemic parasitic diseases. Increasing interaction between wildlife and domesticated animals and humans is also likely to contribute to an increased risk of Epizootic diseases and the emergence of “new” diseases. Pathogen evolution, including drug resistance was considered to be of increasing importance with a high impact in relation to the occurrence of vector-borne disease, epizootic disease, endemic bacteria/viral/fungal disease, the emergence of new diseases and endemic parasitic diseases. The economics of livestock farming, with increasing pressure on profit margins and intensification of production is also likely to impact highly on endemic diseases, especially endemic parasitic diseases while intensification of production could increase the challenge presented by epizootic diseases. However, alternative production systems, which are likely to become more important, would pose challenges for the control of endemic parasitic conditions. Conversely increasing technological developments in relation to surveillance, monitoring and disease control will have a positive impact across all of the disease categories with harmonisation and improved implementation of regulations contributing further to improved disease control, especially in relation to epizootic diseases and zoonoses.

The scenarios developed in the European exercise were based on two critical uncertainties – “the state of human contentedness” and “the rate of environmental change,” which formed the framework, and a range of the prioritised drivers. The implications of these in relation to animal welfare and disease challenges and the research needed to protect against these possible futures were then considered. The Moscow workshop, involving participants from all

four regions in a back-casting exercise separately considered enablers and barriers to arrive at a 2034 preferred future "animal disease minimised or rapidly contained ensuring a safe and secure food supply" and the research needs to ensure we get there.

Important challenges identified include vector-borne diseases, an improved understanding of the role of wild life, antibiotic effectiveness and availability, anthelmintic resistance, gut health and introduction of trans-boundary diseases. The focus of technological developments should be on diagnostic tests, integrated surveillance systems, vaccine development and alternatives to antibiotics and alternative vector control methods. However, development of the necessary technologies to meet future challenges requires an enabling environment with support for the basic science as well as further along the research pipeline, partnerships and collaborations and knowledge transfer mechanisms. Addressing the specific areas and/or maximising the benefits of technological advances will be enhanced if the capacity/structural changes recommended are also addressed.

Conclusions:

Over the next two decades we are likely to experience continued animal disease challenges due, in part, to the current rapid rate of change and political and economic instability while capacity to respond to these challenges appears to be shrinking in many parts of the world. However, technology has been evolving at a rapid pace enabling smarter ways of detecting and responding to challenge. Futures activities help us understand change and prepare for a range of plausible alternative futures. What is important is not trying to predict what will happen, but being more prepared to engage with whatever may happen.

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THE CURRENT SITUATION IN HEALTH AND IN ANIMAL HUSBANDRY IN SLOVAKIA

Jozef Bíreš

State Veterinary and Food Administration of the Slovak Republic, Botanická 17, 842 13 Bratislava, bires@svps.sk

INTRODUCTION

The mission of the State Veterinary and Food Administration of the Slovak Republic as the state administration authority is management, control and direction of activities and actions in the field of veterinary animal health, animal welfare, feed hygiene, ecology, veterinary pharmaceuticals, official control of foodstuffs and hygiene of foodstuffs of animal and vegetable origin, special commodity modes, appropriate laboratory diagnostics and rapid alert system. There are the following priorities:

In the field of animal health:
- implementation of national eradication programs and updates thereof in accordance with applicable legislation and health situation
- improve the system for the identification and registration of animals
- improve the Veterinary Information System
- the update of national contingency plans in accordance with applicable legislation
- surveillance of bluetongue, eradication programs for infectious bovine rhinotracheitis-continued genotyping of female sheep population
- monitoring of diseases of bees

Animal welfare (animal welfare):
- protection of animals used for experimental purposes
- inspections of farm animals
- protection of companion and circus animals
- inspections of animals at the time of slaughter - guides of good practice.

RESULTS

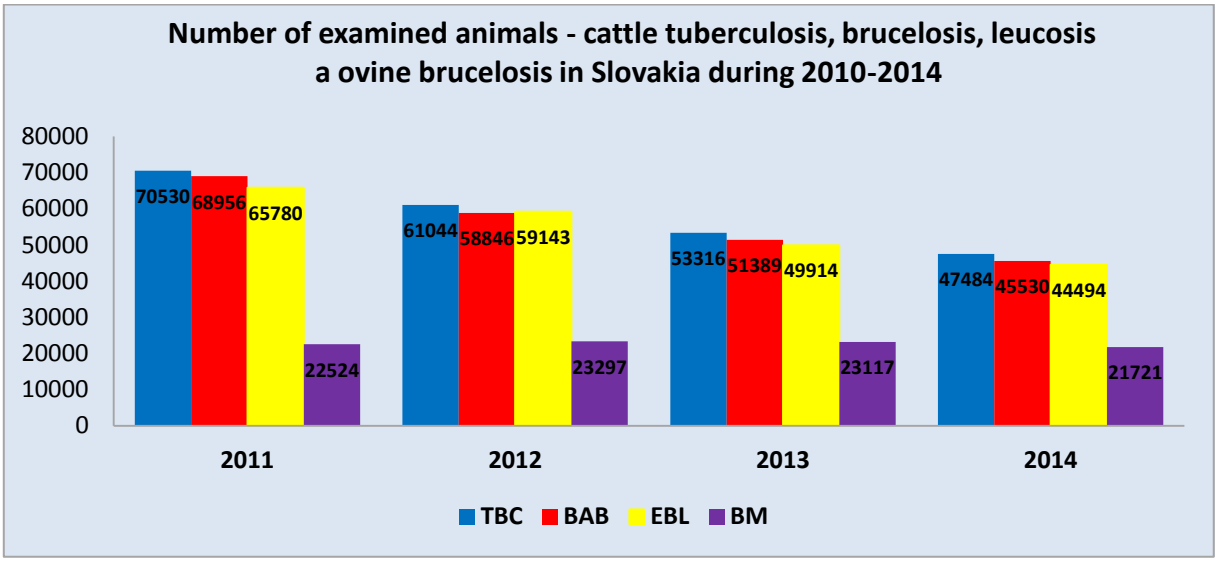
In the area of animal health in 2014 SVFA performed the obligations arising from the Act. 39/2007 Coll. The main task consists in preparing management tools, guidance and coordination of the execution of state management by 40 DVFA in the field of checking compliance with requirements for the identification and registration of animals, the classification of farms, regions and areas for the occurrence of certain animal diseases, the implementation of programs to control, monitoring and eradication of disease, control and compliance with the requirements for the operation of assembly centers, markets with animals, assembly centers for trade with animals, insemination centers, animal holdings and other facilities for breeding and keeping of animals subjected to approval or authorization by veterinary authorities.

In 2014 in the field of animal health SVFA developed and submitted MARD SR fourteen national programs to eradicate, control or survey of animal diseases in the Slovak Republic for approval:
1. The national program for the eradication of rabies in Slovakia for 2014;
2. Survey design (surveillance) Bluetongue (bluetongue) in the Slovak Republic for the year 2014;
3. National control program for classical swine fever in the feral pig population in Slovakia in 2014.
4. The national program for the eradication of American foulbrood for 2014.
5. The national program for the eradication of viral haemorrhagic septicemia fish (VHS) in the Slovak Republic in 2014;
6. The national program for the eradication of bacterial fish diseases in the Slovak Republic in 2014.
7. Survey plan for avian influenza in poultry and wild birds in Slovakia in 2014.
8. The national control program for Salmonella infections in breeding flocks of domestic fowl (Gallus gallus) in the Slovak Republic in 2014;
9. A national control program for Salmonella infection in laying flocks of domestic fowl (Gallus gallus) producing eggs for human consumption in the Slovak Republic in 2014;
10. National control programs for Salmonella infections in broiler flocks of domestic fowl (Gallus gallus) in the Slovak Republic in 2014;
11. National control programs for Salmonella infections in flocks of turkeys in the Slovak Republic in 2014.

12. The plan for the eradication of infectious bovine rhinotracheitis (IBR) in Slovakia for 2014.
13. The program of prevention, monitoring and control of certain transmissible spongiform encephalopathies (TSEs) in the Slovak Republic for the year 2014;
14. Breeding program for resistance in sheep portable / transmissible spongiform encephalopathies - scrapie in the Slovak Republic for the year, 2014.

Classification of holdings, regions and areas

During 2014 Slovak Republic has continued to maintain the status of a country officially free of bovine tuberculosis, bovine brucellosis, enzootic bovine leucosis and ovine brucellosis (Brucella melitensis). To maintain the statute of SR made a total of 47,484 tuberculin cattle, 45,530 serological tests for bovine brucellosis and 44,494 tests for enzootic bovine leucosis during 2014. On ovine brucellosis (Brucella melitensis) were examined in total 21,721 sheep and goats older than 6 months of age and all breeding rams. For reasons of the recognition of the status and to avoid the spread of animal diseases SVFA developed a "Plan VPO 2014" and methodological guidance "Classification holdings for the gradual implementation surveillance networks and health requirements for the movement of live animals and germinal products - Guidance for 2014 from December 20, 2013".



Rabies

Rabies is always fatal disease of warm-blooded animals in case of detection of clinical signs. For this reason, the National Program for the eradication of rabies in Slovakia in 2014 ("rabies eradication program") was aimed at preventing the spread of rabies and for its timely and safe diagnosis in case of risk of its spread through injury by potentially rabid animal. Rabies eradication program was implemented through the compulsory vaccination of domestic carnivores against rabies at the expense of the farmer and through oral vaccination of foxes against rabies carried out in two seasonal campaigns - spring and autumn 2014, paid for from the state budget funds, EU co-financing.

Classical swine fever

Monitoring of classical swine fever (hereinafter "CSF") in domestic pig holdings as well as monitoring of CSF in wild boars population has been carried out in accordance with the national control programs for classical swine fever in feral population in Slovakia in 2013, which was approved by the European Commission and co-financed EU funds.

During the year 2014, the Slovak Republic didn´t detect case of CSF in domestic pig holdings or wild boar.

Aujeszky's disease

Targeted monitoring of Aujeszky's disease (MA) in pig holdings in 2014 was made on the basis of the Plan of veterinary prevention and protection of the national territory in order to maintain free MA status for the Slovak Republic. In September 2006, the last pig holding was successfully eradicated from MA and Aujeszky's disease in recent years has not occurred in any new outbreak.

Infectious bovine rhinotracheitis

Monitoring of IBR / IPV in cattle holdings in 2014 was made on the basis of the approved eradication program - Plan for the eradication of infectious bovine rhinotracheitis (IBR / IPV) in Slovakia in 2014. The program aims

to revitalize cattle holdings from IBR / IPV on the whole territory of the Slovak Republic. Revitalization of cattle holdings will improve the health status and will remove the barriers both in the domestic and foreign trade.

In 2014 SVFA continued with eradication of IBR / IPV on cattle farms. The vaccination was continued in IBR/IPV free holdings and based on the development of individual eradication plans. Monitoring in officially free holdings was realized in 2014 according to the Plan VPO for 2014. The final survey was covered by the budget of the SVFA SR.

Disease of poultry and wild birds

In 2014, monitoring of poultry diseases again focused on the most serious diseases, directly endangering the health of poultry and the health of the human population.

Subjects of the monitoring:

a) Infections caused by Salmonella spp. (within four national control programs)

b) Avian influenza in poultry and wild birds (within the survey plan)

Apart from health problems among birds themselves and considerable financial losses for the poultry farmers these zoonosis represents significant risk for humans.

Salmonella infections

Monitoring of Salmonella infections in poultry holdings shall take place within four programs:

National Control Program for Salmonella infections:

1. in breeding flocks of domestic fowl (Gallus gallus) in the Slovak Republic in 2014
2. in flocks of domestic fowl (Gallus gallus) producing eggs for human consumption in the Slovak Republic in 2014
3. in broiler flocks of domestic fowl (Gallus gallus) in the Slovak Republic in 2014
4. flocks of turkeys in the Slovak Republic in 2014

Bluetongue

• Surveillance of bluetongue in Slovakia in 2014

Targeted monitoring of bluetongue (BT-Net "hereinafter referred to as" BT ") in the Slovak Republic is aimed at:

1. Research, monitoring the disease on territory of the Slovak Republic, which is also the declaration that SR is free of the disease,
2. Gathering of data on the estimated risk of entry of the disease into SR, observation of the rules of prevention against disease introduction and application of the restricted, tightened measures on the movement of animals from restricted zone and through restricted zones.

Preventive measures are performed in accordance with applicable legislation:
• Inspections of imported susceptible animals
• Testing of susceptible animals before moving from restricted zone and when moving through restricted zone
• serological testing of susceptible animals after abortion
• Imports of semen of bulls serologically tested

Transmissible spongiform encephalopathies (TSE)

• Program of prevention, monitoring and control of certain TSE in Slovakia for the year 2014

(hereinafter 'the monitoring of TSE ")

Table 1

The results of monitoring of BSE in cattle for the period from 1.1.2014 to 31.12.2014 in Slovakia

Targeted group- cattle	The number of samples examined in the NRL for TSEs Zvolen	
	Examined	Positive
Dead and slaughtered	7335	0
Urgently slaughtered	100	0
With clinical symptoms on ante mortem examinatiom	7	0
Slaughtered health	19	0
Slaughtered in eradicate BSE	0	0
Suspected	0	0
The sum	7461	0

Table 2

The number of confirmed cases of BSE in cattle in individual years

year														
2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total value
5	6	2	7	3	0	2	1	0	1	0	0	0	0	27

From 2001 to 31.12.2014 27 cases of BSE were confirmed in SR.

Monitoring of TSE in sheep and goats in the SR in 2014 was implemented within the TSE monitoring programs as follows:
- From all healthy slaughtered sheep and goats in a slaughterhouse over 18 months from infected holdings for the eradication of scrapie;
- From all dead and emergency slaughtered of sheep and goats over 18 months;
- From all sheep and goats suspected of being infected with a TSE, regardless of age.

In 2014 were examined 11,657 pieces of sheep and 148goats pieces in the National reference laboratory for TSEs at Veterinary Institute Zvolen (the "TSE NRL for Zvolen") within the monitoring of TSE examined together.

Table. 3

The results of monitoring TSE - scrapie in sheep for the period from 1.1.2013 to 31.12.2014

The targeted group of animals	The number of samples examined in the NRL for TSEs Zvolen	
	Examined	Positive
Slaughtered and dead	9984	9
Urgently slaughtered	6	0
With clinical symptoms on ante mortem examination	0	0
Slaughtered health	0	0
Slaughtered in eradicate	1667	0
Suspected	0	0
The sum	11657	9

Apart from the results of monitoring TSE in the Table. 3 there was confirmed one case of atypical scrapie in sheep originating in the Slovak Republic in the NRL for TSEs in Poland in 2014. The sample was collected from slaughtered - healthy sheep at slaughterhouse in Poland.

Table. 4

The number of confirmed cases of TSE - scrapie in sheep in different years

Year												
2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total value
4	31	9	9/1*	8	27	1*	2/3*	6/4*	0/3*	0/4*	6/4*	102/20*

* atypical scrapie

From 2003 to 31.12.2014 122 cases of scrapie in sheep were confirmed in SR.

Breeding program for resistance in sheep portable / transmissible spongiform encephalopathies - scrapie in the Slovak Republic in 2014 ("breeding program").

In 2014 1434 pieces of rams were examined for genotyping examination from breeding-experimental, breeding and reproduction uninfected holdings with sheep.

Representation of individual genotypes from examined 1,434 rams according to risk groups (examined in the breeding program by risk groups).

- The risk group no. 1 63.46% is detected.
- The risk group no. 2 31.93% is detected.
- The risk group no. 3 0.98% is detected.
- The risk group no. 3 1.88%* (ARQ / ARQ) is detected.
- The risk group no. 4 1.26% is detected.
- The risk group no. 5 0.49% is detected.

CONCLUSION

The Slovak Republic has implemented an efficient, consolidated and within the EU harmonized system of controls in the food chain from farm to table at the national level. In recent years, there has not been recorded any health risk (occurrence of dangerous infections) or food scandal in Slovakia.

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ORAL PRESENTATIONS

Animal welfare, health and behaviour

THE EFFECTS OF HIGH AND LOW CONCENTRATE FEEDING ON DAIRY COWS' FEEDING BEHAVIOUR

M. Soonberg and D. Arney

Department of Animal Nutrition, Estonian University of Life Sciences, Tartu, Estonia

Abstract

In robotic milker dairy systems, where the concentrate and forage components of the ration are offered to the cows separately, lack of control over intakes can result in difficulties balancing the forage and concentrate portions of the diets, leading to problems associated with high concentrate intakes and concomitant low forage intakes. As a pilot study, six cows (two at the highest and two at the lowest yield of the milk production range) were selected. The cows were fed a ration comprising, separately, concentrate feed from a robot and a feeder, and partially mixed ration at the feed barrier. With the low number of samples the results are indicative and descriptive, but it appears from the raw data that individual variation in visiting times and times spent at the feed barrier are greater than the effect of level of production. Cows also spend a significant portion of their time idling at the feed barrier, not actively feeding. It is concluded that care should be taken to presume behaviour from positional data, and there is no evidence that cows with higher and lower milk yields are differentially motivated to feed from a forage source.

Introduction

To ease feeding management, many producers supplement forage with concentrate, based on the average nutrient requirements of the herd (Lawrence et al., 2015). This means that cows whose production is less than average will receive more concentrate than their requirements, and cows with milk production level higher than this average will receive less concentrate than they need. This means that some cows are overconditioned and others are too thin and are not able to reach their full milk yield potential. Overconditioned cows at calving are at higher risk for retained foetal membrane. Which might lead to reduced fertility (Roche, 2006) and early culling.

Cows feed intakes are not only affected by the amount and quality of concentrate given. It also depends on lactation stage and position in the social hierarchy (Berry et al., 2006). Feed intake is at its peak in mid-lactation, increases in early lactation and declines at the end of lactation (Berry et al., 2006). However Friggens et al., (1998) found that lactation stage affects only cows who receive high concentrate total mixed diet. Those on a low concentrate total mixed diet showed no effect on dry matter intake as lactation progressed. In order to explore the comparative feeding strategies of differently fed, and differently yielding cows, their feeding behaviour at the forage feed barrier was observed.

Cattle living in a group have a strict hierarchy. In a feeding area low ranking cows are more likely to be pushed away by high ranking cows and has to fight for their place. Lower ranking cows spend more time standing without feeding (Proudfoot et al, 2009, reviewed by Loceck-Luchterhand et al., 2014) which might be a risk for locomotion problems (Proudfoot et al., 2009), feed at a faster rate (Proudfoot et al, 2009 reviewed by Loceck-Luchterhand et al., 2014) and spend less time in the feeding area than high ranking cows (Val.Laillet et al., 2008 reviewed by Huzzey, et al., 2012). The cows selected for observation in this trial were all over a parity of two, so non were new to the herd and would have had an established social position.

Material and method

The trial was carried out on Märja farm of the Estonian University of Life Sciences, Tartu, Estonia. Multiparous Holstein Friesian cows were cubicle-housed and milked with an automatic milking system (DeLaval). All cows received concentrate at the milking robot and additionally in a partially mixed ration at the feed barrier. Six cows were selected based on their concentrate consumption from concentrate feeding bin. Three cows did not receive any additional concentrate and the other three received higher (2 kg and 4 kg per day) amounts of concentrate. Cows were observed from a gantry over the feeding area, and feeding behaviour at the feed barrier was recorded over a 24 hour period from June 2014 till February 2015.

Descriptive statistics for each parameter were calculated with Microsoft Excel.

Results and discussion

Statistical analyses showed that the mean time spent feeding did not differ much between the treatments. The same thing can be said about standing, walking and drinking behaviours. Because of the low number of samples it is not possible to confirm that cows receiving a higher amount of concentrate spend less time feeding on forage.

Displacing another cow at the feeding barrier can be considered as a normal social behaviour of dairy cows (Bouissou et al., 2001, reviewed by Ruuska, et al., 2014). It increases when less feed is available or stocking density is too high (Chapinal et al., 2011, Collings et al., 2011 and Proudfoot et al., 2009, reviwed by Ruuska et al., 2014). In the trial the oldest cow (V lactation) was the least aggressive. The other cows were also less aggressive towards her. The youngest cow (II lactation) received the most aggressive behaviour toward herself.

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IMPACT OF STRAW DELIVERY FREQUENCY ON THE HEALTH OF FINISHING PIGS AND PEN CLEANLINESS

L. Wahlund, C. Lindahl, A.-K. Lind

JTI - Swedish Institute of Agricultural and Environmental Engineering, Uppsala, Sweden.

Summary

The purpose of this study was to investigate if the delivery frequency of straw impacts the health of finishing pigs and cleanliness of their pens. An observational based case-control study was carried out in two sections at a conventional finishing pig farm. In Section 1, a system for automatic straw delivery was installed and fresh straw was delivered 3 times a day. In Section 2, straw was delivered manually once a day. Both sections received the same amount of straw/day. While no clear impact on the pig's health was observed, increased delivery frequency of straw did improve pen cleanliness.

Introduction

Straw has been found to improve the welfare of pigs as it can act as a recreational and/or nutritional substrate, as well as improve thermal and physical comfort when used as bedding (Day et al., 2008; Tuyttens, 2005). According to the Swedish Animal Protection Law, providing straw is mandatory in pig production and most finishing pig farms in Sweden deliver straw by hand once a day. The purpose of this project was to investigate if an automatic straw delivery system enabling greater frequency of delivery affected the health of finishing pigs and cleanliness of their pens. The objective of this work is to improve animal welfare, health and the working environment in conventional pig production in Sweden.

Material and methods

An observational based case-control study was carried out during two rearing periods on a farm with conventional All-in All-out finishing pig production in two separate sections (42 pens in each section with 10 pigs in each pen). In Section 1 (S1), a system for automatic straw delivery was installed. This system was developed and tested by JTI - Swedish Institute of Agricultural and Environmental Engineering (Lindahl et al., 2008).

In S1 straw (short chopped) was delivered three times a day approximately 10 minutes after feeding, and in Section 2 (S2) straw (long chopped) was delivered manually once a day. In both sections the same amount of straw/day (0.1 kg/pig) was used. Measurements were taken two days per week in both sections after the pigs arrived, and during week 2, 7 and 10 of the study. Dust on surface (DOS) measurements were taken during the entire study period. The measurements taken were: 1) behaviour studies, 2) assessment of animal health (tail biting marks, injuries, body condition score, cleanliness of the animals and lameness), 3) amount and cleanliness of residual straw and overall cleanliness of the pen, 4) occurrence of air born dust and DOS.

Results

In general, both sections had low frequency of tail biting, especially in the first rearing period. In the second rearing period tail biting was more frequent in S1, where 6.4% of the pigs had tail injuries (53 of 834 observed pigs), in comparison with S2, where 3.9% of the pigs had tail injuries (32 of 826 observed pigs). Contrary, the number of wounds and scratches were higher in S2. There were no differences in body score condition between the two sections and there were few observations of lameness. Overall there were no significant differences in the health parameters between the two sections.

There were significant differences ($p<0.0001$) in time spent manipulating straw between the two sections, where pigs in S1 were more active ($p=0.0474$). The pens in S1 were on average cleaner, while the pens in S2 had more residual straw and the straw was dirtier. On average there was more DOS in S1, but this difference was not significant. While mean values for airborne particles (mg/m3) were higher (not significant) in S1 in the first rearing period, this changed with higher airborne particles in S2 in the second rearing period.

Discussion

Earlier studies on the relationships between straw, hygiene and health in finishing pigs are few and ambiguous (Tuyttens, 2005). Certain diseases and injuries have been shown to increase while others decrease. However, straw is

considered to reduce the risk of leg and hoof injuries and mortality in piglets and finishing pigs (Tuyttens, 2005). In this study there were very few health problems and no significant differences in health between the two sections. This could be explained by the fact that the pigs in S1 and S2 received sufficient amounts of straw.

According to Day et al. (2008), pigs provided chopped straw had more tail-biting incidents than those with full-length or long chopped straw. This suggests that pigs were not able to manipulate the chopped straw in the same way as full-length or long chopped straw. Contrary to Day el al. (2008), the results from this study showed no significant differences in tail-biting between the two sections.

Several studies have shown that pigs with access to straw are more active than those without straw (Morgan et al., 1998; Scott et al., 2006; Day et al., 2008), and that pigs with straw devote more time manipulating straw and less time interacting with other pigs. In the present study however, the pigs in S1 spent more time manipulating the straw compared to S2, even though they received the same amount of straw. This is in line with Fraser et al. (1991) who found that pigs are most active when straw is new. Straw or other bedding material often contains dust that pigs and farmers are exposed to. On average there was more DOS in S1. This can be explained by the fact that the straw was delivered three times a day instead of once.

Conclusions

In general the pigs exhibited few health problems, probably due to the fact that both sections received the same amount of straw. While no clear impact on the pigs' health was observed, increased delivery frequency of straw did improve pen cleanliness. The effects of straw length and amount of straw provided needs to be further investigated to clarify any impact this might have on the aggressive behaviour of finishing pigs and any welfare problems such as tail biting and belly nosing.

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MONITORING WELFARE IN PRACTICE ON DUTCH DAIRY FARMS

F.J.C.M. van Eerdenburg[a], J. Hulsen[b], B. Snel[c] and J.A. Stegeman[a]

[a]*Dept Farm animal health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, the Netherlands; F.J.C.M.vanEerdenburg@uu.nl*
[b]*Vetvice, Bergharen, the Netherlands*
[c]*DLV, Deventer, the Netherlands*

Summary

The results of three welfare assessment protocols have been correlated with the Welfare Quality® assessment protocol on 60 dairy farms in the Netherlands. After adaptations to the WQ protocol, an alternative assessment protocol could be constructed of components of the other three. This had a correlation with the adapted WQ of 0.88. Execution of this new welfare monitor takes approximately 1.5 h for a farm with 100 dairy cows. This protocol is now being integrated in the Koekompas (=Cow Compass), a management assistance tool.

Introduction

An increase in welfare is correlated with a higher milk yield (Van Eerdenburg et al., 2013). The Welfare Quality welfare assessment protocol® (WQ) is quite intensive and it takes a long time (up to a day) to assess a farm. Several other protocols haven been designed and published that do not require a full day for a single farm and these have been compared with WQ.

Materials & methods

In this project 3 other welfare measuring protocols: Welzijnswijzer (=Welfare Indicator), Koekompas (=Cow Compass) and the Continue welzijns monitor (=Continuous Welfare Monitor), have been compared with WQ on 60 dairy farms in the Netherlands. Four veterinary practices made a list of their dairy farmer clients. Each was given a score from good to bad, based on the availability of good quality food & water, quality of housing, health and behaviour. Out of the lists, randomly, 60 farms were selected in such a way that in each practice there were 5 good-, 5 average- and 5 bad farms. The farms were visited within 2 weeks for all protocols in order to avoid changes in welfare status over time. The result of the WQ protocol was considered to be the reference and the other three were correlated with WQ (Pearson correlation in SPSS version 20.0). Not only at the level of the end score, but also at principle, criteria and indicator level.

Results & discussion

The results for WQ were: 3 farms with score Not Classified, 52 with score Acceptable and 5 Enhanced, no farm was scored Excellent. This implies that WQ does not have a proper discriminative capacity. Because of this, the correlations with the other protocols were very low and not statistically significant. In relation to the WQ endscore the Pearson correlation was calculated for the principle of Feeding 0.46; Housing 0.15; Health -0.07 and Behavior 0.47. Therefore, the original WQ was adapted in 3 ways: 1) Drinkers: If one of the drinkers is dirty, all drinkers are considered to be dirty. This is not logical because a farm with 10 drinkers will have a lower score than a comparable farm with 5 clean ones just because one of the drinkers is dirty. However, 9 clean drinkers are available for the cows. In the adapted protocol, therefore, a weighted score for cleanliness was introduced. A clean drinker scored 1, partially dirty 2 and dirty 3 points. After giving the score for the rest of the parameters measured, the total is divided by the average score for the cleanliness. 2) Integument alterations: If a cow has one hairless patch (HP) or lesion/swelling, she is taken in the calculations as a cow with a HP or a lesion/swelling, not taking into account how many HP or lesions/swellings she has. In the adapted protocol, therefore, the average number of HP, lesions and swellings per cow is used in the calculations. 3) Qualitative Behaviour Assessment (QBA): The QBA is seriously disputed (Bokkers et al., 2012) and, in the experience of the present study, very difficult to explain to the farmers. Since the aim of WQ is improvement of animal welfare, one has to motivate the farmer to improve the situation on his farm. With the use of the QBA, farmers are not convinced that the result is something to be taken seriously. Therefore, the QBA was omitted. A new score was calculated for the 60 farms: 22 farms scored Not Classified, 31 scored Acceptable and 7 Enhanced, no farms scored Excellent. The Pearson correlations of the 4 principles were: Feeding: 0.85; Housing: 0.45; Health: 0.99 and Behaviour: 0.99. Correlations with the WQ protocol and the other protocols were still very low. However, it was possible to construct a shorter protocol out of the components of the three other protocols tested

(table 1), with a correlation of 0.88. Furthermore, the number of animals that need to be assessed on an individual basis could be reduced substantially as well. Thus reducing the time required for the execution of the protocol substantially (table 2). Assessing welfare of a group of animals can be done with animal based measures and environmental measures. WQ uses mainly animal based measures, like behavior. These measures reflect better what the status of the cow is, but need a substantial amount of time to assess, whereas measuring the environment can be done relatively fast. For some parameters there was a high correlation between the animal based and environmental measures. For example, the number of collisions of the cow with the dividers (during the lying down movement) correlated well with the width of the freestall (r^2 = 0,63; p= 0,03). Measuring the width of the freestall takes just a few minutes, whereas to estimate the number of collisions in a reliable way one needs at least two hours watching cows that lie down. In the new welfare monitor, therefore, the number of collisions is replaced by the width of the freestalls.

Principle	**Parameters measured**
Feed & water	Body condition
	Water supply
Housing	Freestall dimensions
	Softness of bedding
	Cleanliness of the cows
	Access to pasture
	Cows lying outside freestall
Health	Locomotion score
	Skin lesions
	Mastitis
	Other diseases (respiratory/metabolic/fertility)
Behaviour	Avoidance distance at the feedrack
	Possibilities for expression of normal behaviour

Table 1. Parameters measured in the new welfare monitor

	75%	66%	50%
Lameness	5,5	6,8	9,9
Skin Lesions	5,2	6,1	9,3
Diseases	5,9	3,9	8,8
Health (principle)	4,9	4,5	7,9

Table 2: Average deviation in % of the original score for items in the WQ protocol when 75%, 66% or 50% of the animals was scored individually.

It is proposed to change the WQ protocol according to the 3 adaptations described above.The newly developed protocol can be used as a screening tool for welfare problems and to improve the management on a dairy farm.

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PIG WELFARE ASSESSMENT BASED ON PRESENCE OF SKIN LESIONS ON CARCASS AND PATHOLOGICAL FINDINGS IN ORGANS

N. Čobanović, N. Karabasil, N. Ilić, M. Dimitrijević, D. Vasilev, A. Cojkić, Lj. Janković

Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia

Summary

The aim of the study was to assess pig welfare at slaughterhouse based on presence of skin lesions on carcass and pathological findings in organs. The assessment was performed according to the Welfare Quality® protocol. Fatteners (60, in total) were assessed on the basis of the protocol, in two visits at the same slaughterhouse. A 28.3% of the carcasses showed high score of skin lesions and in 26.7% the presence of lesions were negligible or very low. The signs of pneumonia and pleurisy were present in 38.3% and 6.7%, while pericarditis was observed in 3.3% of assessed organs. Milk spots were present in 53.3% of assessed livers.

Keywords: pigs, pathological findings, skin lesions, welfare

Introduction

Skin lesions affect negatively on carcass grading and pork quality. The most common causes of skin lesions are fighting between pigs, inadequate facility design and violent handling (Guàrdia et al., 2009, Karabasil et al., 2011, 2013a, 2013b). Skin lesions on the rear part of the body may be caused by rough handling, while skin lesions on the front part of the body often may be caused as a result of fighting between pigs (Dalmau et al., 2014). The main non-infectious causes of pathological findings in organs are inadequate microclimate conditions and poor biosecurity measures on farms. Pneumonia and pleurisy are the most common findings in pig lungs at slaughter (Sanchez-Vazquez, 2013). Pericarditis occurs as a consequence of lymphoreticular penetration of causative agents of pneumonia or pleurisy (Leps and Fries, 2009). Ascariosis is the most important internal macro-parasitism present in farmed pigs. The aim of the study was to assess pig welfare at slaughterhouse based on presence of skin lesions on carcass and pathological findings in organs.

Material and methods

The study was performed on 60 fatteners (48 barrows, 12 gilts) from the same commercial farm in two visits (30 animals per visit) at the same slaughterhouse. The assessment was performed according to the Welfare Quality® protocol (2009).

Results

From a total of 60 examined pig carcasses, 28.3% were found with skin lesion score 2 and 45% with score 1, while 26.7% were found with skin lesion score 0 (Table 1). Skin lesions with score 2 were more frequent in barrows (20% compared with 8.33% in gilts), mostly on the front part of the carcass (25%). Distribution of skin lesions in different parts of the pig carcass is shown in Table 2.

Table 1. Classification of examined pig carcasses based on skin lesions score

	Male + female	Male	Female
%	**%**	**%**	
Score 0	26.7	10	16.67
Score 1	45	33.33	11.67
Score 2	28.3	20	8.33

Table 2. Distribution of skin lesions in different parts of the pig carcass

		Ears	Front	Middle	Hindquarters	Legs
Score 1	**%**	1.67	23.33	1.67	20	-
Score 2	**%**	3.33	25	-	6.67	-

The signs of pneumonia and pleurisy were detected in 38.3% and 6.7%, while pericarditis was observed in 3.3% of assessed organs. Milk spots were found in 53.3% of assessed livers (Table 3).

Table 3. The number and percentage of pigs with different pathological findings

Health status	**%**
Pneumonia	38.3
Pleurisy	6.7
Pericarditis	3.3
Milk spots	53.3

Discussion

Skin lesions on carcass, found in this study, are not in accordance with Llonch et al. (2012) results, who found 28.3% of the carcasses with high score skin lesions and in 26.7% the presence of lesions were negligible or very low. Guàrdia et al. (2009) assessed skin lesions on pig carcasses based on a five point scale. They reported that 72.4% of examined pig carcasses were scored as seriously damaged, 16.6% as slightly damaged and only 11% carcasses were found to be without any evidence of damage. In this study, skin lesions score were higher in male than female pigs, mostly on the front part of the body. Lesions on the front part of the body may often be caused by fights connected with social ranking after mixing unfamiliar pigs (Dalmau et al., 2014). Karabasil et al. (2013b) reported skin lesions in 29.03% of examined pigs, without classification of examined pig carcasses based on skin lesions score.

The percentage of pleurisy (6.7%) and pericarditis (3.3%) on the herd level was below warning threshold, based on the Welfare Quality® protocol (2009). On the other hand, the percentage of pneumonia (38.3%) and milk spots (53.3%) were above. This indicates a serious welfare problem on the herd level on the farm of origin. The results of pathological findings, found in this study, are in accordance with Kofer et al. (2001), who found pneumonia in 43.7% of cases, pleurisy in 22.7%, pericarditis in 6.8% and milk spots in liver in 45.6%. Contrary to this study Makinde et al. (1993) detected pneumonia in 17.4% and milk spots in 16.4% of assessed organs, while Llonch et al. (2012) found pneumonia in 11.6 % of examined lungs, and milk spots in 3.0 % of assessed livers.

Conclusion

Skin lesions on carcasses along with presence of pathological findings in the organs of slaughtered pigs, can serve as good indicators for the assessment of animal welfare, health status and environmental conditions on the farm of origin.

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WELFARE AND HOUSING ASPECTS OF LAYING HENS WITH INTACT BEAK IN AVIARY SYSTEMS

B. Spindler[1], M. F. Giersberg[1], J. Hartung[1], R. Andersson[2] and N. Kemper[1]

[1]Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour, University of Veterinary Medicine Hannover, Foundation, Germany [2] StanGe, Faculty of Agricultural Sciences and Landscape Architecture Osnabrück, Germany

Summary

Beak trimming is one of the most disputed welfare criteria in the housing of laying hens. The German government has recommended the abolition of beak trimming as soon as possible. Therefore, this paper provides an insight into the welfare and housing conditions of 12 commercial flocks of laying hens with intact beak. The scientific monitoring of the flocks showed that it was particularly difficult to maintain a satisfactory litter quality, favourable ambient climatic conditions, a permanent access to appropriate pecking materials and a high light intensity as well as to rear pullets in good conditions throughout the housing period. While feather damages were detected at all flocks, cannibalism outbreaks were noticed in 8 of the 11 flocks during the laying period. However, the measures taken against cannibalism calmed down the situation in all affected flocks. The results give reason to reconsider behavioural and housing needs of pullets and laying hens.

Introduction

Behavioural disorders such as feather pecking and cannibalism are an important welfare issue in housing laying hens. These behaviours may result in poor plumage conditions, patches of feather loss and skin damages. Egg production usually drops and mortality increases in affected flocks. To reduce the damages of these behavioural disorders, beak trimming is a common practice. However, it is also one of the most disputed welfare criteria in the housing of laying hens. The German government has recommended the abolition of beak trimming as soon as possible, in some parts of Germany by the beginning of 2017 at the latest. Therefore, it is necessary to minimise risk factors in housing and management which are positively associated with feather pecking and cannibalism. Many studies showed that frustration and a wide range of environmental factors, such as light intensity, climate, access to environmental enrichment and diet, effected feather pecking behaviour (Huges and Duncan, 1972; Green et al., 2000; Lugmair, 2009). This paper provides an insight into the welfare and housing conditions of 12 herds of laying hens with intact beak housed under practical conditions in alternative systems.

Materials and methods

The scientific monitoring of the 12 commercial flocks (1,800 - 32,000 hens/flock; Lohman Brown, except 1 Lohman Tradition and 1 Dekalb White flock) involved regular visits during rearing (3 visits) and production (6 visits), in which housing conditions, flock health and production parameters were evaluated. In addition, 50 hens per flock were weighed and inspected individually during each visit. The non-beak-trimmed hens were reared and housed in aviary systems. To prevent feather pecking and cannibalism, a guide defining optimal management and housing conditions was created at the beginning of the study (ML, 2013). An outbreak of cannibalism was defined if 10% of the inspected hens had injuries up to 0.5cm (diameter).

Results

The hens were housed between 73 and up to 99 weeks of life (mean 79.5 week of life). The pullets were reared up to 16 and 17 week of life before they were came into the production stable where the laying hens were housed between 55 and 77 weeks (mean 60.2 week of life).

The evaluation of the housing conditions showed that various environmental enrichments were used during rearing (7 flocks) and laying (10 flocks). Most of the flocks had access to pecking materials, such as pecking stones, hay, straw, plastic elements and wheat. The maintenance of a satisfactory litter quality, favourable ambient climatic conditions and high light intensities during the entire housing period were particularly difficult (Table 1).

Furthermore, the examined hens weighted up to 370g less than recommended by the breeding company's guidelines, though an adequate body condition during the entire rearing and production period is an important factor to

avoid behavioural problems. Although a continuous monitoring of the flock's weight gain is crucial to intervene in time, only about half of the visited farms used automatic barn scales.

Moderate feather damages were detected in all flocks at the end of rearing. During production, feather pecking occurred in all flocks. This resulted in poor plumage conditions in all flocks at the end of the laying period. During production, 8 of 11 flocks showed cannibalism at an average age of 39 weeks. The measures taken against cannibalism calmed down the situation in all affected flocks.

Discussion

According to other studies, the present evaluation in commercial flocks shows that feather pecking is a usual occurrence in the production period of laying hens (Green et al, 2000; Niebuhr, 2006). Management and housing factors were possible associated with an increased risk of skin injuries (Lugmair, 2009).

Conclusion

The results of this scientifically monitored field study indicate that it is possible to house hens with intact beak. However, high requirements on housing and management have to be met. The results also give reason to reconsider behavioural and housing needs of pullets and laying hens.

Acknowledgments

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http://www.ml.niedersachsen.de/portal/live.php?navigation_id=32023&article_id=110604&_psmand=7

Table 1: Housing and management parameters

Rearing period	Number of flocks 10	Production period	Number of flocks 11
Restricted access to scratching area			
- within the first 3 weeks of life	7	- immediate access	3
- within the first 5 weeks of life	1	- within the first week	9
- within the first 9 weeks of life	1	- within the first 3 weeks	2
Provision of environmental enrichment (separate & combined)			
Provision in total	7	Provision in total	10
thereof:		thereof:	
- dust bathing areas in the first 3 weeks of life	1	- pecking blocks	8
		- various plastic elements	5
- egg carton (board)	1	- straw* (thereof 1x bales of straw)	4
- straw	1		
- hay	1	- hay	2
- various plastic elements	1	- hay bricks	3
- separate dust bathing areas	2	- separate dust bathing areas	1
- pecking blocks (cellular concrete)	3	- egg carton (board)	1
		- carrots/ apples	2
		- grains (daily provision)	1
Litter management			
- depth of litter > 1 cm after access to scratching area (n= 8 flocks)	8	- depth of litter > 1 cm after access to scratching area	9
- agglutination of litter in parts of the barn in the 9th week of life (n= 7 flocks)	3	- agglutination of litter in parts of the barn (peak of production)	6
- agglutination of litter in parts of the barn at the end of rearing (n= 7 flocks)	3	- agglutination of litter in parts of the barn at the end of production	6
Light and lighting			
- daylight	2	- daylight	3
- average light intensity (at least during one visit) < 20 lux	8	- average light intensity (at least during one visit) < 20 lux	11
- average light intensity (at least during one visit) < 10 lux	6	- average light intensity (at least during one visit) < 10 lux	11
- differences in light intensity > 8 lux between rearing and production period	3	- light intensity at the end of the production period < 5 lux	9
Ambient climatic conditions during housing			
- ammonia > 10 ppm	2	- ammonia > 10 ppm	4
- deviations from optimal ambient temperature	4	- deviations from optimal ambient temperature	11
- exceeding of recommended air velocity	4	- exceeding of recommended air velocity	3

* According to the farmer

WELFARE, HEALTH AND BIOSECURITY - THE BASIS FOR EFFECTIVE PRODUCTION OF FARM ANIMALS

P. Novak[1], G. Mala[2], K. Tittl[1]

[1] Tekro Ltd., Prague, Czech Republic; [2] Institute of Animal Science, Prague Uhrineves, Czech Republic

Summary

The presented work is focused on developing and validation an objective comprehensive system for evaluation of biosecurity, welfare and health in breeding practice, which is applicable for different species of farm animals. It enables to analyse the actual level of biosecurity on one hand, and on the other hand, to propose measures that are necessary for improving the level of biosecurity in livestock.

Introduction

Animal welfare, health and biosecurity represent an important factor affecting the level of farm animal production at all stages of the livestock. Maintaining mutual balance among animal health, environment and production should be based on fulfilling the basic needs of livestock (welfare) and the principles of biosecurity.

Adherence to animal hygiene in farms is one of the key factors in ensuring a high level of protection of public health against zoo noses and food-borne zoonotic diseases [1]. The higher levels of biosecurity lead to improved animal health and productivity, and to a reduction of the use of antimicrobials [2], which are important features of sustainable animal production.

Comprehensive approach of solution - "from conception to consumption" or "from stable to table"- is necessary to ensure the integrated protection of quality of production at every level of the production chain [1,3,4,5].

Material and methods

This study is based on the analysis of selected indicators of welfare, health and biosecurity of farm animals, which is directly related to the possibility of their evaluation including the expression of potential interactions among above mentioned criteria. The selection of welfare, health and biosecurity indicators, methods of measurement and their evaluation will be used in different livestock production systems.

Results

The objective of a comprehensive assessment system of biosecurity, welfare and health of farm animals will be analysed on the actual level of the selected indicators. The results of the analysis will be the ground for improving the management of the herd/flock as part of an overall management strategy, health, production and reproduction in farms.

In the context of assessing the level of biosecurity will be collected and analysed critical control points aimed at preventing penetration of infectious agents to the farm by people (the entry of foreign persons, hygienic filter, personal hygiene), animals (quarantine, herd turnover, contact with wild animals), health (health status, herd health management), technological systems and equipment (technology of housing, feeding,

watering, waste removal, ventilation), transportation (care of vehicle entry of foreign vehicles) and sanitation (cleaning and disinfection, handling of carcasses).

Evaluating the level of welfare will be focused on the area of housing (animal environment, the possibility of movement, the possibility of rest), nutrition (feeding, water, supplements), health (management, disease prevention, reduction of the risk of injury) and behaviour (possibility of natural manifestations, minimization stress, human - animal relationship).

When determining the critical control points for evaluation of animal health. It will be used from records of production and reproduction performance in monitored farms, including breeding and veterinary evidence (frequency of occurrence of infectious, non-infectious and invasive diseases, including injuries etc.).

Evaluation of the quality of the final products will be based on screening determination of inhibitory substance that lives in meat, raw milk and eggs.

Discussion

Agricultural biosecurity refers to management practices designed to prevent the introduction of pathogens into herds or the spread of pathogens within a herd that could harm the health of the herd. Therapy of disease, in comparison to their prevention is not as efficient or economical [6]. Many diseases in the herd or flock can be prevented by using good animal practices.

In the report of the European Commission [7] is shown the possibility of interrelationship between animal welfare and food safety. It was proved that high welfare standards have both direct and indirect impact on safety and quality food. There is a link between animal welfare and health and public health [8].

Insufficient observance of the principles of biosecurity on farms increases the risk of outbreaks of infectious diseases, including zoonosis and their spread rapidly [9]. Research results indicate that between one third and one half of all human infectious diseases have a zoonotic origin. That is transmitted from animals [10]. The human infections occur by contact with infected animals, but far more common way of infection is by consuming food which are contaminated by pathogenic (disease-causing) microorganisms such as bacteria and their toxins, viruses and parasites is more frequently [10].

Conclusions

Appropriate level of welfare, prevention of diseases and respects for biosecurity standards are a prerequisite for production of healthy and biologically wholesome raw materials and foodstuffs of animal origin as one of important indicators of improved competitiveness and economical use of livestock.

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WELFARE IMPROVEMENT OF LAYING HENS UNDER SEMI-OPEN REARING DURING COLD PERIOD WITH ZN AND VITAMIN C SUPPLEMENTATION

N. Bozakova[1], L. Sotirov[1], K. Nedelkov[1] and M. Simeonov[2]

[1]*Faculty of Veterinary Medicine, Trakia University - Stara Zagora, Bulgaria*
[2] *Institute of foraging crops – Pleven, Bulgaria*

Summary

The purpose of the study was to provide a welfare evaluation of *DeKalb Brown* hens (n=337; 295♀ and 42 ♂) under semi-openrearing system duringthecold winterperiod, afterdietarysupplementationofeither 35 mg/kg zinc (Zn-group)or 35 mg/kg zinc and 250 mg/kgvitamin C (Zn+Vit. C-group).The welfare was scored on the basis of ingestive, gregarious, sexual and agonisticbehaviour, plasma corticosterone and oxidative stress index (OSI).

The welfare score of the control hens during the cold period was 40.00 %, of those supplemented with Zn – 66.67 %, and with Zn + vit. C – 80.00 %.

Keywords: welfare, Zn & vitamin C, behaviour, corticosterone, oxidative stress index.

Introduction

The cold winter temperatures provoke cold stress in hens under semi-open buildings in continental areas and worsen their welfare. Environmental stress provokes excessive formation of free radicals (Halliwell & Gutteridge, 1989). One of approaches to reduce environmental stress in poultry is the dietary supplementation with zinc or vitamin C because following thermal stress, the concentrations of antioxidant minerals and vitamins in the body are reduced (Sahin et al. 2005). Vitamin C is an antioxidant compound with strong antistress and antioxidant effect.

The purpose of the study was to evaluate the welfare of *DeKalb Brown* hens under semi-open rearing system during the cold winter period, after dietary supplementation of either zinc or the combination zinc and vitamin C on the base of hen's behaviour, plasma corticosterone and oxidative stress index.

Material and methods

The experiments were performed with 337 (295♀ and 42 ♂) *DeKalb Brown* laying hens at the age of 38 weeks in 2012 reared under semi-open farming system and divided in 3 groups (n=295, ♀). They were placed in a 21x7 m building, each group in a 7x7 compartment (49 m^2). The first group was untreated (Control). The diet of the second group was supplemented with 100 mg/kg Zinteral 35 (Lohmann animal health, Germany) containing 35 mg/kg zinc oxide (Zn-group). The diet of the third group was supplemented with the same amount of Zinteral 35 and 250 mg/kg vitamin C (L-acidum ascorbicum, China) (Zn + Vit. C-group).

The behaviour was recorded with a 2 video cameras for 12 hours, over 4 consecutive days during the cold period.

Microclimatic conditions were determined by routine methods.

Blood samples for corticosterone determination were collected from 9 female birds on March 20 to assay plasma corticosterone levels (CorticosteroneELISA RE52211,Germany).

The oxidative stress index was determined by method of Armstrong & Browne (1994).

The welfare score was calculated as per Bozakova et al. (2012).

The statistical analysis was performed with the non-parametric Friedman's and Tukey HSD tests

Results and discussion

The average ambient temperature in the hen's living area was 5.67±0.50 ^{o}C, substantially lower that the allowances of 18-25 ^{0}C.

In the Zn-supplemented group there were significantly more feather cleaning, mating and less aggressive hens vs controls, and in Zn + Vit. C- group - more egg-laying, feather cleaning, dust bathing mating (P<0.01); lower number of aggressive hens vs controls, Table 1.

Table 1. Number of *DeKalb Brown* hens, supplemented either with Zn or Zn + vitamin C exhibiting a specific type of behaviour (mean ±SEM, n=295)

N=295	Cold period	Cold period	Cold period
Behaviour	**Control group, %**	**Zn –group, %**	**Zn + Vit.C- group, %**
Feeding	41.68	43.99	42.83
Drinking	7.40	7.40	7.67
Egg-laying	8.44	10.32	13.56**#
Moving	14.87	12.79*	9.71***##
Resting	5.55	5.90	6.55
Feather cleaning	4.31	5.24*	6.4***#
Dust bathing	3.54	3.97	4.74*#
Aggression	5.55	4.55**	3.31***##
Sexual behaviour	5.51	6.32*	6.59**

^^P<0.01; ^^^P#P<0.05; ##P<0.01###P

There were significantly more egg-laying, feather cleaning, dust bathing and less moving and aggressive birds in Zn + Vit. C- group than in the Zn- group, Table 1.

Blood corticosterone and OSI in control hens were higher during the cold than the thermoneutral period (Figure 1, Figure 2). In Zn-supplemented hens, corticosterone and OSI were lower than in controls, and in the Zn.+Vit. C-supplemented hens – even lower (Table 2). The five freedoms were scored and the total poultry welfare score in control hens during the cold period was calculated to be 40.00 %, that of the Zn group – 66.67 %, and Zn + vit. C group – 80.00 %.

The improved welfare of hens supplemented with either Zn or Zn + Vit. C could be attributed to the antioxidant and antistress effect of both supplements**.** Being a co-factor of essential antioxidant enzymes - Cu/Zn superoxide dismutase and inhibiting NADPH-dependent lipid peroxidation (Prasad & Kucuk, 2002), zinc limits the excessive secretion of corticosterone tightly linked to stress and anxiety in birds.

Vitamin C plays a major role in the biosynthesis of corticosterone, a primary glucocorticoid hormone involved in gluconeogenesis to enhance energy supply during stress (Sahin et al. (2002). This way, both supplements act synergically in the reduction of environmental stress-induced high blood corticosterone and contribute to the better welfare of birds.

Conclusions

The welfare score of *DeKalb Brown* hens under semi-open rearing system during the cold period was 40.00 %. In birds supplemented with zinc several behavioural changes were observed, reduced blood corticosterone and oxidative stress index compared to controls, resulting in increased welfare score (66.67%). The co-administration of zinc and vitamin C, increased the welfare score to 80.00% due to the antioxidant and stress-reducing effects of both compounds.

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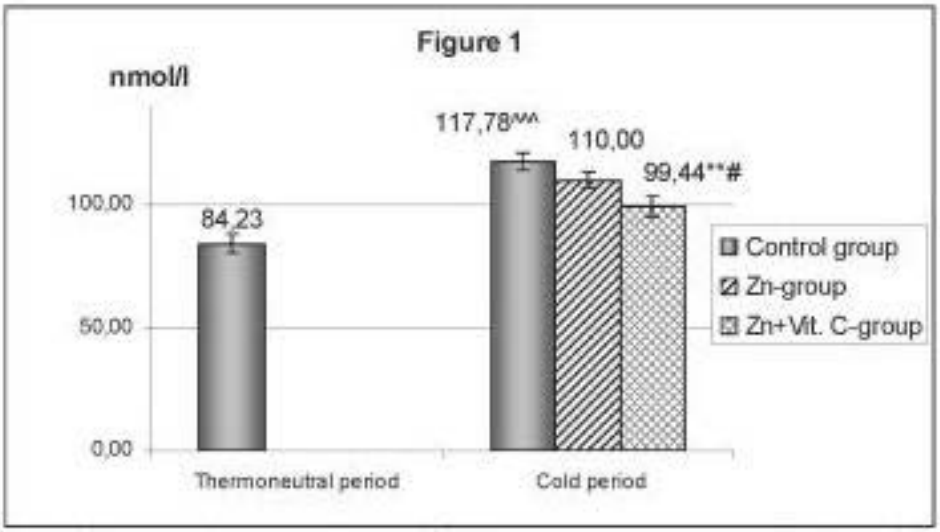

Fig. 1. Blood corticosterone levels in *DeKalb Brown* hens supplemented either with Zn or Zn and vitamin C during the cold period (mean ±SEM, n=9 ♀)^^P<0.01; ^^^P<0.001 : in control group between thermoneutral and cold period; *P<0.05; **P<0.01: between control and experimental groups during the cold period; $^{\#}$P<0.05; $^{\#\#}$P<0.01 $^{\#\#\#}$P<0.001: between Zn- and Zn + vitamin C- supplemented groups during the cold period;

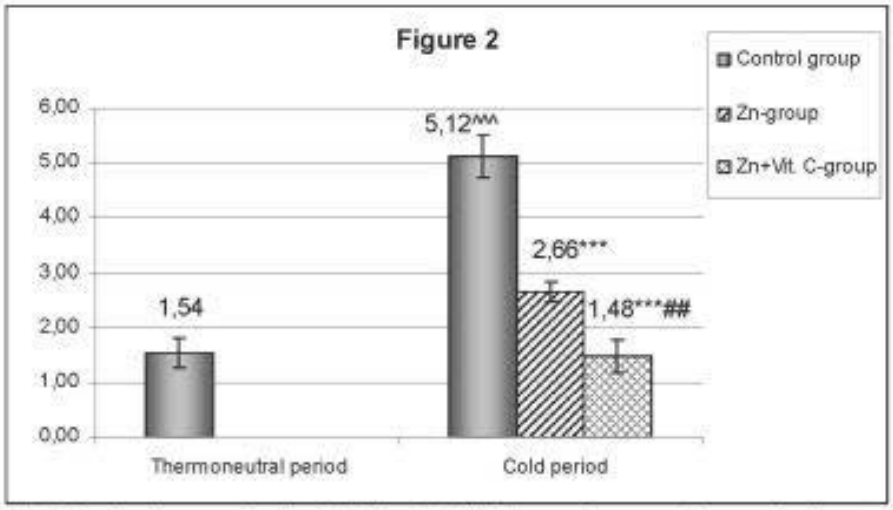

Fig. 2. Oxidative stress index (OSI) in *DeKalb Brown* hens supplemented either with Zn or Zn and vitamin C during the cold period (mean ±SEM, n=9 ♀). ^^P<0.01; ^^^P<0.001 : in control group between thermoneutral and cold period; *P<0.05; **P<0.01: between control and experimental groups during the cold period; $^{\#}$P<0.05; $^{\#\#}$P<0.01 $^{\#\#\#}$P<0.001: between Zn- and Zn + vitamin C- supplemented groups during the cold period;

CALF MANAGEMENT PRACTICES, DISEASES AND ACUTE PHASE RESPONSE

L. Seppä-Lassila[1], M. J. Tenhunen[2], K. Sarjokari[1,3], M. Hovinen[1], M. Norring[1], T. Soveri[1]

[1] *University of Helsinki, Department of Production Animal Medicine, Saarentaus, Finland*
[2] *Ylä-Savon SOTE Joint Municipal Authority, Vieremä, Finland*
[3] *Valio Ltd, Farm Services, Helsinki, Finland*

Summary

Good management practices are crucial in preventing the diseases in calves. We studied the associations between management, calf health and acute phase proteins. Altogether 82 herds were visited and data were collected on 802 randomly selected calves of 0-60 days of age. Calves were examined, and blood samples collected for haptoglobin (Hp) and serum amyloid-A (SAA) analysis, and a questionnaire on calf management was filled in.

The most common signs of disease were diarrhea (13% of calves), cough (11%), increased respiratory rate (10%) and navel-ill (9%). Increased Hp concentrations were associated with diarrhea (p=0.002), increased respiratory rate (p=0.03), farmer's dissatisfaction with the workload (p=0.009), increased number of days the calf spent with the dam (p=0.001) and gradual weaning (p=0.016). Increased SAA concentrations were associated with coughing (p=0.005), gradual weaning (p=0.01), and observed health problems in calves (p=0.02). The number of umbilical diseases on the farm was negatively associated with SAA concentrations (p=0.02).

Hp and SAA showed associations with signs of diseases but also with various management practices suggesting a complex network of immunity, disease and environment.

Introduction

Good management practices are crucial in preventing the diseases in calves. Acute phase proteins (APP) are established markers of inflammation (Horadagoda et al 1999), whose concentrations increase in blood after the tissue injury or infection. APPs are not very specific for inflammation, and increased values have also been associated with decreased growth rate (Orro et al 2006). Their use as general morbidity detectors in slaughter houses has also been tested (Eckersall 2000, van der Berg et al 2007).

The aim of the current study was to evaluate suitability of APPs as an indicator of good management practices.

Material and methods

Herds of >80 cows (n = 184) were selected from Finnish dairy herd recording database. From these 82 volunteering farms with conventional production system, herd staying in the insulated free stall barn at least two years before the study and in automatic milking system farms at least two milking robots with fair stocking capacity, were enrolled. During the farm visits a random sample of calves 0-60 days of age was examined, covering at least 38% of the calves. Altogether 802 calves were included in the study.

Veterinary inspection included examining for increased respiratory rate, changes in respiratory type, presence and type of ocular and nasal discharge, coughing, diarrhea, navel-ill and joint-ill. A blood sample was collected. Also a structured interview on calf management was filled.

Blood samples were analysed for bovine APPs haptoglobin (Hp; haemoglobin-haptoglobin binding assay described by Makimura & Suzuki, 1982, and modified after Alsemgeest et al, 1994) and serum amyloid-A (SAA; ELISA, Phase SAA Assay, Tridelta Ltd., Maynooth, Ireland).

Associations between APPs, signs of disease and management practices were explored by linear mixed models, and farm was included as a random factor. Logarithm transformation was used for Hp and SAA for achieving normal distribution. All the analyses were done using Stata/IC 11.2 (StataCorp, Texas, USA).

Results

Diarrhea was observed in 13%, cough in 11%, increased respiratory rate in 10% of Finnish calves. Navel-ill was discovered in 9% and clear nasal or ocular discharge, purulent nasal or ocular discharge or joint lesion in 2% of calves. In 60% of calves no signs of disease were observed, while 7% of calves had two or more signs of disease.

In the farms mean Hp was 205 mg/l (sd ±83) and SAA 204 mg/l (sd ±33). Calves weaned gradually or spending longer time in calving pen had increased levels of APPs. The detailed results of the regression models are in **Table 1** and **Table 2**.

Discussion

The association of APPs with respiratory tract infections and diarrhea has also been observed before (Pourjafar et al 2011, Tothova et al 2012). However, the negative association with number of observed umbilical disease and SAA concentrations is more difficult to understand; acknowledging umbilical diseases can tell of sharp-eyed farmer and reflect good management, while high number of navel-ill shows different.

The positive association with APPs and farmer's workload or observation of health problems can reflect parts of the same problem: high disease incidence. High incidence rate increases workload, and diseases easier to observe when they are common.

When calves spend longer time with their dams in the calving pen, they are exposed to environmental pathogens and also the conditions are often suboptimal for calves, which might affect the APP concentrations. The bottle fed colostrum protects from diarrhea better than leaving calves to suckle colostrum (Svensson et al 2003), emphazising the importance of colostrum management and need for more studies on hygiene of calving pen.

Increased APP concentrations with gradual weaning are also difficult to interpret. Increased Hp and SAA concentrations are observed after abrupt weaning (Kim et al 2011).

Conclusions

Hp and SAA showed associations with signs of diseases but also with various management practices suggesting complex network of immunity, disease and environment.

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Table 1. Factors associated with haptoglobin concentration in Finnish dairy calves.

Variable	Coeff.	95% Conf. interval	P value	Wald test P value
Diarrhea	0.276	0.103; 0.450	**0.002**	
Increased resp. rate	0.200	0.016; 0.385	**0.033**	
Husbandry workload				**0.01**
Very satisfied	0			
Satisfied	0.260	0.078; 0.441	**0.005**	
Quite alright	0.100	-0.113; 0.313	0.360	
Needs improving	0.303	0.063; 0.543	**0.013**	
Weaning off milk feed				0.086
Abruptly	0			
Gradually	0.130	0.014; 0.246	**0.027**	
Both/mixed	0.035	-0.433; 0.502	0.890	
Calf with dam (days)	0.103	0.041; 0.164	**0.001**	
Age	-0.003	-0.007; 0.0001	0.057	
Gender	-0.054	-.0183; 0.075	0.410	

Table 2. Factors associated with serum amyloid A concentration in Finnish dairy calves.

Variable	Coeff.	95% Conf. interval	P value	Wald test P value
Cough	0.307	0.093; 0.522	**0.005**	
Weaning off milk feed				**0.007**
Abruptly	0			
Gradually	0.201	0.048; 0.355	**0.010**	
Both/mixed	-0.401	-0.961; 0.159	0.160	
No of umb. infections*	-0.140	-0.259; -0.021	**0.021**	
Health problems observed	0.185	0.027; 0.343	**0.022**	
Age	-0.024	-0.028; -0.020	**<0.001**	
Gender	0.053	-0.089; 0.196	0.460	

*Number of umbilical infections in last two months; 0-3 cases per farm.

COMMON HEALTH PROBLEMS AND TRADITIONAL VETERINARY PRACTICES OF SMALL SCALE CATTLE FARMING SYSTEMS IN SRI LANKA

P.H.G.J. De Silva[1] and D.C.A. Kalubowila[2]

[1]Department of Animal Science, Faculty of Agriculture, Aquinas University College, Colombo 08, Sri Lanka; [2] ADM Food Products Pvt. Ltd, Badalgama, Sri Lanka

Summary: A survey was conducted to identify common health problems in cattle and to study the various traditional veterinary practices (TVP) used by cattle farmers in Sri Lanka. Diarrhoea was the most prevalent disease in calves below 1 year of age. Mastitis was the most prevalent disease in cows aged more than 4 years according to 67 % of farmers. Forty two percent of famers only practiced indigenous methods for health problems of cattle. The medicinal preparations consist of herbs, latex, oil extracts, spices, metals, minerals, animal products, spider web, anthill mud and human urine. TVP were widely used as treatments for internal and external parasites, bloat, diarrhoea, anorexia, mastitis, foot and mouth disease, agalactia, retained placenta, infertility, ulcers, wounds and snake bites.

Keywords: Cattle disease, Traditional Veterinary Practices, Herbs

Introduction

Sri Lanka has a well-developed system of traditional veterinary medicine to sustain local livestock production over many centuries to date and same remedies are presently used extensively and effectively. Native veterinary physicians believe that diseases are caused by an imbalance of body humours: vaa (wind), pitha (bile) and sem (phlegm). According to Wasantha, (1994) and Perera et al (2006), there are 4,448 varieties of Weppu (Lethargy) Adappan (Nasal discharge and tremors) diseases, 120 of general diseases, 300 diseases of hepato billariary system, 8 diseases along the spine, 300 diseases of the intestines, 13 diseases of the joints of the legs, 1,448 diseases of the head and four extremities that may occur in cattle. Although this classification of diseases is huge in number, the Sri Lankan traditional veterinary physicians have the knowledge to identify and treat them by using various methods.

The existing conventional disease control programs favor the intensive systems of production with livestock in confinement and not small-scale farmers. Due to high cost of conventional medicines coupled with the lack of knowledge on their use, these drugs are usually out of reach of the small-scale farmers.

Therefore study was conducted to examine the indigenous medicinal practices for the health problems of cattle in small scale farming systems.

Material and methods

The list of small scale cattle farmers registered in veterinary offices was randomly used to collect information; which is a blend of rural and urban livestock farmers. Pre-tested questionnaire was used to collect the data on the use of TVP for cattle diseases from 280 cattle farmers in Western, Southern, Central and North central provinces. Data were analyzed by the Minitab 14.0.

Results and Discussion

Majority of the farmers (72 %) reported Diarrhoea is the most prevalent disease in the calves below one year of age while 56 % of farmers experienced pneumonia in calves at this age. 62 % of the farmers reported that young animals with 1-2 years of age were highly susceptible to pneumonia and 51 % of the farmers reported that calves at this stage were highly subjected to parasitic infections.72 % of farmers experienced bloat in 2-4 years old animals while 5 % of farmers reported brucellosis at this stage. Mastitis was reported as the most prevalent disease (67%) in cow's aged more than 4 years. 10 % farmers' experienced milk fever disease in cows of the same age range.

Results revealed that 42 % of the surveyed farmers use TVP and they do not rely on orthodox veterinary medicinal treatment. About 10 % of farmers combined both traditional and orthodox veterinary services as source of cattle disease treatment. It was observed that different indigenous methods such as medicinal preparations, inhalation, burning of vital points (moxibustion) and praying for gods were used to treat cattle diseases. Some of the medicinal preparations by cattle farmers (87%) used in treatment of cattle are given in Table 1. The commonest route for drug administration in cattle is through the mouth (90%). Other routes of drug administration include nose, ear, and anus.

Some (15%) used charms to treat diseases and they incant and recite charms as a preventive measure during epidemics. Further they (21%) practiced branding of different symbols on the different body parts of the animal to treat diseases associated with reproduction and lactation of cattle (Table 2). TVP were widely used by cattle farmers (10%) to treat internal parasites while 38% of farmers to control external parasites. Plant preparations were used by 45% of the farmers when appetite is reduced in cattle. 26% of farmers used TVP for snake bites and identified practices were feeding of a mixture of extract of *Leucas zeylanica* leaves and *Citrus aurantifolia*. *Carica papaya* latex was applied on the site of bite for insect bites. Plant leaves of *Adathoda vasica*, *Vitex negundo*, *Clerodendrum infortunatum* after boiling were used by 45% of farmers to foment the udder after parturition. 69% of farmers used mixture of grinded 100 g of Azadirachta indica, 25g of Allium sativum and mixed with salt water till the final volume is 125 mL and give orally before parturition to facilitate the parturition. Reductions in milk yield was treated by 74% of farmers with hanging broken black color bangles in neck or massage the udder with boiled mixture of *Ricinus communis*, *V. negundo*, *Psidium guajava* and *A. vasica* leaves. Ulcers were treated by using TVP by 15% of the farmers.

Conclusions Since these methods and practices have been successfully carried out for centuries in Sri Lanka in cost effective and sustainable way, it is important to identify the effective components of these traditional veterinary medicines and practices. If they are found to be effective, more widespread use of these methods would considerably reduce the cost of treatments and to identify more sustainable methods in cattle production.

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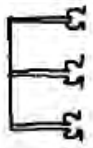
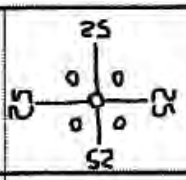
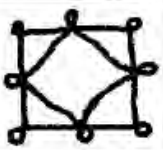
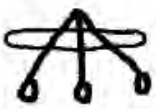
Table 1. Preparations used for different symptoms and diseases of cattle by Sri Lankan Veterinary Physicians

Disease /symptoms	Preparation and administration
Foot and mouth disease	Animals immersed in mud ponds mixed with medicinal plants for several days or apply mixture of grinded *Curcuma longa and A. indica* oil
Anorexia	Residues of *Artocarpus heterophyllus*, paddy straw treated with salt or *Oryza sativa* with straw, mixture of coconut poonac, water and salt with *A. heterophyllus* leaves or rice brand mix with rice washed water and *Allium sativum*, *Carum bulbocastamum*, *Foeniculum vulgare Gaertn* mix with Coconut water are used to feed the animal
Stomachache	200g of fresh entire plant of *Alteranthera sessilis* are cut into small pieces and ground with 240ml of whey. 25ml of common salt water is added to the above mixture and stirred well. More whey is again added to the above mixture till the final volume becomes 375ml. Dosage: 375ml, twice a day, orally.
Bloat	100g of leaves of *Aloe vera* are taken, thorns and outer skin are removed. Then, inner fleshy part of the leaves are squeezed and juice is extracted.120ml of aforesaid juice is ground with 5g of latex of *Ferula asafoetide*. This Paste is dissolved in hot water. More hot water is added till the final volume is 375ml. Dosage: 375ml, twice a day, orally.
Constipation	100g of each carpal of *Allium sativum* and rhizome of *Acorus calamus* are ground with 240ml juice of fresh bark of *Cratewa adansonii*. 25ml of castor oil or 25ml of vinegar is added in to the above thick juice. This mixture is given orally twice a day.
	50 g each of *F. asafoetide* and of *A. sativum* latex are ground well with fresh juice of *A. vera*. Fresh juice of *A. vera* is added in to the above thick juice till the final volume is 375ml. Dosage: 375ml, twice a day, orally.
Diarrhoea	Grind equal parts of *Jatropha glandulifora, Sida codifolia, Treblus asper* and squeeze out the juice. Mix with a cup of sesame oil, citrus juice, juice of *Zingiber officinale and drench.*
Ulcers	Application of mixture of Grinded *A. indica* oil, *Acalypha indica* leaves and *L.zeylanica* leaf, *C. longa*, lime mixture, clay of Ants' house and salt water paste
Internal parasites	*Brassica juncea* and *Azadirachta indica* oil charming, oral administration of extract of *Allium ascalonicum* and *Centrella asiatica* using hot water, firing of charmed *B. juncea* and feeding the mixture of *Acorus calamus and Curcuma longa*
External parasites	Spraying of *A. indica* oil with salt or application of the mixture of *Citrus reticulata* and *Cocos nucifer* oil or application of kerosene and *C. nucifer* oil mixture or external application of grinded *A. calamus*
Deworming	Grind equal amounts young leaves of *Citrus aurantifolia C. sinensis, Atalantia ceylanica, A. indica, Vitex negundo, Caesalipinia bonduc, Gyrinops walla, Cannabis sativa, Monordica charantia*: Mix with a small amount of copper sulphate, make a bolus and administer
Infertility	Heated iron nail added to 175 mL of *Azadirachta indica* oil, Orally give to heat sign shown cows, 30 minutes before mating
Retained placenta	100 g of *Vitex negundo immature leaves mixed with oil* and ground, Add 25 mL of vinegar
Mastitis	100 g of immature leaves of *Tamarindus indica, 100 g of Garcinia gummi, 50 g of Brassica nigra, 50 g* of *Curcuma longa are ground and cooked,* Then make a paste by adding 250 g of wheat flour, Apply the paste on udder, teats and allow it to be there

Table 2: Places of branding for diseases associated with reproduction and lactation of cattle

Infertility of cows					Retained placenta
					
In between the vertebral column & tuber coccygeal area	In between the lumbar & coccygeal area	In between the lumbar & coccygeal area	Rare legs	Front legs	Girth

MILK YIELD IN TUNISIAN HOLSTEIN COWS

Y.Ressaissi [1], M.Ben Hamouda [2]

[1] *Departement of Animal Sciences, National Agronomic Institute of Tunisia*
[2] *National Institute of Agronomic Research of Tunisia*

Summary: Dairy cattle represent an essential component of the Tunisian agriculture system by affording fundamental strategic products. Therefore, it is a primordial sector that should be developed continuously in order to ensure a sustainable development and maintain of the national productivity level. In this context the objectives of this study were to determine the level of milk yield in the Tunisian Holstein herds and to quantify the environment contribution in the daily milk production performances. Analysis have focused on 151 275 test-day records between 2006 and 2011 of 18 144 Holstein cows in their first, second and third parity, calved between 2006 and 2011 and belonging to 116 herds which were divided into 8 groups according to their sizes. Daily average milk yield was calculated and the environment contribution was estimated per herd size group. The average daily milk yield of the whole cows was about 20.19 kg (±8.33 kg) and it has varied from 15.22 kg (±7.97 kg) to 26.21 kg (±8.46 kg) between herd size categories where only 0.07% of cows have reached 50 kg/day. The variance analysis of milk yield has showed a specific effect of every fixed factor within each herd category due to the existential variability in climatic conditions and herd management circumstances for the different production systems encountered in Tunisia. According to this trend, milk yield has varied differently and the productivity level has proved low mainly in small herds. Environmental conditions in Tunisia are characterized by an increased instability revealing hard climatic conditions and a lack in herd management which disadvantage the appropriate expression of the genetic potential of cows, predominantly among small herds where the impact was more pronounced.

Introduction: Livestock is a primordial sector in Tunisia that covers 32% of the national agricultural production, whose 73% are insured by dairy cattle. Holstein cows have greatly take advantage of all agricultural adjustment programs that the self-sufficiency in milk, scheduled for 2011, was achieved much before in 1999-2000 (Elloumi et Essamet, 1997). Dairy performances result from the interaction between genotype and the permanent environment and the Tunisian environment is characterized by considerable physical and climatic variations and most breeders are small farmers with inadequate experience which have emerged diversity in herd management and fluctuation in milk yield (Hammami et *al.*, 2009). The aim of this paper was to quantify the recent milk yield level in Tunisian Holstein herds and to highlight the contribution of the fixed factors.

Materiel and methods: The data used are those of the official milk recording conducted by the Agency of Livestock and Pasture (O.E.P). After edition, the file included 151 275 test-day data recorded on 18 144 Holstein cows in their first, second and third parity, calved between 2006 and 2011 and belonging to 116 herds. Days in milk ranged between the 25th and the 365th day of lactation and were classified into 34 intervals of 10 days. Herd sizes (HS) have varied between 50 and 1712 cows and were classified into eight balanced groups (HS=50, 50 < HS < 100; 100 ≤ HS < 150; 150 ≤ HS < 220; 220 ≤ HS < 300; 300 ≤ HS < 400; 400 ≤ HS ≤ 600; HS > 600). Based on the herd size category, average daily milk yield was calculated and the effect of the non-genetic factors was performed according to this fixed linear model:

$$Y_{ijklmno} = \mu + P_i + CM_j + CY_k + DIM_l + MF_m + H_n + e_{ijklmno}$$

Where $Y_{ijklmno}$ is the observed daily yield of the cow in parity i (i=1, 2, 3), controlled in month j (j=1, ..., 12) during the year k (k = 2006,..., 2011), for days in milk l (l = 1,..., 34), milked with m frequency (m = 1, 2, 3), in the herd of n size (n= 47, 21,17,11,10, 5, 3 and 2 respectively). μ = overall mean; P = the parity effect; CY = the control year effect; CM = the control month effect; MF = the milking frequency effect; DIM = days in milk effect and e = the residual errors. The least squares estimation was carried by the General Linear Model procedure (GLM) of SAS program (Statistic Analysis System, 2003).

Results and discussion: The average daily yield is about 20.19 kg (± 8.33 kg/day) and varies from 15.22 kg (±7.97kg/day) to 26.21 kg (±8.33kg) between herd categories demonstrating that the yield may be more intense in a small herd rather than in a medium herd. Most of cows produce between 10 kg and 30 kg and only 0.07% has reached 50 kg. According to CIWF (2012), Holstein cows produce about 28 kg to 30 kg per day, thus the daily milk yield in Tunisia is considered low. The variance analyses has shown that daily milk yield is specifically explained in

every herd size category. The highest coefficient of determination corresponds to small herds (<50 cows) and the lowest coefficients are relative to large herds (from 400 to 600 cows).This explains a different environmental sensitivity and indicates a more pronounced action of the environment factors in small herds. The herd is an important factor in Tunisian dairy cows where milk production intensity relies on geographical affiliation, annual climate change, breeding systems and all the associated constraints as Hammami et *al.* (2009) have reported. Darej et *al.* (2010) and Bousselmi et *al.* (2010) have also explained that herd performances in Tunisia are closely related to the specific factors within each herd which determine the adaptation level and therefore the capacity of cows in the expression of its genetic productive potential. This shows an insufficient level of management in Tunisia and as result, the productive potential of the cow is directly affected generating dramatic decreases in milk yield.

Conclusion: Dairy performance depends upon genetic background and environmental factors. This study has demonstrated a significant interaction between the environment and genotypes in Tunisia showing that every genotype responds differently to a given environment. Therefore the poor milk yield level results from a lack in breeding and management conditions then the insufficient milk yield is alarming and breeding of Holstein requires better understanding of the environmental sensitivity and optimizing the husbandry in order to enhance productivity.

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IMPACT OF FIRST PARITY LITTER SIZE ON SOW STAYABILITY AND LIFETIME PRODUCTION

E. Andersson§*, J. Frössling*¤, B. Algers*, L. Engblom#, S. Gunnarsson*

**Department of Animal Environment and Health, Swedish University of Agricultural Sciences, PO-Box 234, SE-532 23 Skara, Sweden; ¤ Department of Disease Control and Epidemiology, National Veterinary Institute, SE-75189 Uppsala, Sweden; #Uppsala Clinical Research Center, Uppsala Science Park, Scheele, SE-752 37 Uppsala, Sweden; §Corresponding author: emma.andersson@slu.se, Phone: +46 511 67255*

Summary

To increase production level increase of number of piglets born per litter has been one of the main breeding goals during the last decades. The negative welfare consequences on piglets being born in a large litter are well known while the welfare impacts on sows that produce large litters are more uncertain. The aim of this study was to investigate the effect of first parity litter size on sow stayability and lifetime production.

Sows having a small (<12 piglets total born; SL) or medium (12-14 piglets total born; ML) first parity litter size had significantly ($p < 0.001$) more litters before end of production than sows having a large (>14 piglets total born; LL) first parity litter size. Sows with LL was significant ($p<0.001$) less likely to have a second litter than sows with SL and ML. Sows with SL and ML had the highest lifetime production of weaned piglets.

The results of this study indicate that it is important take first parity litter size into account when assessing sow welfare and profitability in commercial piglet producing herds. Having a SL or ML was found to have a positive effect on sow stayability and lifetime production.

Introduction

During the last decades, one of the main breeding goals in commercial piglet production has been to improve production efficiency by increasing the number of piglets born in each litter. However, this development also has potential negative consequences on animal welfare and production of piglets and sows.

The possible welfare impacts of large litter size on piglets and sows have been reviewed by Rutherford et al. (2013), and they includes an increase in number of piglets that are born dead, a decrease of piglet survival until weaning and larger variation in body weight of the piglets that survives. The short and long terms effects of large litter size on the health and welfare of the sow are more uncertain and have not been studied to the same extent.

The aim of this study is to investigate the effect of first parity litter size on sow stayability and lifetime production.

Material and methods

This study was performed as a retrospective study using data from a sow database established at the Swedish University of Agricultural Sciences. From this, only sows that had produced at least one litter and were registered dead sometime between 2003-2012 were selected. Sows also had to be, in various combinations, crossbreed of Landrace x Yorkshire. To be kept in the dataset herds had to contribute with ≥ 1% of the observations and having mainly crossbreed sows. The final dataset included a study population of 31 311 sows in 22 herds.

The exposure of interest was the size of the sows' first parity litter, i.e. the total number of piglets born. This was divided into three groups; small (<12 piglets; SL), medium (12-14 piglets; ML) or large litter size (> 14 piglets; LL).

To evaluate sow stayability the probability of having a second litter and the number of litters produced at time of death (PL), were analysed. Lifetime production of born piglets (LPBT), lifetime production of piglets born alive (LPBA), lifetime production of weaned piglets (LPW), percentage piglets born dead (PPBD) and percentage piglets dead between birth and weaning (PPDW) were analysed.

The unit of interest was sow and litter size was treated as independent variable. Effect of litter size was tested on one dependent variable at a time. Probability of having a second litter had dichotomous outcome and was analysed using cross tabulation (Chi-square test). All other dependent variables were ordinal and had non-normal distribution and therefore non-parametric tests were used (Wilcoxon rank sum test). The statistical software used was Stata (release 12, StataCorp LP, College Station, TX).

Results

Sows having a SL or ML had a significant ($p < 0.001$) higher median PL than sows having a LL. Sows with a LL had a significant higher median LPBT than sows with ML or SL ($p<0.05$ and $p<0.001$ respectively). Sows having a LL or ML had a significant higher ($p<0.001$ respectively) LPBA than sows with SL whereas sows with SL or ML had a significant higher ($p<0.001$ respectively) LPW than sows with LL (Fig. 1).

Sows having a SL had a median PPBD of 4.3% and a median PPDW of 6.3 %, whereas ML sows had 5.0% and 15.1%, respectively, and LL sows 6.3% and 22.6%, respectively. Thus, LL sows had significantly higher PPBD ($p < 0.001$) and PPDW ($p < 0.001$) than SL and ML sows. ML sows had significantly higher PPBD ($p < 0.001$) and PPDW ($p < 0.001$) than SL sows.

Sows having a LL was significant ($p<0,001$) less likely to have a second litter (OR=0.84; CI 0.78 to 0.90) than sows having SL or ML (OR=1.00 and OR=1.00; CI 0.94 to 1.07, respectively).

Discussion

Sow stayability and lifetime production are important factors in commercial piglet producing herds. Profitability is based on sows' abilities to stay long enough in herd to pay off their own rearing costs and their abilities of deliver weaned piglets. Sow stayability and ability to have a high lifetime production of weaned piglets would then be one of the most interesting results from this study.

It may seem logical that the more piglets a sow give birth to, the more piglets she will wean, and herd profitability will increase. However, our results indicate that the lifetime production of weaned piglets is higher for sows with small or medium sized first litters, compared to sows with large litters. This mainly due to the results that these sows had a higher number of litters produced at time of death and a higher probability of having a second litter.

Sow welfare and production may then be improved by a management strategy of keeping sows with a small or medium sized first parity litter, when planning for removal in herd.

However, the results of percentage piglets dead between birth and weaning indicate that lifetime production of weaned piglets are influenced by management factors such as cross fostering. Further evaluation of litter size effects on lifetime production of weaned piglets, where this type of influence can be controlled, is needed.

Conclusions

Having a small (<12 piglets) or medium (12-14 piglets) litter size in first parity was found to have a positive effect on sow stayability and lifetime production. The results of this study indicate that it is important take first parity litter size into account when assessing sow welfare and profitability in commercial piglet producing herds. Further evaluation of the effects of litter size on health and welfare of sows is needed.

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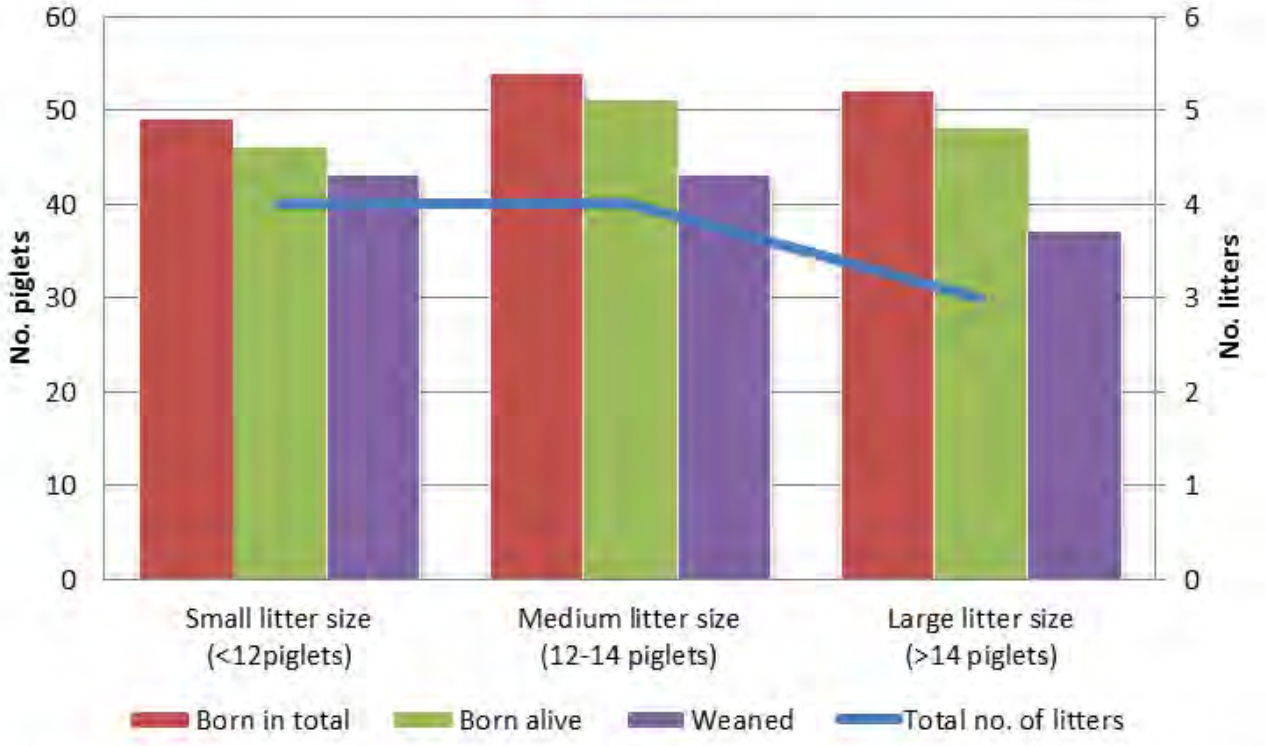

Fig. 1: Lifetime production and removal parity by litter size in first parity.

MANAGEMENT AND PERFORMANCE OF CHICKS HATCHED ON FARM

N. Falke, C. Habig, J.Schulz, J.Hartung, N. Kemper

Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour, University of Veterinary Medicine Hannover (Foundation), Hannover, Germany

Summary

An alternative husbandry system for chickens combines incubation, hatching and growing period at one location. Advantages in comparison to conventional hatcheries are the reduction of cross-contamination by using only one breeder parent flock, no transport stress for hatchlings and a prolonged hatch window, as well as early feed and water intake with possible impacts on intestinal growth by minimal yolk sack use. Within one year, a total of 21 consecutive trials were investigated. Per passage, 126,000 eggs were incubated in two microclimate hatchers for 17.5 days. A total of 2,649,600 eggs of 3 different parent flocks (A-C) were taken into account. Average hatchability was 85.94%. Data varied between 77.17% (flock A) and 92.36% (flock C). Although best hatchability results were shown in breeder flock C, also highest flock mortality was investigated (5.55%). Overall, a mean value of 1st week mortality of 1.12% (0.71% - 1.46%) and a flock mortality of 4.53% (3.48% - 5.55%) were obtained. A positive correlation between 1st week mortality and flock mortality was shown at flock C (r = 0.85732/p = 0.0291).

Introduction

Several economic and commercial issues influence the management of broiler houses today. A possible disadvantage of conventional systems is the necessary transport of one day old chicks to the stables. Hatchlings have to deal with a deprivation in food and water access up to 60 hours by using their yolk sac reserves. But also inadequate air conditioning during transport may adversely affect the health status of young chicks. Transport losses and injuries in chicks with subsequent death are not uncommon. An alternative housing system might improve the start of the short chick's life by transporting only breeding eggs instead of day old chicks.

Materials and Methods

The data collection started with operating of the combined housing system in April 2013 and ended one year later in April 2014. A total of 21 consecutive trials using genetic Ross 308 were investigated. Per passage 120,000 to 132,300 eggs from one breeder flock were incubated in two microclimate hatchers for 17.5 days. After being candled, eggs changed the location to the 36.5 degree pre-warmed stable for hatching on the X-Track system (Vencomatic, Veenendaal, The Netherlands). Although eggs were light screened, infertile eggs were taken into account for the calculation of hatchability. Overall 2,649,600 eggs of 3 different parent flocks (A-C) were included in the analysis. With end of the hatching period performance traits, including mortality, feed and water intake as well as the mean daily weight gain were recorded continuously. The average daily live weight was automatically recorded by two digital weighing scales (DWS-4-ZW, Hotraco Agri, Hegelsom, The Netherlands) that were installed within the stables. Furthermore during 3 hatch period's air temperature and relative humidity data were captured continually every 10 min by a datalogger type Agent (Rotronic Ltd., Ettlingen, Switzerland). Statistical analyses were performed using SAS, Enterprise Guide 6.1 (Statistical Analysis System Institute, Cary, North Carolina, USA).

Results

The mean hatchability of all 21 trials was 85.94 %. Data varied between 77.17 % (flock A) and 92.36 % (flock C). Average rate of hatch for flock A was 84.13% (77.17% - 90.81%), for flock B 82.42% (77.87% - 88.89%) and for flock C 91.02% (89.55% - 92.36%). Although best hatchability results were shown in breeder flock C, also highest mortality 4.53% (3.48% - 5.55%) was investigated in this flock, but also the lowest averaged 1st week mortality of 0.89% (0,71% – 0,99%). The mean of 1st week mortality of all trials was 1.12% and ranged from 0.71% to 1.46%. The highest 1st week mortality was obtained for the hatchlings of flock A (1.46%), but also lower data (0.77%) were recorded, so overall mean value balanced at 1.18%. Mortality rate of flock A behaved equally. The overall mean value was determined at 4.45% (3.48% - 5.45%). In flock B, the 1st week mortality was 1.29% (0.93% - 1.4%) and the flock mortality 4.41% (4.15% - 4.79%). The strength of the relationship between 1st week mortality and mortality, while controlling the effect of the hatchability, was analyzed with a partial correlation measurement. A linear connection

was detected for flock C (r = 0.85732), (p = 0.0291). In flock B mortalities were also positively correlated, but not significant (r = 0.86783), (p = 0.0565). For flock A no correlation was determined (r = 0.57975), (p = 0.1725).

Discussion

Van de Ven et al. (2009) measured for a comparable Patio concept a hatchability of 96.49%, excluded infertile eggs. Yassin et al. (2009) showed in a 2004-2006 ongoing field study involving 3 different hatcheries, an average hatchability of 87%. Bergoug et al. (2013) considered also the age of the breeder parents: young breeders (27 week) achieved a hatchability of 88%: at 40 to 42 weeks of age breeders reached the highest values of 96% before decreasing to 73% for older breeders (59-61 weeks). In contrast, a relatively low value was described by Heier et al. (2001) for the conventional breeding methods with 75.9%. It has to be taken into account that since 1998 the breeding process has been evolved rapidly in recent years. It also should be noted that the higher rate of hatch in combined housing systems is affected by the lack of final inspection regarding second grade chicks.

Conclusions

The results of broilers hatched on farm are comparable to conventionally produced chicken. Similar to Bergoug et al (2013) highest hatchability of 91.02% (89.55% - 92.36%) was achieved in the younger breeders flock C (36-48 weeks breeder's age) and the lowest result of 82.42% (77.87% - 88.89%) in the older breeders flock B (52-55 weeks breeder's age). For the middle age breeders flock A (40-52 weeks breeder's age) hatchability of 84.13% (77.17% - 90.81%) was reached. The minimum value of 77.17% at flock A was possibly influenced by a transport damage of breeding eggs at the beginning of operating. The system provides following advantages: a longer hatch window, early feed and water access, no transport stress for hatchlings and possibly early gut development (Geyra et al., 2001) by marginal yolk sac use.

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MODELLING HYGIENE CONDITIONS IN AUSTRALIAN PIGGERY BUILDINGS

Thomas Banhazi

National Centre for Engineering in Agriculture, University of Southern Queensland, West St Toowoomba, QLD Australia; banhazi.thomas@usq.edu.au

The main aim of the research presented in this article was to model the effects of important housing and management factors on the hygiene level of pig pens and thus understand how top control and potentially improve pen hygiene in commercial piggery buildings. This project aim was achieved by modelling the hygiene levels assessed in a large number of commercial piggery buildings. Hygiene levels were visually assessed in 160 piggery buildings using a standardised 3-step scale system. This assessment system was based on previous publications and was further developed taking into account practical management considerations. Engineering and management characteristics of the piggery buildings were recorded at the time of sampling and these building characteristics were used in the subsequent multi-factorial statistical analysis. The mean faecal contamination of pen floors in all study buildings was 36%. According to the model developed, hygiene levels were affected by the size of the farm (as described by the number of sows), seasons, stocking rate per pen (kg weight/m^2) and management of piggery buildings. Summer conditions and continuous flow pig management resulted in reduced hygiene levels in pig pens. Piggery size positively, whereas stocking rate negatively associated with piggery hygiene. The results highlighted potential strategies that can be used to reduce the negative effects of sub-optimal piggery hygiene on pig production, environment, health and welfare of animals as well as piggery staff. In addition, the factors identified indicated the inherent difficulties associated with managing and controlling dunging patterns on commercial pig farms.

Keywords: manure, dunging, pigs, hygiene, management, season, cleanliness

INTRODUCTION

Since the introduction of partially slatted floors in piggery buildings, the excretory behaviour of pigs has become a crucial factor in the successful management of pig housing systems (Aarnink *et al.*, 1996; Hacker *et al.*, 1994). The pig's excretory activity can affect the pig's and pen cleanliness with obvious consequences for pig health, worker safety and farm productivity (Whatson, 1978). Incorrect dunging patterns in partly slatted pens may lead to performance problems and almost certainly lead to management and labour problems. Previous studies demonstrated a very strong association between pen hygiene (the percentage of solid floor covered by dung) and air quality (Banhazi *et al.*, 2010; Banhazi *et al.*, 2008b; Takai *et al.*, 1998). Unfortunately, very little information is available on the factors affecting the excretory behaviour of pigs as in practice many factors could affect the development of dunging patterns in pig pens (Olsen *et al.*, 2001; Randall, 1980). It is generally accepted that several stimuli act together to produce the pigs dunging pattern in pens (Wechsler and Bachmann, 1998). However, it is not known what factors will influence dunging pattern in Australia under commercial conditions. Therefore, a study was designed to identify the statistically significant factors affecting pen soiling in Australian piggery buildings.

MATERIALS AND METHODS

Details of the design of the study, techniques used for environmental data collection and analysis have been given previously (Banhazi *et al.*, 2008a; Banhazi *et al.*, 2008b). A total of 160 piggery buildings were included in a study, and housing and management information relevant to individual buildings were documented in detail. Environmental information, including temperature and humidity readings were recorded in all buildings using Tinytalk temperature and humidity data loggers (Tinytalk-2, Hasting Dataloggers, Australia) over a 60h period. The dunging pattern in the study buildings were assessed at the time of data collection by classifying the pen cleanliness into three distinct classes, as were done in previous studies (Aarnink *et al.*, 1997; Aarnink *et al.*, 1996). Pen hygiene was deemed to be 'good' if less than 10% of pen floor was contaminated by faecal material (average area covered by dung = 5%). If between 10 and 50% of the pen floor was contaminated with faecal material, then the hygiene level was deemed to be 'fair' (average area covered by dung = 25%). More than 50% floor contamination resulted in the pen classified as having 'bad' pen hygiene (average area covered by dung = 75%). The data collected was forwarded to South Australia for storage and analysis. To facilitate meaningful data analysis, the classification grades were later turned into percentages, as described above. The dependent variable of interest for this study was the extent of floor contamination (%) with manure. The data was analysed using the forward selection procedure in General Linear Model (GLM

PROC) (SAS, 1989). The results presented here are based on the least squares means (± confidence intervals) of fixed effects. As the hygiene standards of pig pens are influenced by many factors, the model was developed at the 90% confidence level to ensure that all important effects likely to influence dunging patterns will be identified.

RESULTS AND DISCUSSION

Table 1 summarizes the basic statistical measures of the raw data collected in the study buildings. The significance of each effect associated with pen hygiene is summarized in table 2. Significant results are shown in figures 1 and 2 and in table 3. The study identified the key factors affecting hygiene levels inside pig building as (1) farm size, (2) season, (3) management, and (4) stocking rate.

Table 1. Level of floor contamination (%) across all study buildings

Parameter	Mean	SD	Range	No. of buildings
Contamination of pen floor by faecal material (%)	36	27	70	112

Table 2. Significance of effects associated with hygiene level in the model developed at the 90% confidence levels

	Probability of the individual effects
Number of sows (farm size)	0.002
Management	0.006
Stocking rate per pen (kg weight/m^2)	0.059
Season	0.086

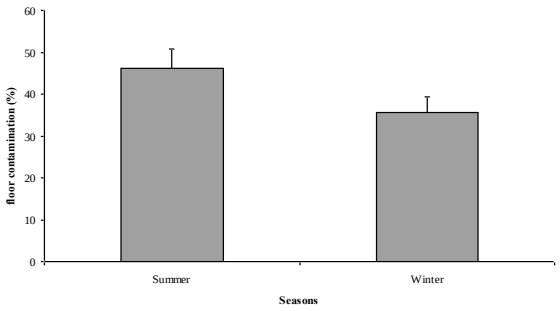

Figure 1: Effect of season on hygiene level (%) in Australian piggery buildings (LS means with SE, p<0.05)

Significantly higher percentage of floor contamination was observed in summer (46%) in piggery buildings than in winter (36%) (Figure 1). In piggery buildings, winter temperatures are lower than in summer, thus pigs tend to use the concreted areas appropriately for resting and the slatted areas for defecating. However, in summer when temperatures are high, pigs are forced to rest on the slatted area in order to keep themselves cool, thus making the slatted area unavailable for defecating. Studies by (Aarnink *et al.*, 2000; Aarnink *et al.*, 2001) have also shown that the fouling of the solid pen area increases with increases in the ambient temperature. A clear "Inflection Temperature" (IT), the temperature at which pen fouling increases, was found for a range of pig weights. This temperature ranged from 25°C for 25 kg pigs to 20°C for 100 kg pigs. Therefore, the main aim of managing dunging patterns in summer should be to discourage pigs to rest on the slatted areas.

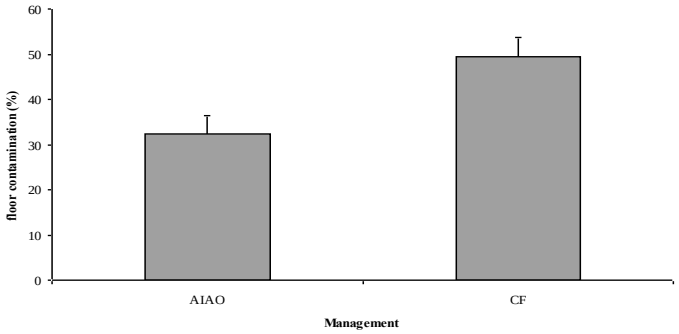

Figure 2: Effect of pig management (all-in/all-out, AIAO vs. continuous flow, CF) on floor contamination (%) in Australian piggery buildings (LS means with SE, p<0.05)

Higher level of pen floor contamination was observed in continuous flow (CF) buildings (49%) when compared to building (32%) managed on the all-in/all-out (AIAO) basis (fig. 2). It is important to consider the management of the buildings, when assessing dunging patterns, as building management will directly influence both the thermal and social environment of pens. In addition, the beneficial effects of regular cleaning between batches will have a direct impact on pen cleanliness in AIAO buildings.

Table 3. The effects of covariates on the level of pen floor contamination (%)

Parameter	Covariate	Slope
Pen hygiene	Number of sows (farm size)	Positive
Pen hygiene	Stocking rate (kg pig/m^2)	Negative

Sow numbers, which was an indicator of farm size, was positively correlated with hygiene levels in the study buildings (table 3). As expected, on larger farms the floor contamination level tends to increase. It has been hypothesized that, on larger farms, because of work pressures, less time is available for cleaning and general maintenance of the pigs' environment. The increased intervals between cleaning episodes create an ideal environment for reduced building hygiene.

Unexpectedly, stocking rate was negatively correlated with hygiene level in grower, finisher and weaner buildings (Table 3). However, further analysis demonstrated that this overall effect was heavily influenced by the close relationship between improved hygiene and increasing stocking rate in weaner buildings (data not shown). In grower/finisher building the relationship was positive indicating that increasing stocking rates will result in greater level of floor contamination. The explanation for these results is not easy, but it could be hypothesised that in weaner buildings the higher stocking rates will result in better self-cleaning of the fully slatted floors, which are typically used in weaner buildings. One of the main benefits of using fully slatted pigpens is to be able to separate the pigs from the excreta. The pigs will ideally deposit and then trample on the excreta forcing it to fall through the slats into a channel or pit below. The success of this system relies on providing conditions that encourage the pigs to trample excrete often, so the floor becomes self-cleaning. Obviously, one of the best ways of achieving this is to increase stocking rates in fully-slatted (weaner) buildings. However, in grower/finisher building the increased stocking rate resulted in reduction in pen hygiene, though this effect was not statistically significant.

CONCLUSIONS

Our results demonstrated that the correct management of air temperature and stocking rate (SR) are the most practically beneficial ways of improving pen hygiene in piggery buildings. Temperature decrease will have a beneficial effect on pen hygiene in partially slatted pens, but there is a lower limit below which temperature cannot be reduced, as it would interfere with thermal comfort. In the same way, SR cannot be decreased drastically, due to potential negative economic impact. Farm size again cannot be manipulated, as the general trend toward larger farm size is driven mainly by economic considerations. In the same way, seasonal effects have to be accepted, but producers must be aware of the increased risks of reduced pen hygiene associated with summer periods. All these and potentially other factors must be taken into consideration, as practical experience demonstrated that dunging patterns are influenced by the combination of many factors under commercial conditions. Only through careful management and design of pigpens will correct dunging patterns be achieved. Care must be taken when designing and importantly

managing the buildings and pens to create a pen environment that is suitable for the development of correct dunging patterns.

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HEALTH AND WELFARE IN ORGANIC LAYING HENS; AN EPIDEMIOLOGICAL STUDY OF EUROPEAN EGG PRODUCING FARMS

Stefan Gunnarsson[1]*, Monique Bestman[2], Christine Brenninkmeyer[3], Paolo Ferrari[4], Jasper Herkens[5], Knut Niebuhr[5], Jan Tind Sørensen[6], and Alice Willett[8]

[1]Dept. of Animal Environment and Health, Swedish University of Agricultural Sciences (SLU Skara), Sweden; [2]Louis Bolk Institute, Utrecht, The Netherlands; [3]Univerity of Kassel, Germany; [4]CRPA, Reggio Emilia, Italy; [5]ILVO-OC, Melle, Belgium; [6]VetMedUni Vienna, Austria; [7]Aarhus University, Denmark; [8]ADAS, Wolverhampton, UK
**Telephone +46 51167 216, Fax +46 511 67 204, e-mail stefan.gunnarsson@slu.se*

Summary

Organic production may give laying hens improved welfare compared to enriched cages or indoor aviary systems. However, an organic system may increase the risk of e.g. parasitism, feather pecking and cannibalism. In order to improve bird health and welfare a European project with in research action CORE Organic II, was conducted. The aim was identify potential risk factors for various health and welfare issues in organic laying hen farms, by an epidemiological study.

At the farm visits data were recorded by the observations at the farm and through structured farmer interviews; the interview record comprised general farm information about the birds, housing conditions, feeding and management procedures. Furthermore, bird use of ranging area and clinical scoring (incl. plumage) was performed. Sampling for red mites and endoparasites was performed. For this cross sectional study, 120 organic layer farms were recruited in eight European countries.

Paddock access time was found to have a significant negative association with the Ascardia galli burden in the laying hens. Increased percentage of feather damage was associated with several management factors. Ranging behaviour was a partly influenced by weather conditions, but increased ranging was promoted by housing and management factors. Thus, the health and welfare status can be improved by refining management routines including feeding strategy.

Introduction

Organic production may give laying hens improved welfare compared to enriched cages or indoor aviary systems, as organic birds are kept at lower stocking density and have access to free range grazing. However, an organic system may increase the risk of e.g. parasitism (helminths and mites), feather pecking and cannibalism in the layers. The prevalence of helminth infection may be high in laying hens kept in organic and free-range production systems, and the infections may be associated with production losses and poor animal welfare (Permin et al. 1999). The poultry red mite (*Dermanyssus gallinae)* can cause irritation and restlessness with a negative impact both on the welfare and the production (Eckert et al., 2005).

Feather pecking consists of forceful pecks and pulls of feathers that are frequently eaten and results in feather loss on the back, vent and tail area (Rodenburg et al., 2013). Apart from being an animal welfare issue, feather pecking is an economic problem, as birds with feather damage need up to one third more feed. Provision of environmental enrichment and access to free range may decrease the risk of feather pecking (Gebhardt-Henrich et al, 2014).

In order to improve bird health and welfare in organic laying hens, a European project within research action CORE Organic II was conducted. The aim was to identify potential risk factors for health and welfare problems in organic laying hen farms focusing on parasites, feather pecking and use of ranging areas.

Material and methods

The study comprised 120 organic layer flocks in eight European countries (Austria, Belgium, Denmark, Germany, Italy, the Netherlands, Sweden and the UK). The farms studied were visited twice during the production cycle and at the farm visits data were recorded through structured farmer interviews and by the observations at the farm. The interview record comprised general farm information about the birds, housing conditions, feeding and management procedures, and information regarding e.g. design and conditions of housing, incl. veranda and free range was recorded by the observers. Furthermore, bird use of ranging area was recorded and at the end of lay visit, 50 hens per

flock were scored clinically regarding plumage, keel bone and feet etc. Sampling for red mites and endoparasites was performed at the visits.

Prior to the farm visits inter-assessor agreement was evaluated after training of all 12 assessors on a training farm. The training was iterated when inter-rater agreement was found to be too low, and training was finished when at least acceptable agreement (kappa >0.40) was achieved for all scores.

In the statistical analysis, the magnitude of the influence of the following factors was investigated: feeding management, pullet rearing, provision of occupational material, use of free range and other flock characteristics.

Results and Discussion

Data were collected from 114 farms at peak of lay, 110 farms at end of lay, and from 56 farms parasitological autopsies were made on at least 15 hens per flock. For hen health parameters, 50 hens were examined per flock at the end of lay and 15 faecal samples taken for helminth egg analysis at each visit (peak and end of lay). One level system was found in 64%, in the farms and 36% had multi-tier system indoor. Range rotation was applied in 28% of the farms, whereas 72 % never changed ranging areas between batches.

Parasites

A total of 907 hens from the 56 flocks were examined post mortem; and 69% of the hens had at least one intestinal Ascaridia galli worm (adult or juvenile). Among the three helminth species, the most common parasite, as determined by detection of worms at necropsy, was A. galli (69 %), followed by Heterakis spp. (32%) and cestodes (13%). Out of the 897 faecal samples examined from the 55 flocks, 66 % of the samples were positive for ascarid/heterakis eggs. No correlation was found between total ascarid worm count post mortem and EPG.

Out of the eight management factors analysed, paddock access time was found to have a significant negative association ($P<0.05$) with the A. galli worm burden in laying hens. None of the eight factors had a significant association with A. galli prevalence, ascarid/heterakis prevalence or ascarid EPG.

Mites infestation was determined in 101 flocks at visit 1 and 107 at visit 2 was used for the analysis. The most prevalent scores were 1-1000 red mites per trap.

Feather pecking

At the end of lay visit 15% of the flocks had severe feather damage, 20% had moderate and 65% had little/no feather damage. Increased percentage of hens with feather damage was associated with longer pre-lay feed was fed, if more different feed phases were fed, in case of lower protein, lower methionine, lower percentage of hens in the veranda, lower percentage of hens in the free range area, more times dewormed, higher no of alternative treatments, no litter replacement or topping, if roughage was provided during rearing, if there was no natural light source and in case of a needle vaccination after rearing.

Increased percentage of hens with body wounds was associated with longer pre-lay feed was fed, if more different feed phases were fed, in case of lower protein, higher degree of blood mites, no needle vaccination at placement, lower calcium and no litter topping.

Use of ranging areas

The overall average maximum percentage of birds on the range was 23.7% of the flock, with a minimum percentage of 0.30% and a maximum percentage of 76.6%. The percentage of birds ranging decreased as the flock size increased ($P<0.001$), and an increase in range area per hen resulted in an increase in the percentage of birds ranging ($P<0.01$), but the ranging decreased as the area of the covered veranda increased ($P<0.001$). Temperature had a parabolic effect on ranging, with the number of birds on the range increasing up to a temperature of 17.7 degrees C ($P<0.001$). A higher percentage of birds were on the range when there was no precipitation or a low wind speed ($P<0.01$). The provision of feed on the range did not significantly affect ranging behaviour. However, the presence of hedges or shelters increased the percentage of birds on the range ($p<0.05$). Bird mortality was not affected by birds' ranging.

Conclusions

This study is the largest epidemiological study of health and welfare in laying hens performed simultaneously in eight European countries, and data from 115 flocks were recorded using the same methodology. Paddock access time was found to have a significant negative association with the A. galli worm burden in laying hens. At the end of lay visit 15% of the flocks had severe feather damage, 20% had moderate and 65% had little/no feather damage. Increased percentage of feather damage was associated with several management factors. Ranging behaviour was a partly influenced by weather conditions, but increased ranging was promoted by housing and management factors. Thus, the health and welfare status can be improved by refining management routines including feeding strategy.

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HOUSING EFFECTS ASSOCIATED WITH IMPROVED PRODUCTION EFFICIENCY IN PIGS

Thomas Banhazi

National Centre for Engineering in Agriculture (NCEA), University of Southern Queensland, West st, Toowoomba, QLD, 4530 Australia; banhazi.thomas@usq.edu.au

The combined effects of air temperature, stocking rate, stocking density and the concentrations of different airborne pollutants were evaluated on the production efficiency of pigs under commercial farm conditions in South Australia. A farrow-to-finish farm with 600 sows, located in South Australia, was used as an experimental site. This farm had been free of *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropeumoniae* for 10 years prior this experiment. Commercial piggery buildings were divided into two separate compartments and the hygiene/air quality conditions were improved in one compartment (experimental group) while the other section was managed according to normal farm procedures (control group). The AIAO building sections were cleaned thoroughly between each batch of pigs to completely remove the accumulation of dirt, dust and dung. The growth rate and environmental variables associated with the experimental and control groups were monitored and compared. The concentrations of airborne particles (total and respirable), airborne microorganisms (total, gram positive and fungi), NH_3 and carbon dioxide (CO_2) were monitored to determine air quality in both the experimental and control building sections. The statistical analysis (using general linear models) identified the reduction of ammonia, airborne particles and improved stocking density as key factors contributing to improved production efficiency. Thus, it was concluded that improving the housing conditions of livestock could result in improved profitability even on farms where it is not possible to directly improve the health of farmed animals.

Keywords: pig houses, environmental quality, farm building, risk factors, dust, bacteria, ammonia, temperature, stocking rate

INTRODUCTION

The major airborne pollutants that farm animals are exposed to in livestock buildings such as ammonia (NH_3), airborne bacteria (total airborne bacteria, gram positive and fungal species), inhalable and respirable particles (Banhazi *et al.*, 2009) have the potential to significantly reduce the animals' production efficiency, health and welfare (Kovacs *et al.*, 1967; Wathes *et al.*, 2004; Done *et al.*, 2005; Lee *et al.*, 2005). These pollutants can attack the animals' immune systems, triggering an inflammatory reaction, and a reduced resistance to respiratory infection (Urbain *et al.*, 1998). Feed intake may also be reduced, resulting in reduced growth rates (Lee *et al.*, 2005). In addition to the potentially negative effects of airborne pollutants on the health and welfare of animals; airborne pollutants can also increase the Occupational Health and Safety risks for farm workers (Banhazi *et al.*, 2009). Additionally, airborne pollutant emissions from livestock buildings could damage the surrounding environment (Banhazi *et al.*, 2008a).

Age Segregated Rearing (ASR) is a method adopted in pig production systems to minimise the transmission of respiratory diseases between successive batches of pigs, but ASR rearing method may also improve air and surface hygiene of piggery buildings (Cargill *et al.*, 1998). In turn, it has been hypothesised that the improved air and environmental quality within piggery buildings will result in growth rate improvements of pigs. Therefore, the aim of this study was to improve environmental conditions within experimental piggery buildings situated on a respiratory-disease-free farm and to determine the effect of these improvements on the growth rate of pigs. To facilitate this experiment, the growth rate (average daily gain, ADG) and air quality (AQ) parameters were monitored in piggery buildings with (AIAO) and without improved management system (continuous flow, CF).

MATERIALS AND METHODS

A farrow-to-finish farm with 600 sows, located in South Australia, was used as an experimental site. This farm had been free of *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropeumoniae* for 10 years prior this experiment. The experimental facilities (a second-stage weaner and a grower building) used in this study were naturally ventilated, with controlled-shutters on both sides and the buildings had partially slatted floors. Approximately 200 pigs were housed in the AIAO sections, which were created at the end of each building using tarpaulin material (Cavacon 5000Q, Tolai marketing, Adelaide, Australia). Approximately 650 grower and 950 weaner pigs were housed in the CF sections of the buildings. The tarpaulin partitions, erected across the buildings, were either attached directly to the roof

line or hung from a wire at the eave-level of the building. This divided the buildings into two separate airspaces and hence air movement between each section was significantly reduced. The AIAO building sections were cleaned thoroughly between each batch of pigs to completely remove the accumulation of dirt, dust and dung. The CF sections of the building were treated according to the existing farm procedure, which did not include regular cleaning. The experimental setup ensured that the animals' genetics, medication, diet and general management (such as feeding, ventilation and effluent systems and husbandry) were identical for both CF and AIAO building sections.

At the age of approximately 6 weeks, 100 pigs were randomly selected and tagged before being divided into two equal subgroups. One group was allocated to each treatment in each building. Conventional weigh-scales (Weigh Crate, Ruddweigh, Guyra, Australia) were used to monitor the ADG of the second-stage weaner (6 to 10 weeks of age) and grower (10 to 20 weeks of age) pigs in both the experimental treatment groups. Tagged pigs were weighed each time they were moved, and when they were approximately 6, 12, 14 and 16 weeks of age. Over approximately a 2.5 year period, data was collected from seven batches of pigs, which were divided into the treatment groups. Approximately once a month, average daily gain (ADG), stocking density (SD), stocking rate (SR), temperature and AQ parameters were determined at the facilities.

The concentrations of airborne particles (total and respirable), airborne microorganisms (total, gram positive and fungi), NH_3 and carbon dioxide (CO_2) were monitored to determine AQ in both the experimental and control building sections. The measurements were averaged for the monitoring period spanning between weight measurements and were treated as representative of the AQ conditions throughout the given period. Concentrations of airborne particles were determined gravimetrically using cyclone samplers for respirable (<5 μm) and Institute of Occupational Medicine (IOM) samplers for total particles (Casella, Ltd., Kempston, U.K.), respectively and the filters were installed at a height of 1.1-1.3 m as described previously (Banhazi *et al.*, 2008b). A standard six-stage bacterial impactor was used to sample the total airborne microorganisms, gram positive bacteria and fungal species (Seedorf *et al.*, 1998). Samples were taken at about midday (1100 h to 1500 h), usually in the centre of the animal building and above the pens. A multi-gas monitoring (MGM) machine was developed to continuously monitor NH_3 and CO_2 gases within the building sections (Banhazi *et al.*, 2008b; Banhazi, 2009). The SR (m^2 pen floor/ pig) was calculated for each group of pigs and similarly the volume of airspace in each building and section was also measured, and the SD (m^3 airspace/pig) calculated. The number of pigs in each section or airspace was recorded at each visit. In all buildings, temperature data was recorded using Tinytalk temperature loggers (Tinytalk-2, Hastings Dataloggers Pty. Ltd., Port Macquarie, Australia) (Banhazi *et al.*, 2008b).

To minimise the natural variation caused by the ADG recorded for the weaner and grower pigs, the percentage change in ADG recorded in the AIAO and CF building sections was analysed as dependant variable using GLM procedure (StatSoft, 2001). The explanatory effects and covariates examined statistically were, total airborne particles (g/m^3), respirable particles (g/m^3), total airborne bacteria (CFU/m^3), gram positive bacteria (CFU/m^3), fungal species (CFU/m^3), NH_3 (ppm), CO_2 (ppm) air temperature (^oC), SR (m^2/pig) and SD (m^3/pig), in the AIAO and CF sections. As the number of data points available was limited, only main effects were tested. The statistical models were developed from the maximum model, by sequentially removing non-significant effects ($P<0.05$, based on type III estimable functions) until only significant effects remained. GLM statistical procedure was used, as it is able to interpret results reliably when handling unbalanced field data (StatSoft, 2001).

RESULTS

Table 1 shows the data used in the second analysis. A summary of the analysis concerning ADG improvement percentages is presented in Table 2, which includes the R^2 value for the model developed.

Table 1 Mean, maximum and minimum percentage of change in ADG and pollutant concentration values recorded in the study buildings (AIAO vs. CF)

Change in variables (%)	Mean	Minimum	Maximum
ADG*	6.1	0.5	11.6
Total airborne particles	48.4	8.8	83.3
Respirable particles	51.2	3.2	88.9
Total bacteria	17.1	3.6	55.5
Gram positive bacteria	22.8	0.5	62.0
Fungi	16.5	5.6	69.7
NH_3	68.8	-3.6	97.3
Stocking density	3.7	-4.4	9.4

*Average daily gain

Throughout the study, an increase in airborne particle concentrations was not recorded in the AIAO sections, although at times, the reduction in the concentration of airborne particles was small (approximately 3 %). The maximum reduction in the concentrations of airborne particles ranged between 85-90%, thus, the AIAO management did not completely eliminate airborne particle pollution. Across all batches of pigs, improvements in ADG varied between 0.5 (essentially no improvement) and 11.6%. On average, the respirable and total airborne particles were reduced by approximately 50% and the concentrations of viable airborne particles (total bacteria, gram positive bacteria and fungal species) were reduced by approximately 20%. The greatest reduction was achieved in the concentration of ammonia, however, on at least on one occasion the ammonia concentration increased by approximately 4% in the AIAO sections.

Table 2. Tests of significance for effects associated with percentage improvement in ADG in the model developed

Effects for weaner sections (Model R^2 = 58.9 %)	ADG	Slope
Reduction in the concentration of total airborne particles (%)	p=0.0327	positive
Reduction in the concentration of respirable particles (%)	p=0.0029	positive
Reduction in the concentration of ammonia (%)	p=0.0425	positive
Improvements in stocking density (%)*	p=0.0044	positive

*Increase in available airspace per pig

Four variables were identified during the second analysis that had a significant positive affect on the percentage-ADG. These variables were the percentage-change in concentration of (1) total airborne particles, (2) respirable particles and (3) ammonia. In addition, (4) the percentage of improvement in SD (i.e. more airspace availability per pig) was identified as the fourth factor. All of these variables were positively associated with ADG improvements. The model developed explained approximately 60% of the variation in ADG improvement.

Neither temperature nor CO_2 concentrations (indicator of ventilation levels) were significantly different between the sections (data not shown). These variables did not influence ADG in the weaner or grower sections either. These findings confirm the reliability of the study results, as according to the analysis these variables did not interfere with the main experimental effect of AIAO versus CF management.

DISCUSSION

The statistical analysis determined the factors that were significantly associated with percentage improvement in ADG (AIAO vs. CF sections). Four covariates were identified as having a significant effect on percentage of ADG improvement and the model developed explained a large percentage of the observed variation (R^2=60%). While approximately 40% of the variation in ADG improvement is still unexplained, the model developed appears to be more robust (Table 2). The covariates identified were the reductions in the concentrations of, ammonia (%), respirable (%) and total airborne particles (%). In addition, improvement in SR (% improvement in available airspace space per pig) was identified statistically as having a significant effect on ADG increase (%).

According to the model, ADG of pigs was positively associated with the reduction in the concentrations of these key airborne pollutants.

The results achieved on this farm demonstrate the benefits of converting existing CF facilities into AIAO production systems, even in high health status herds. During this experiment, the AIAO management system allowed the facilities to be thoroughly cleaned between batches, which resulted in considerably improved air quality. It is likely that the better AQ and improved environmental conditions reduced the stress on the pigs' immune system, resulting in increased production efficiency (ADG) and thus potentially in financial gain.

There was a marked and consistent improvement in AQ in the AIAO sections throughout the experiment. When comparing the concentrations of different airborne pollutants recorded during this study using one-way ANOVA, it was demonstrated that concentrations of almost all airborne pollutants (including ammonia, total and respirable airborne particles) were significantly reduced ($P<0.05$) in both the weaner and grower AIAO sections (results not shown). The percentage-reduction was noticeable for all pollutants in both AIAO sections when compared to their CF equivalents. However, despite these significant reductions, only a handful of covariates were identified by the statistical models, as having a statistically significant effect on ADG. These results also highlight the importance of using appropriate statistical methods when analysing the results of studies implemented on commercial farms.

The SD and SR differences (identified by the GLM analysis as significant) were actually relatively small and statistically non-significant when initially analysed by simple one-way ANOVA. However, according to the analysis, even this relatively small difference in SR had a significant impact on ADG and on the percentage of improvement in ADG. It is still questionable whether the identified effects were a causal effect or simply the identification of a parameter that was consistently better in the AIAO sections. Further experiments are needed to answer these questions. However, based on current results, it appears that improved SR and SD is an important aspect of improved pig management. The reduced stress caused by greater available space and the potentially reduced heat stress due to reduced crowding (and thus better cooling opportunities for individual pig) might have contributed to this observed ADG improvement. In addition, it is also likely that the combined impact of a small SR and SD improvement per pig would add up to a significant improvement when assessed on a pen and/or on building level.

CONCLUSIONS

In summary, the experiment demonstrated the potentially positive effects of improved environmental conditions in piggery buildings. The statistical model identified factors that had an affect on ADG in piggery buildings. The pig in the AIAO sections had significantly higher ADG when compared with pigs housed in their paired CF sections. SR and SD were positively correlated with ADG while the concentrations of all airborne pollutants were negatively correlated. Careful management of these factors could lead to the improved financial performance of the farm. It is important to note that during this study a large and significant reduction in the concentration of airborne pollutants was achieved under commercial farming conditions. It would be expected that an ADG improvement would be experienced on other farms as well after reducing the concentrations of these pollutants. However, the extent of AQ improvements might vary between farms, and thus, might not translate to statistically significant ADG increase elsewhere. Especially in piggeries where high levels of building hygiene are maintained, additional improvements in hygiene and reduction in airborne pollutants may be difficult to achieve. Nevertheless, even ADG improvements that are non-significant statistically could result in significant economical gains on commercial farms.

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THE EFFECT OF THREE BEDDING MATERIALS ON ACID-BASE BALANCE OF ARTERIAL BLOOD OF HORSES

A. Kwiatkowska-Stenzel[1], D. Witkowska[1], J. Sowińska[1], A. Stopyra[2], A. Wójcik[1], T. Mituniewicz[1]

[1]Department of Animal and Environmental Hygiene, University of Warmia and Mazury, Olsztyn, Poland; [2]Department of Internal Medicine, University of Warmia and Mazury, Olsztyn, Poland

SUMMARY

Bedding used in stables is an important factor affecting the quality of the air horses breathe, which in turn, influences the condition of their respiratory tract. Acid-base balance demonstrates the efficiency of gas exchange and therefore the condition of the respiratory tract.

In the investigation 8 healthy horses were used. The investigation was divided into 3 three-week long stages, during which straw (S), peat moss with shavings (PMS) and crushed wood pellets (CWP) were used. After application of each of the bedding the arterial blood was collected and the following parameters were analyzed: pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), oxygen saturation (O_2SAT), total amount of carbon dioxide ($ctCO_2$) and the concentration of bicarbonate (HCO_3). The results were analyzed statistically.

The various stages of research influenced significantly the differentiation of arterial blood parameters in terms of acid-base balance. When CWP was used the mean pH value of blood (7.44) was similar to when PMS (7.43) was used. The obtained values were higher ($p \leq 0.01$) than blood acidity when using S (7.39). In case of pCO_2 mean CWP value (49.23 mmHg) was higher ($p \leq 0.05$) than the mean S value (44.11 mmHg). PMS value (46.50 mmHg) was intermediate. The opposite trend was observed in the evaluation of pO_2, which was the highest when using S (109.04 mmHg), and the lowest when CWP (93.41 mmHg). The highest values of HCO_3 and $ctCO_2$ were found when using CWP (33.99 mmol/l, 35.49 mmol/l). They were higher ($p \leq 0.01$) than S (27.29 mmol/l, 28.68 mmol/l) and PMS (29.74 mmol/l, 31.07 mmol/) values. The lowest O_2SAT was found when using CWP (93.06%). The obtained value differed ($p \leq 0.05$) from the data obtained when S (97.49%) and PMS (97.21%) were used.

The obtained results indicate that for the horses' respiratory system, the least favourable bedding was crushed wooden pellets.

INTRODUCTION

The bedding material is one of the technological elements in stables. According to Regulation of Polish Ministry of Agriculture and Rural Development (14) it is recommended to use bedding materials in all keeping systems for horses. In Poland as well as in the other parts of the world the most popular bedding material used in stables is straw. Taking into consideration horses behavioural needs, straw is the most acceptable material for laying down (11, 13, 18), and also provides occupation for animals during their stay in the stable (18). On the other hand straw has number of disadvantages such as weak absorption of water and ammonia (1, 3, 10). Straw bedding is also characterized by high dustiness (6). In comparison with alternative bedding materials the dustiness of straw can be twice or even three times higher (2). Comparing straw with other bedding materials have also shown its higher endotoxin contamination (12, 16). Different problem of usage of straw, especially oat kind may be eating it by horses which makes impossible full feed intake control and may also lead to horses colic. On the other hand sharp blades of barley straw may cause skin irritation of horses (17).

The disadvantages of straw mentioned above lead to searching new, alternative bedding materials. One of alternative bedding materials used in stables are straw pellets. Comparing with straw and wood chips this material is characterized by the lowest dustiness (6). Thanks to technology used for producing pellets they are less microbiologically contaminated than for example straw.

Growing popularity as a bedding material, is gaining peat. It is characterized by perfect water absorption. However it constitutes a perfect environment for the development of pathogens. A serious disadvantage of this bedding is also its high dustiness and dirtying up of horses (1).

Bedding used in stables is an important factor affecting the quality of the air horses breathe, which in turn, influences the condition of their respiratory tract. Acid-base balance demonstrates the efficiency of gas exchange and therefore the condition of the respiratory tract. The aim of the study was a qualitative assessment and analysis of impact of

straw, peat moss with shavings and crushed pellets beddings used in the stables on health of horses, with particular emphasis on the respiratory system.

MATERIAL AND METHODS

In the investigation 8 healthy horses were used. The investigation was divided into 3 three-week long stages, during which straw (S), peat moss with shavings (PMS) and crushed wood pellets (CWP) were used. After application of each of the bedding the arterial blood from the external carotid artery was collected (24 samples) and the following parameters were analyzed: pH, partial pressure of carbon dioxide (pCO_2), the partial pressure of oxygen (pO_2), oxygen saturation (O_2SAT), the total amount of carbon dioxide ($ctCO_2$) and the concentration of bicarbonate (HCO_3). Measurement of acid-base balance was performed on Rapidlab analyzer 348 (Siemens). The determination was performed in whole blood drawn to the capillaries with lithium heparin using reagents compatible with the Siemens company analyzer. The values of the investigated traits (±SD) were processed statistically using Statistica 10.0 PL software. The verification of the results was conducted with the use of orthogonal single analysis of variance. To determine the significance of differences between the analyzed features Duncan's test was used.

RESULTS AND DISCUSSION

The various stages of research influenced significantly the differentiation of arterial blood parameters in terms of acid-base balance. The obtained data is presented in the table 1. When CWP was used the mean pH value of blood (7.44) was similar to the value obtained during usage of PMS (7.43). The obtained values were higher ($p \leq 0.01$) than blood acidity when using S (7.39). However all obtained results were within reference values (4). In case of pCO_2 mean CWP value (49.23 mmHg) was higher ($p \leq 0.05$) than the mean S value (44.11 mmHg). PMS value (46.50 mmHg) was intermediate. The opposite trend was observed in the evaluation of pO_2, which was the highest when using S (109.04 mmHg), and the lowest when CWP (93.41 mmHg). Ferro et al. (5) stated that horses showing RAO symptoms with a normal values of pCO_2 had a normal pO_2. But when the pCO_2 was higher the pO_2 was statistically lower. A similar tendency was observed in the own study. The highest values of HCO_3 and $ctCO_2$ were found when using CWP (33.99 mmol/l, 35.49 mmol/l). The obtained results were slightly higher than the reference values (8, 15). They were also higher ($p \leq 0.01$) than the values obtained during usage of S (27.29 mmol/l, 28.68 mmol/l) and PMS (29.74 mmol/l, 31.07 mmol/) values. The lowest O_2SAT was found when using CWP (93.06%). The obtained value differed ($p \leq 0.05$) from the data obtained when S (97.49%) and PMS (97.21%) were used. The obtained results indicate that during usage of CWP the oxygen saturation was lower than the reference value. Not sufficient oxygen saturation is the effect of hypoxia of organism, it can accompany inflammation states of respiratory tract or chronic respiratory diseases such as RAO (7). Kirschvink et al. (9) in their study concerning the influence of cardboard bedding material on respiratory tract stated a substantial improvement of pCO_2 and pO_2 and deterioration among these parameters when using straw. Although the CWP bedding appeared to be the least favourable it should be mentioned that most of the obtained results of acid-base balance were within reference values. Slight exceed of some values were rather not connected to pathological states, because the horses stayed healthy during the whole investigation period.

CONCLUSION

The obtained results indicate that for the horses' respiratory system, the least favourable bedding was crushed wooden pellets. It could be contributed to its high dustiness, which may cause mechanical irritation of respiratory system.

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Table 1. Average values of parameters of acid-base balance of arterial blood of horses ($\bar{x}$ ±SD) during stages of the investigation

Parametr		Bedding material / Stage of the investigation		
		Straw/S	Peat moss with shavings/ PMS	Crushed wood pellets/ CWP
pH	Average	7.39^{B}	7.43^{A}	7.44^{A}
	SD	±0.02	±0.03	±0.03
pCO_2 mmHg	Average	44.11^{b}	46.50	49.23^{a}
	SD	±2.00	±2.04	±6.12
pO_2 mmHg	Average	109.04^{a}	99.74	80.41^{b}
	SD	±21.56	±20.85	±34.65
HCO_3 mmol/l	Average	27.29^{B}	29.74^{B}	33.99^{A}
	SD	±1.67	±1.34	±4.17
$ctCO_2$ mmol/l	Average	28.68^{B}	31.07^{B}	35.49^{A}
	SD	±1.70	±1.33	±4.33
O_2SAT %	Average	97.49^{a}	97.21^{a}	93.06^{b}
	SD	±1.14	±1.18	±8.66

A,B – significant differences P≤ 0,01 between investigation
a,b – significant differences P≤ 0,05 between investigation

USING A PIG SCREAM SOUND MONITOR TO GIVE AN INDICATION OF THE PIGS' IMPAIRED WELFARE DUE TO FEED DEPRIVATION

J. Vandermeulen[1], C. Bahr[1], S. Ott[2,3], E. Tullo[4], I. Fontana[4], M. Guarino[4], C.P.H. Moons[3], F.A.M. Tuyttens[3,5], T.A. Niewold[2], D. Berckmans[1]

[1]M3-BIORES – Measure, Model & Manage Bioresponses, KU Leuven, Leuven, Belgium; [2]Livestock-Nutrition-Quality, KU Leuven, Leuven, Belgium; [3]Departement of Animal Nutrition, Genetics and Ethology, Laboratory for Ethology, Ghent university, Belgium; [4]Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, Milan, Italy. [5]Institute for Agricultural and Fisheries Research (ILVO), Melle, Belgium;

Summary

Aspects of on-farm animal welfare could be automatically and continuously monitored trough sensor technology. Monitoring sounds produced in a pig house could potentially indicate situations of impaired animal welfare. The objective of this study was to investigate whether a 24-hour feed deprivation as a stressor can be detected by analysing the number of pig screams. A newly developed pig scream sound monitor was used to count the number of screams in one compartment containing 24 pigs. Two trials of 15 days were held, in which half the pigs were subjected to 24 hours feed deprivation. The number of detected screams were analysed with Friedman tests. The number of screams was higher during the night of feed deprivation compared to the other nights ($p = 0.006$). This indicates that monitoring of pig screams is promising to detect situations which impair animal welfare such as periods of hunger.

Introduction

Automated continuous monitoring of animals through sensor technology could make a substantial contribution to on-farm animal welfare (Berckmans, 2008). Several examples exist in literature, such as detecting lameness in cattle through camera technology (Viazzi et al., 2014) or monitoring respiratory diseases in pigs through detecting cough sounds (Van Hirtum et al., 1999, Finger et al., 2014).

In literature an automated monitoring of high frequency calls by pigs was developed (Schön et al., 2004). These calls were shown to be related to stressful situations such as restrictive feeding (Schön et al., 2004).

The aim of the present study is to see if there is a difference in number of screams during a 24 hour feed deprivation, using a pig scream sound monitor detector. The assumption was that the change in behaviour would change the number of screams and that the scream monitor would detect this.

Materials and Methods

Animal and housing

Two trials, each with 24 growing pigs (Piétrain Plus × Rattlerow Seghers), were conducted for a duration of 15 days per trial at Agrivet research farm, Merelbeke, Belgium. The average weight of the pigs was 20.9kg at the start and 32.2kg at the end of the first trial, and 31.5kg and 43.0kg respectively, in the second trial. Pigs were divided into four pens (1.60m x 2.35m) with six pigs each. The pens with one feeder space and one nipple drinker were located in the same compartment. In each trial, two different randomly chosen pens were subjected to feed deprivation for 24 hours starting at noon on day 11 of the trial. The experiment was approved by the Ethical Committee of the Faculty of Veterinary Medicine at Ghent University (EC2012/125).

Scream sound monitor

The sound in the compartment was recorded with one microphone (C-4 Small Diaphragm Condenser Mic, Behringer, Germany) positioned in the middle of the compartment at a height of 1.5 m with a precision of 16 bit and a sampling frequency of 22050 Hz. Subsequently, the screams were monitored with an in-house developed pig scream sound monitor algorithm. This monitor provided the number of screams detected per hour in the compartment. The hourly values of the two trials were summed together, resulting in the total number of screams detected per hour for the two trials together.

Statistical analysis

The number of screams per hour and per day were subjected to statistical analysis to show an animal reaction to the 24 hours feed deprivation on day 11. Only periods during the night-time hours were analysed because of two reasons. (1) All screams and sounds due to interaction with the researchers were eliminated in that way. (2) The time instant when the animals regained access to the feeder was taken out, because considerable intensity of vocalisations by the animals were expected due to competition for feed. The night-time hours were defined from 22:00 to 06:00.

To detect a difference in the number of screams during the night time hours of the feed deprivation day, a Friedman test was conducted on two datasets. The first dataset consisted of the number of screams during the night time hours for all nights excluding the feed deprivation day. The second dataset included the feed deprivation day. It was assumed that the number of screams in the former dataset would be similar. Hence a significant outcome of the test would reject this. Lastly, it was assumed that the number of screams in the latter dataset would not be similar. Hence a significant test would prove this.

Results

The Friedman test on all days excluding the feed deprivation day was non-significant ($p = 0.413$). However, the Friedman test on all days including the feed deprivation day was significant ($p = 0.006$), meaning that the number of screams did not differ between any of the nights, apart from the night of feed deprivation. Furthermore, the number of screams during the night of feed deprivation was twice the average of the other nights. This can be seen in Figure 1.

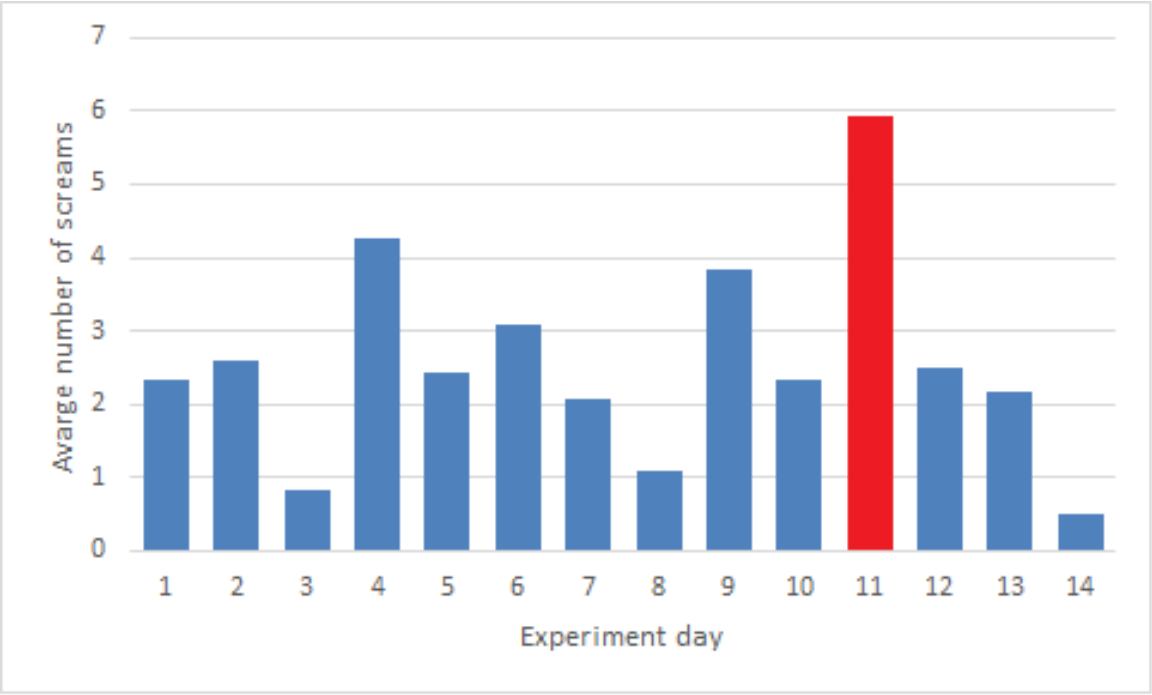

Figure 1: The number of screams in the compartment during the night. Day 11 was the night of the feed deprivation. This indicated that during the feed deprivation night the screaming occurred more often due to a limitation in feed associated with hunger.

Discussion

The results indicate that the vocalisation changed during feed deprivation and that this could be detected with an automated pig scream sound monitor. The feed deprivation presumably impaired pig welfare by causing hunger. In literature it was similarly proven that detecting high frequency calls of pigs could indicate a case of restricted feeding (Schön et al., 2004).

Conclusions

These results show that an automated scream monitor can be used to detect feed deprivation. The number of screams per hour during the feed deprivation period was significantly higher compared to the number of screams during the nights before and after the deprivation period. It ought to be investigated further, whether these promising

results may be further generalised to other situations of impaired welfare. An unknown event could impair the animal welfare without the farmer knowing. Therefore, a scream monitor could warn the farmer so that the farmer can take timely actions to prevent further suffering.

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UTILIZATION OF LAYER-TYPE COCKERELS FOR COMMERCIAL MEAT PRODUCTION

P.H.G.J De Silva[1], Y.M. Wickramasinghe [2] and D.C.A. Kalubowila[3]

[1]Department of Animal Science, Faculty of Agriculture, Aquinas University College, Colombo 08, Sri Lanka
[2] Department of Agricultural Systems, Faculty of Agriculture, Rajarata University of Sri Lanka, Puliyankulama, Sri Lanka
[3] ADM Food Products Pvt. Ltd, Badalgama, Sri Lanka

Summary: A study was conducted to evaluate the effects of genotype and sex on growth performance and carcass quality of broiler and layer male chicken. There was a significant effect of genotype on body weight, feed intake and FCR (p<0.05). LM had higher % of blood, feather, shank, head and internal organ compared to BM and BF (p<0.05). BM and BF had significantly higher values for dressing % (p<0.05). LM had least amount of subcutaneous fat in neck (1.03 ± 0.12), subcutaneous fat in thigh (0.88 ± 0.05) and abdominal fat (1.77 ± 0.10) despite of slaughtering ages (p<0.05). Genotype had been shown to significantly influence on colour of *P. major* and *B. femoris* muscles. Moreover LM had least shear force value (p<0.05).

Keywords: Layer cockerel, Broiler, Growth, Carcass, Meat

Introduction

It is estimated that annually about 8 million day old layer chicks are produced in Sri Lanka (Livestock Statistical Bulletin, 2014) thus resulting similar number of day-old-male layer chicks too. The number of day old male layer chicks likely to be increased day by day with the increase of Sri Lankan egg consumption. Therefore elimination of these day old layer cockerel chicks is a great problem for hatcheries. However sustainable solutions for the egg industry enabling the utilization of these males are needed. The scope for utilizing cockerels as a source of animal protein for human consumption is high. Male chicks from layer strains reared for meat have desirable flavour and less abdominal fat (Murawska, *et al*., 2005; Murawska and Bochno, 2007). But studies done on fattening of laying-type cockerels are few. Therefore this study was conducted to develop an ethically justifiable production system for layer-type males and to evaluate growth performance and carcass quality of brown layer cockerel verses broiler chickens.

Material and methods

A total of 180 one-day-old Broiler male (BM), female (BF) and brown layer male (LM) chicks were distributed into 9 floor pens in a completely randomized design. Broilers were reared to 8th weeks and others reared till 16th weeks and all birds were fed the same standard broiler commercial diets adlibitum. The body weights, feed consumption and FCR were recorded at weekly intervals. Two birds from each replicate were randomly selected at 4th, 6th, 8th, 12th and 16th weeks for carcass trait assessment. Abdominal fat content around vent and subcutaneous fat content in (clavicocervical (neck) and sartorial femoral (thigh)) were measured. Instrumental colour measurements (CIE L* a* b*) of *Pectoralis major* and *Biceps femoris* were taken as described by Souza *et al*., 2011. Tenderness was measured following a procedure similar to Abdullah and Matarneh., (2010). The data were analyzed by ANOVA and mean separation was done by using Duncan's multiple range test.

Results and Discussion

Genotype was significantly (p<0.05) affected body weight during 16 weeks of age. There was an increasing trend in body weight as the birds advanced in age but the mean body weight values were increased marginally with the increase in the age of LM. At the age of 4, 6 and 8 weeks, LM, in comparison to broiler chicken (BM or BF) of the same age, had a 3.9, 3.5 and 3.3 times smaller body weight, respectively. BM had 6.25 % mortality within the 8 weeks of age while BF had 5 % mortality during first week of age but LM had zero mortality until the end of the experiment. Livability of the birds was 100%, indicating that the LM responded well. BM and BF consumed more feed than LM (p<0.05) at all stages of growth. Mean values of weekly FCR affected by genotype, sex and age and FCR increased with advancing age for both genotypes (p<0.05). At 8th week, BM had the significantly lowest FCR (2.06 ± 0.03) but FCR of BF (2.18 ± 0.02) lower than LM but was insignificant (p>0.05).

There was a significant effect in % of blood on genotype and sex at 4th, 6th and 8th weeks of age (p < 0.05). LM had higher blood % compare to BM and BF. Similar tendency by genotype and sex was observed for feather %. There

were no significant ($p > 0.05$) genotype and sex differences found among the % of shank and head. The % of internal organs was significantly higher in LM than BM and BF ($p < 0.05$). The greater by product weight in LM could be associated with total body weight and different growth rate between broiler and layer male chicken. The % of the external and internal by product was highest at 4th weeks of slaughtering regardless of genotype and sex ($p < 0.05$). However, with increased slaughter age of chicken there was a significantly better dressing % regardless genotype ($p < 0.05$). Moreover BM and BF had significantly higher values for dressing % ($p < 0.05$).This finding suggests that the differences in carcass traits across genotype probably arise from metabolic differences and from differences in the onset of fattening.

Genotype has significant effect on subcutaneous fat in thigh, neck and abdominal fat despite slaughtering ages ($p < 0.05$). LM had lower fat content in carcass over the rearing period presumably as a consequence of their greater physical activity likely to affect muscle metabolism and reduced the fat deposition.

LM had recorded highest L* value for breast (60.58 ± 1.18) and thigh (49.41 ± 1.08) muscle at 8th week of slaughtering age ($p<0.05$). Least b* coordinate recorded by BM (4.48 ± 1.09) while BF had significantly higher b* (5.27 ± 0.64) for breast muscle ($p<0.05$). Conversely, a* and b* for *B. femoris* muscle of BM and BF had significantly lower values ($p<0.05$). It was found that genotype was significantly affect on shear force (SF) value ($p<0.05$). BM (1.67 ± 0.65) and BF (1.52 ± 0.71) had significantly higher SF value at 8th week of age than LM (1.20 ± 0.54). The SF values of LM at 16th week was higher than BM and BF at 8th week of age ($p<0.05$).

Conclusions

This study concludes that variation in the genetic makeup, sexual differences and slaughtering age of chickens accounted for observed differences in growth and carcass quality characteristics. Considering all these attributes in the performance and carcass quality, the egg industry can take advantage considering the availability of these light egg-type males in the market.

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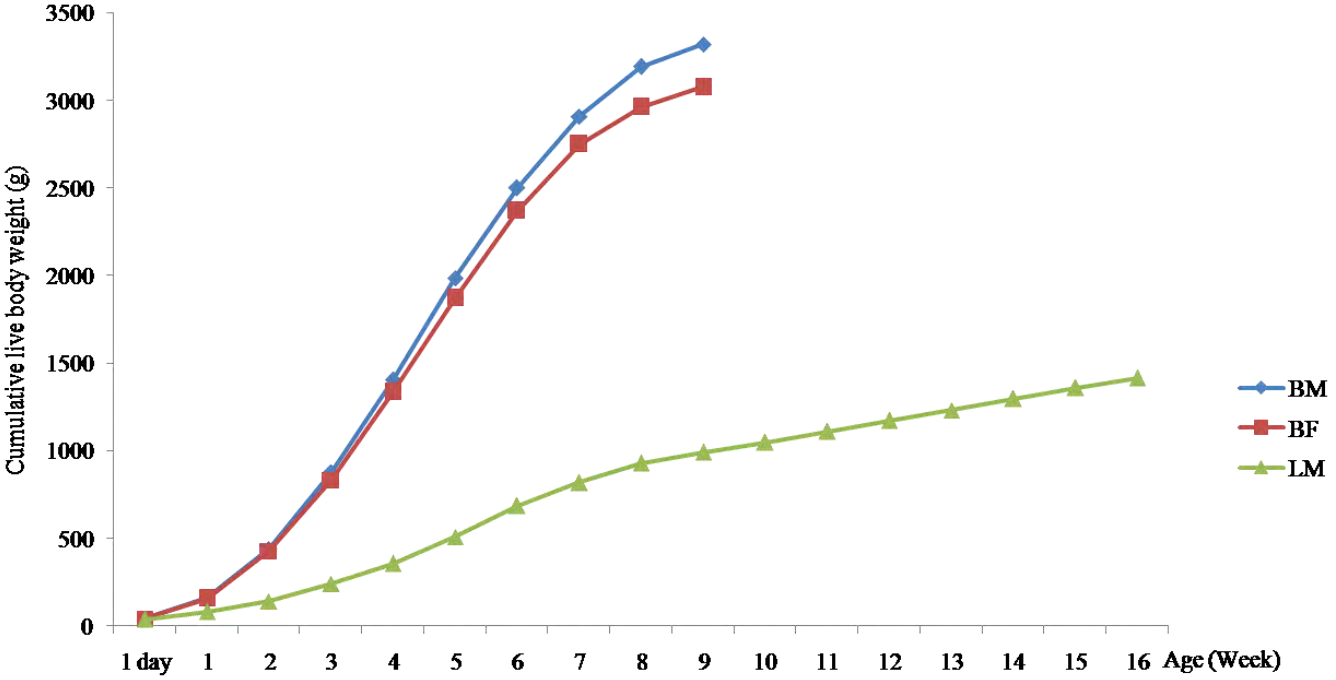

Figure 1: Mean values of weekly cumulative live body weight (g) of experimental birds

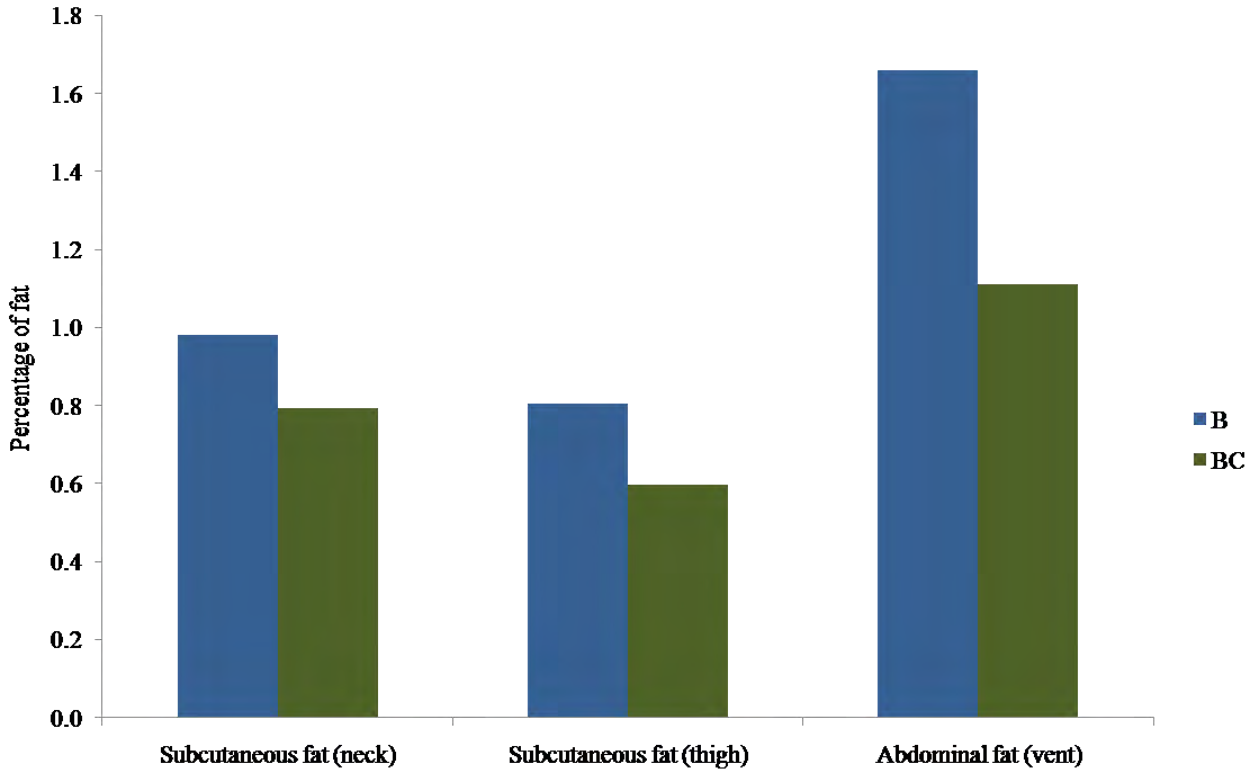

Figure 2: Effect of different genotype on percentage of fat content

EVALUATION OF SOME ANIMAL WELFARE INDICATORS ON BEEF CATTLE IN SILVOPASTORAL VS EXTENSIVE SYSTEMS

S. Huertas, P. Bobadilla, J. Piaggio, F. Vila, J. Baruch & A. Gil

OIE Collaborating Center on Animal Welfare and Production Systems
Department of Biostatistics, Faculty of Veterinary. University of the Republic of Uruguay

Summary

Beef cattle production in Uruguay was traditionally made in extensive conditions (pasture based); nevertheless recent increase in the price of the land, accelerated development of agriculture, and the need to increase the productivity per hectare have made new mixed production models, such as silvopastoral systems which integrate forestry plantations (*Eucaliptus spp*) with extensive beef production. In other regions these systems have proved to be beneficial for cattle production, by providing protection from extreme weather and a richer environment, hence improving animal welfare. The objective of this work is to compare animal based welfare indicators between silvopastoral systems and the traditional extensive production of beef cattle. A herd of 40 Hereford steers, 18 months old, average weight of 314kg. Located in Lavalleja, Uruguay, South America. Were randomly assigned to two groups, one group went to a silvopastoral system paddock and the second to a traditionally extensive system paddock, both of them with the same animal density, and similar pasture composition. Weather conditions were recorded. According to the Welfare Quality® Protocol adapted to local conditions, animal based indicators were periodically assessed for both groups: ocular and nasal discharge, diarrhea, body condition, cleanliness, and lameness. All data was processed for statistical analysis in Stata 11 (Stata. Inc). No significant differences ($p<0.05$) were found between treatments, regarding the animal based indicators assessed. No significative differences in weather or pasture were found between paddocks. Provided that these indicators have very low occurrence frequencies in traditional extensive production systems, and based on these preliminary results, we cannot state the existence of differences in the two systems compared.

Introduction

Beef cattle production in Uruguay as well as in many American countries has been traditionally made in extensive conditions (pasture based). Recent increases in the price of the land, accelerated development of agriculture, and the need to increase the productivity per hectare have made new mixed production models to become more common, such as silvopastoral systems which integrate forestry plantations (*Eucaliptus spp*) with extensive beef production systems (Polla, 2000).

In many regions of the world these systems have proved beneficial for cattle production, by providing protection from extreme weather, mitigating the effects of climate change on animals, and promoting further improvements in productivity in a richer environment (Murgueitio, 2008; Tarazona et al. 2012).

The welfare can be measured considering animal based and environment based indicators. Indicators measured in the animal such as body condition, gain weight, presence or absence of visible lesions were registered (Huertas *et al.* 2011).

Animals at silvopastoral systems show a better adaptation both to the environment as to the people. Shadow allows animals to dedicate more time to graze and ruminate, consuming more food and decreasing water requirements, improving conversion efficiency. Likewise, there has been an improvement in reproductive behaviour (Ocampo *et al.* 2011).

In Uruguay, there are few studies on this subject, the main objective of this work is to evaluate the effect of silvopastoral systems on animal welfare comparing indicators between silvopastoral and the traditional extensive production system.

Materials and methods

A sample size of 40 Hereford steers, 18 months old, average weight of 314 kg (SD 27.9) were randomly allocated 20 in 100 hectares of natural grass (NG) and in 200 hectares of silvopastoral system (SPS) of 3 years old *Eucalyptus*

globulus, planted in a 4x2 meters design. Both paddocks had the same animal density, and similar pasture composition. Weather conditions were also recorded.

Studied animals were weighed individually every 45 days on a scale Tru-test MP600 placed at the exit of the squeeze chute, animal welfare indicators were registered according to the *Welfare Quality*® protocol adapted to local conditions (Huertas *et al.* 2009, 2011). All data was processed for statistical analysis using Stata 11 (Stata. Inc).

Results and discussion

The results presented here are still preliminary. Data was obtained during a period from August-December 2014. In comparing weights of the animals in both treatments, no significant differences were found ($p<0.05$). Remarkably, during the months in which the observations were made rainfall and temperature were recorded above the country average, allowed a better performance in both group of animals. Regarding AW indicators evaluated, no significant differences between treatments.

Conclusion

These results do not allow to establish differences between treatments in animal performance. Provided that these indicators have very low occurrence frequencies in traditional extensive production systems, and based on these preliminary results, we cannot state the existence of differences in the two systems compared. Maybe under adverse weather conditions differences can increase. However, the research is at initial stage, so future observations can alter the results obtained up to now and some differences between treatments may occur.

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TRIMMING FETLOCK HAIR EFFECT ON EQUINE HOOF THRUSH

J. Baruch, F. Vila, S. Huertas, A. Gil

Departamento de Bioestadística. Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay

SUMMARY

According to different authors the fetlock hair acts as a mechanism of draining from the equine member, avoiding the accumulation of humidity on the solar part of the hoof from rain, sweat or shower. This humidity is one of the risk factors for the equine hoof thrush causing lameness and pain. This factor is also described as foot rot for other species, like in sheep in which as equally as in horses the *Fusobacterium necrophorum* is isolated from the hoof. The objective of this study was to determine if there is a statistical association between trimming the fetlock hair and hoof thrush. During 2012-2013, 200 Thoroughbred horses from 20 different farms were evaluated for: trimmed fetlock hair and presence of any sign of hoof thrush. The association between variables was evaluated by Chi2 test (α=0.05). Of the 200 horses 26.0% had the fetlock hair trimmed and 20.5% presented signs of hoof thrush. There was a significant association between these two variables. In spite of this there was no difference in prevalence between farms or sex. Many farmers trim the fetlock hair because they claim that fetlock swelling can be detected easier, or just because they say it makes the horse look better, without considering that trimming it has a statistically significant risk factor for this pathology.

INTRODUCTION

Uruguay has approximately 425.000 horses, occupying the second place in the world ranking of horses per capita. According to a study from UruguayXXI (2012), 87% of the horses belong to farms and are dedicated to farm work, 7-8% belong to exposition breeds, 4-5% are sport horses and 1-2% are for tourism and education purposes (5).

Track horses represent 39%, endurance 22%, raid (Uruguayan endurance sport) 11% and polo the 5%. In Uruguay exportation of sport horses, goes up to 700 animals that represents US dollars 5millons. Meat horse and sub productsare US 23 million per year (5).

Total horse industry and related activities account for US 335 million annually in Uruguay. This includes salaries, competition money etc. Another relevant fact is that competition horses directly employ around 18 thousand people without considering indirect job positions (5).

There are several racetracks in Uruguay, the biggest one is *"Hipódromo Nacional de Maroñas"* with approximately 2000 horses and *"Las Piedras"* both in the international circuit.

The fetlock hair is a tuft of hair that comes from the fetlock joint. According to different authors, it acts as a draining mechanism from the equine limb, preventing the accumulation of humidity from rain, sweat or shower in the solar part of the hoof (6).Humidity is a risk factor for equine hoof thrush. Thrush is defined as a degenerative condition of the frog involving the central and lateral sulci, which is characterized by the presence of black necrotic exudates and a foul odor. This pathology is generally associated with unhygienic conditions such as an accumulation of moist sawdust, manure, and other organic material (4, 3, 7, 2).

All these factors create a favorable environment for bacteria commonly isolated from the affected hoof, *Fusobacterium necrophorum (F.n.)*. Humidity is also described as a risk factor for foot rot in other species, like in sheep (3).

Footrot in sheep, goats, cattle and pigs, is caused by the synergic action of *F.n.* and *Dichelobacter nodosus (D.n.).* In horses,seems to be caused only by *F.n.* as showed by Petrov (2013).

There are few scientific data on the association between trimming the fetlock hair and hoof thrush. The objective of this study was to determine if there is an association between those characteristics.

MATERIALS AND METHODS

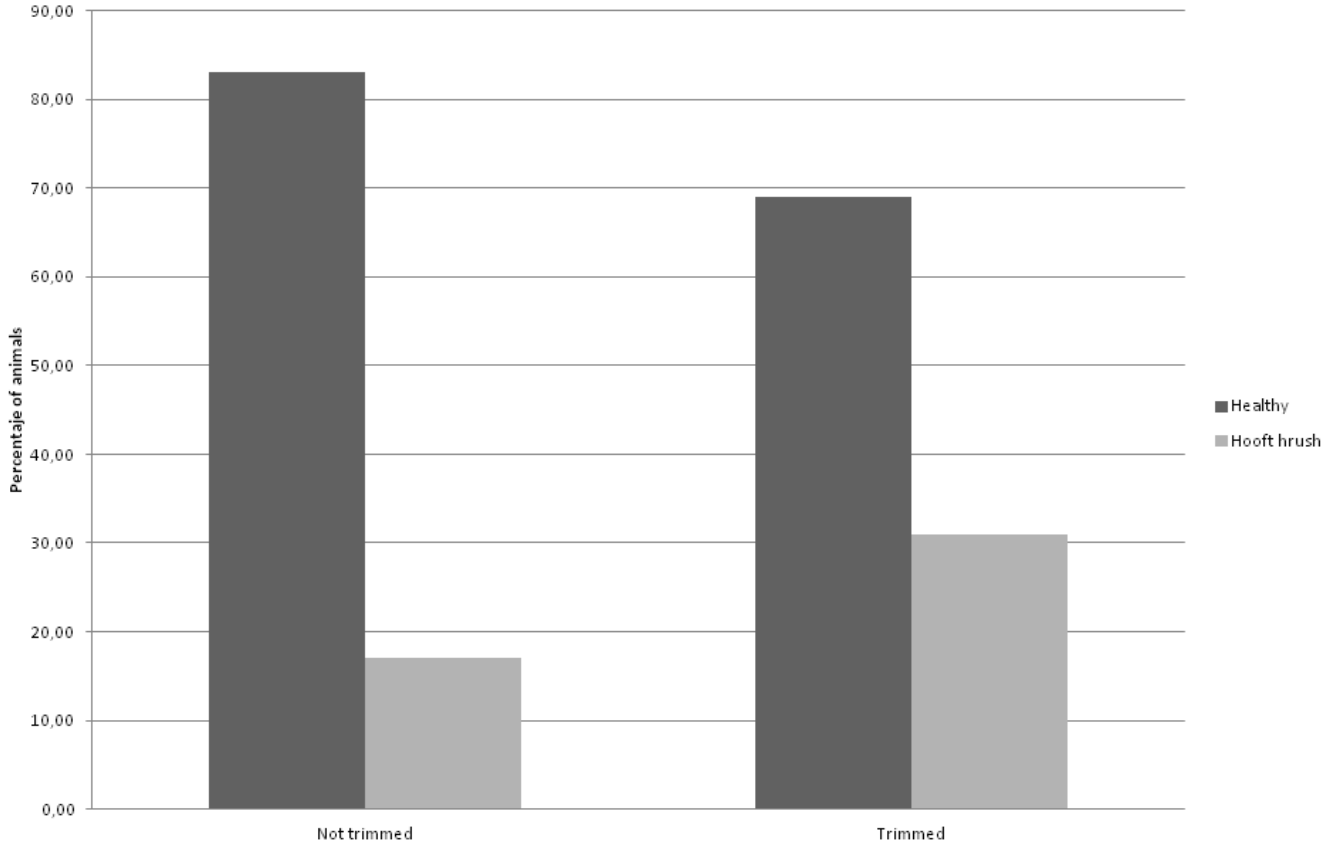
During 2012-13, 200 Thoroughbred horses from 20 different farms belonging to the *"Hipódromo Nacional de Maroñas"* racetrack were evaluated for trimmed fetlock hair and presence of any sign of hoof thrush. The selection criteria was the predisposition of the owner to receive the research team. Data was collected by one researcher previously trained for the task. The association between variables was evaluated by Chi2 test (α=0.05).

RESULTS

The average of animals per farm was 10 (minimum 1 to maximum 35), from which 102 were males and 98 females. The ages ranged from 2 to 7 years with a mean of 2.9 years.

From a total of 200 animals observed, 26.0% (n=52) had the fetlock hair trimmed and 74% (n=148) did not. The 20.5% (n=41) presented signs of hoof thrush and 79.5% (n=159) did not. Figure 1 shows a higher proportion of animals with signs of hoof thrush in those who had the fetlock hair trimmed. There was a significant association between these variables (α=0.05). Despite this, there was no difference in prevalence between farms or sex.

Figure 1



DISCUSSION

Fetlock hair are part of the horse and acts as a natural drain from the leg avoiding excessive humidity of the hoof (3, 7). There would be no reason to trim it, but many trainers trim the fetlock hair because they claim that fetlock swelling can be detected easier, or just due to aesthetical purposes, those were some of the answers when asked the reason for trimming.

As mentioned by some authors, humidity is a risk factor to hoof thrush (6, 4, 2) confirming our hypothesis that trimming the fetlock hair promote the hoof thrush, according to our results.

No significant differences between males and females were found, indicating that the management in this racetrack seems to be the same to both sex. Similarly, there is no difference between the proportions of hoof thrush among farms, suggesting also the same criteria on animal handling procedures.

CONCLUSION

Owners, trainers, vets and general people working with horses should consider the welfare of the animal beyond the look of the leg, and avoiding the elimination of natural ways in which the defense mechanism of the horse works.

If we consider the economic importance of the horse industry in Uruguay we think there should be more investigation about the welfare of horses and not just training and nutrition related research.

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THE INFLUENCE OF HOUSING SYSTEM ON VOLUNTARY ACTIVITY OF HORSES

K. Gulbrandsen[1], A.H. Herlin [2]

[1]Equine Studies Program, Swedish University of Agricultural Sciences, Flyinge, Sweden
[2]Dept. Biosystems and Technology, Swedish University of Agricultural Sciences, Alnarp, Sweden

Summary

The influence of three housing regimes were investigated on physical activity (motion index) and lying behaviour by the use of accelerometer technology (IceTag®; IceRobotics Ltd, UK) during 10 days per treatment. The housing regimes were: Single box with horses turned out three to four hours during daytime and in a box during night (BOX NIGHT), single box from 07:00 to 16:00 and in a group outdoors between 16:00 and 07:00 (OUTDOORS NIGHT) and loose housed in a group (LOOSE). The plan was to study five horses in each treatment BOX NIGHT and OUTDOORS NIGHT and subsequently move them to the housing regime LOOSE. Due to failure of equipment and of horses being unable to adapt to the loose housing system some data and horses were omitted and replaced. Thus, a total of 12 horses were studied, six of them were only in one housing system and six of them changed housing system. Horses in the BOX NIGHT had less than half of the activity than the horses in the LOOSE and OUTDOORS NIGHT. There was no difference at the group level, but individual responses could be seen. Daily lying time was about 100 ±40 minutes in the BOX NIGHT horses and less than half in OUTDOORS NIGHT and LOOSE horses 42 ± 19 and 37 ±26 minutes respectively. More freedom will result in more voluntary exercise and less lying.

Introduction

Freedom of movement allowing for voluntary, low intensity exercise is essential for welfare and it may also contribute to maintaining fitness. However, the usual housing alternatives used for horses have low or moderate opportunities of providing the horse voluntary physical activity. Voluntary activity of horses in a horse activating loose housing system depends on the horse's opportunities for moving such as the layout, placement of functional elements and feeding frequency (Rose-Meierhöfer et al. 2010a). There is also an effect of group size on activity (Rose-Meierhöfer et al. 2010b). Chaplin and Gretgrix (2010) found the total time spent active of horses kept in paddock to be the twice the time of horses kept in a yard and the fully and partially stabled horses being less than half of the yard kept horses. Recumbent lying is observed to be longer in horses kept in single boxes during the night than in loose housing (Hoffman, 2012) or in horses turned out at night (Lindberg et al. (2013). There is an effect of increased voluntary locomotor activity on bone density and improved fitness (Graham-Thiers et al 2013). The aim of this study was investigate the effects of housing systems which provides different opportunities for voluntary exercise on horse activity and recumbent resting.

Materials and Methods

The study was performed at the equine center "Flyinge" in southern Sweden using horses, of Swedish Warmblood breed, included in the equine studies program and used daily. The studied housing regimes were: Single boxes with horses turned out in paddocks, together with a few other horses, three to four hours during daytime (BOX NIGHT), single boxes from 07:00 to 16:00 and turned out in groups of between 16:00 and 07:00 (OUTDOORS NIGHT) and loose housed in an Active-Stable system® (LOOSE). The studies were performed during two periods. The two housing systems using single boxes (BOX NIGHT and OUTDOORS NIGHT) were studied in the first period. Horses were then moved to the Active-Stable system® (LOOSE) in the second period. Five horses were each allotted to the initial two single box treatments in period 1 but three horses of each single box treatment could fulfil the treatment in period 2. Four horses were thus added to the LOOSE treatment. A total of twelve horses were studied. In total, six horses were able to complete the both treatments while six horses only completed one treatment. The reasons for this were equipment failure and horses not being able to cope with the LOOSE treatment.

The activity of the horses was recorded during ten days in each housing system by the use of accelerometer technology (IceTag®; IceRobotics Ltd, Edinburgh, UK). The behavioural parameters produced by the IceTags were motion index and lying time. Data were compiled in Excel (means and standard deviations) and students T-test, used for the statistical analysis.

Results and discussion

Activity – motion index

The three horses, that moved from BOX NIGHT to LOOSE increased motion index significantly ($p<0.05$) from 6335 (± 1160) to 17460 (± 1891). The average motion index index for all five horses in the BOX NIGHT group was 6690 (± 2588). The three horses that initially was kept in OUTDOORS NIGHT had here an motion index of 15087 (± 3014) and in the LOOSE 12768 (± 2182) (n.s.). There was a significant ($p>0.05$) difference in activity between the BOX NIGHT compared to the OUTDOORS NIGHT horses. The higher activity level in the housing systems with more freedom is suggested to be beneficial to the athletic horse.

Lying time

The average total lying time in all BOX NIGHT horses was 100 (±40) minutes, in the OUTDOORS NIGHT horses 42 (± 19) minutes and in the LOOSE horses 37 (±26) minutes.

Horses kept in OUTDOORS NIGHT and moved to LOOSE did not change in lying time. The importance of the different lying times and the effect of housing system on the lying time is not fully understood but horses have the ability to rest standing and there is very little known on the horses need for recumbent resting.

Conclusions

Housing systems for horses providing more freedom resulted in more voluntary exercise and less lying. The implications of the longer lying times in horses kept a major part of the time in boxes are not fully understood.

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AMMONIA AND HYDROGEN SULPHIDE CONCENTRATIONS AND EMISSIONS IN LARGE-SCALE UNINSULATED LOOSE-HOUSING COWSHEDS IN TEMPERATE CLIMATE CONDITIONS

A. Kaasik

Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia

Summary

The mean ammonia concentration in the indoor air of large scale uninsulated loose housing cowsheds was 2.43 ppm, varying on a monthly basis within the range of 0.0 to 9.7 ppm. The average emission of ammonia in dairy cows barn was 5.8 kg (emission factor 3.2%) and of hydrogen sulphide 0.31 kg per animal per year. The annual average indoor temperature in barns was 11.0 °C (on a monthly basis the range was -5.8 to 22.6°C), and relative humidity was 70.3% (on a monthly basis the range was 52.2 to 96.8%). As the indoor temperature in uninsulated housing depends directly on the outside temperature, the total emissions of pollutants during the cold season of the year were significantly lower compared to the warmer period.

Introduction

The first large scale (more than 300 dairy cows) uninsulated dairy farm was founded in Estonia in the year 2003. Since then, all new or renovated dairy farms have used this housing system. Today there are more than 150 farms of this type. Due to the excellent ventilation of these buildings the barn microclimate would be expected to be acceptable, but there is no precise overview about main pollutants (ammonia and hydrogen sulphide) concentrations and emissions in this type of housing.

Estonia is in a temperate climate zone. The annual mean air temperature is 5°C. Commonly the coldest month is February, with a mean temperature of -5°C, and the warmest month is July with a mean temperature of 18°C. The annual mean relative humidity is about 80-83% (EMHI, 2011).

Ammonia is formed in housing from manure, especially from urea in urine. The main source of hydrogen sulphide in animal housing is also manure. Ammonia and hydrogen sulphide formation from manure is a microbial process. For H_2S formation the environment should be anaerobic. NH_3 and H_2S emission from manure depends directly on the composition of the feed ration and also on the balance of nutrients, available for rumen microorganism protein and energy requirements (Swensson, 2007). The concentration of ammonia and hydrogen sulphide in indoor air is affected by the indoor (and therefore outdoor) temperature and the ventilation rate. NH_3 and H_2S emissions in the colder period of the year are significantly lower than other periods, when the living conditions for microorganisms are not optimal (Zhang et. al., 2007, Burton et. al., 2007, Smits et. al., 2007).

Material and methods

The measurements were carried out in three uninsulated (2) or semi insulated (1) large-scale loose-housing dairy farms. The measurements were carried out, and data were collected, once a month in the period from October 2012 till October 2013. For contaminant assessment the concentrations (ppm) of ammonia (NH_3), hydrogen sulphide (H_2S) and carbon dioxide (CO_2) were determined, and temperature (°C) and relative humidity (%) were also measured. The duration of each measurement was 24 hours, the concentrations of carbon dioxide, ammonia and hydrogen sulphide were measured once per minute. The interval between each measurement of temperature and relative humidity was once per hour. The list of measuring devices used and their technical parameters are given in table 1.

Table 1: List of measuring devices used

No	Device	Parameter measured	Description
1	Dräger X-am 7000	CO_2; NH_3; H_2S	(Dräger Safety GmbH) Electrochemical NH_3 analyser, measuring range 0-200 ppm; electrochemical H_2S analyser, measuring range 0-20 ppm; infrared CO_2 analyser, measuring range up to 50,000 ppm
2	Jerome 631-x	H_2S	Measuring range 0.003-50 ppm
3	Rotronic HygroLog	Temperature; air humidity	(Rotronic AG)

To calculate the ventilation capacity the carbon dioxide mass balance method was used (equation 1).

$Q_v = P / C_{in} - C_{out}$, where, Equation 1

P = Gas emission g per hour. For the CO_2 coefficient, 330 g/h per dairy cow was used (CIGR, 1984);

C_{in} and C_{out} = CO_2 concentration (ppm) in the barn and outside.

Results and discussion

The mean emission of ammonia in dairy cows barn was 5.8 kg (emission factor 3.2%) and for hydrogen sulphide 0.31 kg per animal per year. The average ammonia emission per cow per year on Danish farms with same housing technology is 5.4 kg, with an emission factor of 3.8% (Poulsen, 2012). The average ammonia concentration in the indoor air was low, 2.43 ppm, varying on a monthly basis in a range of 0.0 to 9.7 ppm. The annual average indoor temperature in the barns was 11.0 °C (on a monthly basis in a range of -5.8 to 22.6°C) and relative humidity 70.3% (on a monthly basis in a range of 52.2-96.8%). In figure 1 are shown relationships between ammonia and hydrogen sulphide emissions in uninsulated loose housing cowsheds and the inside temperature. There was strong positive correlation between ammonia emission and temperature (r = 0.81). The correlation between H_2S emission and temperature was weaker (r = 0.48).

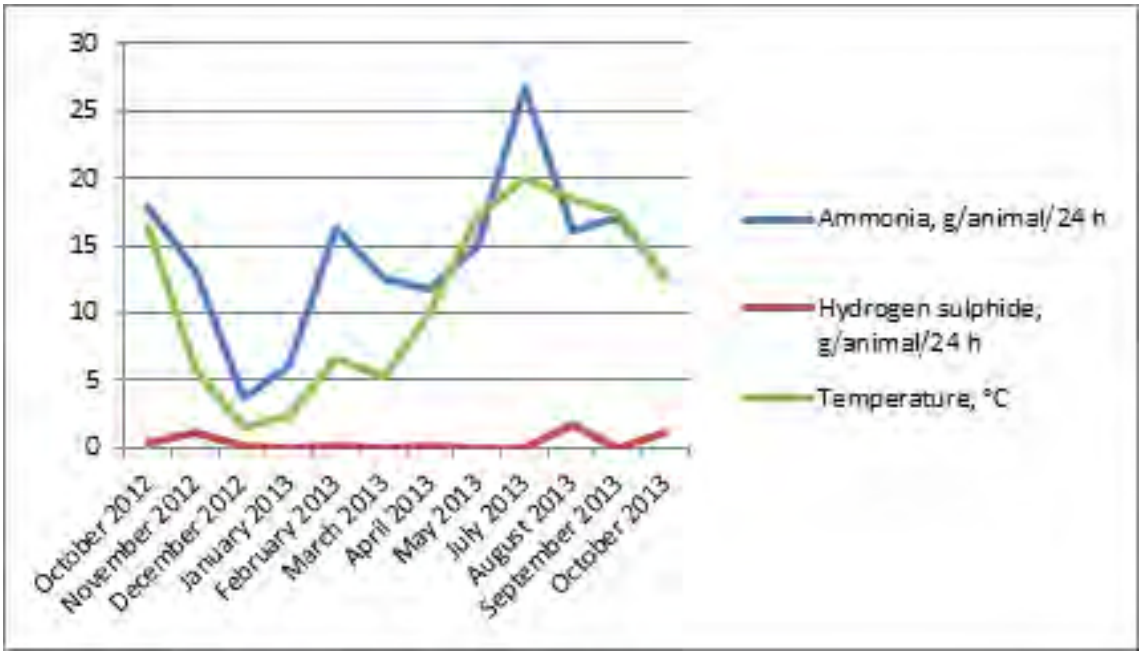

Figure 1. Relationships between NH_3 and H_2S emissions and indoor temperature

Conclusions

The average ammonia and hydrogen sulphide concentrations in the indoor air of large-scale uninsulated loose-housing cowsheds are low. As the indoor temperature in an uninsulated barn depends directly on the outside temperature, the total emission of pollutants during the cold season of the year was significantly lower compared to the warmer period. However, the concentrations of pollutants were higher in the cold period because the volume of ventilation was reduced due to the closure of ventilation openings and curtains during the cold season.

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Antibiotic resistance

OCCURRENCE OF CARBAPENEM RESISTANT AND CARBAPENEMASE PRODUCING ENTEROBACTERIACEAE (CPE) ISOLATED ON GERMAN PIG-FATTENING FARMS

Nicole Roschanski[1], Anika Friese[1], Annemarie Käsbohrer[2], Lothar Kreienbrock[3] and Uwe Roesler[1]

[1] Free University Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany; [2] Federal Institute for Risk Assessment, Department Biological Safety, Berlin, Germany; [3] University of Veterinary Medicine Hannover, Institute for Biometrics, Epidemiology and Information Processing, Hannover, Germany

1. Introduction

As carbapenems are mostly considered as drug of last choice for the treatment of serious infections in human, the increasing spread of carbapenem resistance among Enterobacteriaceae is quite alarming. During the last couple of years a wide variety of carbapenemases has been isolated from cases of human infections, but just a few studies have reported their occurrence in livestock and livestock associated surroundings. However, since recently Enterobacteriaceae carrying *bla*$_{VIM-1}$ genes have been isolated in German animal husbandries (Fischer et al., 2012; 2013), the monitoring of CPE in livestock became a major topic within the European Union.

2. Materials and Methods

Within the here described study a collection of 238 pooled feces and boot swab samples, chosen from a cross-sectional study including 58 pig-fattening farms throughout Germany was investigated. The bacteria were selected on MacConkey agar plates containing 0.125 µg/ml Meropenem. Enterobacteriaceae which were able to grow on these plates were further investigated by using different phenotypic- as well as genotypic approaches.

3. Results

Four *Escherichia coli*, two *Enterobacter cloacae* and one *Proteus penneri*, showing either resistance or reduced susceptibilities against carbapenems, were isolated from five different farms.

Two of the *E. coli* strains, derived from one farm, contained the carbapenemase gene *bla*$_{VIM-1}$. The remaining Enterobacteriaceae did not show the presence of such a resistance gene. In these cases other resistance mechanisms, leading to reduced carbapenem susceptibility, were detected.

4. Conclusions

Until now, CPEs within German pig-fattening farms show a low prevalence (1 out of 58 farms, 1.72%). Furthermore, non-CPEs showing increased carbapenem tolerance have been detected. These findings indicate that Carbapenem resistant Enterobacteriaceae might be present in animal husbandries. To prevent a further spread of these bacteria between farms and farm animals, an understanding of the routes of introduction together with consequent monitoring programs will be necessary.

SURVEY OF LISTERIA MONOCYTOGENES IN RAW AND TREATED MANURES FROM TWO PIG FARMS

A-M. Pourcher [1,3], C. Ziebal [1,3], E. Boscher [2,3], S. Picard [1,3], M. Perrot, J. Desneux [1,3], A. Diara [1,3], M. Denis [2,3]

[1] Irstea-Rennes, Rennes, France ; [2] Anses, Unité HQPAP, F-22440 Ploufragan, France ; [3] Université Européenne de Bretagne, Rennes, France

Summary

Land application of manure and manure by-products may increase the environmental risks through dissemination of pathogens. This study aims to evaluate the impact of two biological manure treatments on the levels of enterococci and *L. monocytogenes* and to estimate if physical and chemical characteristics of the effluents have an influence on the levels of the bacteria. Samples were collected monthly for one year in raw manure and in liquid fraction of the treated manure stored in a lagoon. Additional samples were collected in soil around the lagoon for the enumeration of *L. monocytogenes*. Strains of *L. monocytogenes* were serotyped by PCR and differentiated with RFLP-PFGE analysis. Enterococci level decreased by 3-4 $\log_{10}$ after treatment whereas *L. monocytogenes* was less impacted by the manure treatment. Except the temperature, no parameter was correlated with the levels of *L. monocytogenes*. The 252 isolates were divided into three serogroups present in the three matrices and were divided into 44 genotypes. Nine were found both in manures and lagoons suggesting that these strains could be of porcine origin; 17 only in lagoons and/or soils, suggesting an environmental origin of the strains.

Introduction

In order to limit surface water eutrophication from agricultural non-point sources, pig manure may need to be treated before spreading. However, these treatments which are initially designed to reduce P and N levels, have a relatively low impact on pathogens. Among them, *L. monocytogenes* is of particular interest due to its high prevalence in pig feces (Boscher *et al*., 2011). Moreover, the presence of *L. monocytogenes* in lagoon effluents (liquid fraction obtained after decantation or mechanical separation of treated manure) at a frequency higher than that of the raw manures, suggested that the lagoons, used to water crops, may be a favorable environment for the survival of *L. monocytogenes* (Pourcher *et al*., 2012). This study aims to acquire new data on the impact of manure treatments on enterococci and *L. monocytogenes* and to estimate the influence of physical and chemical characteristics of the effluents on *L. monocytogenes.*

Material and methods

Two pig farms were sampled. The treatment consisted of storage of raw manure in a tank, followed by a mechanical separation step and by an aerobic digestion of the liquid fraction. Treated manure was separated in a settling tank (farm 1) or dehydrated using a filter band press (farm 2). The liquid fraction was removed and sent to a lagoon. Raw manure and lagoon effluent were sampled monthly for a year. Supplementary samples were collected over the year in soil located around the lagoons and in lagoons. For each farm, 12 samples were collected in manure tank and 20 in soil and lagoon.

Enterococci were enumerated on selective Slanetz–Bartley agar incubated at 37 °C for 48 h. Colonies were confirmed on Bile Esculin agar incubated at 44 °C for 2 h. *L. monocytogenes* was enumerated by using MPN method, with One-Broth medium incubated at 30 °C for 48 h and RAPID'L.Mono™ incubated at 37 °C for 48 h. Microbial analyses were performed on three replicates. A total of 252 strains of *L. monocytogenes* were serotyped by PCR and differentiated with RFLP-PFGE analysis.

Results and discussion

The mean monthly values of the chemical and physical parameters of the four effluents are reported on Table 1. Manure 1 contained less organic matter (OM) and TKN than manure 2 whereas ion contents were in the same order of magnitude in the two manures.

Table 1. Mean values of the chemical and physical parameters of manures and lagoons

parameter	Manure 1	Manure 2	Lagoon 1	Lagoon 2
pH	7.7	7.8	8.8	8.9
OM g/L	16.9	26.4	1.0	2.5
TKN g N/L	2.9	4.1	0.1	0.2
PO_4^{---} g P/L	0.04	0.06	0.1	0.08
Mg^{++} g/L	0.04	0.01	0.1	0.06
Ca^{++} g/L	0.2	0.07	0.04	0.05
K^{+} g/L	2.0	2.3	2.4	2.6
NH_4^{+} g/L	1.4	1.9	0.004	0.1
Temperature °C	12.5	13.4	11.3	10.9

The lagoon effluents were less concentrated than the manures and have a higher pH. The biological treatment and the separation step of the treated manure led to a reduction of OM in the effluent stored in the lagoon.

Enterococci levels were stable in manures and lagoons (Fig.1A) during the year of the study. Their concentrations in lagoons, regardless the farm and the date of sampling were *ca.* 3 Log_{10} lesser than in manures.

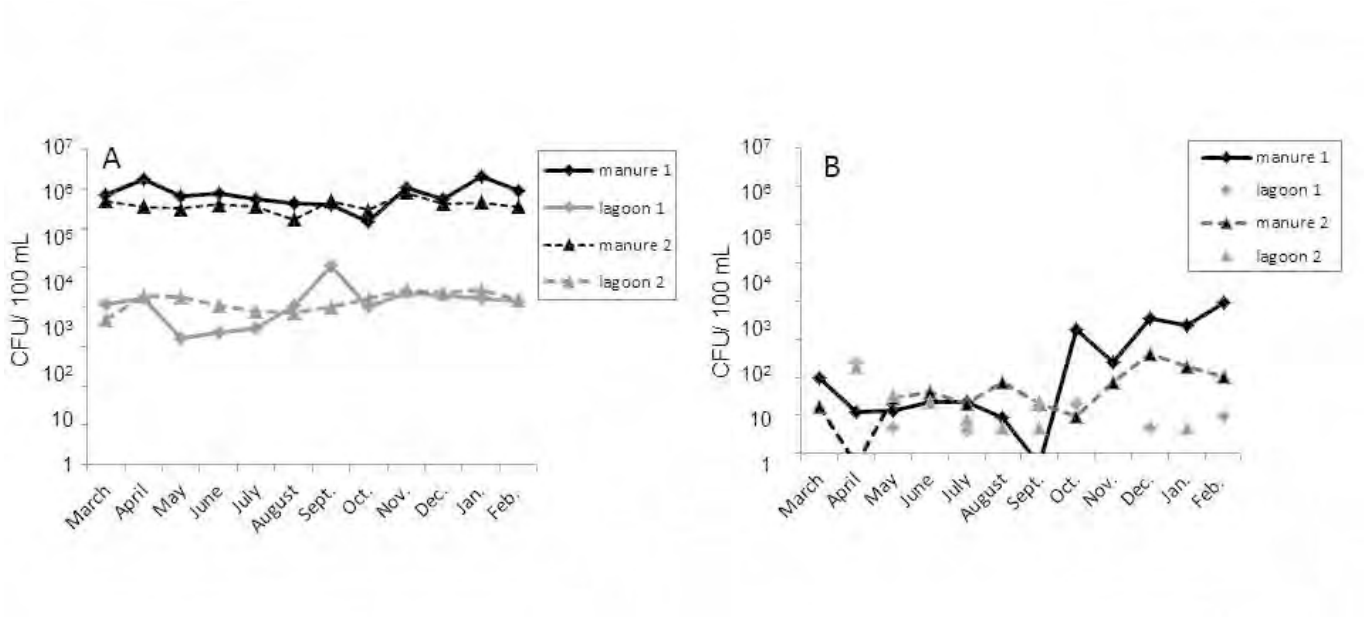

Figure 1. Average concentrations of enterococci (A) and *L. monocytogenes* (B) in manures and lagoons during one year.

L. monocytogenes was present in 91.7 % of manure, 58.3% of lagoon and 50% of soil samples. Its presence in manure is due to carriage in pigs. Indeed, Boscher *et al.* (2011) reported a carriage of *L. monocytogenes* of feces of sows in 46% of the farms and a carriage in pigs in 25% of farrow-to-finish farms. When *L. monocytogenes* was detected, its level ranged between 0.5 and 10^3 MPN/100 mL (Fig. 1B). The difference of levels between manures and lagoons was lesser (1.6 log_{10}, farm 1 and 0.4 log_{10}, farm 2) than that observed for enterococci. The highest levels of *L. monocytogenes* were observed during the coldest months in manures. This is in agreement with the data reported by Boscher *et al*. (2011) who observed a highest frequency of detection of *L. monocytogenes* in pig feces at the fall/winter season. Except the temperature in the manures, none of the factors correlated with the levels of *L. monocytogenes*. The 252 isolates were divided into three serogroups present in the three matrices. Serogroups IIa and IIb dominated in farm 1, whereas serogroup IVb was dominant in farm 2 (Fig. 2A). The serotypes 1/2a, 1/2b and 4b have been also recovered from feces of pigs and from French pork-processing plants (Chasseignaux *et al.*, 2001, Boscher et *al.*, 2011).

The isolates were divided into 44 genotypes.

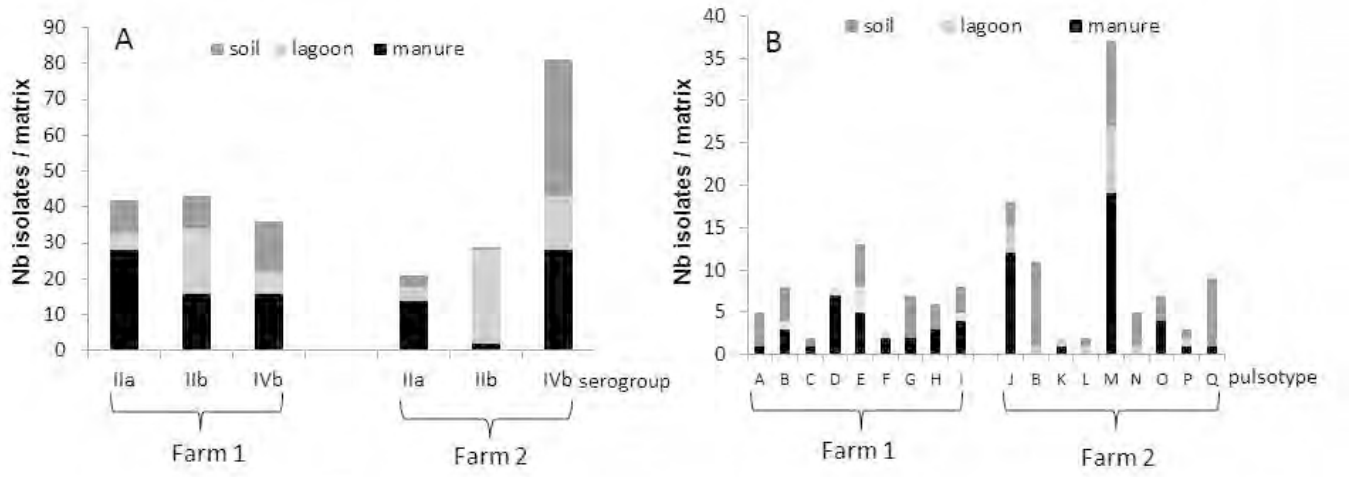

Figure 2 : Serogroups (A) and pulsotypes (B) found in 2 or 3 matrices in each farm

Nine were found both in manures and lagoons suggesting that these strains could be of porcine origin (Fig. 2 B). Seventeen were found only in lagoons and/or soils, suggesting an environmental origin of the strains.

Conclusion

Biological treatment of manure has little effect on *L. monocytogenes* levels. The detection in lagoons through the study period of pulsotypes not found in manures, suggests their capacity to survive in these manure by-products which could thus contribute to their spread in the environment.

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MSSA AND MRSA CO-COLONIZATION DYNAMICS AND CLONAL DIVERSITY IN PIGS

A. Friese[1], U. Roesler[1], B. Kraushaar[2], A. Fetsch[2]

[1]Institute for Animal Hygiene and Environmental Health, Freie Universität Berlin, Robert-von Ostertag-Straße 7-13, 14163 Berlin, Germany; [2]Federal Institute for Risk Assessment, Department Biological Safety, Diedersdorfer Weg 1, 12277 Berlin, Germany

Introduction

Methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) are colonizers of skin and mucosa. Studies about MSSA and MRSA in human patients showed a competition of these two microorganisms for colonization space in the anterior nares. Moreover, in humans one clone can be found rather than differing types of MSSA and MRSA. Concerning a potential control strategy, this could also be important for pigs. Therefore, the aim of this study was to investigate the colonization dynamics of both MSSA and MRSA in pigs over a longer time period and investigate their clonal diversity.

Materials and Methods

Eighteen pigs were sampled three times every ten weeks with a nasal swab. Additionally, environmental samples such as swabs of walls, toys, brushes, watering places and troughs were taken. All samples were investigated for MSSA and MRSA, respectively. *Spa*-typing was done with up to five MRSA and MSSA isolates, respectively, found per sample and time point. Of almost 400 MSSA and MRSA isolated, 62 isolates were further investigated by microarray.

Results

We found three pigs to be non-carriers and twelve were colonized with both MRSA and MSSA. In 14 out of 54 samples taken from all animals over all-time points MSSA were found, only. In comparison, nine samples were exclusively MRSA-positive. *Spa*-types of MSSA and MRSA were mostly different with CC398 associated *spa*-types within MRSA isolates, whereas CC9 associated *spa*-types predominate as MSSA in both, pigs and their environment. Moreover, strains of the same clonal lineage showed a high genetic identity despite their origin.

Conclusions

The results do not support the hypothesis of a competitive colonization of MSSA and MRSA in the anterior nares of pigs. Rather we found a changing status. Hence, highly identic clones of MSSA and MRSA, respectively, are present in the anterior nares of pigs and their environment.

MOLECULAR CHARACTERIZATION, PHAGE TYPES AND ANTIMICROBIAL SUSCEPTIBILITIES OF SALMONELLA ENTERICA SUBSP. ENTERICA SEROVAR ENTERITIDIS FROM CHICKENS AND CHICKEN MEAT IN TURKEY

H. Kalender[1], S. Şen[2], H. Hasman[3], R. S. Hendriksen[3], F. M. Aarestrup[3]

[1] Department of Bioengineering, Faculty of Engineering, Firat University, Elazig,Turkey ; [2] Etlik Veterinary Control Central Research Institute, Ankara,Turkey; [3] National Food Institute, Technical University of Denmark, Copenhagen, Denmark

Summary

The aim of this study was to examine *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S.* Enteritidis) strains isolated from chickens and chicken meat in Turkey by Pulsed-Field Gel Electrophoresis (PFGE), phage typing and plasmid profile analysis, and determine the occurrence of antimicrobial resistance of *S.* Enteritidis strains. A total of 38 *S.* Enteritidis strains (17 fecal and 21 meat isolates) were analysed by PFGE after digestion of DNA with *Xba*I restriction enzyme. Seven different PFGE profiles were observed. A predominant type (X1) was found in 27 (71%) of *S.* Enteritidis strains. Five different plasmid profiles were observed among the strains. Twenty five (65.78%) of the strains had the same plasmid profile (P2). The most common phage type was PT4, which was found in 15 (39.4%) of the strains. In antibiotic susceptibility test, twenty-one isolates were susceptible to all antimicrobial agents tested whereas seventeen were resistant to one or more antimicrobials.

Introduction

Enteritidis is one of the most common causes of human salmonellosis worldwide and poultry and poultry products are considered to be a major source of infections. Forty-seven percent of *Salmonella* strains isolated from humans between 2000 and 2002 in Turkey belonged to *S.* Enteritidis and this serovar is also the most common one isolated from chickens and chicken meat (3,4).

Phage typing and antibiotic susceptibility testing are traditional methods used for epidemiological studies. However, molecular techniques such as pulsed-field gel electrophoresis (PFGE), plasmid profiling and ribotyping have recently been used for subtyping of *S.* Enteritidis isolates (1,7). Development of resistance in zoonotic bacteria constitutes a public health risk, and *Salmonella* resistant to multiple antimicrobial agents have emerged worldwide recently (1, 2).

This study was conducted to determine the occurrence of antimicrobial resistance and occurrence of resistance genes, phage types, and molecular variation of *S.* Enteritidis isolates from chickens and chicken meat in Turkey.

Materials and Methods

A total of 38 *S.* Enteritidis isolates were examined in this study. Isolates originated from chicken fecal (17) and chicken meat (21) samples in Turkey. Serotyping and phagetyping were performed.

Susceptibility to antimicrobial agents was determined as minimum inhibitory concentration (MIC) determinations using a commercially prepared,dehydrated panel (Trek Diagnostic, England). The presence of *tem, int-1, aadA, sul1, aac(3)-IIa* and *ant(2)-Ia* genes was performed by PCR.

Plasmid DNA was purified by an alkaline lysis method. *Sma*I enzyme (Fermentas) was used for digestion of plasmid DNA.

Pulsed-field gel electrophoresis was performed using XbaI (Fermentas) according to CDC PulseNet protocols.

Results

Twenty-one (55%) of the 38 isolates were susceptible to all antimicrobial agents tested. All isolates were susceptible to chloramphenicol, ceftiofur, amoxicillin+clavulanic acid, ciprofloxacin, colistin, florfenicol, tetracycline and trimethoprim. Twelve isolates were resistant to nalidixic acid and had reduced susceptibility to ciprofloxacin. Two isolates were resistant to ampicillin, while six isolates were resistant to gentamicin, spectinomycin, streptomycin, and sulphamethoxazole. Seven different PFGE pattern were observed among the 38 isolates and their phylogeny is

shown in Fig.1. The most common PFGE pattern was X1(71%). The most common phage type was PT4, which was found in 15(39.4%) of the isolates. Other phage types were PT7, PT16, PT1, PT6, and PT35 (Table 1).

All six streptomycin-resistant isolates gave positive reactions for *aadA, int-1,* and *sul1*. PCR also confirmed the location of the *aadA* genes in the class 1 integron structure. Both ampicillin resistant isolates were positive for *tem*. All six gentamicin-resistant isolates were negative for *aac(3)-IIa* and *ant(2)-Ia* genes.

A total of five different plasmid profiles were found among the isolates. The predominant plasmid profile was P2 (65%).

Discussion

In the present study, most of the isolates shared the same PFGE pattern. A predominant PFGE type has also been found in other studies on *S.* Enteritidis (5, 6). The predominant PFGE type contained different phage types, which is consistent with the results of other researchers (2, 5). Phage typing was able to further subdivide the PFGE type, whereas the opposite was not the case. This thus, supports the use of phage typing as a valuable tool for epidemiological typing of *S.* Enteritidis. It was previously reported that the combined use of molecular and phenotypic techniques allowed more accurate discrimination within *S.* Enteritidis strains (5).

In the present study, the predominant phage type was PT4. This phage type has been frequently reported from both poultry and humans in recent years (1, 5) and is also the predominant phage type among human isolates from Turkey (1). Thus, our results indicate that both human and poultry isolates share the same phage type in Turkey.

We found a frequent occurrence of resistance to nalidixic acid among the isolates.The observed resistance to quinolones is a potential public health risk because this group of antibiotics often is the drug of choice for treatment of infections in humans. This study is first to identify resistance genes among *S.* Enteritidis isolates from animals in Turkey. Our results confirm the presence of resistance genes associated with integrons in *S.* Enteritidis isolates that have also been reported by others (4, 7).

Conclusions

This study showed the diversity of *S.* Enteritidis strains found in chickens and chicken meat in Turkey. Plasmid profiling, phage typing and PFGE were efficient for discrimination *S.* Enteritidis isolates.

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Table 1. Phage Types, Plasmid Profiles, Antimicrobial Resistance, and Pulsed-Field Gel Electrophoresis (PFGE) Patterns of 38 *Salmonella enterica* Serovar Enteritidis Isolates from Broilers and Chicken Meat in Turkey.

Isolate No.	*Source*	*Geographic origin*	*PFGE pattern*	*Phage type*	*Plasmid profile*	*Resistance pattern*[a]
1	Broiler chicken	Eastern Turkey	X2	PT1	P0[b]	-
2	Broiler chicken	Eastern Turkey	X2	PT1	P0	-
3	Broiler chicken	Eastern Turkey	X2	PT1	P4	-
4	Chicken meat	Southern Turkey	X3	PT16	P4	GEN, SPE, STR, SMX
5	Chicken meat	Southern Turkey	X3	PT16	P4	GEN, SPE, STR, SMX
6	Chicken meat	Southern Turkey	X3	PT16	P4	GEN, SPE, STR, SMX
7	Chicken meat	Southern Turkey	X3	PT16	P4	GEN, SPE, STR, SMX
8	Chicken meat	Western Turkey	X1	PT7	P2	NAL
9	Broiler chicken	Eastern Turkey	X1	PT4	P2	-
10	Chicken meat	Western Turkey	X1	PT7	P2	NAL
11	Chicken meat	Western Turkey	X1	PT7	P2	NAL
12	Broiler chicken	Eastern Turkey	X1	PT4	P2	-
13	Chicken meat	Western Turkey	X1	PT7	P2	NAL
14	Chicken meat	Western Turkey	X1	PT4	P2	-
15	Broiler chicken	Eastern Turkey	X1	PT4	P2	-
16	Broiler chicken	Eastern Turkey	X1	PT4	P2	-
17	Chicken meat	Western Turkey	X1	PT7	P2	NAL
18	Broiler chicken	Eastern Turkey	X1	PT4	P2	-
19	Broiler chicken	Eastern Turkey	X1	PT4	P2	-
20	Broiler chicken	Eastern Turkey	X1	PT4	P2	-
21	Broiler chicken	Eastern Turkey	X1	PT4	P2	-
22	Chicken meat	Southern Turkey	X1	PT4	P5	GEN, SPE, STR, SMX
23	Broiler chicken	Eastern Turkey	X1	PT4	P2	-
24	Layer chicken	Eastern Turkey	X1	PT4	P4	-
25	Chicken meat	Western Turkey	X1	PT6	P2	NAL
26	Chicken meat	Eastern Turkey	X1	PT6	P2	-
27	Layer chicken	Eastern Turkey	X1	PT4	P2	-
28	Broiler chicken	Eastern Turkey	X1	PT4	P2	-
29	Broiler chicken	Eastern Turkey	X1	PT4	P2	-
30	Chicken meat	Western Turkey	X1	PT7	P2	NAL
31	Chicken meat	Southern Turkey	X1	PT7	P5	GEN, NAL, SPE, STR, SMX
32	Broiler chicken	Eastern Turkey	X1	PT4	P2	-
33	Chicken meat	Western Turkey	X1	RDNC	P2	NAL
34	Chicken meat	Western Turkey	X1	RDNC	P2	NAL
35	Chicken meat	Eastern Turkey	X4	PT16	P1	-
36	Chicken meat	Eastern Turkey	X5	PT6	P2	-
37	Chicken meat	Eastern Turkey	X6	PT35	P3	AMP, NAL
38	Chicken meat	Eastern Turkey	X7	RDNC	P3	AMP, NAL

[a]AMP, Ampicillin; GEN, gentamicin; NAL, nalidixic acid; SPE, spectinomycin; STR streptomycin, SMX, sulphamethoxazole
[b]No plasmids detected

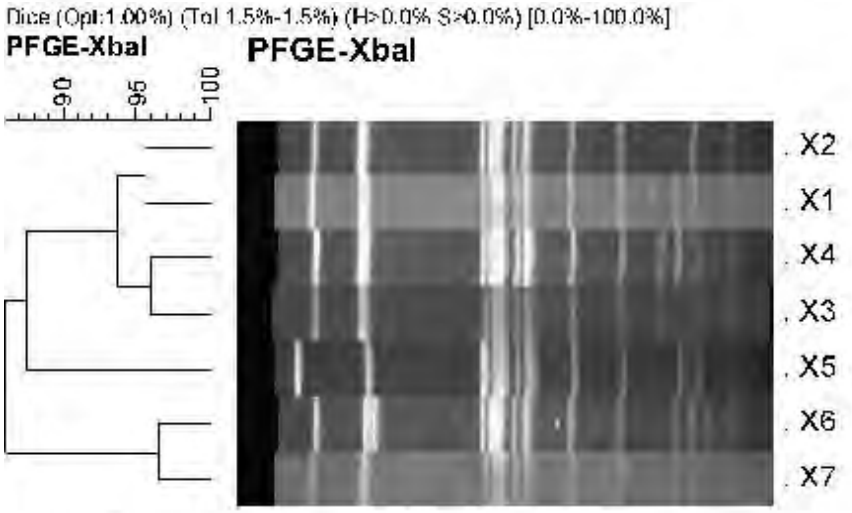

Fig. 1. Phylogeny of pulsed-field gel electrophoresis patterns of 38 *Salmonella* Enteritidis isolates obtained from broilers and chicken meat in Turkey.

EMISSION OF ESBL/AMPC-PRODUCING E. COLI FROM BROILER AND TURKEY FATTENING FARMS IN GERMANY

H. M. Lu[1], H. Laube[1], A. Käsbohrer[2], A. Friese[1] and U. Roesler[1]

[1] Free University Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany; [2] Federal Institute for Risk Assessment, Department Biological Safety, Berlin, Germany

Introduction

Extended-spectrum beta-lactamases (ESBL) reduce dramatically the effectiveness of modern extended-spectrum cephalosporins and monobactams. The aim of this study was to comparing investigate the occurrence of these microorganisms in the air and on soil surfaces close to ESBL/AmpC positive broiler and turkey fattening farms including the possible spread of these bacteria via the faecal rout and/or via exhaust stable air in the environment.

Materials and Methods

We investigated seven German broiler and turkey fattening farms, each, three times during one fattening period. Therefore, slurry samples, exhaust air samples and various surfaces in the vicinity of the barns were investigated. Additionally various samples inside the barns including samples of animals, environmental samples and air samples were taken in parallel. For phenotypic detection of ESBL/AmpC-producing E. coli all taken samples were cultivated on MacConkey-agar with 1µg/ml Cefotaxime. The occurrence of different ESBL/AmpC-genes was confirmed by PCR and if necessary by sequencing.

Results

Preliminary results show a wide spread of ESBL/AmpC-producing E. coli in pig and broiler farms as well as an emission in their surroundings. All slurry samples originating from broilers were positive for ESBL/AmpC-producing E. coli. Additionally 28.8 % of all boot swab taken from various surfaces in the surrounding of the broiler farms turned out to be positive for ESBL as well as 10 % of the exhaust air samples. In contrast, the so far investigated turkey farms show a significantly lower contamination of slurry samples and of the surfaces in the vicinity of the barns.

Conclusions

Faecal and airborne emission of ESBL/AmpC-producing organisms from broiler barns and in a significant lower extend also from turkey barns seems to be a possible source for the spread of ESBL in the environment. Further analyses on that topic are necessary.

OCCURRENCE OF DECREASED DISINFECTANT SUSCEPTIBILITY AMONG ESBL- / AMPC- PRODUCING E. COLI ISOLATED FROM BROILER

T.Li[1], N. Roschanski [1], A. Friese [1], U. Rösler[1]

[1]*Institute of Animal Hygiene and Environmental Health, Freie Universität Berlin, Germany*

Introduction

Disinfectants are widely used in different areas and an inappropriate use might cause the emergence of disinfectant-resistances, leading to further disinfection failure. Beyond this, the co-selection of disinfectant- and antibiotic- resistance has been reported in case of various bacteria. Due to the fact that the presence of ESBL/ AmpC dramatically limits the therapeutic options, the appearance of disinfectant-resistances in these kinds of bacteria might contribute a health thread for humans as well as animals.

Materials and Methods

90 ESBL-/ AmpC-producing *E. coli*, isolated from cloacal swabs of chicken out of seven broiler fattening flocks throughout Germany were investigated. For each strain the minimal inhibitory concentration (MIC) of three different disinfectants – Benzalkonium chloride (BKC), Chlorhexidine (CHX) and Triclosan (TCL) was determined. In addition, the presence of 5 different *qac* genes (*qacE, qacEΔ1,qacH, qacF* and *qacG*) was investigated.

Results

Compared to three *E. coli* reference strains, 23 samples showed decreased susceptibility to single or multiple disinfectants (8/CHX; 7/TCL; 3/BKC+CHX; 5/CHX+TCL). However, none of the strains was able to survive at concentrations recommended for disinfection with commercially available products.

The *qacEΔ1* gene was found in 18.89% samples, the frequency of *qacH* 6.67% was low while *qacE, qacF* and *qacG* were not detected. However, all isolates showing an increased MIC against BKC contain none of the investigated *qac* genes, whereas three BKC sensitive isolates contain *qacH* as well as *qacEΔ1* genes.

Conclusions

ESBL-/ AmpC- producing *E. coli* possessing decreased susceptibility to disinfectants have been detected within chicken in German broiler fatting farms. However, none of the detected MIC values indicate that accurately performed disinfection procedures will lead to ineffectiveness. In addition, no correlation between the presence of *qac* genes and the increased disinfectant tolerance has been observed.

TRANSMISSION OF ESBL-/AMPC-PRODUCING ENTEROBACTERIACEAE ALONG THE ENTIRE PRODUCTION CHAIN OF BROILERS

K. Daehre[1], M. Projahn[1], P. v. Tippelskirch[2], S. Orquera[2], T. Alter[2], A. Friese[1], U. Roesler[1]

[1] *Freie Universität Berlin, Institute of Animal Hygiene and Environmental Health, Berlin, Germany*
[2] *Freie Universität Berlin, Institute of Food Hygiene, Berlin, Germany*

Introduction

The occurrence of extended-spectrum ß-lactamase (ESBL) and/or plasmid-mediated AmpC ß-lactamase- producing *Enterobacteriaceae* in broiler fattening farms was shown in previous studies. The detection level of these antibiotic resistant bacteria was already high in cloacal swabs of one-day old chicken. This leads to the hypothesis of an early entry or emergence of these resistant bacteria within the broiler production chain, maybe with a strong impact of the hatcheries' management or the parent flocks.

Material and Methods

We are investigating this hypothesis by tracking seven broiler flocks along the entire production chain starting from the parent flocks. Several samples originating from the eggs/animals as well as from their direct environment in the hatchery/barn are collected. First, the presence of ESBL-/AmpC-producing *Enterobacteriaceae* is investigated in the parent flocks, only positive flocks are included in this study. The hatching eggs of the particular parent flocks are traced at three different time points and at specific locations of hazard inside the hatchery. After that the chicken from the same batch of hatched eggs as investigated before will be analysed three times during their fattening period in the farm, from arrival to the end. Then the same flocks are sampled at the slaughterhouse.

Results

The analysis of the first broiler chain showed no findings of ESBL-/AmpC-producing *Enterobacteriaceae* within the hatchery. Here the resistant bacteria initially appeared in the middle of the fattening period. In contrast to this in the second investigated chain ESBL-/AmpC-producing *Enterobacteriaceae* were already found in the hatchery.

Conclusion

By now a precise conclusion about the transmission of the resistant bacteria along the broiler production chain cannot be given. More production chains will be investigated and further analyses will lead to information about molecular characteristics and relationships of different isolates. These results of the ongoing project will be presented.

THE INFLUENCE OF ESBL-PRODUCING E. COLI FROM THE CHICKEN FARM TO SURROUNDING RIVERS

L.L. Gao, T.J. Chai, L.M. Wei

College of Veterinary Medicine, Shandong Agricultural University, Taian, China

Summary
The dissemination of drug-resistant bacteria from animal farms to aquatic environments can pose a potential threat to public health. In this study, antimicrobial resistance, resistance genes, and genetic similarity of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli of different origins were analyzed to track the spread of drug-resistant bacteria of animals.

Introduction

Extended-spectrum β-lactamases (ESBLs) are enzymes that able to hydrolyze the penicillins, the first-, second-, and third-generation cephalosporins, and aztreonam except cephamycins or carbapenems [1], which mainly exist in Enterobacteriaceae, especially in Escherichia coli (E. coli) and Klebsiella spp. [2]. Since firstly isolated in the 1980s, a growing number of ESBL-producing bacteria were gradually found in various environments, which has posed a great threat to public health [3].

Water environments have been believed to be the reservoir where bacteria from different origins are able to mix, and therefore resistance genes can spread and evolve [4]. Drug-resistant bacteria in water environments have become a potential risk to public health.

Up to date, a lot of research has been focusing on prevalence characterization and resistance mechanisms of the ESBL-producing E. coli [5]. However, little information about emissions of animal-origin ESBL-producing bacteria to nearby water bodies is available. In this study, we selected a big laying hen farm and its nearby river to isolate the ESBL-producing E. coli, and then resistance characteristics and genetic similarity of isolates were analyzed, with the aim to better understand the dissemination of drug-resistant bacteria of animal origins to nearby waters.

Material and Methods

From December 2012 to June 2013, 10 fresh fecal samples from each chicken house were collected each visit (10×5). At the same time, water samples were also collected from upstream 10m, downstream 10m, 50m, and 100m away from discharge outlet of the farm, and 9 water samples from one site were collected every time (9×4).

All the fecal samples were streaked EMB Agar plates and incubated at 37°C overnight. The water samples were filtered through 0.45 pore size membranes, and then the filters were placed on EMB agar plates incubated for 24h at 37°C. For each sample, the non-replicate colony with typical E. coli morphology and size was identified by both classical biochemical methods and API 20E system.

E. coli isolates were firstly screened by growth on MacConkey agar medium with cefotaxime, and further analyzed by phenotypic confirmatory test. Antimicrobial susceptibility of ESBL-producing strains to 18 antimicrobials was detected with ATCC25922 for quality control.

The DNA of ESBL-positive E. coli were extracted by the boiling method and then subjected to multiplex PCR to analyze the presence of SHV, TEM and CTX-M genes [6].

The genetic similarity was determined using ERIC-PCR.

Results

There were 29 non-replicate ESBL- producing E. coli identified from 150 manure samples and 108 water samples, including 16 strains from manure and 13 isolates from water. The ESBL- positive E. coli from the five chicken houses were 6, 3, 4, 2 and 1respectively. All the ESBL- producers from feces and water was resistant to at least 6 out of 18 antibiotics tested. The resistance rates of 16 fecal isolates to AMP, CXM and ATM were all 100%. All the ESBL-producing E. coli from water samples were resistant to CF, TE, SXT, AMP, CXM and ATM.

Among the 16 ESBL-producing E. coli from feces, 4 strains only carried TEM gene and 10 isolates contained CTX-M gene alone, and 2 strains carried both TEM and CTX-M genes. From the thirteen ESBL-producing E. coli from water, CTX-M gene was detected in 11 isolates followed by TEM gene in 6 isolates, and 4 isolates contained both TEM and CTX-M genes. SHV gene was not detected in this study.

The similarity between isolates from water and those from feces ranged from 65% to 100%. The strain TW002 from downstream water had 100%similarity with 2 fecal isolates TF032 and TF044, and downstream water isolate TW071 had 100% with TF081 from feces; isolates TW009, TW059, and TW051 from downstream water had above 90% similarities with the strains TF103, TF081 and TF065 from feces respectively. The only isolate TW017 from the upstream river water had about 65% similarity with fecal isolates.

Discussion

Of the fecal samples from the five houses, the isolation rate of the ESBL- producing E. coli was 10.7%, which was similar to that in Portugal, but lower to the results reported in Belgian [7]. The main reason may be related with the different medicine practices. In this study, the prevalence rate of the ESBL producer in downstream water samples was 14.8%, higher than that of the upstream and aquatic environment in Hangzhou, China [8]. Wastewater effluents from animal farm may be the major contributor.

All the ESBL-producing E. coli from feces and water showed multi-drug resistance (MDR), resistance to at least 6 antibiotics. Through the detection of resistance genes, CTX-M and TEM genes were both found, and the CTX-M gene was the prominent ESBL genes in both manure isolates and downstream water strains, which was consistent with previous reports [Ho et al., 2011].

The similarity of the isolates from feces and water samples were between 65% and 100%, indicating the multiple resources of the water isolates. 5 ESBL- producing E. coli from downstream water samples had above 90% similarities with those from the chicken feces. These results indicated that ESBL- producing E. coli in animal feces can spread through wastewater, leading to surrounding water environment contamination.

Conclusion

Comparison of resistance phenotype, resistance genes and genetic similarity between fecal isolates and water strains indicated the dissemination of drug-resistant bacteria to nearby water environments.

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OCCURRENCE OF ESBL-/AMPC-PRODUCING ENTEROBACTERIACEAE IN TURKEY FATTENING FARMS IN GERMANY

H.M. Lu, U. Roesler, A. Friese

Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany

Introduction

Previous studies showed the occurrence of ESBL-/AmpC-producing *Enterobacteriaceae* in different farm animals. Up to the present these resistant bacteria were not investigated systematically in turkeys. This cross-sectional study is to ascertain the prevalence of ESBL-/AmpC-producing *Enterobacteriaceae* in a representative survey distributed over Germany.

Materials and Methods

In this study 48 turkey farms are investigated, which are selected depending on the turkey population in each federal state in Germany. Pooled feces, dust samples and boot swabs are parallel collected in two different age groups. On the one hand these samples are taken in the rearing unit of a farm, on the other hand in the fattening unit.

In most of the samples ESBL-/AmpC-producing *Enterobacteriaceae* are detected qualitatively after enrichment and also quantitatively. MacConkey-Agar with 1µg/ml Cefotaxime is used for selection. All ESBL-/AmpC-suspected microorganisms are confirmed by disc-diffusion-assay and detecting ESBL- (CTX-M, TEM, SHV) and AmpC-genes (CMY).

Results

So far 38 of 48 turkey farms are evaluated statistically. In nearly 60% of the investigated farms a positive ESBL-/AmpC-status could be detected, which means that at least one of the ten samples within one farm was ESBL-/AmpC-positive. Regarding all samples approximately one third of the samples were positive for ESBL-/AmpC-producing *Enterobacteriaceae.*

There is no significant difference between the prevalence in samples originating from the rearing units and the fattening units.

Conclusions

This study is to collect data of ESBL-/AmpC-producing *Enterobacteriaceae* in fattening turkey farms in Germany. In comparison to similar previous studies in poultry, especially broiler, the prevalence of ESBL-/AmpC-producing *Enterobacteriaceae* is lower and the predominant ESBL-/AmpC-gene was CTX-M.

INFECTIOUS DISEASES OF CARRIER PIGEONS AND THEIR RESISTANCE TO SELECTED ANTIBIOTICS

F. Zigo, D. Toropilová, L. Takáč, M. Tomko, M. Chripkova, M. Vasiľ

University of Veterinary Medicine and Pharmacy, Košice, Slovak Republic

Summary

The aim of this study was to monitor the health status of carrier pigeons (*Columba livia*) coming from two independent holdings during the race season. The two holdings differ in the way the prevention and treatment was carried out. In the A holding – the treatment and prevention was based on the use of antibiotics, while in the B holding – natural medicine agents in the form of extracts were used. 80 pigeons from each holding were collected faecal samples and swabs of the cloaca, oropharynx, crop and infraorbital sinuses. After laboratory analyses of infectious agents, followed by testing with the disc diffusion method for resistance to selected 13 antibiotics. In the A holding - there was an increased resistance (>50.0%) *E. coli* and *E. columbae* strains to ampicilin and *S. gallolyticus* strains to neomycin.

Introduction

Pigeons are during the race season exposed to various adverse effects. Increased stress during the race is an important factor that significantly affects their health. The accumulation of large numbers of pigeons in one place increases the potential for transmission of infectious agents which may later sign on the increased incidence of disease. The aim of this study was to characterize the most common diseases pigeons and determine the sensitivity of isolated bacteria of the oropharynx swabs, crop and cloaca to the tested antibiotics.

Methods

During the race season were monitored by two different holdings of pigeons with different ways of prevention and treatment. In the A holding - the treatment and prevention was based on the use of broad-range antibiotics based tetracycline, ampicillin, erythromycin and streptomycin, while in the holding B - natural medicine agents in the form of extracts from the garlic, organic acids and ethereal oils were used. 80 pigeon samples were taken per holding from infraorbital sinuses and cloaca. Clinical examination was performed according to Jantošovič et al. (1998). Determination of coccidiosis, trichomoniasis, ectoparasitosis and endoparasitosis was performed according to Letkova et al. (1997). Bacteriology analyses of *Staphylococcus spp., Streptococcus spp., Enterococcus spp., and Pasteurella spp.* were centred on the cultivation, identification and taxonomic classification according to Vasil et al. (2012). The diagnostics for *Escherichia* and *Salmonella* was performed after multiplication in buffered peptone water and culturing on the Mac Conkey medium according to Stenzel et al. (2014). To culture mycoplasmas were used mycoplasma-selective agar plates and broths according to Turcsányi et al. (2012). The resistance to 13 antibiotics was assessed with the disc diffusion method according to the CLSI (Clinical and Laboratory Standards Institute, USA). Bacteria were rated as resistant or sensitive according reference zone by manual from producer (Oxoid Ltd. Basingstoke, Hants, UK). In the overall assessment of the isolated species of bacteria ($n \geq 6$) was judged the overall resistance within a specific species to individual kind of antibiotics. Value of resistance from isolated strains were valuated as sensitive (S) ≤ 10.0 %, moderate sensitivity (MS) 10,0 – 40.0 % resistant (R) 40.0 – 50.0 % and very resistant (VR) ≥ 50.0 %

Results and discussion

According to Bergman (2013), the most common diseases pigeons include coccidiosis, trichomoniasis and respiratory infectious which is also confirmed in the studied holdings A and B (**Graph 1 Comparison of the most common diseases of pigeons in the monitored holdings A and B).**

In a study conducted Stenzel et al. (2013) from 683 pigeons in Poland were isolated from cloacal swabs *E. coli, S. faecalis* and CNS, which are considered the natural digestive tract commensals. The most frequently bacteria isolated from cloacal swabs in the holding A were *E. coli* (37 strains), *E. columbae* (29 strains), *E. faecalis* (21strains), *S. gallolyticus* (17 strains) and *S. faecalis* (12 strains). In the same holding swabs from the oropharynx and crop was recorded the occurrence CNS (7 strains), *S. aureus* (6 strains) and *E. coli* (7 strains). In holding B were at a higher rate from cloaca swabs isolated *E. coli* (56 strains), *E. columbae* (26 strains), *S. gallolyticus* (33 strains), *E. faecalis*

(27) and *S. faecalis* (24 strains). CNS (19 strains), *S. aureus* (9 strains) and *E. columbae* (6 strains) were the most frequently isolated from swabs of the oropharynx and crop.

Table values are expressed resistance isolates from both holdings during the race season.

The presented data in indicate a very high level of drug-resistance (VR ≥50.0%) of the isolates *E. coli* and *E. columbae* to ampicillin isolated from the A holding, in which the prevention and treatment carried out with broad-spectrum antibiotics and are consistent with the results reported in other countries (Futagawa-Saito et al. 2007; Stenzel et al. 2013). The *E. coli, E. columbae* and *E. faecalis* isolates were most sensitive to cephalotin and oxacilin. From isolates *S. gallolyticus* was observed in holding A resistance (40.0 – 50.0 %) to penicillin and streptomycin as well very high level of drug-resistance (VR ≥50.0%) to neomycin.

Species of bacteria	n		AP	AX	CX	CE	ER	LI	NE	NO	PE	ST	OX	CP	TE
E. coli	**A**	*41*	**VR**	MS	MS	S	S	MS	MS	S	S	MS	S	S	MS
	B	*56*	**R**	MS	S	S	S	MS	S	S	S	MS	S	S	MS
E. columbae	**A**	*32*	**VR**	MS	S	S	S	MS	S	S	MS	MS	S	S	MS
	B	*38*	**R**	MS	S	S	S	MS	MS	S	S	MS	S	S	MS
E. faecalis	**A**	*29*	MS	MS	S	S	MS	MS	MS	MS	S	MS	S	S	**R**
	B	*27*	MS	MS	S	S	MS	MS	MS	MS	S	MS	MS	S	MS
S. gallolyticus	**A**	*19*	MS	MS	MS	MS	MS	S	**VR**	MS	**R**	**R**	S	S	MS
	B	*29*	MS	S	S	S	MS	S	MS	MS	MS	MS	S	S	MS
S. faecalis	**A**	*12*	MS	MS	S	S	MS	MS	MS	MS	S	MS	S	S	**R**
	B	*24*	S	S	MS	MS	S	S	MS	MS	MS	MS	S	S	MS
*CPS**	**A**	*10*	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	S	S	MS
	B	*18*	MS	S	MS	S	MS	S	MS	MS	MS	MS	MS	S	MS
CNS[1,2]	**A**	*11*	MS	S	MS	S	MS	MS	S	MS	MS	MS	S	S	MS
	B	*27*	MS	MS	MS	S	MS	S	MS	S	MS	S	MS	S	MS

Legend: AP - Ampicillin (10 µg); AX - Amoxycilin (25 µg); CX - Cloxacilin (5 µg); CE - Cefaperazone (30 µg); ER - Erythromycin (10 µg); LI - Linkomycin (15 µg); NE - Neomycin (10 µg); NO - Novobiocine (5 µg); PE - Penicillin (10 U); ST - Streptomycin (10 µg); OX - Oxacilin (5 µg); CP - Cephalotin (30 µg); TE- Tetracykline (10 µg), CNS* – *S. xylosus, S. wameri, S. simulans, S. schleiferi,* CPS* - *S. aureus and S. intermedius* n – number of positive samples, A – holding A, B - holding B

Tab.1 Value of resistance to antibiotics in isolated bacteria in the holdings

Conclusion

In summary, the drug-resistance of the bacterial strains isolated from pigeons at the holding A is higher than the holding B. It may pose a real risk to human health due to the easy transmission of the bacteria from birds to humans which is facilitated by long-term exposure to the microorganisms.

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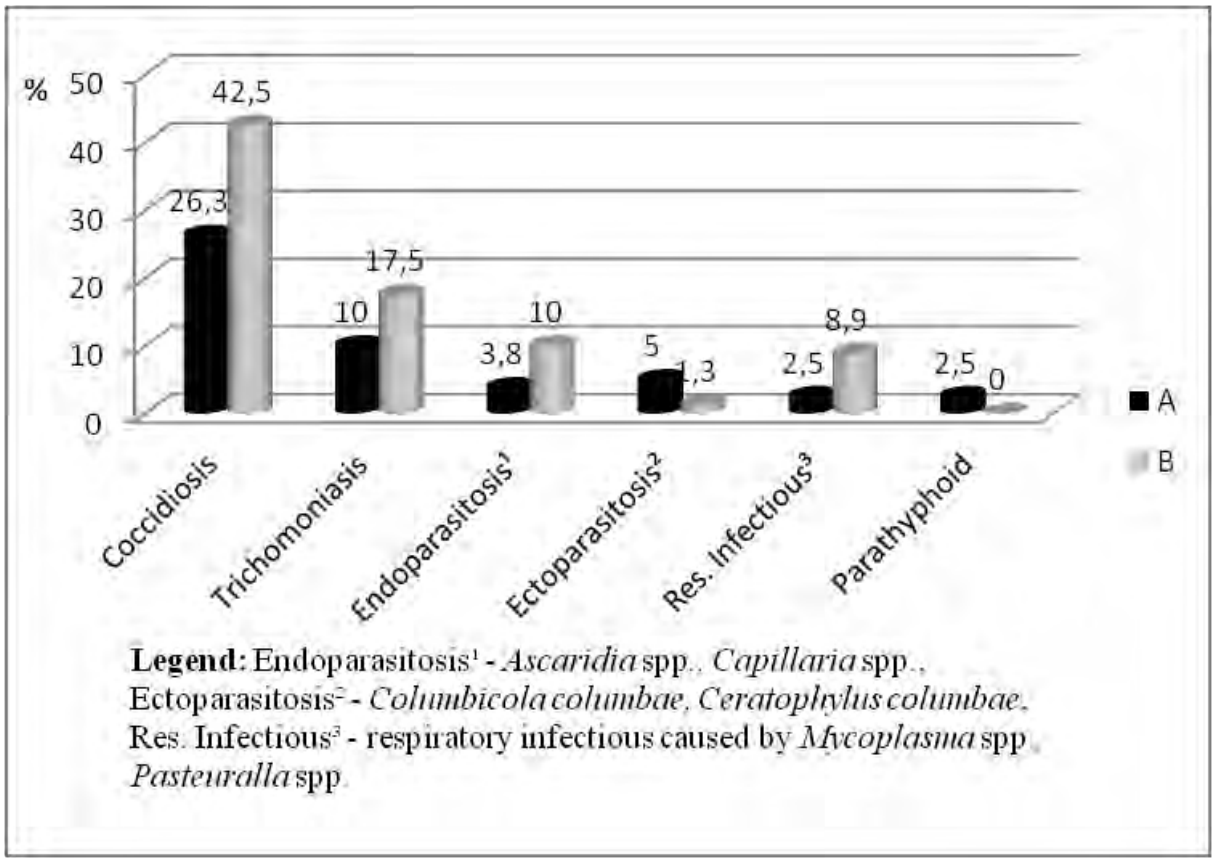

Legend: Endoparasitosis[1] - *Ascaridia* spp., *Capillaria* spp.,
Ectoparasitosis[2] - *Columbicola columbae, Ceratophylus columbae,*
Res. Infectious[3] - respiratory infectious caused by *Mycoplasma* spp.,
Pasteuralla spp.

TRACKING OF LISTERIA MONOCYTOGENES IN THE AIR AND ON SURFACES FROM THE BROILER FARM TO THE ABATTOIR

M. Dobeic [1,3], M. Golob[2], D. Kušar[2], M. Pate[2], Š. Pintarič [1,3], I. Zdovc [2,3]

[1]University of Ljubljana, Veterinary Faculty, Institute for Environmental and Animal Hygiene with Ethology, [2]Institute of Microbiology and Parasitology, Gerbičeva 60, SI-1000 Ljubljana, Slovenia; [3]Center of Excellence NAMASTE, Jamova 39, SI-1000 Ljubljana, Slovenia

Summary

Major sources and routes of transmission of *Listeria monocytogenes* were monitored from the broiler farm to the abattoir. To determine the source of contamination, the isolates were characterized by classical and molecular serotyping and by pulsed-field gel electrophoresis (PFGE). PFGE genotyping was used to determine whether *L. monocytogenes* was introduced into the slaughterhouse with animals or, perhaps, there was an endogenous source of *L. monocytogenes* in the abattoir. The results showed the presence of six types of *L. monocytogenes* in the abattoir, however any positive sample was not isolated at the broiler farms. On the surfaces in the abattoir, 10% of samples were positive for *L. monocytogenes.* Using the cyclone sampling method, 44% of samples in the abattoir were positive for the airborne *Listeria* spp.; among them, 28% were confirmed as airborne *L. monocytogenes*.

Introduction

Listeria spp. are ubiquitous and widely spread in the environment, thus different pathways of their dissemination from the livestock to the meat processing industry should be recognized (1). The latter are mostly supported by animals and their products, water, soil, air and humans (2). Listeriae prefer humid and cold areas with the strong affinity to form biofilms. They are highly resistant and spread also by the bio-aerosols (3). Studies of the transmission of listeriae in the real conditions are not showing a clear picture of this phenomenon. Therefore, the aim of the present work was to monitor the possible routes of transmission of *L. monocytogenes* from environment to the meat processing industry or if there exist the persistent or endogenous types of *Listeria* spp.

Materials and methods

Sampling was performed in 2 broiler farms and 2 poultry abattoirs to determine the surface and airborne *Listeria* spp. with the smearing and cyclonic method. On broiler farms 90 samples of litter faeces and 10 air samples were taken, while in the abattoirs 60 samples from surfaces and 50 air samples were taken. Surface samples were taken on the floor of evisceration room, packing machine, the water of stunning and plucking machine, evisceration machine, table for carcass processing, chicken wings and workers gloves. The air was sampled from evisceration and cold room and from live animals unloading facility. The isolates were characterized by the classical and molecular (PCR-based) serotyping and by the pulsed-field gel electrophoresis (PFGE) (4). Dendrograms with 1% tolerance were created using the UPGMA (Dice coefficient) algorithm. The criterion for the classification of isolates into subtypes was the 80% consistency between pulsotypes.

Results

On broiler farms none (n=0) of samples was positive on *Listeria* spp. neither in faeces or in the air. On the surfaces of abattoir 5 samples (8%) were positive on *L. monocytogenes.* Among them 2 samples (3%) were positive on the floor of evisceration, 1 sample on the surface of packing machine, 1 sample on chicken wing and 1 sample on chicken breast skin. Other samples were negative. In the air of poultry slaughterhouse, 22 samples (44%) were positive on *Listeria* spp. Among them 14 samples (28%) were positive on *L. monocytogenes* and 9 samples (18%) were positive on *L. innocua*. Samples of *L. monocytogenes* were positive in evisceration room (n=7), animals unloading facility (n=2), and in bleeding room (n=5).

Results of the PFGE typing of *L. monocytogenes* isolates (Fig.1) showed that 6 types could be found in the abattoir, namely they grouped into 3 subtypes comprising 10 (9 from the air, 1 from the surface), 4 (2 from the air, 2 from the surface) and 2 (both from the air) isolates, respectively, and three additional subtypes each represented by a single isolate (all from the surface). They all showed the same serotype (classical 1/2a, molecular IIa) with the exception of one isolate (L398) that showed a different serotype (4b, IVb) and was in general the most divergent from all of the rest. This isolate, together with L395, was obtained from a different abattoir than all the others.

Discussion

In experiment we were unable to confirm the transfer of *L monocytogenes* from broiler farm to the abattoir, since we were not able to confirm any positive case of *L monocytogenes* on broiler farms. We have identified a significant number of positive samples *Listeria* spp. on surfaces of abattoirs. Even more interesting is the intensive load of the air by these bacteria. Given the positive samples were identified on surfaces and in the air of places in abbattoirs with most intense processing this confirms that the main source of contamination with listeria are live animals. However, due to the absence of positive samples of Listeria on broiler farms and contemporary the *Listeria* spp. burdening of the abattoirs where the same pulsotypes of *Listeria* spp. were identyfied this indicates to a high probability of the existence of endogenous types of *Listeria* in abattoir. It was shown that the air and surface isolates in abattoirs can share a pulsotype, therefore their connection could be deducted. Since the isolates were not all obtained from the same period but nevertheless exerted the same pulsotypes, namely the air isolates from the two biggest clusters with 10 and four representatives, respectively, were from 2011 and the surface isolates from 2012, it can be concluded that the endogenous *L. monocytogenes* can not be neglected and the transmission route form the air to surface presumed.

Conclusions

We were unable to confirm the transfer of *L monocytogenes* from broiler farm to the abattoir, however due to the same pulsotypes, endogenous types of *Listeria* in a abattoir can not be neglected.

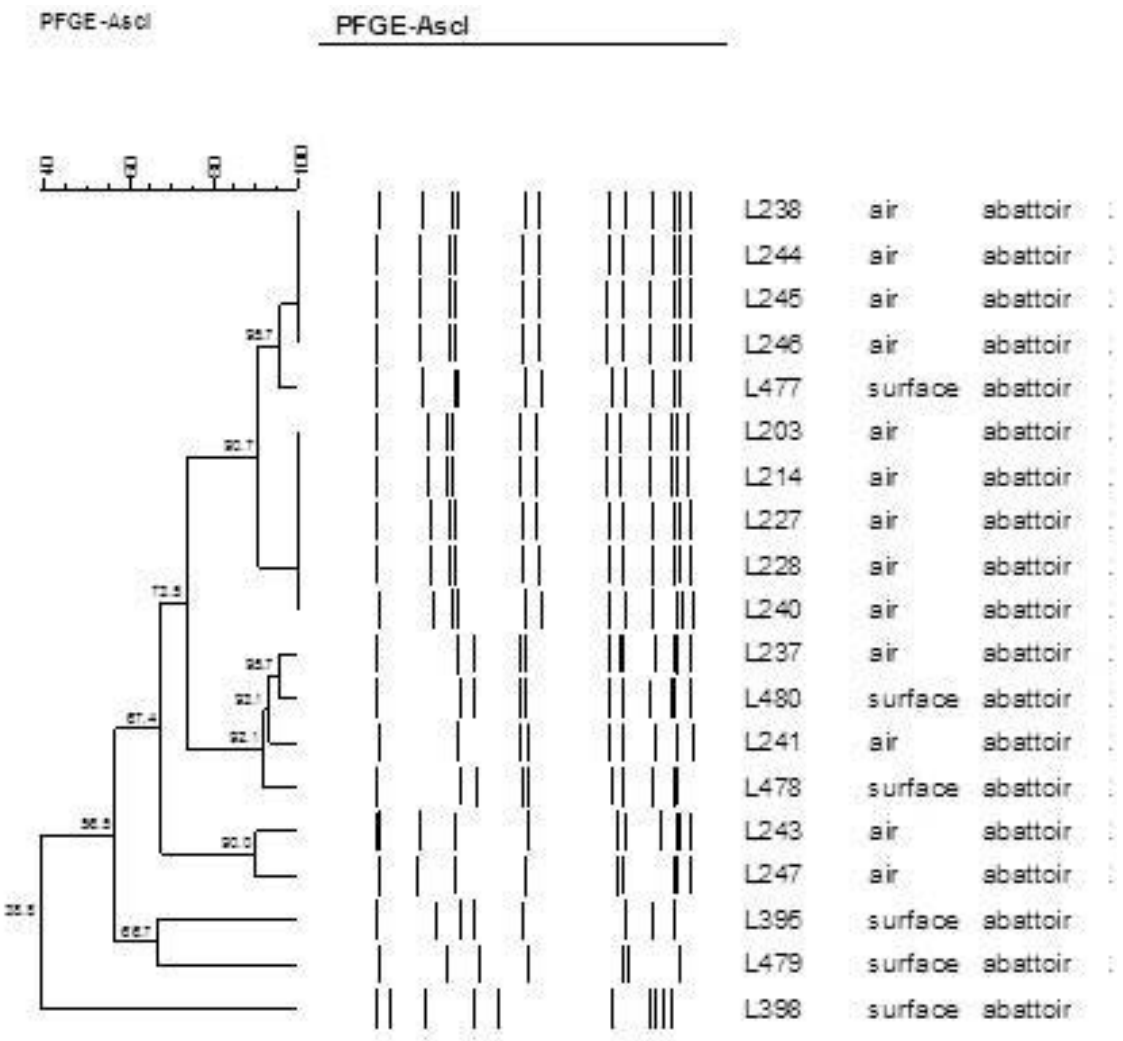

Fig.: 1. Dendrogram of the PFGE typing of *L. monocytogenes* isolates

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SUSCEPTIBILITY TO DISINFECTANTS OF RESISTANT AND NON-RESISTANT BACTERIA

F. Kirchner[1], M. Reinhardt[1], C. Cuny[2], Y. Pfeifer[3], U. Truyen[1], S. Speck[1]

[1]Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Leipzig, Germany; [2]Robert Koch-Institute, National Reference Center for Staphylococci and Enterococci, Wernigerode, Germany
[3]Robert Koch-Institute, Division of Nosocomial Infections, Wernigerode, Germany

Introduction: Increased antimicrobial (AB) resistance has been observed among many bacteria leading to treatment failures in human and veterinary medicine. It has been shown that some resistance mechanisms are common against both antibiotics and biocides hence one could hypothesise that AB-resistant bacteria are likewise disinfectant-resistant. Referring to that we determined minimum inhibitory concentrations (MICs) of AB-resistant and non-resistant bacteria for biocides used in health care setting, veterinary medicine, animal husbandry, and food production.

Materials and Methods: Disinfectants used were ethanol, sodium hypochlorite, sodium hydroxide, peracetic acid, formic acid, glutaraldehyde, and benzalkonium chloride. MICs were determined after 72 h of incubation according to the guidelines of the German Veterinary Society (DVG). In addition, the most effective neutralising agent was selected for each biocide. Besides the 2 Gram-positive and 3 Gram-negative reference strains recommended by the DVG, 11 *Staphylococcus* isolates (MRSA, MSSA, from human/animal infection/colonisation), and 12 *Enterobacteriaceae* (origin: food, human infections, control strains) including ESBL-producing *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella* Typhimurium were tested, including, whenever possible, AB-resistant and non-resistant isolates of the same species.

Results: MICs determined for MRSA, MSSA, and *Enterobacteriaceae* bacteria including ESBL-producer were similar to MICs determined for the recommended susceptible reference strains. A trend towards slightly lower susceptibility to ethanol, sodium hydroxide, glutaraldehyde, and formic acid was observed for Gram-positive compared to Gram-negative bacteria.

Conclusions: MRSA, ESBL-positive and non-resistant bacteria investigated in here were similarly susceptible to the disinfectants tested. This indicates that there is no *per se* disinfectant resistance in AB-resistant bacteria. However, more comprehensive data, particularly on the bacterial resistance mechanisms to biocides are needed.

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Environmental pollution and animal health

LEVELS OF LEAD IN SLAUGHTERED ANIMALS TISSUES

T. Youssef[1], Y. Hefnawy[1], H. Hassan[2]

[1] *Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Egypt*
[2] *Medical Biochemistry, Faculty of Medicine, Assiut University, Egypt*

SUMMARY

In recent years, much attention has been focused on the concentrations of lead in foods in order to check for those hazardous to human health. A total of 168 samples of liver, kidney and muscles were collected from cattle and buffaloes slaughtered in Assiut abattoirs. The samples were subjected to preparation & for measurement the level of lead by using Atomic Absorption/Flaming Emission Spectrophotometer (Shimadzu model AA 630-02). Concerning buffaloes samples, livers showed variable levels of lead, where the mean values ± SD were 0.38 ± 0.16 µg/g wet weight. Whereas in kidneys samples the mean value ± SD were 0.30 ± 0.17 µg/g wet weight. The corresponding values of the lead in muscles of buffaloes were 0.09 ± 0.07 µg/g wet weight.

As for cattle samples, the investigated livers were found contaminated with lead where their mean values ± SD were 0.40 ± 0.13 µg/g wet weight. On the other hand, kidneys samples were also positive for lead under investigations where their mean values ± SD were 0.38 ± 0.30 µg/g wet weight. Muscles samples contained a mean values ± SD of 0.07 ± 0.08 µg/g wet weight. Analysis of variance showed a significant to very high significant differences in lead levels among the examined cattle livers, kidneys and muscles. As for buffaloes, non significant differences lead contents were observed only between livers and kidneys. The public health hazards of the determined lead in tissues of buffaloes and cattle were discussed. Furthermore, the hygienic measures adopted to prevent contamination of meat with heavy metals as well as to protect the consumer were outlined.

Introduction

The toxicity of lead is attributed to the fact that it interferes with the normal function of enzymes. The edible species have been widely analyzed for Lead **(Miranda *et al.*, 2005; Abou Donia, 2008 and Iwegbue, 2008)**. Lead is toxic to the blood, the nervous, urinary, gastric and genital systems. Furthermore, it is also implicated in causing carcinogenesis, mutagenesis and teratogenesis in experimental animals **(Pitot and Dragan, 1996)**. The aims of the present study were to determine the levels of lead, in muscle, Liver and kidney of cattle and buffalo slaughtered in Assiut city.

Materials and Methods

Collection of samples: A total of 168 samples of liver (part of caudate lobe), kidney and muscles (part of diaphragm) were collected from 23 male cattle and 33 male buffaloes (2-3 years old) slaughtered in Assiut abattoirs. Each sample was about 50 grams weight and was individually placed in polyethylene bags and labeled with the date, kind, age and sex of each animal. The collected samples were immediately taken to the laboratory in an ice box where they were kept deeply frozen at -20°C until preparation, digestion and analysis.

Preparation of the samples: According to **A.O.A.C. (1975)**. The prepared samples were for measurement by using the Atomic Absorption/Flaming Emission Spectrophotometer (Shimadzu model AA 630-02), using an air acetylene flame and hallow cathode lamp.

Statistical analysis: Mann-Whitney Rank Sum test was used to compare between any two groups with skewed data.

Results

Table (1): concentrations levels of Lead in the examined samples

Lead (µg/g wet weight)	buffalo's liver	buffalo's kidney	buffalo's muscle	cattle's liver	cattle's kidney	cattle's muscle
mean ± SD	0.38±0.16	0.30±0.17	0.09±0.07	0.40±0.13	0.38±0.30	0.07±0.08*
Range	0.03-0.57	0.03-0.66	0.01-0.33	0.13-0.75	0.11-1.36	0.01-0.31

Table (2): concentrations levels of Lead in liver, kidney and muscles samples of cattle & buffaloes

animal	Lead (µg/g wet weight)	liver site	kidney site	muscle site	liver v kidney significance	liver v muscle significance	kidney v muscle significance
cattles	mean±SD	0.38±0.16	0.30±0.17	0.09±0.07	*	***	***
cattles	range	0.03-0.57	0.03-0.66	0.01-0.33	*	***	***
buffaloes	mean±SD	0.40±0.13	0.38±0.30	0.07±0.08*	N.S	***	***
buffaloes	range	0.13-0.75	0.11-1.36	0.01-0.31	N.S	***	***

* Significant ($p < 0.05$)
** Highly significant ($p < 0.01$)
*** Very highly significant ($p < 0.001$)

Discussion

From the summarized results given in Table (2), it is evident that there is no significant difference in lead concentrations in buffalo liver versus buffalo kidneys, and very highly significant difference in buffalo livers versus buffalo muscles, as well very highly significant difference in buffalo kidneys versus buffalo muscles. The statistical results indicated that there is a very high significant difference in lead concentrations in cattle liver versus cattle muscle, as well as, a very high significant difference in lead concentrations in cattle kidneys versus cattle muscles and significant difference in cattle liver versus cattle kidney. The obtained results agree with the findings of many researchers **(Abou Donia, 2008 and Iwegbue, 2008).**

The maximum lead concentration must not exceed 0.5 mg/kg of cattle offal and 0.1 mg/kg for muscles (**Egyptian standard, 2007**)

Lead levels higher than the monitoring standards obtained by Egyptian Standards **EOSQC (2007)** which were detected in 17% in buffalo livers, 13% buffalo kidneys and 13% buffalo muscles, while in cattle higher levels were detected in 17% of livers, 21% of kidneys and 17% of muscles.

Conclusion

The information given by the achieved results proved that all the examined livers, kidneys and muscles of both buffaloes and cattle were found contaminated with lead. Some tissues showed higher levels of lead than that recommended by the Egyptian Standards. Monitoring programme must be carried out periodically to determine the changing risks to health from animal food products in Assiut Province in order to inform any adopted management strategy, as well as Monitoring programme must be carried out periodically to measure heavy metals in soil and plants intended for animals to assess their load of lead to avoid their hazards on animals and humans.

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BLOOD COMPOSITION OF COWS KEPT IN THE COOPER INDUSTRY AREA IN POLAND

R. Kołacz, Z. Dobrzański, P. Cwynar, K. Pogoda-Sewerniak, R. Kupczyński, W. Janeczek

Department of Environmental Hygiene and Animal Welfare, Faculty of Biology and Animal Science, Wrocław University of Environmental and Life Sciences, J. Chełmońskiego 38C, 51-630 Wrocław, Poland

Biochemical parameters of blood represent an indicator elements of environmental pollution, especially when the animals are kept in the extensive (pasture) system. The aim of the study was to compare the blood metabolic profile of dairy cows kept near the cooper industry area, where the tailing pond "Żelazny Most" (Low Silesia, Poland) is located. It occupies an area of approx. 1,400 ha, and the volume of waste stored in the pond is approx. 500 million m^3. "Żelazny Most" is a source of metal pollution for all of the surrounding villages, where the metal dusts contaminate meadows and pastures. The blood samples were taken from clinically healthy cows (age 4-6 years), kept in the pasture system. The grazers are located in I zone (2-4 km from the tailing pond) – 11 cows and in the II zone (located more than 5 km from the tailing pond) – 10 cows. The blood was collected only one time and using the kinetic method the samples were aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT) were determined. Using the colorimetric method the blood determination inlouded: calcium (Ca), inorganic phosphorus (P), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu). Moreover, an antioxidant status was determined using the colorimetric method of total antioxidant capacity (TAS) and the activity of glutathione reductase (GR). The results showed that the distance from the tailing pond affected a significant increase in the concentration of Ca, Fe and Zn, TAS and GR in the serum of cows, especially in I zone, what may suggest that the flotation waste pond has the influence on the examined parameters in blood.

CLIMATE CHANGE AND LIVESTOCK IN DEVELOPING COUNTRIES

J.A. Saltijeral, G. Ruiz Lang

Departamento de Producción Agrícola y Animal, Universidad Autonoma Metropolitana-Xochimilco, Mexico City, Mexico

Summary

Veterinary professionals of the second decade of the century must be educated with a wide environmental awareness and a deep knowledge of bioethics. Also veterinarians should promote animal production in small and medium scale.

Introduction

Such productions have less environmental impact and provide animal welfare. Climate change threatens food security especially in less developed countries. This phenomenon promotes migration and causes loss of biodiversity. The aim of this paper is to cover a curricular gap in the curriculum of the Bachelor of Veterinary, pretending that the graduate is able to visualize production and animal welfare from an environmental perspective.

Material and methods

The paper proposals are:

- The environment as a complex system,
- Bioethics and global ethics, and
- Conservation medicine approach

Results

In many countries, consumers are fed up with being deluded by the agribusiness. Instead of using public money to subsidize factory farms – as in the United States and European Union – consumers want reasonable policies that promote ecologically, socially and ethically sound livestock production. Extensive farming is a production model that usually has negative effects on biodiversity, and generates climate change. It also has a high social cost because it does not generate jobs and profits of this economic activity does not benefit the domestic market (Klein, 2014).

In the other hand, intensive industrial production in many places In the world deprives millions of chickens and pigs animal welfare. They confined in small spaces and exposed to physical and psychological suffering, and are unable to perform their natural movements (animal rights). This production model is only justified by economic gain of capitalism. It is very important that the future veterinarians will be able to visualize animal production and animal welfare from an environmental perspective (Bok, 2014).

Discussions

The livestock sector generates more greenhouse gases, mainly the intensive and large type productions. The 18% measured in equivalent carbon dioxide (CO_2), than the transport sector, generates 65 percent of anthropogenic nitrous oxide, which represents 296 times the Global Warming Potential of CO_2. Most of this gas comes from manure. It responsible for 37 percent of all methane produced by human activity (23 more times more harmful than CO_2), and 64 percent of ammonia, which contributes significantly to acid rain. Uses 8% of global water. An estimated 20,000 liters of water are needed to produce 1 kg of beef (Leff, 2012). The process of expansion of livestock in third world countries and Latin America represents a threat to the sustainable development of the region. An excellent option environmentally friendly is to impulse conversion of extensive livestock systems into semi-stabled, with introduction of grass cutting and silvo-pastoral fodder banks, also set biogas plants to recycle manure. Agro ecology promotes systems for sustainable livestock production. Family farming preserves traditions, folklore and local culture and conservation of natural resources. For many poor farmers in developing countries livestock are also a source of energy as draft power and an essential source of organic fertilizer for crops (Morin, 2006).

The developing countries are those most affected by climate-related disasters such as heat waves, heavy rains and flooding. This was declareted at the global conference, at Lima Peru in 2014, on climate change sponsored by the UN attended by delegates from 200 countries. The work of this conference will culminate in Paris. Next December, this year. This expert affirmed that among the countries affected were the poorest, which are least responsible for

climate change. Family farming gives the possibility to increase food production, employment and sustainable rural development activities. Farmers or family farmers should be supported by local governments to promote their access to natural and productive resources, technical assistance, access to credit and insurance. All this will promote food security in the countries of the southern hemisphere, these experts say that cannot carry out agriculture and cattle ranch without power, communications, infrastructure, safety and health (Jonsen, 2003).

Leff (2012) affirms that natural resources should be managed with a comprehensively and environmental development perspective. In this approach the concepts of environmental eco-technological rationality and productivity arise from the perpesctives of cultural, ecological and technological productivity.

Conclussions

The curriculum proposals for veterinary education in this paper are:

1) Environment as a complex system. In recent decades, the science of environment insists its complexity. The same images of the planet seen from space, have helped us to see the land with forests, oceans, atmosphere and living things linked by numerous agencies in a common whole (Morin, 2006). We propose the use of systems theory to understand environment.

2) Bioethics and global ethics. The term "bioethics" (from the Greek bios, life and ethos, ethics) is a new name, first used by the American oncologist Van Rensselaer Potter suggests that bioethics is the systematic study of human behavior in the area of science human and health care, as this behavior in the light of moral values and principles (Potter, 1988) is examined. He suggests the need to overcome the current split between science and technology and humanities part of another.

3) Conservation medicine approach. The current concept of health not only considers the human being, but encompasses animal and ecosystem health. This means that human health, health of domestic and wild animals and ecosystem health promotes ecological health (Pedersen, 2009). This leads to the integration of veterinary medicine, human medicine and environmental health under one approach called Conservation Medicine (CM).

The recommendations made in this paper are mainly aimed at the universities of developing countries, but obviously it is important that veterinary schools in rich countries also take it into account.

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Environmental stress as cause of diseases

EFFECT OF HIGH LEVELS OF AMMONIA IN AIR ON STRESS RESPONSE TO ADRENOCORTICOTROPIN AND FORCED RUNNING IN RABBITS

M. Dyavolova, P. Moneva, I. Yanchev and D. Gudev

Department of Biochemistry and Physiology, Institute of Animal Science, Kostinbrod, Bulgaria

Abstract

The aim of the present study was to evaluate stress response in rabbits using heterophil/lymphocyte ratio as indirect stress marker. Twenty male rabbits of the New Zealand White breed at the age of 4 months were randomly allocated into two groups: control - reared under low air ammonia levels (1.4-14.6 ppm) and experimental – reared under higher ammonia levels (28-57 ppm). The rabbits of both groups were exposed to forced-running stress for 15 min on day 37 of the trial and two weeks later each received i.m. injection of 0.1 mg synthetic ACTH. The animals were sacrificed two days after termination of the trial and adrenal glands were excised and weighed. Ammonia changed H:L ratio in control but not in experimental rabbits following exposure to forced running and ACTH. Heterophil to lymphocyte ratio increased in control but not in experimental rabbits at 2h following ACTH injection ($P<0.01$), relative to basal value. The rabbits under high ammonia levels had heavier adrenal glands than control rabbits ($P<0.01$). The results are interpreted to suggest that ammonia modifies glucocorticoid secretion or glucocorticoid-induced increase in H:L ratio.

Keywords: rabbit, stress, heterophil/lymphocyte ratio, adrenal weight.

Introduction

Atmospheric ammonia in confinement facilities is a major harmful pollutant which affects recovery rate of heterophils (Murata and Hornito, 1999). Exposure of nursery pigs to 35 ppm of ammonia has been shown to double lymphocytes and monocytes count.

Thus, the aim of the present study was to evaluate stress response in rabbits using H:L ratio as a stress marker.

Materials and Methods

Twenty New Zealand male rabbits at the age of 4 months were randomly allocated into two groups (control and experimental) - 10 rabbits in each. The rabbits were reared individually in wire-floor cages in which feed and drinking water were supplied ad libitum. Air temperature, relative humidity and CO2 levels were within the following limits - 16-24°C; 40-70% and 480-1260 ppm. During the 51 days long experiment the rabbits of the control group were reared under low air ammonia levels (1.4 – 14.6 ppm), whereas the experimental rabbits were kept under higher levels of naturally occurring ammonia (28-57 ppm) via window closing. Thirty seven days after starting of the experiment all rabbits were exposed to 15 min forced running. Blood samples were collected by ear venepuncture before and following exposure to stress. Two weeks later the rabbits of both groups received (i.m.) 0.1 mg adrenocorticotropin 1-24 in 0.5 ml of saline. Blood was taken before and following adrenocorticotropin (ACTH) injection at 1 and 2 h. The acute effect of air ammonia on adrenal function was assessed by spreading liquid ammonia on the floor, immediately after the end of forced running session and ACTH injection which resulted in an increase in air ammonia level up to 158 ppm. All rabbits were sacrificed two days after termination of the trial and adrenal glands were excised and weighed. Peripheral blood leukocytes were counted on smears. Air ammonia was recorded via AeroQual S200 Monitor, equipped with ammonia sensor head (0-100±0.1ppm). The results of one factor statistical analysis are expressed as means ± S.E.M. and were analyzed by ANOVA.

Results and discussion

Heterophil to lymphocyte ratio in the control rabbits declined at 20 min following forced running while in the experimental rabbits it remained unchanged indicating that ammonia influenced H:L ratio in response to stress (Fig.1). Control rabbits unlike experimental rabbits had higher H:L ratio at 2 h following ACTH administration showing once again that ammonia is implicated in the modulation of H:L ratio (Fig.1).

Glucocorticoids are known to increase heterophil to lymphocyte ratio (Dhabher et al., 1995). Heterophil to lymphocyte ratio in control rabbits increased both at 1h ($P>0.05$) and 2h ($P<0.01$) following ACTH administration. The observed increase of H:L ratio in control rabbits at 2 h following ACTH injection was most probably short in duration since we did not find any increase of cortisol at 1 and 2h following ACTH injection in our previous experiments with similar experimental design (Dyavolova et al., 2014). The lack of significant H:L ratio increase at 1 and 2 h following ACTH administration in the experimental group suggests that ammonia is implicated in glucocorticoid secretion or glucocorticoid-induced increase of H:L ratio. Ammonia is known to increase NO synthesis (Swamy et al., 2005) and NO was reported to modulate cortisol and corticosterone secretion (Adams et al., 1992). Consequently, H:L ratio may turn out to be unreliable stress marker in animals reared under high ammonia level.

The experimental rabbits had heavier adrenal glands ($P<0.01$) relative to the control rabbits (Fig.2). Adrenal hypertrophy in the hyperammonemic rabbits was obviously not due to increased secretion of glucocorticoids, because of the similar basal values of H:L ratio between the control and experimental rabbits (Fig.1). Ammonium chloride ingestion was reported to decrease serum cortisol level (Llansola et al., 2013). Consequently, the higher adrenal weight in the experimental rabbits was most probably due to hypertrophy of the adrenal zona glomerulosa. This view is in agreement with the reported zona glomerulosa hypertrophy in ammonium chloride treated rats (Lina and Kuijpers, 2004).

Conclusion

Exposure of control and experimental rabbits to forced running did not cause any increase in H:L ratio. Ammonia prevented H:L ratio increase in experimental rabbits at 2 h following ACTH injection and resulted in adrenal hypertrophy.

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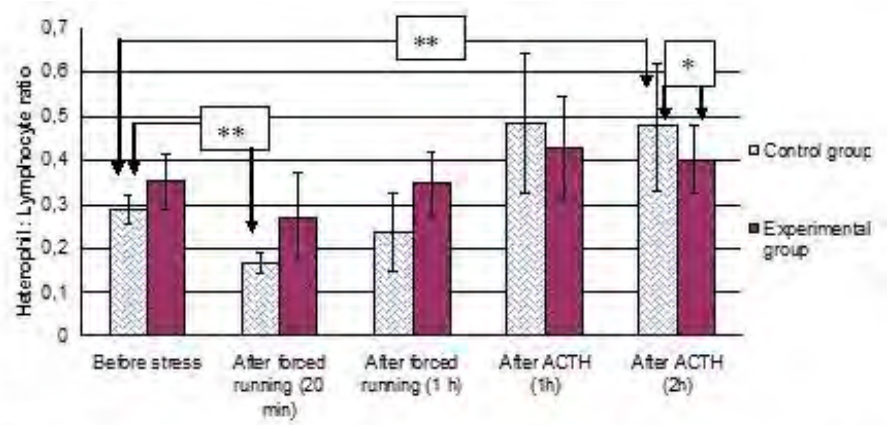

Fig. 1.Effect of forced running and ACTH injection on heterophil to lymphocyte ratio in rabbits, reared under low and high ammonia levels.
* P<0.05, ** P<0.01

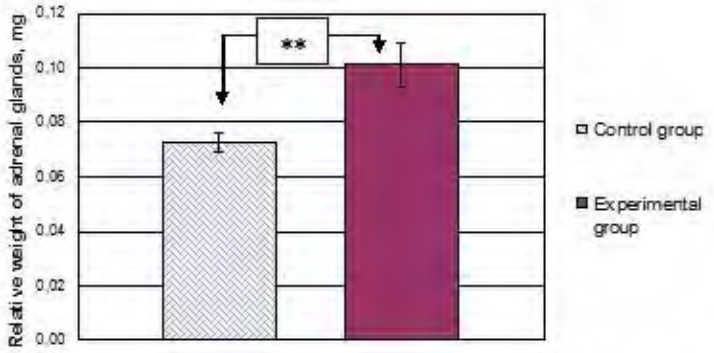

Fig.2. Effect of ammonia on the relative weight of adrenal glands in rabbits reared under low and high ammonia levels. ** P<0.01

EFFECT OF STRESS DURING LAIRAGE AND STUNNING ON QUALITY OF PORK CARCASS, MEAT AND "HAM"

P.H.G.J De Silva[1]and D.C.A. Kalubowila[2]

[1]Department of Animal Science, Faculty of Agriculture, Aquinas University College, Colombo 08, Sri Lanka; [2] ADM Food Products Pvt. Ltd, Badalgama, Sri Lanka

Summary: A total of 300 Duroc × (Land Race × Large White) castrated boars were subjected to treatments: 1. MS (mechanical stunning- non penetrative iron pole) with stress (MSWS); 2. MS without stress (MSS); 3. ES (electrical stunning -250 V, 1.25 A, 50 Hz, 8–12 sec) with stress (ESWS); 4. ES without stress (ESS) during lairage. ESS method resulted significantly higher (P<0.01) bleeding % and water holding capacity (WHC) but resulted significantly lower (P<0.05) carcasses temperature, drip loss and dressing %. The either method of ES significantly (P <0.01) reduced the stunning time, hemorrhages in internal organs, incidence of bruises, injuries, *E coli*, *Staphylococcus*, and *Total plate count* in carcasses. A faster rate of muscle pH decline was found in MSWS (P<0.05). Highest L* and a* values were observed in hind leg meat from MSWS (P>0.05).

Keywords: Stress, Lairage, Stunning, Pork, Ham, Quality

Introduction

The most important single defect of pork meat is pale, soft and exudative (PSE) represent a major problem to the pork industry. Its poor processing characteristics and appearance make it unacceptable to both processors and consumers. The methods used to handle and process pigs in slaughter plants are well-known to have a large impact on carcass and meat quality of pigs and animal welfare (Gispert et al., 2000; Mlynek et al., 2012). As well as stress immediately prior to slaughter, stunning method and bleeding method play important roles in the conversion of muscle to meat (Brown et al., 1998; Velarde et al., 2000). Therefore appropriate pre slaughter handling and slaughtering method of pigs are very important, not only from a welfare point of view, but it also affects carcass and pork meat quality and is consequently linked to economic implications for processor. A study was conducted to investigate the effect of stress during lairage and stunning for the quality of pork carcass, meat and ham under commercial conditions.

Materials and Methods

Three hundred crossbred castrates (live weight 86±6.9 kg) of the same genotype (Duroc × (Land Race × Large White)) fed and handled at identical conditions, randomly allocated into four treatments: 1. MS with stress (MSWS); 2. MS without stress (MSS); 3. ES with stress (ESWS); 4. ES without stress (ESS) during lairage. As physical characters live weight, carcass weight, dressing %, stunning time, bleeding time, blood aspiration in lungs, injuries, bruising and blood spots in meat were determined. Carcass temperature was measured by digital thermometer (TESTO106), pH measured using a digital pH meter (Checkit Micro pH WP 01) in the *Longissimus dorsi* muscle and WHC measured by centrifuging method. L*, a*, and b* values were assessed one hour after excision with a Minolta Chroma meter CV-300. The experimental meat samples were analyzed for *Staphylococcus aureus* on baird parker medium spread plates incubated at 37 ^{0}C, *E coli* on petirifilm^{TM} *E coli* count plate incubated at 35 ^{0}C, TPC on petirifilm^{TM} aerobic count plate incubated at 37 ^{0}C and *Salmonella* was determined according to Gray and Patrick (1995). The data set was analyzed by an analysis of variance (SAS Institute Inc., N.C., USA).

Results and Discussion

Both hot and chilled carcass weights, as well as dressing percentage, were significantly lower (p<0.05) in ESS pigs could be due to the complete bleeding in carcasses. Either method of ES have significant short stunning time and bleeding time (P<0.05). ESS pigs, bleeding percentage was significantly (p<0.01) higher (70.58 %) in 3 min than MSWS pigs (37.23%). The faster rate of muscle pH decline in MSWS group could be due to increased glycolytic rate of muscle compared with others. MSWS pigs was resulted significantly higher (p<0.05) carcass temperature and drip loss while lower (p<0.01) WHC (Table 2). High temperature in muscle of MSWS pigs could be due to severe stress and impulsive behavior during stunning.

Grandin, (1994) was implied that high "excitability" create pre-slaughter stress during stunning result elevated glycolytic metabolism in pigs just prior to slaughter leads to production of heat which will elevate the pig's body temperature. Van der Wal et al., (1997) indicated that combination of rapid pH drop and high temperature results in denaturation of muscle proteins that bind water which leads to the reduced WHC. Meat of the ESS pigs, was significantly lower L* and a* than other groups ($p<0.05$). It was observed that higher incidences of bruises, hemorrhages, injuries and skin blemishes in carcasses, blood spot on meat and blood aspiration in liver, lung, heart which were significantly higher in MSWS pigs ($p<0.01$) due to physiological stress of lairage and stunning induced capillary rupture because of the increased blood pressure immediately after stunning resulting high residual blood. The either method of ES significantly ($P <0.01$) reduced *TPC*, *E coli* and *Staphylococcus* count in carcasses. This may be attributed to the proper bleeding causing less blood to retain in the carcass. The bacterial count was positively affected by the amount of residual blood left in the carcass. However, *Salmonella* weren't observed in any method.

Highest injected brine percentage to silver side muscle (15.7 %) and WHC (63.3%) was observed in hind legs obtain from ESS ($P<0.05$) pigs. Brine injected % and WHC has shown significant difference between stunning method ($P<0.05$). Highest tenderness (0.04KN) recorded in ham from ESS animals ($P<0.05$). There is significant difference ($P<0.05$) in tenderness of ham between MS and ES method with stress. Highest "L" (59) and "a" (11.5) values and cook loss (17.86 %) was observed in ham produced from MSWS.

Conclusion

Possible sources for the observed variation in carcass and meat quality may relate to the stress cause during lairage and stunning method. The study showed that by using ES without stress system it is possible to increase carcass and meat quality of pork.

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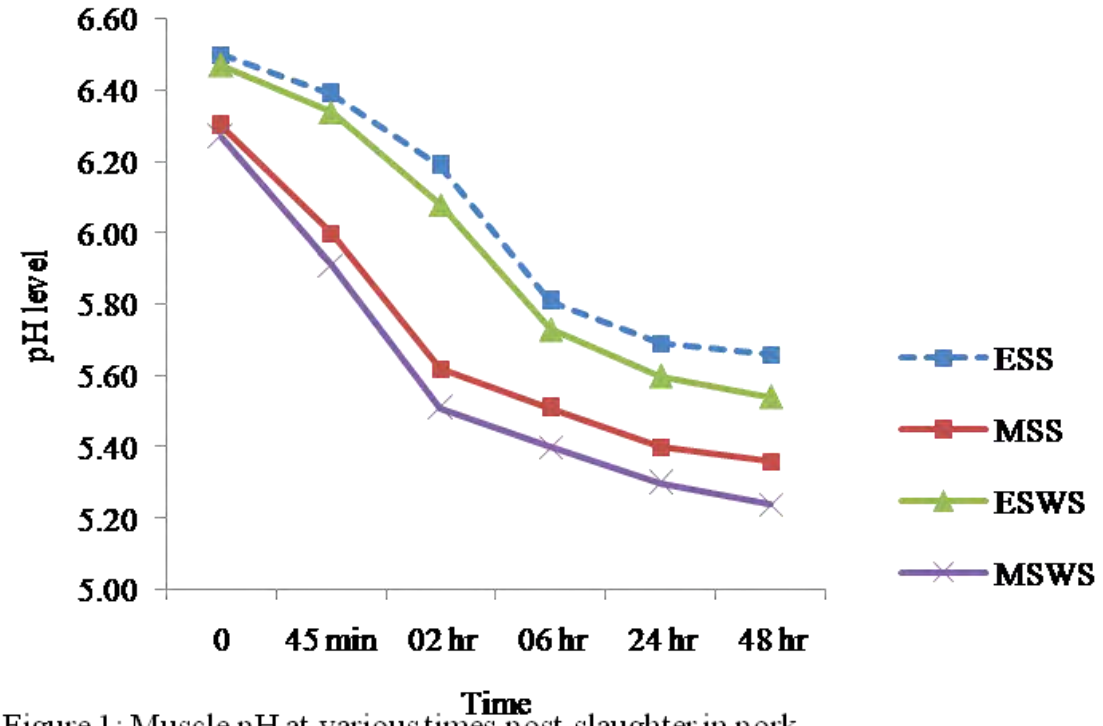

Figure 1: Muscle pH at various times post-slaughter in pork

Table 2 Effect of stress during lairage and stunning methods on meat quality of pork

Carcass characteristics	ESS	ESWS	MSS	MSWS	P value
Temperature (°C)	34.49 [a]	35.12 [b]	37.36 [c]	37.85 [d]	0.02
Colour					
L*	46.12 [a]	46.83 [a]	48.06 [b]	49.71 [c]	0.03
a*	6.71 [a]	6.78 [a]	7.41 [b]	8.12 [c]	0.04
b*	1.20 [a]	1.18 [a]	0.90 [b]	0.85 [b]	0.21
Drip loss (%)	13.47 [a]	15.93 [b]	20.24 [c]	22.86 [d]	0.01
WHC (%)	48.15 [a]	46.22 [b]	41.38 [c]	38.27 [d]	0.01

*Means within rows showing different superscripts are significantly different ($p<0.05$)

ACETYL SALICYLIC ACID PROTECTS AGAINST HEAT-STRESS DAMAGE IN CHICKEN MYOCARDIAL CELLS, POSSIBLY VIA AN INDUCTION IN HSP27 EXPRESSION

Di Wu[1], Jiao Xu[1], Erbao Song[1], Shu Tang[1], Xiaohui Zhang[1], Yingjun Lu[1], N. Kemper[2], J. Hartung[2] and Endong Bao[1*]

[1] College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China
[2] Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

Summary: We investigated whether acetyl salicylic acid (ASA) protects chicken myocardial cells from heat-stress mediated damage in *vivo* and whether the induction of Hsp27 expression is connected with this function. Pathological changes, damage-related enzyme levels, and Hsp27 expression were studied in chickens following heat stress with or without ASA administration. Our findings indicate that pretreatment with ASA protects chicken myocardial cells from acute heat stress *in vivo* with almost no obvious side effects, and this protection may involve an enhancement of Hsp27 expression.

Introduction

Modern poultry species such as the broiler chicken are highly sensitive to heat stress due to the feather cover, lack of sudoriferous glands, and fast growth. CK-MB and LDH are widely regarded as indicators of acute myocardial injury [1]. Hsp27 overexpression is able to attenuate cardiac dysfunction in transgenic mice [2]. It is possible that ASA confers heart protection by up regulates expression of different HSP family members, including Hsp27 [3]. As Hsps (especially Hsp27) are widely believed to protect against cell injury caused by various stress factors, it is reasonable to consider that ASA may protect against myocardial injury from heat stress by modulating Hsp expression.

Materials and Methods

270 SPF chickens were randomly divided as the HS (heat stress) group, the ASA-HS (aspirin administrated before heat stress) group, and the ASA (aspirin administration) group. Chickens in the ASA-HS and ASA groups were administered aspirin (Sigma, USA) orally at 1 mg/kg body weight 2 h in advance. Chickens in the HS and ASA-HS group were heat stressed in a preheated air chamber at 40 ± 1°C with 60% ~ 70% humidity over a series of time periods (0 h, 1 h, 2 h, 3 h, 5 h, 7 h, 10 h, 15 h, and 24 h). Birds were allowed free access to food and water ad libitum. 10 birds from each group were sacrificed humanely by decapitation at each time point. The LDH and CK-MB activities and Hsp27 level were measured according to the manufacturer's instructions and commercially ELISA Kit (MBS700383, MyBioSource, USA) Heart tissue samples were obtained, fixed then cut into 5-μm-thick serial sections and were stained with H&E. Immunohistochemistry studies were also performed.

Results

CK-MB levels in the heat stressed chickens in the HS group remained significantly increased after heat exposure for 1 h, 2 h, 5 h, 7 h, 10 h, and 24 h. CK-MB levels in the chickens mostly unchanged in the ASA-HS group. Chickens in the ASA group presented with the least variation in serum CK-MB levels. The changes in the serum LDH levels showed a similar but more sensitive trend as that observed for CK-MB.

Pathological changes in the HS group were much more serious than that in the ASA-HS group, while there were no obvious pathological changes in myocardial fibers.

Although Hsp27 expression did vary in all three groups, it was apparent that Hsp27 expression continuously increased in ASA-HS group and ASA group, compared to HS group. Therefore, ASA treatment must be responsible for the steady increase in Hsp27 expression over time.

In the HS group, prior to heat exposure, Hsp27 (brown) was mainly located in the cytoplasm. Following heat exposure, there was an especially strong signal in the nucleus. In the ASA-HS group, Hsp27 signals were located in the cytoplasm and the nucleus of myocardial cells. However, Hsp27 signals became stronger in the cytoplasm and the nucleus of myocardial cells at later time points following heat exposure. In the ASA group, Hsp27 was detected in the nucleus and cytoplasm of myocardial cells over the course of exposure.

Discussion

Heat stress causes considerable damage to chicken myocardial cells in this study, and the responding CK-MB and LDH activity suggests that the extent of myocardial cell damage was serious and got worse over time. The myocardial cell damage in chickens stressed after ASA oral administration was decreased compared with animals not treated with ASA. Furthermore, the levels of CK-MB and LDH in the serum of chickens given ASA were lower following heat stress. Together this suggests that ASA may prevent myocardial cell damage caused by heat stress.

In the present study, ASA treatment reduced myocardial cell injury and efficiently induced Hsp27 expression. The expression of Hsp27 in the heart tissues of the heat stressed chickens in HS group varied over time. Furthermore, insufficient Hsp27 expression in heat-stressed myocardial cells was accompanied by myocardial damage suggesting that abundant Hsp27 expression may be required to protect cells from heat stress damage.

Previous studies have reported that heat stress and other classic stress factors always lead to relocalization of Hsp27 into the nucleus [4]. Although, in our study, Hsp27 was induced more efficiently by ASA than heat stress, and relocalization of Hsp27 into the nucleus was not observed if ASA was the only inducing factor. This suggests that the mechanism of ASA-induced Hsp27 protection may differ from classic heat stress responses. However, the detailed mechanism of this form of protection remains to be determined.

Conclusion

The results of this study suggested aspirin was able to protect chicken myocardial cells from heat stress caused injury *in vivo*. Meanwhile, aspirin also was able to inducing more Hsp27 expression which has been reported to be important in cardiovascular disease protection and stress resistance. Translocation of Hsp27 caused by heat stress was not repeated by aspirin administration. This suggested aspirin induced Hsp27 expression through another pathway differ from heat stress. Aspirin possess a potential ability to be a novel anti-heat stress medicine.

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Figure Legends

Fig. 1 The variations of heart-damage related enzymes and Hsp27 expression in myocardial cells after heat exposure

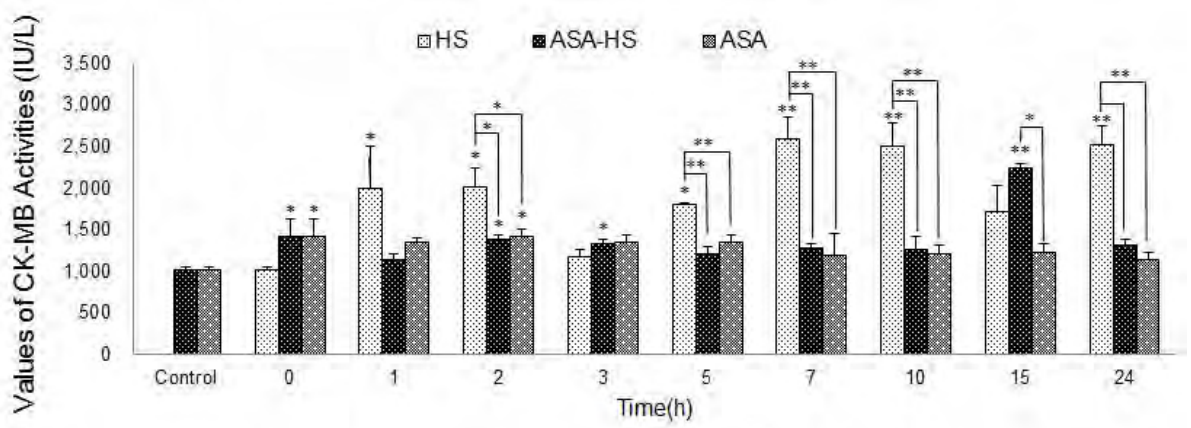
* indicates significant differences of LSD (Least Significant Difference) with significance at the $P < 0.05$ level.

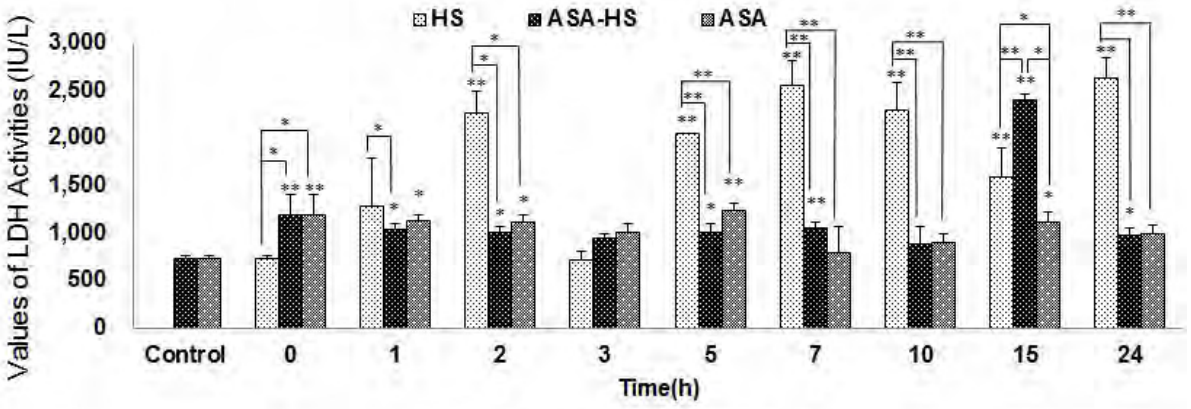
** indicates significant differences of LSD (Least Significant Difference) with significance at $P < 0.01$ level.

Fig. 2 Representative histopathological images of chicken myocardial cells following heat exposure and localization of Hsp27 determined by Immunohistochemistry

(a): Hematoxylin and eosin staining. Scale bar = 10 μm. HS group:

(b): Immunohistochemistry staining and Mayer's hematoxylin counterstaining of heart tissues. Scale bar = 10 μm.


HS
ASA-HS
ASA
Values of CK-MB Activities (IU/L)
3,500
3,000
2,500
2,000
1,500
1,000
500
0
Control
0
1
2
3
5
7
10
15
24
Time(h)


HS
ASA-HS
ASA
Values of LDH Activities (IU/L)
3,000
2,500
2,000
1,500
1,000
500
0
Control
0
1
2
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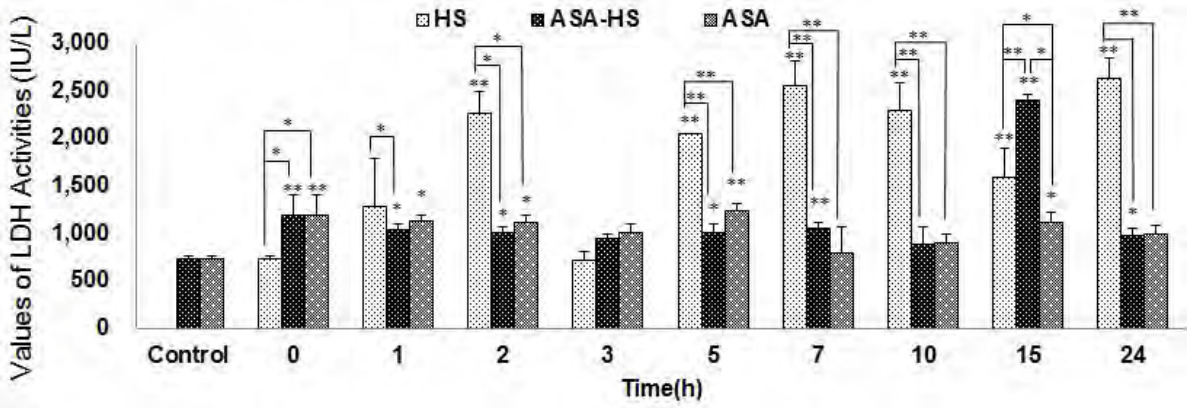
10
15
24
Time(h)


HS
ASA-HS
ASA
Values of LDH Activities (IU/L)
3,000
2,500
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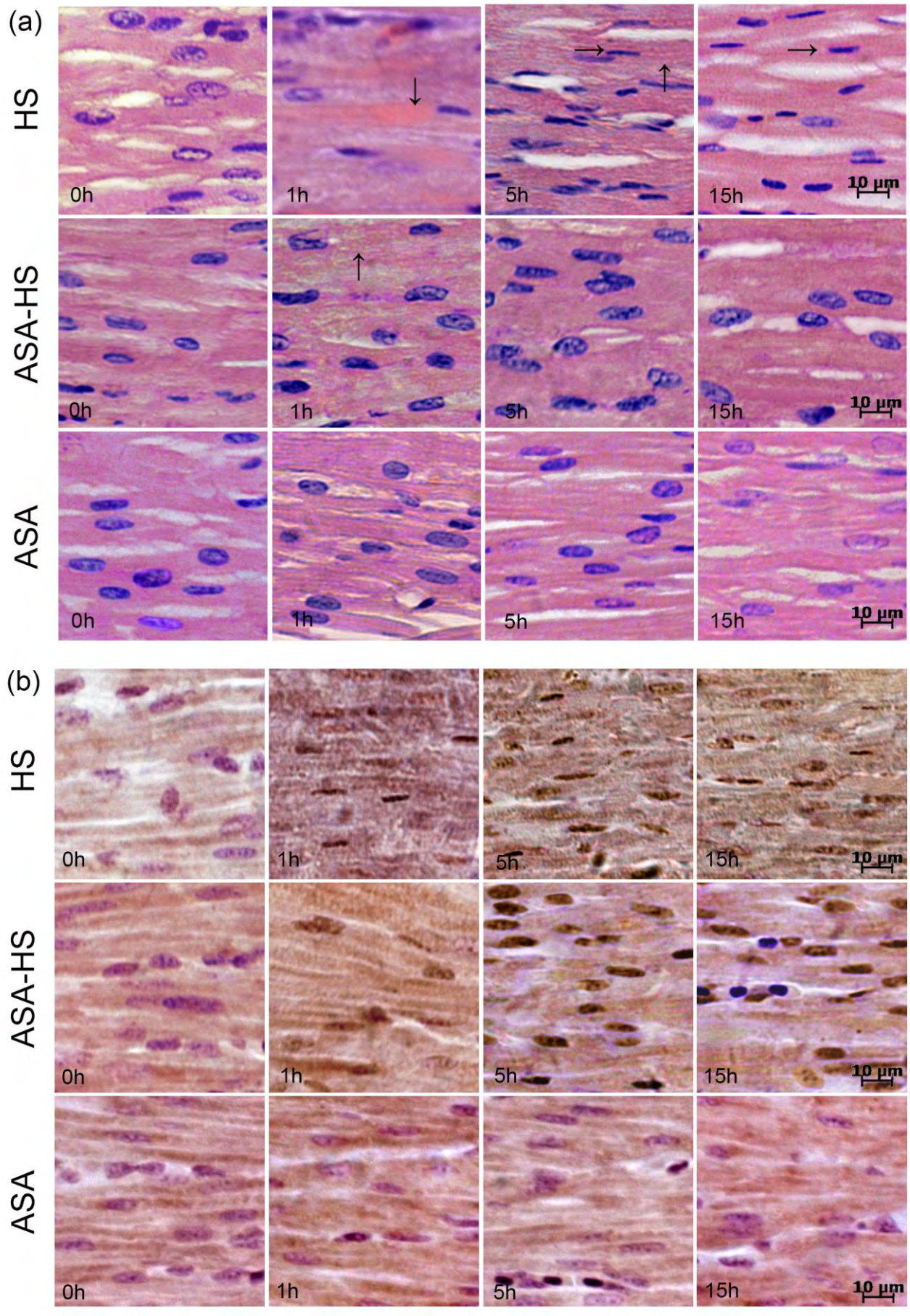
(a)
HS
ASA-HS
ASA
0h
1h
5h
15h
10 μm
(b)
HS
ASA-HS
ASA
0h
1h
5h
15h
10 μm

REDUCTION EFFICIENCIES FOR BIO-AEROSOLS BY BIOLOGICAL AIR CLEANING SYSTEMS INSTALLED WITHIN PIGGERIES

C. Habig, J. Hartung, N. Kemper

Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

Summary

Due to the increasing intensification of livestock farming, installation of air cleaning systems is mandatory in some areas to protect the surroundings from emissions. The aim of the study was to detect whether and to what extent bio-aerosols can be reduced by air cleaning systems and how far their amount increases by these systems. Comparison of reduction efficiencies was made for a trickle-bed reactor and a three-step system. While the trickle-bed reactor was visited 20 times for sampling, 10 dates of measurement were realized for the three-step system. In regular intervals, bio-aerosols were collected three times (30 min each) per date using impingement techniques. Measurements were made in parallel before (raw gas) and after (clean gas) the systems. Under conditions of proper operation, both systems showed reduction efficiencies for total bacteria of more than 80%. While the amount of *Staphylo*- (94%), *Strepto*- (81%) and *Enterococci* (93%) was lowered by the three-step system at any time, the number of colony-forming units could not always be reduced by the trickle-bed reactor, and was even higher in the clean than in the raw gas in some cases. Similar observations were made for the Methicillin-resistant *Staphylococcus aureus*, which were reliably reduced by the three-step system (89%), but not by the trickle-bed reactor at any time. The reduction efficiency for endotoxins was in the range of 8% to 88% for the three-step system, and between 24% and 100% for the trickle-bed reactor. The results show that microorganisms and endotoxins in the exhausted air of piggeries can be reduced by biological air cleaning systems under conditions of proper operation.

Introduction

The growing demand for livestock products led to an intensification of production systems with higher numbers of animals kept in specialized stables. Beside high amounts of ammonia, dust and odours, these facilities are regarded as a source of microorganisms too. Therefore, installation of air cleaning systems (ACS´s) is mandatory in some regions to protect the surrounding population and environment from emissions. The aim of the project was to detect whether and to what extent bio-aerosols can be reduced by ACS´s and how far the amount of microorganisms and endotoxins in the outgoing air increases by these systems.

Material and methods

Two types of ACS´s installed in two conventional fattening piggeries in Germany were investigated. One of the systems combined three steps of physical and chemical washing as well as biofiltration through a wall of root wood pieces. The second system was a trickle-bed reactor (TBR) which reduces pollutants by biofiltration at the surface of plastic packages that are continuously sprinkled by process water. In 2- to 3-week intervals ten samplings were carried out at the three-step system (TSS) between March and August 2010. The TBR was examined from July 2012 to May 2013 with a total of 20 samples taken in regular intervals. Bio-aerosols were collected in parallel before (raw gas) and after (clean gas) the ACS at three times (30 min each) per date. Raw gas samples were taken in the central exhaust air duct of the piggery before the air stream passes the ventilators. A sampling tube was designed to assure an isokinetic sampling. For the collection of bio-aerosols at the clean gas side where the purified air flows from the ACS to the environment, a square sampling hood (1 m²) with a central tube was used. In the raw as well as in the clean gas section, a fan and a flow straightener were attached at the end of the sampling tube to provide an adjustable air flow and constant air velocity of 4 m/s. A vacuum pump was used to lead the exhausted air through AGI-30 Impingers filled with 30 ml of *phosphate buffered saline* solution. Afterwards, samples were cooled down, pooled and processed within the next 24 hours. The analyses included the total bacteria count, the number of *Strepto*-, *Staphylo*- and *Enterococci*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterobacteriaceae*, Pseudomonades, Actinomycetes, molds and endotoxins.

Results

The mean concentrations of the fractions of different bacteria and endotoxins cultivated from the samples collected at the raw and clean gas side of the TSS and TBR are shown in Table 1. The reduction efficiencies for total bacteria highly depended on the impact of external factors and were in the range of 74% to 97% for the TSS and 35% to 98% for the TBR, with mean values of 88% and 85%, respectively. While *Strepto-* and *Enterococci* were reduced by the TSS at a high level of 94% at any time, the TBR reached lower reduction efficiencies of 84% and 90% on average. Furthermore, a strong variation of *Strepto-* and *Staphylococci* colony-forming units was assessed for the TBR. The reduction ratios were between 0% and 100%, but noting also 50% and up to 757% more bacteria after passing the TBR. In contrast, *Staphylococci* were always lowered by the TSS (mean value: 81%). MRSA were cultivable from samples collected in raw as well as in clean gas. Within the measurement periods, the number of MRSA in the exhausted air was relatively low, with highest values of 1600 cfu/m³ raw gas. The TSS reached a mean reduction efficiency of 89% for MRSA. At the TBR, MRSA were found in eight raw gas samples and were completely eliminated in six of these cases. During two measurement days, MRSA were detected solely or in a higher number in clean than in raw gas. *Enterobacteriaceae* were not detectable by standard cultivation methods in samples from the TSS and only once in raw gas collected at the TBR. However, genetic material from coliform bacteria was found in some samples, indicating a small proportion of *Enterobacteriaceae* on the total bacteria count. While thermophilic actinomyces couldn´t be counted anywhere for the TBR, the TSS reduced their counts by 97%. Both systems showed a strong variability in the reduction of mesophilic actinomyces, with even a higher number of colony-forming units in clean than in raw gas. Similarly, the number of molds was either decreased or increased through the systems or only detectable in clean gas. Pseudomonades were determined in six raw gas samples collected at the TSS. They were reduced at three times and equal before and after the system or higher in the clean gas samples at the other days. At the TBR Pseudomonades rarely appeared either in raw or in clean gas. The endotoxin content was very low for the TSS with a maximum of 23 EU/ml in raw and only 10 EU/ml in clean gas. With 92% on average, endotoxins were reliable reduced by the TBR, except for one day, with a lowering capacity of only 24%.

Discussion

Differences in reduction efficiencies were shown between the ACS´s and for the single system, depending on the operational status. The combination of several stages seems to be beneficial for the capacity of air cleaning. To maintain a high reduction efficiency of ACS and prevent these systems from disturbances, frequently controls are necessary.

Conclusions

The present results show that microorganisms and endotoxins in the exhausted air of piggeries can be reduced by ACS´s under conditions of proper operation.

Table 1. Mean concentrations of the fractions of different bacteria and endotoxins cultivated from the samples collected before (raw gas) and after (clean gas) the three-step system and the trickle-bed reactor, respectively.

	Three-step system		Trickle-bed reactor	
	Raw gas [cfu/m³]	Clean gas [cfu/m³]	Raw gas [cfu/m³]	Clean gas [cfu/m³]
Total bacteria	80497	8540	86681	10432
Streptococci	21592	1192	8517	4004
Staphylococci	17181	2408	3800	441
Enterococci	4629	204	1950	183
MRSA[1]	394	40	84	17
Enterobacteriaceae	bld	bld	6	bld
Pseudomonades	72	68	107	305
Actinomycetes 25 °C	3076	1396	63	24
Actinomycetes 50 °C	1048	48	bld	bld
Molds	414	212	294	248
	Raw gas [EU/m³]	Clean gas [EU/m³]	Raw gas [EU/m³]	Clean gas [EU/m³]
Endotoxins	12	6	11	1

[1] MRSA: Methicillin-resistant *Staphylococcus aureus*
bld: below the limit of detection

EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON THE SURVIVAL OF AIRBORNE BACTERIA

A.J.A. Aarnink, P. Hoeksma

Wageningen UR Livestock Research, Wageningen, the Netherlands

Summary

The objective of this study was to investigate the effects of temperature (T) and humidity (RH) on the survival of different types of bacteria in the air. The microorganisms studied were *Escherichia coli* (*E.coli*; Gram -), *Enterococcus mundtii* (*E.mundtii;* Gram +), and *Mycoplasma synoviae* (*M.synoviae*; no cell wall). Wet and dry suspensions of these three microorganism species were aerosolized. From the results it can be concluded that the tested airborne bacteria in wet aerosols, in this experimental setup, survive long enough to be transmitted over a long distance at different temperatures and humidity levels. The impact of the procedures for preparing dry aerosols on the viability of microorganisms isn't clear and should be further studied.

Introduction

Since the outbreaks of infectious animal diseases such as Avian Influenza and Q-fever in the Netherlands many residents in areas with intensive livestock farming are increasingly concerned about the negative effects of livestock production on human health. Opposition against intended expansion and relocation of livestock production facilities is growing. Pathogenic microorganisms emitted from livestock buildings may cause animal and human diseases by airborne transmission. However, many knowhow gaps in this field still exist; real health risks still have to be quantified and differentiated per infection and farm type.

Microorganisms can emit in wet or in dry aerosols. Wet aerosols are mainly generated from the respiratory tract. Dry aerosols originate from dust sources, of which manure, skin particles, feed, and bedding material are reported to be the main sources (Aarnink et al., 1999; Heber et al., 1988); (Cambra-López et al., 2011). Airborne microorganisms are exposed to meteorological factors, particularly temperature, humidity and solar radiation (Dungan, 2010). These factors may have significant effects on the survival of these micro-organisms. Furthermore, airborne microorganisms might be protected from outside influences by (dust) particles coagulated with the viable particle.

The objective of this study was to determine the survival rate of bacteria at different temperatures and relative humidity levels. This paper describes the results of a laboratory study with three groups of bacteria, gram-positive, gram-negative and mycoplasma, represented by *Enterococcus mundtii, Escherichia coli* and *Mycoplasma synoviae* respectively.

Material and methods

The effect of temperature (T) and relative humidity (RH) on the survival of three different types of airborne microorganisms was assessed in a 3x3 experimental set up for T = 10, 20 and 30^{o}C and RH = 40, 60 and 80%. Airborne microorganisms were sampled with glass impingers (AGI-30), which were filled with 20 ml buffered peptone water, at 0.5, 10, 20 and 30 min after aerosolizing 3 ml wet bacteria suspension or 1 ml freeze dried bacteria suspension mixed with 150 mg of dust. From the counts of culturable microorganisms in the subsequent samples the half-life time of the three microorganisms was calculated. Uranine was used as a tracer. All treatments (combinations of T and RH) were done in triplicate.

Two identical stainless steel climate controlled isolators (L = 1.6 m, W = 0.93 m, H = 1.1 m; V = 1.64 m^3) were used as aerosolization space. All bacteria concentrations after aerosolization were corrected for the uranine decrease which is related to physical losses of bacteria, mainly caused by deposition of the aerosols on the floor and walls of the isolator.

Results

The results show that the biological decay of the bacteria concentration in the first half minute after wet aerosolization was variable with clear differences between bacteria types and climatic conditions. The decay of *E.coli* and *M.synoviae* was larger than of *E.mundtii* ($P<0.01$). The decay was influenced by temperature and relative humidity in a variable way. *E.coli* showed, on average, a larger decay at higher relative humidity ($P<0.01$). The effect of temperature was not consistent, while it interacted with relative humidity. *E.mundtii* showed a smaller decay at higher temperatures (not significant) and higher levels of relative humidity ($P=0.01$). *M.synoviae* showed a tendency towards a larger decay at higher temperatures ($P=0.06$) and had a relatively large decay at 40% humidity, however, the effect of humidity was not significant ($P=0.13$).

The concentrations of the three bacteria in the isolator showed different changes in the course of time from 0.5 min after wet aerosolization onwards, with different effects of temperature and relative humidity. For *E.coli* and *E.mundtii* a significant effect of temperature level on the decay was found ($P<0.05$) and there was a tendency towards a temperature effect on the decay of *M.synoviae*. On average, *E.coli* showed larger decay than *E.mundtii* and *M.synoviae*. At medium temperature (20°C) the decay of *E.coli* was significantly ($P<0.05$) larger than at 10 and 30°C. Contradicting to this, *E.mundtii* and *M.synoviae* showed lowest decay at 20°C. *E.mundtii* showed highest decay at 30°C and intermediate at 10°C. *M.synoviae* showed similar decay at 10 and 30°C. No significant effects of relative humidity on the decay of the three tested bacteria were found.

After wet aerosolization, the half-life time of E.coli was lowest at 20°C (1.97 min), highest at 30°C (6.04 min), and intermediate at 10°C (3.17 min). On the contrary, half-life time of *E.mundtii* and *M.synoviae* was highest at 20°C (20.3 and 28.1 min), lowest at 30°C (5.65 and 4.93 min), and intermediate at 10°C (9.08 and 5.37 min).

After dry aerosolization only viable counts were found of *E.mundtii*. The decay of *E.mundtii* in the first half minute after dry aerosolization was not influenced by temperature. The effect of relative humidity, however, was significant ($P<0.01$). The largest decay was found at low relative humidity. The concentration of *E.mundtii* showed only small changes between 0.5 and 30 min after aerosolization.

The half-life times of dry aerosols of *E.mundtii* were not influenced by temperature but they were substantially affected by relative humidity ($P<0.05$), with the lowest half-life time at 60% humidity (16.6 min), highest half-life time at 80% humidity (90.9 min), and intermediate half-life time at 40% humidity (32.9 min).

Conclusions

1. The studied bacteria, *E.coli, E.mundtii* and *M.synoviae*, representing Gram-, Gram+, and bacteria without a cell wall, respectively, stay airborne and alive for several minutes after aerosolization within a wide range of temperatures (10-30°C) and relative humidity (40-80%).
2. Two phases of decay are observed: 1) a fast initial decay during and directly after spraying, probably caused by the rapid evaporation of the water from the aerosols; 2) a slow decay in the air after the initial phase.
3. *E.mundtii* bacteria survive longer in dry aerosols than in wet aerosols.
4. *E.coli* and *M.synoviae* hardly survived the preparation procedures for dry aerosols. The impact of these procedures on the viability of microorganisms isn't fully clear and should be further studied.

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Available on request (andre.aarnink@wur.nl).

SEEKING SELECTION SIGNATURES IN PRODUCTION AND REPRODUCTION TRAITS IS IMPERATIVE TO EXPLOIT LIVESTOCK CAPABILITIES

Maryam Javed, Asif Nadeem

Institute of Biochemistry & Biotechnology, University of Veterinary & Animal Sciences, Lahore, Pakistan; maryam.javed@uvas.edu.pk

Abstract

Introduction: Being an agrarian country, Pakistan is primarily dependent upon livestock for its economy. Role of animal sector is not only crucial for determining national GDP but also pivotal as provider of necessary food items and their byproducts. It has been estimated that species of livestock can give milk about 29.472 million tones and beef about 1.115 million tons annually. Among different species of livestock, buffalo stands out as an efficient converter of poor quality roughages into highly valuable products as milk and meat. Buffalo contributes about 68% of total milk produced in Pakistan. Production potential of these animals has been admired internationally. But production capacity has been found reversely related with fertility. More would be the production poorer would be the fertility. This opens the opportunity to genetically study the significant loci for production and reproduction traits. To test this hypothesis, present research was planned to study CYP11b1 and CYP19A1 genes as candidate for milk yield and silent estrus behavior in buffaloes.

Animals, Materials and Methods: Selected animal species was Riverine Buffalo. Blood samples were collected from true representative of river buffalo. Primers were designed from coding regions of the two genes and them these were amplified and sequenced. Comparison of sequences provided genetic variations and then statistically analyzed.

Results: In CYP11b1, seven variations were identified and in CYP19A1, four novel variations were identified. After statistical analysis, one of CYP11b1 and two of CYP19A1 were found significantly associated with milk yield and silent estrus behavior respectively.

Conclusions: It was concluded that animals with high milk yield are depicting signs of poor fertility in terms of silent estrus behavior.

Keywords: Polymorphism, Milk production, Fertility, Buffalo, Association

Introduction

Genetic characterization of production traits with candidate genes has been the major selection criteria for animals with superior genetic potentials both in production and reproduction traits. In many parts of the world, improvements have been made in many of the breeds on the basis of novel selection signatures in significant parts of the genes and still our quest for new markers is in the run. In present study, CYP11b1 and CYP19A1 were studied at genomic level for identification of novel polymorphisms in the coding region of the gene (Khatib et al. 2006). These genes have been found associated with milk yield, quality and reproductive traits in farm animals (Kataoka et al. 2000). Several QTLs controlling milk production traits have been reported in these two genes (Heyen et al. 1999; De Koning et al. 2001; Rodriguez-Zas et al. 2002; Olsen et al. 2002; Awad et al. 2010; Schopen et al. 2011). The present study explored the association of a single nucleotide polymorphism (SNP) in the both genes with milk fat content in river buffaloes of Nili-Ravi breed. Polymerase chain reaction technique was performed for genotyping the animals. A total of fifteen SNPs were identified. Identified selection signature can serve as genetic marker for section of superior buffaloes to enhance the production and reproduction potential of our animals.

Materials and Methods

Sampling strategy

A total of 50 animals of Nili-Ravi buffalo breed were selected from government and private livestock farms (Buffalo Research Institute, Pattoki; Livestock experimental Station, Okara). Animal were categorized into two groups. Group-1 included animals in first month of their second lactation with milk fat content more than 8% (n=50). In group-2, animals were selected with same cyclic stage (first month of second lactation) but with milk fat content less than 8% (n=50). Then selected animals were subjected to blood sampling. 10mL blood was collected from each animal in

EDTA added vaccutainer. Blood was immediately transferred to the ice cooler and was shifted to Molecular biology and Genomics lab. In Institute of Biochemistry and Biotechnology, University of Veterinary and Anima Sciences, Lahore for further processing.

Genomic DNA extraction, PCR amplification and sequencing

DNA was extracted by using organic DNA extraction protocol reported by Maryam et al. (2012) with some modifications. Specific sets of primers (table-1) were used to amplify the all exons of OLR1 gene. Then PCR amplicons were purified and sent for DNA sequencing.

Bioinformatics analysis

Identified sequences were aligned with CYP11b1 and CYP19A1 genes sequence reported in cattle (AC_000162.1) and total of fifteen SNPs were identified (table-2, fig-1). These variations were tested for Hardy Weinberg Equilibrium and only one (P17H) was found obeying HWE and was selected for association analysis.

Results

CYP11b1 and CYP19A1 genes were studied for the identification of biomarkers. Khatib et al. (2006) identified the genetic variation in this gene that was associated with milk fat content. In present study, total fifteen polymorphisms were identified (table-2, fig-1). Out of these 15, four were intronic and remaining eleven were exonic. From these eleven, five were synonymous and were not changing any amino acid. Remaining six were non-synonymous. Ratio of transition and transversion is 1.15:1.

Results of single marker analysis depict the distribution of alleles indicated that CYPb-5[P= 0.1306 >0.05], CYPb-6 [P= 0.0913>0.05], CYPa-10 [P= 0.1014>0.05], CYPa-11 [P= 0.2403>0.05] and CYPa-13 [P= 0.1410>0.05] were non-significant and following Hardy-Weinberg equilibrium indicating that the alleles were randomly distributed throughout the population, no migration had occurred, no bottlenecks happened. While for loci CYPb-1 [P= 0.0001<0.05], CYPb-2 [P= 0.0010<0.05], CYPb-3 [P= 0.0005<0.05], CYPb-4 [P= 0.0013 <0.05], CYPa- 7[P= 0.0011<0.05], CYPa-8 [P= 0.0010<0.05], CYPa-9 [P= 0.0015<0.05], CYPa-14 [P= 0.0021 <0.05], and CYPa-15 [P= 0.0002 <0.05] as probability value of Chi-square test was below 0.05, suggesting that population at these polymorphic sites was indicating significant deviations from Hardy-Weinberg equilibrium. These results have been given in table-2. All of mutations were novel and were not reported before. Most of variations were identified in exon-4. Khatib et al. (2006) also reported associated mutations in exon-4.

Discussion

Out of total fifteen variants, one was found obeying Hardy Weinberg Equilibrium (HWE). Description of identified variations has been mentioned in table-2. Chi square testing was performed on these variations and P-value (>0.05) was calculated. Results of genotypic and allelic frequencies are given in table-4 and 5. The allele frequencies identified at this position were not consistent with those of Khatib et al. (2006), Komisarek & Dorynek (2009) and Wang et al. (2012) who reported 0.46, 0.43 and 0.42 for allele A and 0.54, 0.57 and 0.58 for allele C in US, Polish and the Israeli Holstein cattle populations, respectively. However, they are consistent with the frequencies reported by Schennink et al. (2009) with 0.71 and 0.29 for alleles A and C in an experiment with a Dutch Holstein population. Allele C was found associated with high milk fat %age.

HWE analysis revealed significance of identified loci in local buffalo population. Genotypic and allele frequency were also calculated. Kataoka et al. (2000) studied CYP11b1 and found similar genotypic frequency. The present study is an example of candidate gene approach to find some novel variations at population level. This study is first step in finding some probable markers for milk fat %age in Nili-Ravi buffalo that can be used in future selection and breeding program.

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Table-1: List of Primers

Sr#	Primer Name	Primer Length	Product Size	Primer Sequence (5'-3')
1	*CYP11b1* F1	24	385	CA CACAGATTCACCACTTTCCCTTCC
2	*CYP11b1* R1	21		CCACACCCAGGCATTGTAGTT
3	*CYP11b1* F2	20	465	GATATTGAATCCCAGCTCCT
4	*CYP11b1* R2	20		CATCTTCCCATTCACTCCTA
5	*CYP11b1* F3	19	508	GTTGGGTTGATTTGTTGTC
6	*CYP11b1* R3	21		GGACCTCTAATGTAGAACCTG
7	*CYP11b1* F4	20	490	AGTCTGGGTGTAATTCTGAC
8	*CYP11b1* R4	20		CTTTACAGCGATGTCTAGTG
9	*CYP19a1* F1	19	390	GCTCCACTAGACATCGCTG
10	*CYP19a1* R1	20		CAGTGAGAAGCCCACACATC
11	*CYP19a1* F2	21	512	CCAACTCTCTCACACAAGGAC
12	*CYP19a1* R2	20		GGACTTGGAACAAGTTAGGG
13	*CYP19a1* F3	20	497	GGATCTGGAGGAAAAGAAGG
14	*CYP19a1* R3	18		GCAAAGGCAATGTAGTGA
15	*CYP19a1*F4	19	453	CCTAACTCAAGGTCACAGC
16	*CYP19a1* R4	19		GGACAAGCCAATTTAAGAC
17	*CYP19a1* F5	19	574	CTTGGAATCACATGGTAGT
18	*CYP19a1* R5	21		GAGATTCTAGTCCATGAAATC

Table-2: Polymorphic sites detected in the *CYP11b1 (CYPb) and CYP19a1* (CYPa) region

Genetic variants	Transition/ Transversion	Chi test (P>0.05)
CYPb-1	Transversion	1.2051 **
CYPb-2	Transition	0.0010 *
CYPb-3	Transversion	0.0005 *
CYPb-4	Transversion	0.0013 *
CYPb-5	Transversion	0.1306 **
CYPa-1	Transversion	0.0913**
CYPa-2	Transition	0.0011*
CYPa-3	Transversion	0.0010*
CYPa-4	Transition	0.0015*
CYPa-5	Transition	0.1014**
CYPa-6	Transition	0.2403**
CYPa-7	Transition	0.0000
CYPa-8	Transition	0.1410 **
CYPa-9	Transition	0.0021 *
CYPa-10	Transversion	0.0002 *

**Significant*

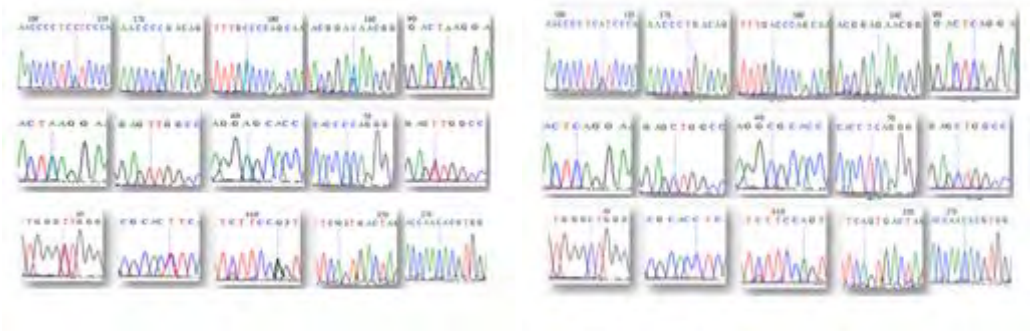
***Non-significant*

Table-3: Allele Frequency for all Loci of *CYP11b1 and CYP11a1*

SNP ID	Allele Frequency		Minor Allele Frequency
CYPb-1	**C**	**A**	0.2771
	0.7229	0.2771	
CYPb-5	**A**	**C**	0.0120
	0.9880	0.0120	
CYPa-1	**A**	**C**	0.0723
	0.9277	0.0723	
CYPa-5	**T**	**C**	0.2530
	0.7470	0.2530	
CYPa-6	**T**	**C**	0.0241
	0.9759	0.0241	
CYPa-8	**G**	**A**	0.2289
	0.7711	0.2289	

Table-4: Genotypic Frequency for all Loci of *CYP11b1 and CYP11a1*

0.2683	0.1951	0.5366
0.5484	0.0645	0.3871
0.4516	0.0968	0.4516
0.2439	0.5854	0.1707
0.2195	0.5122	0.2683
0.5366	0.1951	0.2683



Wild Type **Mutations**

Fig-1: Sequences of *CYP11b1 and CYP11a1* genes showing DNA sequence variation

Prevention and hygiene in cattle production

MICROBIOLOGICAL CONTAMINATION OF DAIRY COW BARN AIR AND MILK ACCORDING TO SEASONS

S. Žužul[1], M. Ostović[2], I. Šandrić[3], S. Menčik[4], Ž. Pavičić[2], K. Matković[2]

[1]Fifth-year student, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia; [2]Department of Animal Hygiene, Behaviour and Welfare, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia; [3]Likomed veterina Ltd., Sinj, Croatia; [4]Department of Animal Husbandry, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

SUMMARY

In this study, bacteriological air contamination in a dairy cow barn was assessed according to microclimate parameters in various seasons; the results obtained were then compared with the microorganism count in raw milk. Total bacterial count in the barn air was significantly higher (P<0.05) in warm period and *vice versa*, accompanied by the same trend in the microorganism count recorded in raw milk. High positive correlation was found among bacterial count in barn air, barn air temperature and microorganism count in milk (P<0.05 all). In conclusion, bacterial count in barn air and consequently postsecretory milk contamination depend on the season, i.e. barn air temperature.

Keywords: dairy cow, microclimate, air bacteria, milk quality

INTRODUCTION

Milk is one of the essential foodstuffs of animal origin in human diet. Because of its composition, milk is ideal food for all age groups and has a protective role in human health. However, milk may also be harmful for human body, in particular when it is contaminated with microorganisms, since it is a medium favouring their growth. Milk contamination with microorganisms can be secretory, occurring *via* mammary glands, or postsecretory, arising from the environment [1]. Milk quality is directly influenced by the microorganism count irrespective of the route of contamination [2]. The aim of the study was to investigate whether bacterial count in a dairy cow barn air relative to the microclimate influences microorganism count in fresh raw milk.

MATERIAL AND METHODS

The study was conducted at a dairy cow farm in all seasons. Cows were kept in tie-stall of medium length (1.8x1.3 m) with straw bedded floor and milked at the site into cans. Udder disinfection was performed before and after milking. Lighting and ventilation in the barn was combined, natural and artificial. Cows were fed concentrated and voluminous feed twice daily. Bacteriological air contamination (CFU/m^3), microclimate parameters, air temperature (ºC) and relative humidity in the barn (%), and microorganism count in milk (CFU/mL) were determined 3 times *per* month (at morning milking, at the beginning, middle and end of month) in April, August, October and December. Bacterial count in the barn air was determined by air sampling (Merck MAS-100, Merck KgaA, Germany) into Petri dishes with nutrient agar (Biolife, Italy) for determination of total aerobic mesophilic bacterial count and their incubation at 37 ºC for 24 hours. Microclimate parameters were measured by a portable digital instrument (Testo, Germany), while microorganism count in raw milk was determined by standard laboratory methods. The data collected were analysed by use of the Statistica v.12.5 software (StatSoft Inc., 2014).

RESULTS

Table 1. Microclimate parameters in the barn throughout study period

Parameter	Month					
April	August	April + August	October	December	October + December	
Mean±SD						
Air temperature (°C)	21.33^{a,b} ±0.58	26.00^{a,c,d} ±1.00	23.67* ±0.29	19.00^{c,e} ±1.00	12.33^{b,d,e} ±1.53	15.67* ±1.16
Relative air humidity (%)	60.00^{a,b} ±2.00	63.67^{c} ±3.22	61.83* ±2.57	70.00^{a} ±4.36	73.00^{b,c} ±2.00	71.50* ±1.50

n=3 measurements *per* month for both parameters; [a,b,c,d,e]values in the same row marked with the same letter showed statistically significant between-month differences at the level of $P<0.05$; *values in the same row differed statistically significantly at the level of $P<0.05$

Table 2. Total microorganism count in barn air and raw milk throughout study period

Parameter	Month					
April	August	April + August	October	December	October + December	
Mean±SD						
Bacterial count in barn air (CFU/m^3)	2.03×10^{4a} ±868	$2.96\times10^{4a,b,c}$ ±1380	$2.49\times10^{4*}$ ±267	$2.29\times10^{4b,d}$ ±925	$1.84\times10^{4c,d}$ ±872	$2.07\times10^{4*}$ ±191
Microorganism count in raw milk (CFU/mL)	1.30×10^{4a} ±1000	$1.97\times10^{4a,b,c}$ ±1155	$1.63\times10^{4*}$ ±1041	$1.53\times10^{4b,d}$ ±1528	$1.20\times10^{4c,d}$ ±1000	$1.37\times10^{4*}$ ±1258

n=3 measurements *per* month for both parameters; [a,b,c,d]values in the same row marked with the same letter showed statistically significant between-month differences at the level of $P<0.05$; *values in the same row differed statistically significantly at the level of $P<0.05$

DISCUSSION

Optimal air temperature in dairy cow barns is 5 to 20 ºC, production temperature 5 to 28 ºC, and optimal relative air humidity 60% to 80% [3]. Study results showed air temperature and relative humidity levels to be within the production limits for dairy cows throughout the study period. Total bacterial count in the barn air was significantly higher ($P<0.05$) during warm season and *vice versa*, which could be attributed to determination of aerobic mesophilic bacterial count, whose growth is favoured by air temperatures of 20-45 ºC. Bacterial count in the barn air ranged from $1.84\text{x}10^4$ CFU/m^3 air in December to $2.96\text{x}10^4$ CFU/m^3 air in August, which was within the range determined by Matković *et al*. [4]. Total milk microorganism count of ≤100,000 CFU/mL met the standard quality requirements [2] and followed the total barn air bacterial count pattern (lowest and highest milk bacterial count recorded in December and August: $1.20\text{x}10^4$ CFU/mL and $1.97\text{x}10^4$ CFU/mL, respectively). High positive correlation was found among the barn air bacterial count, microorganism count in raw milk and barn air temperature ($P<0.05$ all). Between barn air temperature and relative humidity there was a high negative correlation ($P<0.05$), whereas no such correlation was recorded between barn air relative humidity and other parameters, i.e. barn air bacterial count and microorganism count in raw milk ($P>0.05$ both).

CONCLUSIONS

The results obtained in this study suggest a conclusion that season, i.e. barn air temperature, influences bacterial count in barn air, and both of these parameters influence postsecretory bacterial contamination of raw cow milk.

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THERAPEUTIC APPROACH TO MASTITIS AT DRYING

Helio Langoni[1], Anelise Salina[1], Benedito Donizete Menozzi[1]

[1]*Department of Veterinary Hygiene and Public Health, São Paulo State University, Botucatu, São Paulo, Brazil*

SUMMARY

Mastitis is a problem for the milk industry with economic losses. Its control is important and the treatment at drying is a recommendation to promote healing of pre-existing infection and to prevent new infections during the lactation. Was used cloxacilin benzathine formulation at drying off in subclinical mastitis cases where the pathogens were *S. aureus* (n=50) and coagulase negative *Staphylococcus* - CNS (n=120). Immediatelly post-partum a colostral secretion was collected for microbiological examination. CNS cure was obtained in 30 teats (25%), that corresponds to 75% of microbiological cure and for *S. aureus* mastitis cases the isolation of agent occurred in 20 teats post-partum, resulting in 60% of efficacy.

INTRODUCTION

Mastitis remais an severe problem for the dairy-farm. Besides the economic losses we must consider the public health aspects because many microorganisms involved in their etiology can cause human disease's especially due the production of enterotoxins by CNS and also by *Staphylococcus aureus*. It is caused by many microorganisms being *S. aureus* among the contagious pathogens one of the most important (1). CNS, *Corynebacterium bovis* and *Streptoccus agalactiae* are also frequent as contagious pathogen (2). The environmental pathogens as *Escherichia coli, Klebsiella pneumoniae*, *Enterobacter spp, Streptococcus uberis* and *Streptococcus dysgalactiae* are present where the cows lives and can be transmitted between or during the milking (1).

The mastitis control programs aims to reduces the infection at a low level considering the health of the mammary gland by de elimination of pre-existing infection and to the prevention of new cases of mastitis, as well as by the reduction of the infection duration time. The dry cow therapy is an important component in mastitis control to promote healing of pre-existing infection and prevent new infections during lactation (3). Cure rates range from 67% to 100% against the major causative pathogens of subclinical mastitis in dry period (4).

Various cloxacilin benzathine containing intramammary suspensions are available for use at drying off. Cloxalin is effective against penicillin-resistant and sensitive staphylococci (*S. aureus),* the most prevalent Gram positive pathogen involved in persistent udder infections which are targeted by the drying off treatment. The concentration of cloxacilin in the glandular tissue of udder was verified (5), and the results showed that the concentrations measured in the perfusate samples were bellow the limit of quantification, indicating limited absorption of the antibiotic from the glandular tissue. Concerns the field testing of different products for effectiveness agaisnt *S. aureus* during the dry period showed cure rates greater than 80% (6). The intramammary application of long-acting antibiotics is an essential element in the drying off and must be recommended for the mastitis control (7). Considering the importance of the mastitis control the purpose of this study was to evaluate the antibiotic treatment at drying off.

MATERIAL AND METHODS

This study was conducted in a commercial dairy farm with mechanical milking system with an average of 120 Holstein cows on lactation, during the herd milk quality monitoring period of 2011-2014. The status of udder health was known from results of bacteriological tests conducted monthly on milk samples with CMT positive grade 3+ or from the frequency and type of udder pathogen from milk samples, collected before or during course of clinical mastitis cases. Heifers and adult cows were enrolled in the peripartum period, diagnosed microbiologically by milk secretion cultivation obtained on the last day of lactation, after washing and drying the teats with individual disposable paper towels and disinfection with iodine alcohol 3%. Duplicate quarter samples were taken at the same time for bacteriological tests prior the treatment in sterilized vials and frozen until send to the laboratory for performing cultures in bovine blood agar base 5% and agar MacConkey with incubation for up to 72 hours. Were subjected to the study, and analysis cases where the involved pathogens were *S. aureus* and CNS sensitive in vitro to cloxacilin. Each cow was treated with the same product (benzathine cloxacilin 600 mg - Orbenin Extra Dry Cow®) in all quarters. Immediately post partum, a sample of colostral secretion was collected proceeding in the same way for microbiological examination.

RESULTS AND DISCUSSION

For *S. aureus* mastitis cases the results showed the reisolation the agent only in 20 teats (40%) post partum (60% of efficacy). For CNS, the cure was obtained in 30 teats (25%), with 75% of microbiological cure. Results of the present study are in agreement with other studies (8). Similarly the results agree with (9) by using the same product at drying off on farms with a low and a high prevalence of heifer mastitis in the Netherlands.

CONCLUSION

We can conclude by the importance of the mastitis treatment at drying and also that the used formulation can be suggest in the control mastitis program.

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INTRAMAMMARY INFUSION OF LACTIC ACID BACTERIA AND THERAPEUTIC EFFECTS ON MASTITIS IN DAIRY COWS

H. Nagahata[1], M. Horiguchi[1], A. Tamiya[1], S. Gondaira[1], H. Higuchi[1] & K. Kawai[2]

[1]Animal Health Unit, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 069-8501, Japan;
[2]Department of Animal Health, School of Veterinary Medicine, Azabu University, Sagamihara 229-8501, Japan

Summary

The objective of the study was to characterize the immune responses of mammary gland by intramammary infusion of LAB in cows, and to evaluate the therapeutic effects of LAB to lactating cows with mastitis. Twelve Holstein cows were used to analyze the cellular and immunological effects of mammary glands by infusion of *Bifidobacterium breve*(3x10^{9}cfu) and 32 cows with subclinical mastitis for therapeutic trials. Ten to 30 fold increases in numbers of SCC were found in milk from quarters at 1-2 days after infusion of LAB. Significantly increased concentrations of LF, IgG and IgA, and Increased cytokine responses (IL-1**β**, TNF-**α,** IL-8,-12) on milk leukocytes were found in cows at 1-3 days after infusion. The therapeutic trials of intramammary infusion of LAB on mastitis were conducted: bacteriological cure rates were 62.5% for environmental streptococci, 50% for CNS, 0-20% for *Staph. aureus* at 7-14 days post infusion. SCCs in quarter milk with no bacteria growth were significantly decreased at 14 days. Intramammary infusion of LAB may be a possible approach for non-antibiotic treatment for mastitis.

Introduction

Development of alternative approaches to antibiotics is of increasing concern for control of mastitis. The uses of lactic acid bacteria (LAB) and their bacteriocin were proposed as possible alternative approaches for treatment of bovine mastitis (1,2). This study was performed to investigate the immune responses of mammary gland of dairy cows followed by intramammary infusion of LAB, and therapeutic effects of LAB infusion on subclinical mastitis of dairy cows were evaluated.

Materials and methods

Cows: 44 Holstein Friesian cows, mid lactation stage, were selected based on their somatic cell counts (SCC) and the presence of mastitis-causing pathogens in milk from dairy cows. Based on the bacteriological and SCC data on 3 days before the study, 24 quarters with subclinical mastitis showing mastitis causing pathogens >250 CFU/ml and 30-150 x 10^4 cells/ml in quarter milk and 8 quarters with no pathogen and <20 x 10^4 cells/ml in milk were selected.

Milk samples and Parameters: Quarter milk samples were collected on the day before infusion (day-1), prior to infusion (day 0) and on days 1,3,5,7,14 and 21 days post-infusion. SCC was determined by a cell counter and milk compositions were measured. Bacteriological analysis of quarter milk was performed by the procedure described (3). N-glucosaminidase (NAGase) activity in milk was measured by fluorometric assay (3). Milk Lactoferrin (LF), IgG and IgA were measured using kits.

Lactic acid bacteria (LAB): LAB, *Bifidobacterium breve,* was propagated and was adjusted to 1 x 10^9 CFU/ml and used as a live LAB or heat-killed LAB. LAB (3ml, 3x 10^{9}CFU) were infused into the teat following regular milking.

Chemiluminescence (CL) : CL response in milk was measured in a luminometer (3). CL counts (cpm) were read after addition of opsonized zymosan (opz 10mg/ml).

Cytokine mRNA and cytokines: mRNA expressions of TNF-α, IL-1β, -6, -8 and Lf on somatic cells and cytokines TNF-α, IFN-γIL-1β, -8 and -12 in milk collected from LAB-infused quarters were determined using kits.

Results

Significant increase in SCC and NAGase activity in quarter milk were found at 1-2 days post infusion and their values decreased after 3-5 days. SCC in quarter milk of cows after infusion of LAB showed 7 to 30-fold increase compared to prior to infusion, and peaked at day 1-2 after infusion, thereafter SCC decreased and reached to the pre-infused values on the day 7. LF concentrations in infused quarter milk significantly ($P<0.05$) increased on day 3 to day 5 after LAB infusion than those of pre-infused values. IgG in infused quarters appeared to be increased and IgA levels in infused quarters on day 4 to day 7 were significantly ($P<0.05$) higher than pre-infused values.

OPZ-stimulated CL response in LAB infused quarters increased significantly on day 2 to day 4 after infusion compared to the pre-infused values. mRNA expressions of IFN-γ, TNF-α, IL-1β, -8, and Lf were found to be up-regulated in somatic cells from LAB infused quarters on days 1. Cytokines IFN-γ, IL-1β, TNF-α, IL-8 and -12 in milk were detected on day 1 after LAB infusion. Increased IL12 and TNF-α were found in milk from cows with mastitis than that of recovered at 21 days after LAB infusion.

The therapeutic trials of intramammary LAB infusion were evaluated in dairy cows with mastitis: bacteriological cure rates (<250cfu/ml) were 62.5 %(5/8) for environmental streptococci, 50%(5/10) for CNS, 0-20%(1/6) for *Staph. aureus* at 7-14 days post infusion. Mean SCCs in 8 quarter milk with no bacteria growth were significantly decreased at 14 days post infusion.

Discussion

Marked increases of SCC and OPZ-induced CL responses found in LAB infused quarters, indicating that LAB induces large influx of PMN into LAB-infused quarters, and enhanced CL response appears to be associated with the increased numbers and potentiated functions of migrated neutrophils in quarters. These results suggested that intramammary LAB infusion promotes the clearance of the pathogens causing mastitis from quarters and alleviate the local infection in affected quarters from cows with subclinical mastitis.

The up-regulations of cytokines IL-1β、TNF-α, IL-6, -8 and NF-*k*B mRNA expressions on somatic cells from LAB-infused quarters were found on days 1 after infusion than those of LAB pre-infused. These results suggested that LAB stimulates immune system and modulates immune functions of the mammary gland.

The therapeutic trials of intramammary LAB infusion were evaluated in dairy cows with subclinical mastitis: the bacteriological cure rates were 62.5 % for environmental streptococci, 50% for coagulase-negative staphylococci, and 0-20% for *Staphylococcus aureus* at 7-14 days post infusion. SCCs in quarters with no bacteria growth were significantly decreased at 14 days post infusion.

Conclusions: Intramammary LAB infusion promotes the clearance of the pathogens causing mastitis from quarters and alleviates the local infection in affected quarters from cows with subclinical mastitis. Intramammary infusion of LAB may be a possible approach for non-antibiotic treatment for mastitis.

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THE EFFECTIVENESS OF DIFFERENT PRE-, AND POST DIPPING TEAT DISINFECTANTS AGAINST PROTOTHECA ZOPFII ALGAE

P. Kovacs[1]; A. Kovacs[1], G. Szita[2], V. Jurkovich[1], E. Brydl[1], L. Konyves[1]

[1]Department of Animaly Hygiene, Herd Health and Veterinery Ethology, Szent Istvan University Faculty of Veterinary Sciences, Budapest, Hungary;
[2]Department of Food Hygiene, Szent Istvan University Faculty of Veterinary Sciences, Budapest, Hungary

Summary: Several before and post milking teat dipping products were tested against *Prototheca zopfii* algae. The effectiveness of both type of products were evaluated on wet and dry teats. During our experiments we found that on dry teats the pre and post milking teat dipping products are much more effective than on wet teats. From the four different pre dipping products, polyhexamethylene biguanide (PHMB) didn't show any effectiveness against the algae. Chlorhexidine was much more effective, but both the dry and the wet "teat" hosted algae after the treatment. The didecyl-n methyl ammonium chloride and the PVP-iodine based products were very effective. No algae could be retrieved from the "teats" after these products. We have examined nine different of post milking products with 2 different type of active substances. We have examined 4 different products with lactic acid in different or unknown concentration. One unknown concentration product was totally effective while the 3% cc product was almost 100% effective. The other two products were unable to kill the algae on the wet teat, while they were effective on the dry teats. 5 different iodine based products were also tested. The wet teat also altered the effectiveness of these products, but it was still better than the lactic acid, and they were very effective on the dry teats.

Introduction: Since the first identification *Prototheca zopfii* became the 4th-5th most common mastitis pathogen in Hungary. Without an effective treatment the only way to control this disease is prevention, and in this, the before and after milking teat disinfection plays a major role.

Materials and methods: For our experiment we collected four different pre dipping and nine different post dipping products from Hungarian dairy herds. We wanted to provide the exact same conditions every time, to standardize the experiment, therefore we made artificial teats (fingers of plastic gloves filled with cotton swabs) and after proper disinfection these teats were used. Every teat was dipped into a *Prototheca zopfii* broth, and the disinfectants were used on wet and dry teats as well. In case of the pre dipping products we waited as long as it was advised in the manual of the products. With the post dipping products we performed two different methods. We gave the products 30 minutes and the teats were used as they were and we also tried to wipe it with a paper towel to act like the cleaning mechanism of the bedding material after lying down. Then the teats were pushed to Sabouraud agar plates. After 48 hours of incubation the number of alga colonies was counted on the agar plates. We standardized the test area on the plates, the colonies were counted on these marked test areas. Every experiment was repeated 3 times and the average of the 3 results were used in the evaluation.

Results: We compared four different pre dipping products. The polyhexamethylene biguanide (PHMB) was totally ineffective (wet teat (WT) 183 colonies, dry teat (DT) 28 colonies), while the chlorhexidine was much more effective (WT: 8, DT: 1) but didn't kill all the algae on the teat. In both cases the wet teats have much worse results than the dry treats. The didecyl-n methyl ammonium chloride and the PVP-iodine based products were able to kill all the algae on the wet and the dry teats too (WT: 0, DT: 0). From the nine post dipping products four contained lactic acid in different concentrations. One of them was effective while the other three had moderate effectiveness.

- - Lactic acid cc unknown(1): WT: 12, DT: 0; WT+swipe: 80, DT+swipe: 0
- - Lactic acid cc unknown(2): WT: 0, DT: 0; WT+swipe: 0, DT+swipe: 0
- - Lactic acid 1%: WT: 127, DT: 0; WT+swipe: 3, DT+swipe: 0
- - Lactic acid 3%: WT: 0, DT: 1; WT+swipe: 1, DT+swipe: 0

The five iodine based post dipping products had better effectiveness, especially on dry teats.

- - complex iodine: WT: 0, DT: 0; WT+swipe: 50, DT+swipe: 0
- - PVP iodine: WT: 13, DT: 0; WT+swipe: 13, DT+swipe: 0
- - PVP iodine 5%: WT: 0, DT: 0; WT+swipe: 0, DT+swipe: 0
- - iodine 5%: WT: 0, DT: 0; WT+swipe: 0, DT+swipe:1
- - iodine 3600 mg/kg: WT: 16, DT: 0; WT+swipe: 0, DT+swipe:0

Conclusions: In case of teat disinfection the agent is only one of the things which have an effect on the result. For the teat preparation, before milking, PVP iodine or didecyl-n methyl ammonium chloride based products are advised to use. For post dipping both the lactic acid and iodine can be good, but the manual must be checked to see the concentration or the form of the active substance (min. 3% lactic acid or 5% PVP iodine). If the teats are washed before the disinfection, they must be dried properly, or the effectiveness of the products will be lower than expected. Before, during and after the post milking teat dipping the washing of the floor or the milking units is not advised because it also can have a negative effect on the disinfection.

CHLORHEXIDINE BASED TEAT DIPS - A COMPARISON OF DISINFECTIVE PROPERTIES

E. Vavrova[1], M. Machacek[2], Z. Sladek[1], V. Trojan[3]

[1]Department of Morphology, Physiology and Animal Genetics, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech republic
[2]University of Veterinary and Pharmaceutical Sciences, Palackeho trida 1-3, 612 42 Brno, Czech Republic
[3]Department of Plant Biology, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech republic

Summary

The aim of this study was to compare disinfective properties of three chlorhexidine based teat dips. The samples were obtained from teat skin of 12 clinically healthy dry cows of Holstein-Friesian breed. After cleaning their teats with wet towels, we used sterile swabs to wipe the teat skin to get the control samples. The swabs were put into test tubes with BC7 medium. Four cows were treated with pink dense, pink sparse and blue sparse dips respectively. After 30, 60, 90, and 120 minutes, we took samples the same way previously. Each time 4 samples of every concentration were gained. These samples were examined by modified suspension method according EN on Malthus AT machine in the Institute for State Control of Veterinary Biologicals and Medicines in Brno. Swabs were cultivated in 4ml of BC7 medium for 24 hours at 37°C. Antibacterial properties of distinct disinfections are shown by the changes of electric conductivity of the medium. Each suspension was inoculated to blood agar and cultivated for another 24 hours, so that the colony forming units (CFU) could be counted. The bacteriostatic properties are proven by decline of CFU in the samples obtained in certain exposure times compared to control sample. To declare the disinfective properties, we need the decline to be 3 logarithmic orders or more. The results have shown sufficient bacteriostatic effect of all three dips. In 30 minutes after dipping, the CFU of blue sparse dip decreased in 3.77 logarithmic orders. Both pink dips have a great bacteriostatic effect – decline in 3.77 logarithmic orders. The onset of the effect appeared 30 minutes after exposure and went on until the end of sampling.

Introduction

Many disinfectants have been developed specifically for the dairy industry to prevent the spread of infectious diseases (Boddie et al., 1997). Teat dipping with a proven disinfecting teat dip has been demonstrated to be one of the most effective mastitis control practices (Buchalova and Rauer, 2013). Teat dips are commonly used before and after milking to reduce new infections induced by mastitis-causing bacteria in lactating dairy cows (National Mastitis Council, 1996). The dips are designed to effectively reduce infection caused by environmental bacteria as well as contagious bacteria. After milking the teat canal remains opened for at least 15 minutes allowing pathogenes to enter. Application of the teat dip immediately after milking kills the significant amount of the pathogens on teats and reduces the possibility of the pathogenes entering the teat canal. The barrier dip is most beneficial when the cows are predominantly housed inside the barn (Foret et al., 2006).

Material and methods

The experiment was performed at a Mendel university farm in Žabčice. 12 healthy dry cows of Holstein-Friesian breed were placed in the parlour. The teats of all cows were cleaned with wet towels and sterile swabs were used to wipe the skin of the teats to get the samples of bacterial colonization (control). The swabs were put into test tubes with 4ml BC7 medium. Teats of four cows were treated with pink dense, pink sparse and blue sparse dips respectively. Teat dips were provided ready to use. 30, 60, 90 and 120 minutes after teat disinfection, we took the samples as we did in the beginning of the trial. Each time 4 samples of every concentration were taken. The analyses were made in the laboratory of Institute for State Control of Veterinary Biologicals and Medicines in Brno. The samples were examined by modified suspension method according EN on Malthus AT machine. The test tubes were put into this machine and their contents were cultivated for 24 hours at the temperature of 37°C. Electric conductivity gauging in these tubes is possible thanks to their metalic parts so antibacterial properties of distinct disinfection concentrations are shown by the changes of electric conductivity of the medium. After cultivation the suspensions obtained from the tubes were inoculated to blood agar and cultivated on it for another 24 hours at the temperature of 37°C. The next day colony forming units (CFU) were counted. The bacteriostatic properties are proven by decline of CFU in the control sample compared to exposure times for each chlorhexidine based teat dip.

Appropriate medium is inoculated with suspension of a microorganism in order to achieve total repression of microorganism under experimantal conditions – GE (germicidal effect). GE is sufficient, if the CFU between control sample and exposure sample declines in more than 5 logarithmic orders (the disinfectant is killing microbs) in certain exposure time. When the decrease is in 3 logarithmic orders, the disinfectant has bacteriostatic properties (supression of bacterial gowth).

Results and discussion

The results of our experimant have shown zero metabolic activity of microorganisms present in the samples from teat skin of cows treated with pink dense and pink sparse dips. It means that these two products have bacteriostatic properties. There was no substrate usage leading to changes in the conductivity of the medium. This information points to fact that there were no microbes present. The cultivation on nutrient substance activated the microorganisms and this lead to their growth and colony forming. There was an sporadic metabolic activity in the samples swabbed from teat skin after dipping with blue sparse product. The detection times were put down so the graph shows the accurate progress. All of the teat dips have sufficient bacteriostatic effect. The cultivation of the samples has shown that the pink dense dip has the best disinfective properties. The colonies were small and separated. On the nutrient substances with samples of pink sparse and blue sparse dips, there were coherent layers of microbes. The most important thing is that only spores survived the chlorhexidine treatment. The fact that chlorhexidine doesn´t kill spores is well known.

Conclusion

The primary purpose of using a barrier plus germicide teat dip is to reduce IMI caused by both environmental and contagious pathogens. This study demonstrates positive effects from the use of all chlorhexidine teat dips used. The high viscosity in barrier products also reduce contact with bacteria colonizing the teat skin and the teat canal respectively.

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COMPARED EQUIPMENT FOR ANALYSIS OF SHEEP MILK

A. Saltijeral O., J. E. Guerra L.**, L.A. Lopez J.**, M. Luna L.**, C. G. Ruiz L., J. L. Saltijeral G.

**Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana, Mexico*
***Facultad de Agronomía. Universidad Autónoma de Sinaloa, Mexico.*

Introduction

In Mexico, production of sheep's milk is relatively recent compared to Europe, Asia and Africa. In our country this production occupies a very little place in the national and international context. However, to develop it, it could be the basis of an industry that represent solutions to various problems of market, employment and nutrition.

In Mexico sheep milk is mainly used for making cheese, until recently the most important nutritional properties of milk from sheep that make those who consume to benefit from high quality nutrients were not known. Is important that producers and technicians involved in milk production known analytical techniques to measure the quality of the milk. Given the proliferation of devices in different parts of the world. The aim of this study was to compare two different equipment.

Material and Methods

Traditional methods of analysis, quantitative and qualitative are laborious; require time, labor and cost are high. For this we have developed fast and reliable techniques for assessing control the quality of agricultural products.

Samples was collected from sheep milk of different breeds (East Friesian and Lacaune) from the Sheep Producers Association of Milk, SPR de RL de C.V. Rio Frio, Ixtapaluca, State of Mexico, during the months from April to June. Using the methodology described in NOM -155 - SCFI - 2012 for proper sampling (Diario Oficial / SE, 2012)

Two equipment in relation to the reference methods were compared; one was the principle of infra-red (spectrophotometry) using the 133B Milko- Scan (Foss Electric®) and the second method was by the principle ultrasonic analysis using the Lactoscan SA50. (Milkotronic®). The percentage of fat, protein, lactose, solid no fat and freezing (it is finally only by ultrasonic analysis) was measured. Samples of milk were taken daily once a day for 30 days in 35 dairy ewes. Measurements were performed in accordance with the following regulations: fat (ISO 488 / IDF 105 : 2008) protein (ISO 8968 / IDF 020 : 2001), and lactose (ISO 2262 / IDF 198 : 2007) plus freezing point (ISO 5764 / IDF 108 also sheep milk samples collected using reference methods (methods Gerber, Microkjeldahl, Lane and Enyon) published in the ISO / IDF and NOM standards in the dairy laboratory analysis.

Milk analyzers were calibrated to measure the composition of sheep milk using values obtained from fat, protein, and lactose by reference methods, making subsequent adjustments to calibration. JMP® 10software was used for comparative statistical analysis “Student t “between the methods of milk.

Results

Ultrasonic analysis (UA) has a higher degree of correlation with the reference methods that infrared method (IRM) Using test " t student " highly significant differences (P > 0.01) in protein and lactose between the two methods of analysis used before calibration and fat after addition of protein between the ultrasonic analysis and reference methods calibrated before calibrating and lactose between infrared analysis and reference methods before calibrating, and significant differences (P > 0.05) between fat analyzers and the reference methods.

Table No. 1. Analysis of milk components and comparison between two equipment

Components	Reference methods	IRM	% difference	UA	% difference
Protein	4.90	6.52	+ 33.1	3.40	-30.6
Fat	8.3	8.2	-1.2	8.96	+7.9
Lactose	4.54	5.07	+11.7	6.06	+33.5
Solid Non Fat	17.74	19.79	+2.05	18.42	+0.68

Conclusions

IRM underestimate the amount of fat and overestimates the amount of protein. UA underestimates the amount of protein and overestimates the amount of lactose. Comparison with reference methods in the other parameters has no significant differences. So both methods are recommended for use, even for small production is preferable to use the UA and large industries IRM. Important factors are the amount of sample required (Lactoscan need 25 ml / Milko -Scan only 5 ml); the time it takes to process them (Lactoscan, 1 min / 20 sec Milko -Scan), plus the cost of equipment, since as shown in Table 2 huge differences in the cost of each equipment.

Table No. 2. Average cost of milk analyzers on the market.

Equipment	Principle	Brand	Parameters measuring	Price*
Bentley 150	infra-red	Bentley	F, P, L, S	54 000
Lactoscan SPL-60	ultrasonic	Milkotronik	F, P, L, M, NFS, FP, WA, D, T	2 000
Milko-Scan Minor 6	infra-red	Foss Electric	F, P, L, NFS, TS, FP	45 000
LactiCheck 1	ultrasonic	Gaytec	F, P, L NFS, WA	4 000
Milko-Scan 133B	infra-red	Foss Electric	F, P, L, NFS, TS	38 000
Lactoscan SA50	ultrasonic	Milkotronik	F, P, L, M, NFS, FP, WA, D, T	3 000
F = Fat; P = Protein; L = Lactose; M = Minerals; NFS= Nonfat solid; TS =Total solid; FP = Freezing point ; WA = Water added; D = density; T =Temperature of the sample				

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Preventive veterinary medicine

PERFORMANCE OF CROSSBRED PIGS BY INCLUDING SUGARCANE PRESS MUD AS AN ALTERNATE FEED IN SWINE RATION

S. Sahu, B. H. M. Patel, M. Singh, A. K. Verma, G. Singh, S. Kumar, T. Chandrasekar, R. Maurya

Livestock Production and Management Section, Indian Veterinary Research Institute, Bareilly, India

Summary: An experiment was designed to explore the sugarcane press mud (SPM), a waste product of sugarcane factory as an ingredient in the swine ration on growth traits, nutrient digestibility and economy of crossbred (Landrace × *Desi*) pigs. A total of 28 weaned piglets were randomly divided into 4 treatment groups *viz.,* T_0: Control diet without SPM, T_1: Concentrate with 5% SPM, T_2: Concentrate with 10% SPM, T_3: Concentrate with 15% SPM inclusion, maintaining 3 barrows: 4 females under each group. Iso-N_2 and caloric diets were formulated for weaner (10-20kg), grower (20-50kg) and finisher (50-80kg). Growth parameters along with feeding cost per kg gain were recorded at each stage and digestibility trial was performed 60 days after start of the experiment. ADG (kg) was significantly ($P<0.05$) lower in T_2 (0.354 ±0.046) and T_3 (0.349±0.059) than T_0 (0.446±0.081) and T_1 (0.424 ±0.039) group and FCR was poor for the SPM treated groups only during weaner stage, while on subsequent stages above parameters were found comparable. Fortnightly measurements of body length, body height, chest girth and flank to flank length were statistically similar among the groups. Intake and digestibility of DM, OM, CP, NFE and Total carbohydrate during digestion trial was non significant between the treatments but calcium intake and its absorption as % of intake was significantly higher ($P<0.05$) for T_3 than rest of the groups. Absolute mean value for feed cost/kg live weight gain at the end of the study was lowest for T_3 group (Rs. 69.50) and the corresponding value for T_0, T_1 and T_2 were Rs. 74.75, 74.97 and 74.37, respectively. It indicates SPM can be included in the diet upto 15% level.

Introduction

Agro-industrial by-products as alternate source of feed for pigs are constantly in search to replace a portion of the energy, protein or other nutrients in a complete feed to reduce the cost of compound feed. Many researchers tried to replace maize with molasses [1] and with Jaggery Filter Cake (JFC) [6]. There are some other locally available by products whose study in the animal nutrition are limited. Sugarcane press mud (SPM) is one such agro-industrial by-product available plentily in sugarcane factories and generally an organic waste for the agricultural field. Feeding SPM to animals have been tried very recently by few workers [7]. However, scientific study on feeding SPM as an alternate feed in the swine diet was limited. Therefore the present study was carried out to study the effect of SPM on different growth traits of crossbred Landrace pigs.

Material and methods

The experiment was conducted at Swine Production Farm, IVRI, India from January 2012 to July, 2013. 28 crossbred (Landrace ×Desi) piglets nearly 14 kg body weight were randomly distributed in four different groups maintaining 3 male (barrow):4 female in each group. They were housed in a well ventilated shed under RCC roof and on cement concrete floor. All the piglets were dewormed and vaccinated as per standard protocols before the start of experiment. Standard [5] balanced ration (iso-caloric and iso-nitrogenous diet) was prepared by Feed Technology Unit for three different stages, *viz.* weaner (10-20kg), grower (20-50kg) and finisher (50-80kg). Weighed quantity of feed was offered daily twice at 9:30 AM and 4:00 PM. The residues were collected and weighed individually at 9:00 AM on next day in all the groups. Various parameters *viz.* total feed intake, average daily dry matter intake (DMI), feed:gain, total feed cost and feed cost in Rs. /kg body wt. gain were recorded in each stage (weaner, grower, finisher and cumulative period) of the animals. The data obtained were analysed using SPSS soft ware.

Results

During the weaner period T_0 (0.446±0.081 kg) and T_1 (0.424±0.039 kg) showed significantly higher ($P<0.05$) ADG. The total feed (DM basis) consumed was comparably less in T_0 (12.48 ±2.16 kg). Average DMI were significantly higher ($P<0.05$) in T_1 than other groups. Feed: Gain ratio was linearly increased though non significant and the total feed cost (Rs.) was marginally higher in T_1. ADG during the grower period ranged from 0.491±0.091 kg in T_2 to

0.545±0.115 kg in T_0. The total feed (DM basis) and average DMI consumed was comparably less in T_0 (88.10 ±5.72 kg). Feed: Gain ratio was statistically similar, but feed cost/kg BW gain was very close between T_0 (Rs 68.95±3.24) and T_3 (Rs 69.09±5.23). ADG and the days to achieve finishing body weight were comparable among the groups. The total feed (DM basis) consumed was comparably less in T_2 (119.65 ±10.04 kg) than others. Feed: Gain ratio was marginally better for T_3 (4.08±0.27), than other groups. Further, the total cost and feed cost/kg BW gain involved in feeding was lowest in T_3. Cumulative mean values showed non significant ($P<0.05$) results between the treatments. ADG and feed: gain was more for T_0 and feed cost/kg BW gain was lowest in T_3, but these were statistically non significant among groups.

Discussion

Decreased weight gain and ADG during the initial and grower stage might be due to a sudden change in the ration by incorporation of an unusual feed which could have changed the normal gut microflora and might have lowered digestibility of all nutrients which was reflected on growth [2] or might be due to a more CF or TA in the SPM treated groups which could have affected the digestibility of newly weaned pigs as their low digestive capacity [4]. During finisher stage and for the entire experimental period comparable value might be due to fully developed GIT which is capacious enough to digest the CF thus might have made the digestibility at par with the T_0 group animals [3].

Conclusions

As SPM inclusion in the diet upto 15% level reduces the rearing cost without causing any adverse effect on growth parameters, so can safely be used in swine ration.

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DETECTION OF BACTERIA FROM GENUS STAPHYLOCOCCUS FOR CHECKING OF SURFACE DISINFECTION FROM ANIMAL SHELTERS

Silvia-Cornelia Antoniu, Constantin Lupescu

Institute of Diagnosis and Animal Health, Bucharest, Romania

Summary

Introduction

The disinfection is one of the most important, efficient and inexpensive component of the complex of measures for prevention and control of transmissible diseases, maintaining the appropriate level of production and getting salubrious animal products.

The checking of surface disinfection from animal shelters consists of determining the degree of survival of microorganisms from environment of animals after contact with agents of disinfection.

The bacteria from Genus *Staphylococcus* are the indicators because they have very similar resistance properties to the agents of decontamination as well as pathogens.

Animal, material and methods

The scope of this paper is to develop a method of detection of bacteria from Genus *Staphylococcus* in the samples of sanitation taken on disinfected surfaces from animal shelters. The method is based on inoculation on/in the selective culture media (Giolitti-Cantoni bullion, Baird-Parker agar / Chapman agar) and confirmation of typical and atypical colonies by microscopic exam and biochemical tests (catalase and oxidase tests).

Results

The results obtained after the analysis of 2358 samples of sanitation, taken on disinfected surfaces from 252 objectives represented by shelters for birds, bovines, sheep and pigs, in the period 2012-2014, were reported as positive results 17,81% (bacteria from Genus *Staphylococcus* present/ decontaminated surface of 100 cm^2 or swab) and negative results 82,19% (bacteria from Genus *Staphylococcus* absent/ decontaminated surface of 100 cm^2 or swab).

The disinfection of each objective was considered suitable when all the samples collected from objective had a negative result or inadequate – when at least one sample had a positive result.

Conclusions

The method proposed is qualitative (the analyte is represented by staphylococci in generally and the measurand is the detection without enumeration) and more simple in comparison with the methods for the enumeration of coagulase-positive staphylococci (pathogenic species for human and animals by consumption), applicable in food and feed microbiology.

Introduction

The disinfection is one of the most important, efficient and inexpensive component of the complex of measures for prevention and control of transmissible diseases, maintaining the appropriate level of production and getting salubrious animal products (3).

The checking of surface disinfection from animal shelters consists of determining the degree of survival of microorganisms from environment of animals after contact with agents of disinfection.

The bacteria from Genus *Staphylococcus* are the indicators because they have very similar resistance properties to the agents of disinfection as well as pathogens. They are considered “more suitable” as indicators for necessary disinfection effectiveness in paratuberculosis and tuberculosis control, because they have a higher resistance than coliform bacteria (2).

Animal, material and methods

The scope of this paper is to develop a method of detection of bacteria from Genus *Staphylococcus* in the samples of sanitation taken on disinfected surfaces from animal shelters by swabs with diluents (product available commercially or diluents prepared in the lab as peptone water and peptone saline solution). For sampling, the operator wiped the surface to be tested, delimited by a template with an area of 100 cm^2, by swab in 2 plans (transversal and longitudinally) and washed it in the tube with diluent (1 ml), that represent the sample to be tested. It is important that sampling surface to be at least 1/10000 of total disinfected surface and represented by inaccessible points for disinfection operations (30%) and area in contact with animals (70%).

The method consisted in cultivation on/in the selective culture media (Giolitti-Cantoni broth, Baird-Parker agar / Chapman agar) and confirmation of typical and atypical colonies by microscopic exam and biochemical tests (catalase and oxidase tests).

The sample cultivation took place in 2 steps: 1. Inoculation in Giolitti-Cantoni broth and incubation at 37 °C, for 24±2 h/48±2 h in anaerobic conditions; 2. Subculture any tubes showing any blackening or black precipitate after 24±2 h or all tubes that do or not do develop a black precipitate after 48±2 h by spreading onto Baird-Parker agar (that is a moderately selective and differential medium for coagulase-positive stafilococci isolation) or Chapman agar (mannitol salt agar) (that is selective due to high quantity of sodium chloride, that inhibit the majority of bacterial species with exception of halotolerant species as *Vibrio spp.* and differential because of mannitol that, when is fermented, gives rise to acids, that modify medium pH and therefore the color of pH indicator - red phenol - from pink to yellow) and incubation at 37 °C, for 24/48 h in aerobic conditions.

On Baird-Parker agar: typical colonies are black or grey, shining and convex (1mm to 1,5 mm in diameterafter incubation for 24 h and 1,5mm to 2,5 mm in diameterafter incubation for 48 h) and surrounded by a clear zone which may be partially opaque. After incubation for at least 24 h, an opalescent ring, immediately in contact with the colonies, may appear in this clear zone; atypical colonies have the same size as typical colonies and may be: shining black colonies with or without a narrowe white edge, the clear zone is absent or barely visble and opalescent rig is absent or hardly visible or grey colonies free of clear zones.

On Chapman agar: yellow colonies mannitol-positive and pink colonies mannitol-negative.

The confirmation of typical and atypical colonies was performed by:

1. The microscopic exam for differentiation of cocci (GRAM-positive) to another rod-shape bacteria or moulds.
2. The catalase test for differentiation of bacteria from *Micrococcaceae* Family (positive) to bacteria from *Streptococcaceae* Family (negative).The reaction is positive when the effervescence appear immediately (gas bubbles).
3. The oxidase test for differentiation of bacteria from *Staphylococcus* Genus (negative) to bacteria from *Micrococcus* Genus (positive), using reagent for oxidase available commercially.

Results

- The 252 objectives were represented by shelters for birds (51.19 %), bovines (38.10 %), sheep (2.38 %) and pigs (8.33 %);

Table 1

Objectives	2012	2013	2014	TOTAL
Birds	86	28	15	129 (51.19 %)
Bovines	15	75	6	96 (38.10 %)
Sheep	4	2	0	6 (2.38 %)
Pigs	5	1	15	21 (8.33 %)
TOTAL	110	106	36	252

- The sampling was performed from 42.85 % prophylactic disinfected objectives and 57.15 % necessary disinfected objectives;

Table 2

Objectives	2012	2013	2014	TOTAL
prophylactic disinfected	75	14	19	108 (42.85 %)
necessary disinfected	35	92	17	144 (57.15 %)
TOTAL	110	106	36	252

- The results obtained after the analysis of 2358 samples of sanitation, taken on disinfected surfaces from 252 objectives represented by shelters for birds, bovines, sheep and pigs, in the period 2012-2014, were reported as positive results 17.81 % and negative results 82.19 %;

Table 3

Samples	2012	2013	2014	TOTAL
Positive	179	241	0	420 (17.81 %)
Negative	1200	450	288	1938 (82.19 %)
TOTAL	1379	691	288	2358

- The disinfection was considered suitable in 59.92 % objectives and inadequate – in 40.08 % objectives.

Table 4

Objectives	2012	2013	2014	TOTAL
Suitable disinfected	79	36	36	151 (59.92 %)
Unsuitable disinfected	31	70	0	101 (40.08 %)
TOTAL	110	106	36	252

Discussions

- Bacteria from *Staphylococcus* Genus are cocci, GRAM-positive, isolated or grouped “staphylo” (as bunch of grape), giving a positive reaction for catalase and negative reaction for oxidase.
- A positive result means the presence of bacteria from *Staphylococcus* Genus /decontaminated surface of 100 cm^2 or swab and a negative result is correlated with the absence of them.
- The disinfection of each objective was considered suitable when all the samples collected from objective had a negative result or inadequate – when at least one sample had a positive result.

Conclusions

1. The method proposed is qualitative.
2. The analyte is represented by the bacteria from *Staphylococcus* Genus, thatGRAM-positive cocci, isolated or grouped, giving a positive reaction for catalase and negative reaction for oxidase.
3. The measurand is the detection of presence, without enumeration, of stafilococci on determined (100 cm^2) or undetermined decontaminated surface.

4. The method is more simple in comparison with the methods for the enumeration of coagulase-positive staphylococci (pathogenic species for human and animals by consumption), applicable in food and feed microbiology.
5. The checking of surface disinfection from animal shelters based on the results of samples collected from disinfected objectives: the disinfection was considered suitable when all the samples collected from objective had a negative result or inadequate – when at least one sample had a positive result.

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COMPARISON OF FOOT PAD DERMATITIS IN TWO LINES OF TURKEY HENS IN ORGANIC FARMING IN GERMANY

C. Habig, B. Spindler, N. Kemper

Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

Summary

Due to the growing criticism of conventional livestock farming, the demand for organic products steadily increases. Foot pad dermatitis is one of the main problems in fattening poultry. The aim of the current study was to evaluate the occurrence of foot pad dermatitis in two lines of turkey hens kept under organic husbandry conditions in Germany. A total of 15 British United Turkeys (B.U.T.) 6 and 11 Kelly Broad Breasted Bronze (Kelly BBB) flocks from nine organic farms were investigated. Within two farm visits (rearing and fattening period) 50 turkeys were weighed and scored for foot pad dermatitis. Furthermore, approximately 200 pairs of feet per flock were examined at the slaughterhouse. A total of 6,431 pairs of feet were investigated. While the mean foot pad score did not differ significantly between the lines from week six to eight, flocks of the B.U.T. 6 line were scored higher for foot pad dermatitis than Kelly BBB between 14 and 20 weeks of age. Foot pad lesions occurred significantly more often and more severe with increasing age in B.U.T. 6, but not in Kelly BBB. No significant differences were observed between the lines for the mean live weight within the 16th to 18th week of age. None of the B.U.T. 6 flocks reached the goal weights given by the breeding company. In contrast, most of the Kelly BBB flocks reached and even exceeded the performance goals at the end of the fattening period. Differences in foot pad health between turkeys of the Kelly BBB and the B.U.T. 6 line under organic husbandry conditions can not only be attributed to higher body weights of B.U.T. 6, but on other, hitherto unknown factors.

Introduction

Due to the public criticism of conventional farming, green products have gained popularity in the market, because organic animal husbandry is considered to be more animal-friendly and saves resources. Beside behavioral disorders, foot pad dermatitis is a common condition amongst commercially grown turkeys. As less data exist for organic farming, the aim of the study was to assess the occurrence and severity of foot pad dermatitis in two lines of turkey hens kept under organic husbandry conditions in Germany.

Material and methods

A total of 15 British United Turkeys (B.U.T.) 6 and 11 Kelly Broad Breasted Bronze (Kelly BBB) flocks from nine organic farms in Germany were investigated. While most of the farmers kept turkey hens, only data collected from female turkeys were used for further analyses. The average flock size was 1,497 turkeys. Within at least two farm visits, as far as possible, 50 turkeys were randomly chosen for weighing and foot pad scoring. Moreover, approximately 200 pairs of feet per flock were scored after slaughtering. According to the scheme of Hocking et al. (2008) the left and right foot per bird was evaluated separately. Score 0 was given for macroscopically unaltered foot pads. Hyperkeratotic and/or small necrotic lesions were scored 1. Score 2 was assessed if less than one quarter of the sole pad was affected by necrosis. Moderate to severe foot pad lesions with up to or more than half of the foot pad area covered by necrotic cells were scored with 3 and 4, respectively.

Statistical analyses were carried out using SAS, version 9.3. For the data to be comparable between the lines, five dates encompassing several weeks of age were defined. Date one included data collected up to five weeks of age. Week six to eight were considered as date two. Data of flocks visited between weeks of age 14 to 17 belonged to date three. If the examination took place at the end of the fattening period from week 18 to 20 or later, date four and five were assigned, respectively. As data of turkeys younger than six and older than 20 weeks were only present for one of the lines, the first and fifth dates were excluded from statistical analyses. Foot pad scores were analysed using the mean of both feet per bird. Significance of differences between means of B.U.T. 6 and Kelly BBB at the three dates was determined using a two-sample t-test. The date, turkey line and their interaction were regarded as fixed

effects in the statistical model. Results of the analyses of variance were considered significant when P-values were less than 0.05.

Results

The total number of pairs of feet examined within the frame of the project was 6,431. A calculated weighed kappa value of 0.6059 indicated a moderate to substantial agreement between the scores given for the left and right feet per bird. While the mean foot pad scores did not differ significantly between the lines at date two, significant differences were observed between the 14th and 20th week of age, with higher scores detected for B.U.T. 6 (Table 1). Although the overall effects of date and date by line interaction were not statistically significant, flocks of the B.U.T. 6 line showed higher degrees and frequencies of foot pad dermatitis with increasing age (Table 1). No significant differences were detected between Kelly BBB and B.U.T. 6 for the mean live weights measured in the 16th, 17th and 18th week of age (Table 2). Flocks of the B.U.T. 6 line were approximately one kilogram heavier compared to Kelly BBB in week 16 and 18. In contrast, the bronze turkeys had almost 500 g higher mean body weights than B.U.T. 6 in week 17. None of the B.U.T. 6 flocks kept under organic husbandry conditions found in the present study reached the goal weights given by the breeding company (Aviagen™ Turkeys, 2012) under conditions found in large commercial operations. While all of the Kelly BBB flocks had live weights lower than the declared performance objectives of Kelly Turkeys (personal communication) at the beginning of the fattening period (date 2), most of these flocks reached and even exceeded the performance goals with increasing age (date three to four).

Discussion

In accordance to Chavez and Kratzer (1972) and Hafez et al. (2004) we found significant differences in foot pad health between flocks of the B.U.T. 6 and Kelly BBB line, whereas the mean foot pad score given for B.U.T. 6 was higher than the score of Kelly BBB. While these findings were traced to the higher live weights of the heavy B.U.T. 6 line compared to Kelly BBB by the other researchers, only slight differences were detected within the present study.

Conclusion

In conclusion, differences in foot pad health between turkeys of the Kelly BBB and the B.U.T. 6 line under organic husbandry conditions found in the present study have to be traced to other, hitherto unknown factors than the body weight.

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Table 1: Number of investigated flocks (N), means, their standard deviations (SD), Minima (Min), Maxima (Max) and significant differences between the two turkey lines Kelly BBB and B.U.T. 6 for the mean foot pad scores calculated at the 2nd, 3rd and 4th date.

Date	Kelly BBB (1)					B.U.T. 6 (2)					1 - 2
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	P-value
2	6	1.67[a]	0.678	1.03	2.62	5	2.03[a]	0.439	1.53	2.58	0.1990
3	5	1.52[a]	0.484	1.09	2.22	13	2.64[b]	0.437	1.96	3.61	<0.0001
4	10	1.71[a]	0.431	1.06	2.48	12	2.53[b]	0.360	2.02	3.03	0.0001

a, b: means within a column with no common superscripts differ significantly (lowercase superscripts: $P < 0.05$)

Table 2: Number of investigated flocks (N), means, their standard deviations (SD), Minima (Min), Maxima (Max) and significant differences between the two turkey lines Kelly BBB and B.U.T. 6 for live weights (kg) measured in the 16th, 17th and 18th week of age.

Week of age	Kelly BBB (1)					B.U.T. 6 (2)					1 - 2
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	P-value
16	2	7.93	1.739	6.70	9.16	9	8.93	0.716	7.95	9.80	0.1805
17	2	8.95	0.286	8.75	9.16	2	8.47	0.646	8.01	8.92	0.5998
18	3	9.25	0.932	8.39	10.2	2	10.4	1.474	9.34	11.4	0.1938

IMPLEMENTATION OF A CONTINGENCY PLAN FOR THE CONTROL OF AN AFRICAN SWINE FEVER SPORADIC OUTBREAK IN PORTUGAL

S. Madeira[1], C. Pinheiro[2], R. Ribeiro[1], F. Boinas[1]

[1]Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Departament of Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal; [2] Veterinary Surgeon, Portugal

Summary

This work describes the successful implementation of a contingency plan to control a sporadic outbreak of African swine fever (ASF), which occurred in Portugal in 1999, in only 15 days after disease suspicion. The key factors to this successful campaign were the rapid actions developed by the Official Veterinary Services (DGV), collaboration with other Institutions and actions with the producers.

Introduction

ASF is a frequently highly contagious and lethal disease of pigs, although sub-acute and chronic forms could occur. As there is no vaccine its control is based on sanitary measures only. The disease is spread by contact, fomites and Ornithodoros ticks.

ASF was first detected in Portugal in 1957, eradicated in 1958 and re-introduced in 1960 when it became endemic in the country. After 1991, ASF persisted almost exclusively in the south of Portugal. Here prevails a traditional free-ranging extensive system using the local black pig breed "Alentejano" and dry-stone animal housing, which is the habitat of *O. erraticus* complex ticks, vector of ASFV in the Iberian Peninsula [1].

In 1993, Portugal was considered free of ASF but after 6 years, a sporadic outbreak occurred in Alentejo region in November 1999.

This work describes the implementation of a contingency plan carried out by DGV which lead to a rapid and successful elimination of the disease.

Material and methods

In November 5th 1999, a suspicion of ASF infection in a traditional pig herd in Almodôvar county, southern Alentejo (fig. 1 and 2), was reported to the DGV. Serum and organ samples of dead animals were sent for diagnosis to National Reference Laboratory.

Three days after the suspicion, the epidemiological investigation identified a neighbouring herd with direct contact to herd 1 through a broken fence. A third risk herd (herd 3), owned by the same producer, was also identified and the 3 herds were considered as an epidemiological unit and quarantined.

ASF antibodies were confirmed by the laboratory on the 9th November, and all herds were depopulated on the 3 following days, using a captive bolt for previous stunning and carcasses were destroyed by incineration, followed by burial. A 3km protection zone was established on the 12th November, with a preventive slaughter of all pigs within the area being completed in 5 days. Cleaning, desinfection and desinsectization of the premises was also applied and introduction of pigs was not allowed until DGV decision.

ASF virus isolation was confirmed on November 15th and a reinforced surveillance zone of 10km was established. On the 18th November, clinical inspection and fulfillment of epidemiological questionnaires was imposed, coupled with blood sampling of all adult pigs in this area, each herd being sampled twice with a 30 day interval.

Animal movement restriction was implemented in the reinforced surveillance zone and in a general surveillance area of 14 000Km^2, defined on the basis of *O. erraticus* distribution and trade historical data. Pigs couldn't leave the restricted area unless for immediate slaughter with a transit document issued after a clinical evaluation done by a veterinarian.

As a large infestation with *O. erraticus* ticks was found in the outbreak farm, a survey for identification of infested herds in the area and collection of tick samples for virulogical analysis was implemented and surveillance was extended to all domestic pigs in Alentejo being also reinforced the wild boar surveillance.

Results

The index farm had an ASF outbreak in 1987 and in 1991 ASF serology showed negative results. No pigs were present until 1997, when repopulation started. In 1990 herd 3 was serologically positive, being stamped out and depopulated.

Post-outbreak epidemiological investigation revealed links between the 3 herds, either by direct contact, introduction of animals or same ownership. A total of 108 animals were slaughtered immediately after serological confirmation and before viral isolation, based on tick presence and epidemiological investigation. Preventive stamping-out in the protection zone lead to the slaughter of approximately 1000 animals from 99 owners.

In the surveillance zone, sampling of 8135 animals of 159 herds revealed one positive herd, epidemiologically linked with the outbreak farm, which was immediately depopulated.

The extended surveillance program included sampling in abattoirs (6373 sera), of extensively farmed pigs (9499 Alentejano breed pigs sera), of wild boars (1781 sera), and pre-movement tests (64 770 sera), all with negative results.

The total cost of this campaign was of 683 235 Euros (€). The expenditure in personnel and consumables was 139 976€, the laboratory cost including the *O. erraticus* project was 217 655€ and compensatory payments was 325 604€, which corresponds to around 50% of the total cost of the campaign.

The outbreak was controlled in 2 weeks and Portugal was considered free of disease in 1 December 2000.

Discussion

The key factors for this successful campaign were the rapid actions developed by DGV, its collaboration with the diagnosis laboratory, municipal and police force authorities, and the actions with the producers. The chain of command was well defined with efficient communication between the national and local centres of control, and between the field brigades. All agents implicated had experience in the field. Measures were implemented without any previous warning of the local producers to avoid any illegal movement and slaughter of pigs for domestic consumption. Compensatory payments were made up to one and a half month after stamping out.

ASF virus was isolated from 4 herds and from ticks in the premises of the index farm [2]. Viral persistence in ticks was considered the most probable cause for the outbreak as no pigs from other origins were introduced in these farms, no potentially infected food was fed to pigs, and wild boar serology was always negative.

Most of the tick-infested farms identified by the *O.erraticus* survey were destroyed, and the re-use of outbreak farms infested with ticks was prohibited. Only in 2002 European legislation [3] laid down specific measures for the repopulation of infested farms, which was only possible up to 5 years after the occurrence of an ASF outbreak.

Conclusions

Although historical, this case study can be a valuable example to official authorities which are now facing the same situations with the current dissemination of ASF in Eastern Europe and Russian Federation. Also, it constitutes a model approach that can be followed with other infectious diseases of animals.

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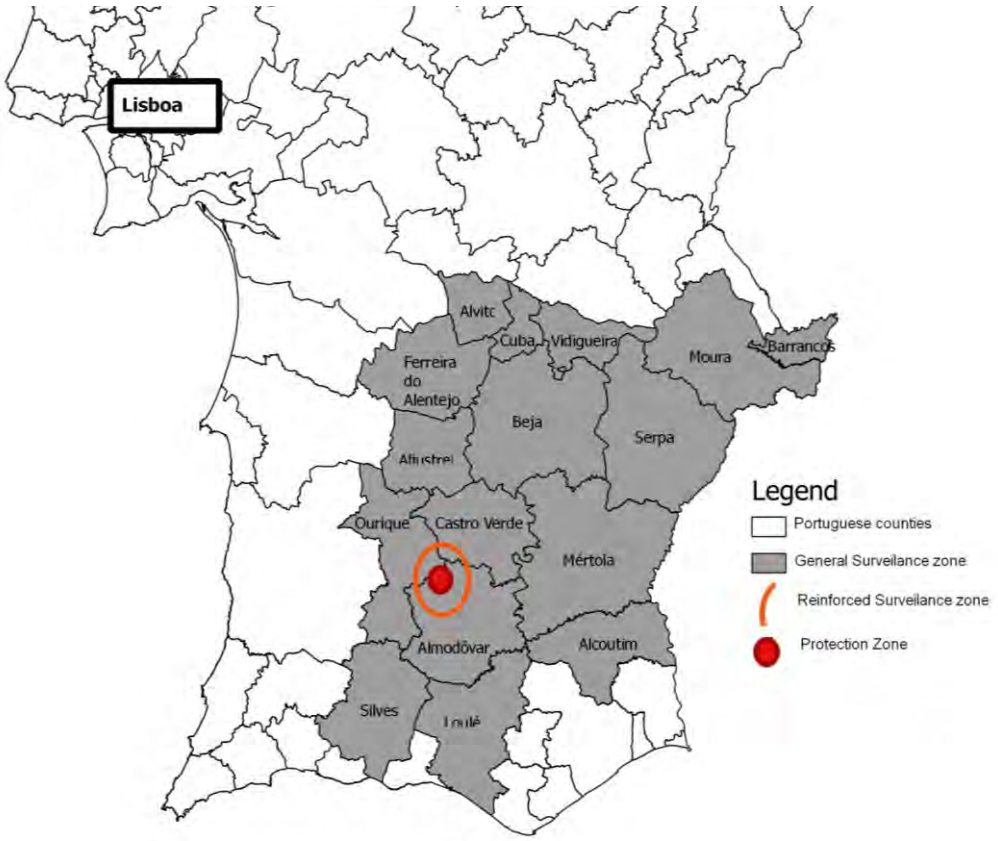
Lisboa
Alvito
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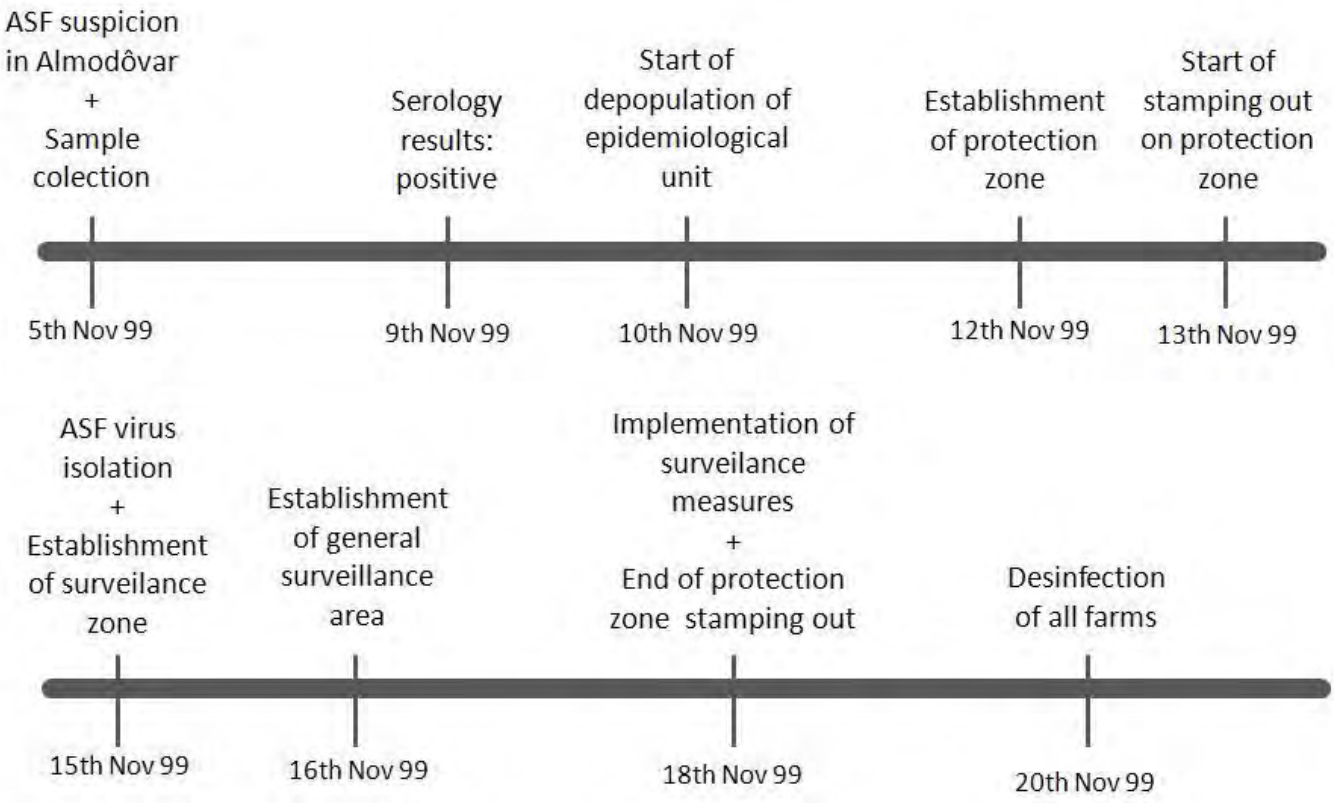
Ferreira do Alentejo
Beja
Serpa
Aljustrel
Ourique
Castro Verde
Mértola
Almodôvar
Alcoutim
Silves
Loulé
Legend
Portuguese counties
General Surveilance zone
Reinforced Surveilance zone
Protection Zone


ASF suspicion in Almodôvar + Sample colection
5th Nov 99
Serology results: positive
9th Nov 99
Start of depopulation of epidemiological unit
10th Nov 99
Establishment of protection zone
12th Nov 99
Start of stamping out on protection zone
13th Nov 99
ASF virus isolation + Establishment of surveilance zone
15th Nov 99
Establishment of general surveillance area
16th Nov 99
Implementation of surveilance measures + End of protection zone stamping out
18th Nov 99
Desinfection of all farms
20th Nov 99

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTION RISK FACTORS: A STUDY IN 109 FARROW-TO-FINISH HERDS

C. Fablet[1], C. Marois-Créhan[2], V. Dorenlor[1], F. Eono[1], E. Eveno [1], V. Tocqueville[2], S. Gorin[3], S. Quéguiner[3], L. Bigault[4], B. Grasland[4], G. Simon[3], N. Rose[1]

[1] Anses, Unité Epidémiologie et Bien-Etre du Porc, B.P. 53, 22440 Ploufragan, France [2] Anses, Unité Mycoplasmologie et Bactériologie, B.P. 53, 22440 Ploufragan, France [3]Anses, Unité Virologie Immunologie Porcines, B.P. 53, 22440 Ploufragan, France [4] Anses, Unité Génétique Virale et Biosécurité, B.P. 53, 22440 Ploufragan, France

Summary Factors associated with porcine reproductive and respiratory syndrome virus (PRRSV) infection were investigated in a cross-sectional study carried out in 109 French farrow-to-finish herds. Samples (tracheo-bronchial mucus and blood) were taken from a random sample of 4, 10, 16 and at least 22 week-old pigs (45 pigs/herd). Serum samples were tested by ELISA for PRRSV antibodies. Infection by *Mycoplasma hyopneumoniae* (Mhp), *Actinobacillus pleuropneumoniae*, swine influenza viruses (SIV) H1N1 and H1N2 and porcine circovirus of type 2 were detected by specific serological assays or PCR tests. Data related to herd characteristics, biosecurity, management and housing conditions were collected by a questionnaire during a herd visit. Climatic conditions in the nursery and fattening rooms, where the oldest sampled pigs were housed, were measured over 20 hours. A herd was deemed to be PRRSV positive when a minimum of one pig of 10, 16 or at least 22 weeks old tested positive by ELISA. Factors associated with PRRSV seropositive status of the herd were identified by logistic regression. Large herd size, the lack of disinsection in the gestation facilities, on-farm semen collection, a short time-period for gilt quarantine and a low temperature setpoint for the ventilation controller in the fattening room significantly increased the odds of a herd being seropositive for PRRSV. Infection by Mhp and H1N2 SIV were associated with a PRRSV positive status.

Introduction Porcine reproductive and respiratory syndrome virus (PRRSV) is widespread in pigs reared in large and confined populations in most swine producing countries worldwide. While significant progress on PRRS and PRRSV knowledge has been made since PRRS emergence, the epidemiology of the disease is not fully understood. A more thorough understanding of the epidemiology of the disease is important for a more efficient control of PRRSV at the herd and area scales. The aim of the present study was to identify and quantify the effects of PRRSV infection related factors in farrow-to-finish herds.

Material and methods Data and sera used were collected in 109 pig herds in the framework of a cross-sectional study on respiratory diseases in western France (November 2006 to February 2008). Blood samples were collected from four different batches of pigs in each herd, aged 4, 10, 16 and at least 22 weeks (15 pigs/batch). Sera from batches of 10, 16 and at least 22 week-old were tested for PRRSV antibodies (ELISA test 2XR-IDEXX). Sera from the oldest pigs were tested for *Mycoplasma hyopneumoniae* (Mhp) and *Actinobacillus pleuropneumoniae* serotype 2 and serogroup 1-9-11 antibodies (DAKO ELISA-Kitvia, Swinecheck App2-Biovet and Swinecheck App1911-Biovet, respectively). Antibodies against European swine influenza viruses (SIV) H1N1 and H1N2 were searched in sera from the oldest pigs by a haemagglutination inhibition test [1]. The porcine circovirus type 2 genome load in sera from batches of 4, 10 and 16 week-old pigs was quantified by real-time PCR [2]. Tracheo-bronchial swabs were taken from 10 of the 15 pigs selected from the batches of 4, 10 and 16 week-old animals and placed in 2 ml of buffered peptone water broth. Mhp DNA was identified in all swabs by modified nested-PCR [3]. Data related to herd characteristics, biosecurity, management and housing conditions were collected by a questionnaire. Temperature, relative humidity, CO_2 concentration, respirable dust level in the nursery and fattening rooms, where the oldest sampled pigs were housed, were measured over 20 hours starting at 4:00 pm on the day of the on-farm visit [4]. Ammonia concentrations were measured at pig nose level on the day of the visit. A herd was deemed to be PRRSV positive when a minimum of one pig of 10, 16 or at least 22 weeks old tested positive by ELISA. Factors associated with PRRSV seropositive status of the herd were identified by logistic regression [5].

Results Out of the 109 herds, 65 (59.6%, confidence interval at 95% [50.2%-69.0%]) were considered infected by PRRSV in the growing-finishing steps. Factors associated with PRRSV seropositive status of the herd retained in the final model logistic model are presented Table 1.

Discussion The odds for a herd to be PRRSV positive increased with herd size, as already been reported [6]. This might be due to a greater risk of virus introduction from outside the herd through increased number of direct or indirect contacts in large herds, greater risk of virus transmission within the herd [7]. On-farm semen collection increased the odds for a herd to be PRRSV positive. Experimental studies showed that PRRSV and viral RNA may be detected in semen from infected boars [8]. Most boar studs in western France are currently PRRSV negative. Hence, they represent a limited risk for virus transmission. By contrast, farm-boars are often housed with pregnant sows providing opportunities for booster infections. On-farm semen collection is therefore likely to be at risk for virus transmission to sow herd and persistence in infected herds. We found that rather than isolation, a long duration of the quarantine reduced the odds of a herd to be positive. Even though pigs can remain persistently infected for several months, Charpin *et al.* [9] showed that PRRSV-infected pigs were no more infectious after 42 days. Increasing the time spent in quarantine facilities may thus reduce the probability of introducing infectious animals. Lack of disinsection in the pregnancy facilities increased the odds for a herd to be PRRSV positive. Insects have been shown to mechanically transmit PRRSV from infected to susceptible pigs [10]. Controlling the living vectors would help to reduce the possibility of pigs being exposed to the virus by indirect route. Infections by two respiratory pathogens were associated with PRRSV status of the herd. These results must be interpreted cautiously because the study design did not allow to establish the time-sequence of events. The observed associations do not imply a causal relationships. These findings might indicate that these pathogens shared common risk factors.

Conclusions Recommended measures aimed at a better control of PRRSV infection would include proper biosecurity measures to minimize the risk of virus introduction and management practices minimizing direct and indirect virus transmission within the herd.

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EFFICIENCY OF ANTIBACTERIAL ORGANIC CHELATE COMPOUNDS IN BRACHYSPIRA HYODYSENTERIAE CHALLENGED PIGS

András Adorján[1*], Pál Rafai[1], Zoltán Papp[1], Endre Brydl[1], László Jakab[1], Péter Kovács[1], Viktor Jurkovich[1], László Makrai[2], Gyula Balka[3], Melinda Kovács[4], Árpád Bata[5], László Könyves[1]

[1]Faculty of Veterinary Science, Szent István University, Department of Animal Hygiene, Herd-health and Veterinary Ethology, István u. 2. H-1078 Budapest
**e-mail: Adorjan.Andras@aotk.szie.hu*
[2]Faculty of Veterinary Science, Szent István University, Department of Microbiology and Infectious Disease, Hungária krt. 23-25 H-1143 Budapest
[3]Faculty of Veterinary Science, Szent István University, Department of Pathology, István u. 2. H-1078 Budapest
[4]Faculty of Agricultural and Environmental Sciences, University of Kaposvár, Department of Physiology and Animal Hygiene, Guba Sándor u. 40 H-7400 Kaposvár
[5]Dr. Bata Ltd., Bajcsy-Zs. u. 139 H-2364 Ócsa

Summary

Our aim was to establish a new, reliable and reproducible challenging method for the swine dysentery (SD) in order to use it for investigation of two developed non-antibiotic feed additives (Dys-1, Trial D-1).

Introduction

The SD is a widespread, serious disease of the growing pigs which can cause mucous or bloody diarrhoea and consequent death (sometimes reach 30%) (4) or production losses. The SD was first written down in 1921 by Whiting et al. (7) however the role of the *Brachyspira hyodysenteriae* (*B. hyodysenteriae*) was proved only in 1971 (6). Nonetheless the reliable and reproducible induction of the SD is not exist until now, the results in the literature is very variable (2) and the pigs can harbour the *B. hyodysenteriae* without any clinical signs. (1,3) Therefore at first a new infection model was established by us from the existent knowledge about this illness.

Material and methods

The research contain three period: observation (9 days), introductory (19 days) and pilot (4 weeks) period. 24 hungarian white×danish landrace F1 pigs (14,6±2,2 kg) were allocated into four groups (Control, T2, Dys-1, Trial-D1) separately from each other on the zero day. Control group was fed with pig starter feed in the whole research but the other three groups' feed was changed after the observation phase. From the starting of the introductory phase the Dys-1 and Trial D-1 started to eat their non-antibiotic feed additives until at the end of the research. Except the Control group T2 toxin (2mg/feed kg dose) was also fed in the introductory and the first week of the pilot period. After the first two periods all of the pigs was inoculated twice (24hrs interval) with freshly collected colon scraping from clinically ill pig in SD. For the successful induction of the disease proton pump-inhibitor (esomeprasol, Nexium, 40mg/pig) and starvation were also used for the gastric pH increasing.

The weight of the pigs was measured every weeks individually. The leftover of the feed was measured weekly too and the given food was also recorded in every dispension time. In the pilot period the signs of the SD and/or the faeces quality twice daily was observed and categorized in a 5 grade scoring system (5) which was contracted into two: A (mild diarrhoea) and B (serious diarrhoea) category for better interpretation.

Results

All of the pigs became ill and produced the typical signs of the SD. One member of the Control group spontaneously died 7 days after the onset of the SD. His pathological examination showed diffuse, fibrinous-necrotic colonitis with mucous-bloody content.

In the table 1 the first day and duration of the serious diarrhoea (B category) and the average faeces points in the groups can be followed. The correspondence between the used treatment in the groups and the frequency of the signs was evaluated with Fisher exact probe. This probe proved that recorded quantity of the signs in the groups depend on the used treatment and are not random findings. The Fisher exact probe also verified the greater chance

of the serious diarrhoea (B category) in the Control group comparing it to the other groups and the comparison was significant in case of T2 group (p=0.004)

The average weight and weight gain of the groups was represented in the table 2. The weight and weight gain before the challenge matched to the normal growing up and the deviation was not significant in neither groups. The weight gain was stopped in each animal after the infection and also some animals start to lose weight in some part of the pilot period. Except the Dys-1 group which can put on weight even though the infection. The statistical analysis could not prove the Dys-1 effectiveness against the other groups, but the tendency (p=0.12) was encouraging. The Control group weight gain pointed similarly to the literature that the SD without treatment or not from the beginning can worsen the production of the pigs.

During the thorough pathological examination of the colon emerged that only clinically ill animals at the extermination can showed pathological alterations. The bacteriological and hystological examinations represent the same findings as well. From the sick animals' colon content the *Brachyspira spp*. was successfully cultured and isolated as well as they were found in large numbers on the hystological colon sections. Nonetheless the degree of the necrosis of the colon in the Dys-1 group was lower than other groups in spite of the *Brachyspira* presence differed only slightly from the others.

Discussion

The results of our study proved that our infection model was successful nonetheless small changes was suggested in the future. For example the proton pump-inhibitor was not necessary to use with the same time of starvation. The T-2 toxin treatment was also successful, but its level in the feed had to be checked more frequently. The closure of the challenge is suggested to do at the 10-14th days after the inoculation. The non-antibiotic compounds, namely Dys-1 and Trial-D1, cannot inhibit the clinical appearance of the swine dysentery. However the Dys-1 group produce both better performances and better pathological findings. For this reasons the Dys-1 further investigation on more animals will be advisable.

Conclusions

The successful induction of the SD is feasible in large group of animals, but the role and effects of the used interactions on the pig's intestinal health needs further investigations in the future for better understanding of the SD appearance. Therefore blood and gut content from a small group of the animals were collected for our further examinations.

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Groups	The first signs of serious diarrhoea (days)	Accumulated days of diarrhoea (days)	Duration of serious diarrhoea (days)	Accumulated faecal scores
Control	8,3±6,12	16,4±4,6	9,0±7,09	198
T2	5,8±2,16	18,3±9,7	6,8±6,17	167
Dys1	6,3±4,27	17,5±9,4	8,3±7,00	193
Trial D1	5,3±3,98	17,7±6,3	9,2±7,14	194

Table 1 First appearance and duration of diarrhoea and accumulated faeces scores

Groups	At start of the experiment	At the time of experimental infection		The end of the experiment	
	average weight (kg)	average weight (kg)	average daily weight gain (kg)	average weight (kg)	average daily weight gain (kg)
Control	14,67±1,37	34,30±2,35	0,70±0,05	37,70±10,18	0,12±0,59
T2	14,58±2,20	35,22±4,05	0,74±0,12	36,50±13,41	0,04±0,40
Dys1	14,58±1,91	33,72±5,64	0,68±0,17	45,33±13,65	0,40±0,34
Trial D1	14,33±2,16	33,22±5,72	0,67±0,20	38,75±12,79	0,19±0,45

Table 2. Average live weight and daily weight gain of groups.

BEHAVIOR OF L. MONOCYTOGENES STRAINS IN SWINE MANURE MICROCOSM AS DETERMINED BY MOLECULAR AND CULTURE ASSAYS

J. Desneux[1,2], A. Biscuit[1,2], S. Picard[1,2] and A-M. Pourcher[1,2]

[1] *IRSTEA, 17 avenue de Cucillé, 35044 Rennes, France*
[2] *Université européenne de Bretagne, France*

Summary

Pig manure can be biologically treated leading to by-products such as liquid fractions stored in a lagoon. Manures and lagoons may contain pathogenic bacteria which can be disseminated in soil after spreading. The survival of pathogens depends on extrinsic and intrinsic factors such as their ability to enter into a viable but non cultivable state (VBNC). The aim of this study was to compare the behavior of *Listeria monocytogene*s which can enter VBNC state, in pig manure and in lagoon microcosms. Two strains of *L. monocytogenes* belonging to two serogroups and isolated from two piggeries were inoculated in flasks containing a manure and a lagoon effluent stored at 8°C and 20°C during 63 days. The bacteria were quantified by 3 methods: Plating on PALCAM medium, qPCR and qPCR associated with Propidium MonoAzide which quantifies both live and VBNC bacteria. Regardless of their origin and of their serogroup, the strains showed similar kinetics of decrease which depended on the type of matrix and on the temperature. The VBNC bacteria appeared within the first hours of contact regardless of the matrix. Their proportion increased over time in the two matrices. This study highlights that lagoon effluents used for watering plants, create more favorable conditions than manure for VBNC cells of *L. monocytogenes.*

Introduction

Pig manure which is usually stored in pits before being sprayed onto land, can be biologically treated to lower the load of nitrogen. One of the by-products of the treatment consists of a liquid fraction stored in a lagoon (lagoon effluent). Both manure and lagoon may contain pathogens, the persistence of which depends on a variety of environmental and intrinsic factors of the pathogens including their ability to enter into a viable but non cultivable state (VBNC). Among these pathogens, *L. monocytogenes* has a high prevalence in pig feces (Boscher *et al*., 2012). Moreover, their frequency of detection has been reported to be higher in lagoon effluents (after biological treatment) than in raw manures (Pourcher *et al*., 2012), suggesting that the lagoon effluents may be a favorable environment for the survival of *L. monocytogenes.* The lagoon effluent used to water crops may therefore play a role in the transfer of this pathogen in the environment. This study aims to compare the behavior of *L. monocytogene*s which can enter VBNC state, in laboratory manure and lagoon microcosms.

Materiel and method

The experiments were performed with two strains (L111 and L120) rifampicin resistant (rif-mutant) isolated from a pig manure and a lagoon effluent which differed by their genotype and their serogroup. Manure and lagoon effluent were sampled in a manure treatment unit located in Brittany (France). Both strains were inoculated separately in triplicate in manure and in lagoon effluent microcosms incubated at 8°C and 20°C. The samples were collected at 0, 7, 21, 42 and 63 days after inoculation of *L. monocytogenes*. At each time point, three quantifications were performed: count by cultural method using Palcam medium, quantification by molecular methods after DNA extraction using Taqman qPCR (Nogva *et al*., 2000) and quantification by qPCR associated with Propidium MonoAzide ($qPCR_{PMA}$) to highlight the presence of VBNC cells. The conditions for PMA analyses were respectively for the manure and the lagoon effluent : a final concentration of 55 µM or 20 µM, an incubation in the dark of 5 min or 20 min of and an exposition to light of 55 min or 20 min.

Results and discussion

Regardless of the experimental conditions (temperature, matrix) and whatever the quantification method used, the behavior of the two strains was similar (figure 1). The most important factor was the temperature. Indeed, the persistence of the strains considerably increased at 8°C. The results also show an effect of the matrix. At 20°C, the decrease of *L. monocytogenes* concentration after 63 days of storage was higher in manure (5 Log_{10}) than in lagoon

effluent (4 Log_{10}). Conversely, at 8°C the maximum decrease was observed in lagoon effluent (4 Log_{10} *vs* 3 Log_{10} in manure).

Conclusion

The serotype and the origin of the strains does not influence their behavior as both strains showed similar kinetics of disappearance for the conditions tested in this experiment. VBNC cells which appear during the first hour of contact with the matrices were more numerous in manure suggesting that the raw manure was more stressful that the lagoon effluent. Low temperature increased the survival of *L. monocytogenes* and reduced the formation of VBNC cells. The high proportion VBNC cells in the manure and in the lagoon indicate that the culture-dependent methods highly underestimate the real number of *L. monocytogenes* and thus the risk of dissemination of this pathogen in the environment.

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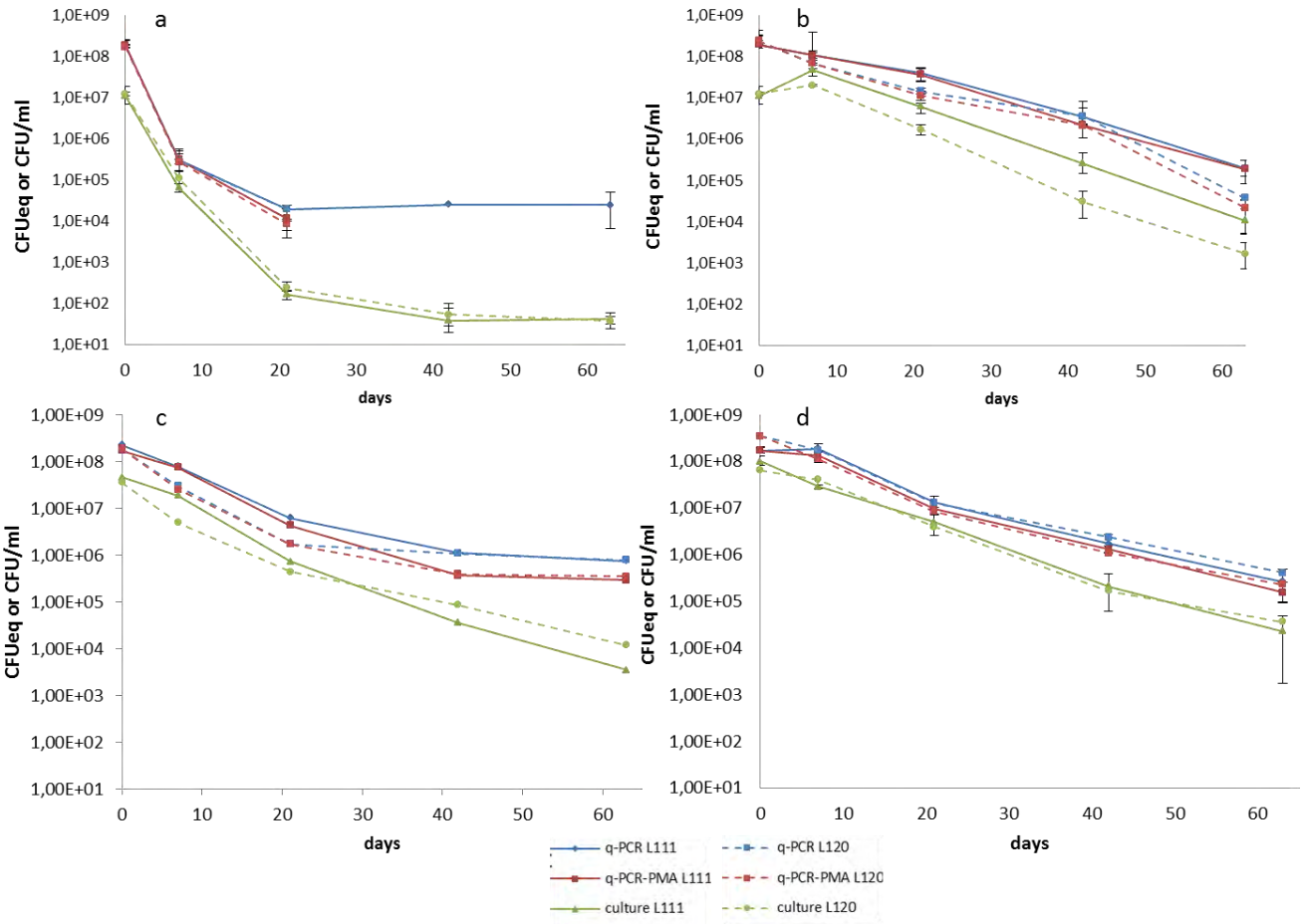

Figure 1: Average concentrations of strain L111(solid line) and strain L120 (dotted line) in manure and lagoon effluent at 8°C and 20°C estimated by qPCR, $qPCR_{PMA}$ and culture. a: manure at 20°C; b: manure at 8°C; c: lagoon effluent at 20°C; d: lagoon effluent at 8°C.

VBNC cells which appeared in the first hour of contact with the matrix, reached up to 99.8% of the viable bacteria (table 1).

Table 1: Percentage of VBNC cells in manure and lagoon effluent microcosm at T0 and after 63 days at 8 and 20°C.

strains	temperature	Time (day)	Manure	Lagoon effluent
L111	20°C	0	94.1 (0.2)[a]	72.9 (0.4)
L120	20°C	0	92.2 (0.8)	76.0 (0.3)
L111	20°C	63	99.5 (0.2)	99.1 (0.1)
L120	20°C	63	99.8 (0.2)	99.3 (0.3)
L111	8°C	0	94.3 (0.2)	40.9 (0.3)
L120	8°C	0	94.8 (0.2)	81.7 (0.3)
L111	8°C	63	94.3 (0.1)	85.2 (0.2)
L120	8°C	63	92.2 (0.3)	84.2 (0.3)

[a] standard deviation

At the beginning of the experiment, the proportions of VBNC cells were higher in the manure than in the lagoon effluent. After 63 days, their proportion was less important at 8°C in lagoon effluent. It is noteworthy that, expect in manure at 8°C, the proportion of VBNC cells increased over time. It should be noted that the VBNC cells can, when they are in favorable conditions become cultivable and regain their virulent characteristics (Olivier, 2010).

CHARACTERISATION OF MYCOPLASMA SUIS INFECTIONS IN SPLENECTOMISED AND NON-SPLENECTOMISED PIGS

S. Dietz, S.-L. Mack, J. Stadler, C. Jannasch, K. Hoelzle, L. E. Hoelzle

Institute of Animal Science
University of Hohenheim, Stuttgart

Summary
The present article summarizes the differences in clinical and haematological parameters between M. suis infected splenectomised and non-splenectomised pigs. Moreover, different shedding routes of M. suis were observed to get more information about the pathogenesis.

1. Introduction

The haemotrophic Mycoplasma suis is the major cause of infectious anaemia in pigs and responsible for important economic losses worldwide. As M. suis cannot be cultured in vitro experimental infections of pigs play an essential role for pathogenesis research. The aim of the study was to clinically characterise the course of experimental infection using the highly virulent and red blood cell (RBC) invasive M. suis strain KI3806 in splenectomised and non-splenectomised pigs and to correlate clinical and haematological parameters with M. suis blood loads. In addition shedding of M. suis via urine, faeces, saliva, nasal and vaginal secrets was investigated to get insights into potential transmission routes.

2. Materials and Methods

Seven days after housing, 7 pigs were splenectomised according to the method of Heinritzi (1984). The piglets were kept in one stable during the experiments.

One week after splenectomy all pigs (14 pigs, 7 splenectomised, 7 non-splenectomised) were experimentally infected subcutaneously with 1 mL M. suis containing porcine blood (1 x 10^8 M. suis/mL as determined by quantitative PCR; Hoelzle et al., 2007) and were clinically and haematologically monitored and recorded according to a score system as described previously (Hoelzle et al., 2009). Accordingly, pigs were euthanized after reaching clinical scores of at least 5. Haematological parameters, i. e. RBC count, haemoglobin, PCV, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using the Scil Vet ABC tool (Scil Animal Care Company GmbH). Biochemical serum parameters, i. e. glucose, iron, bilirubin, urea, creatinine, alpha amylase, and pancreas lipase were analysed using the Hitachi 911 Chemistry Analyser (Roche). EDTA- anticoagulated blood, spontaneous urine and feces, as well as salivary, nasal, and vaginal swabs were taken 2 days before infection and on days 2, 4, 6, 8 post infection (DPI) or on the day of euthanasia, respectively. Air samples were collected in the stables close to the experimental animals. Dust swabs were taken from horizontal surfaces in the stables and water samples from the drinking troughs. M. suis-infection and shedding were characterized by qPCR and ELISA.

3. Results

All splenectomised pigs were PCR-positive 2 DPI with maximum mean bacterial load of 1.61 x 10^{10} M. suis/mL blood on 8 DPI. They developed severe anaemia and massive hypoglycaemia by 8 DPI. The non-splenectomised pigs became PCR-positive within 23 DPI and reached maximum mean load of 1.64 x 10^5 M. suis/mL blood. They developed mild anaemia, massive skin alterations and seroconverted within 35 DPI. Shedding was found in saliva as well as nasal and vaginal secrets from day 6 DPI on with a quantity of 3.4 x 10^2 to 2.7 x 10^5 M. suis/swab. In urine M. suis DNA could be detected in 100.0 % of the samples from day 6 DPI on with a quantity of 4.7 x 10^2 to 6.3 x 10^5 M. suis per mL. When shedding patterns were correlated to the median bacterial blood loads shedding was observed at loads of 2.0 x 10^9 – 7 .0 x 10^{10} M. suis per mL blood.

No M. suis DNA could be amplified from feces. Dust and water samples of the pig drinking troughs were positive for M. suis on days 2 and 6 post infection, air samples were M. suis – negative throughout the experiment.

4. Discussion

The higher virulence is reinforced by RBC-invasiveness and induction of eryptosis (Groebel et al., 2009; Felder et al., 2011). Experimental infection of splenectomised pigs with M. suis KI_3806 resulted in severe, acute infectious anemia, where as in non-splenectomised pigs clinical signs were characterised by mild anemia and a cutaneous manifestation. The higher virulence of M. suis KI_3806 was reflected by a short incubation time, an extremely fast pathogen proliferation, and clinical manifestation even in non-splenectomised pigs. In addition, we detected M. suis DNA in different sample material during the early phase of experimental infection. However, due to the lack of an in vitro cultivation system we could not determine whether M. suis DNA detected in urine, saliva, nasal and vaginal secrets or environmental samples represent viable bacteria. Remarkably, no M. suis shedding via feces could be detected. Future investigations should include experimental transmission routes and the use of lower virulent M. suis strains to analyze the late and clinically inapparent stages of infection.

5. Conclusion

The study demonstrated that splenectomised pigs show a fulminant course of infection and in contrast, non-splenectomised pigs show a mild course, which resembles the situation in naturally infected pigs. Moreover, our results indicate that blood independent direct transmission as well as indirect transmission via environmental contamination could play a role in the epidemiology of M. suis infections.

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UDDER HEALTH IN RELATION TO COW'S SEASONAL KEEPING

I. Kocina, V. Antane

Faculty of Veterinary Medicine, University of Agriculture, Jelgava, Latvia

Summary

Subclinical udder infections remain essential problem in dairy herds in Latvia, the incidence of subclinical mastitis is up to 30%. The aim of this study was to evaluate somatic cell count (SCC) in cow milk in relation with cow seasonal keeping and pathogens presence in the udder. Milk was sampled from 16 cows of similar age and productivity, from clinically healthy udder quarters. Our study confirms that interaction between cow seasonal keeping and pathogenic bacteria presence in udder quarter significantly affect SCC in milk ($p<0.05$).

Introduction

Mastitis causes severe economic losses due to decreased milk production and increased management costs. A subclinical cow, certainly represents a possible source of infection for other cows (Fourichon et al., 2001).It has been described that housing conditions and management significantly affect cow's udder health and milk quality (Demelash, 2005; Lusis et al., 2006).

Various methods of early diagnostics and evaluation of mammary gland diseases have been investigated, developed and compared, still a universally recognized indicator of the udder health is somatic cell count (SCC) in milk. That is why in the present research, SCC in milk was used as the indicator for the udder functional and health status evaluation.

Material and methods

The experimental part of the study was carried out in 75 dairy cow herd during two year cycle. During the summer–autumn period cows were grazing in the day pasture, in winter-spring they were kept in a barn.

Milk was sampled two times in grazing, two times in housing period from 16 cows of similar age and productivity in the middle stage of lactation, from clinically healthy udder quarters.

Microbiological examination was carried out samples of all quarters in those cows that showed CMT positive result even in one quarter. Standard procedures to identify pathogenic microorganisms in milk were performed in compliance with IDF (1981) and method described by Quinn et al. (2000). SCC was estimated by Somacount Analyser, complying with LVS EN ISO 13366-3:1997.

The obtained research data were statistically processed by using SPSS program 11.0 and Microsoft Excel packages.

Results and discussion

A comparative evaluation of SCC in milk was carried out in housing and grazing period. The obtained results (Tab.1) suggest that the mean SCC in milk samples is 233 710 (566.31) ml^{-1}; in housing period 169 530 (419.94) ml^{-1}, in grazing period 302 880 (685.72) ml^{-1} considerably higher ($p<0.05$).

Table 1. **Somatic cell count in milk in housing and grazing period**

Seasonal keeping	Number of samples	Average value thousand/ml^{-1}	Standard deviation	Min value thousand/ml^{-1}	Max value thousand/ml^{-1}
Housing period	111	169.53	419.94	0.00	2972.00
Grazing period	103	302.88	685.72	0.00	4332.00

SCC in milk in housing and grazing period

Results of our previous studies carried out in different herd also suggest that SCC in cow milk is significantly higher in grazing period (Kociņa, Antāne, 2000). Most possible, this increase is facilitated by the effect of intensive environmental factors causing udder irritation and activation of the animal immune response. It is noted that SCC in the cow milk is used to be very changeable even increased in not infected quarters (Kociņa, Antāne, 2000; Lusis et al., 2006).

In present study following pathogens were identified: *Coagulase Negative Staphylococcus* in 52.9% of samples, *Streptococcus uberis* in 26.5% of samples, *Staphylococcus aureus* in 20.6% of samples.

In present study to analyze the variation of SSC in cow's milk seasonal keeping and pathogenic mastitis agents were evaluated as interrelated factors (Table 2).

Table 2: **Somatic cell count in milk during housing and grazing period in relation with the pathogenic agents identified in the udder quarters**

Quarter milk	Housing period			Grazing period		
	Number of samples	SCC thousand/ ml^{-1}	Standard deviation	Number of samples	SCC thousand/ ml^{-1}	Standard deviation
Without pathogens	34	121.46	240.33	35	358.34	701.71
CoNS	11	581.22	101.46	11	238.55	181.99
Str. uberis	8	375.00	812.52	3	2197.00	930.55
S. aureus	5	817.00	402.46	4	232.33	397.22

SCC in milk during housing and grazing period in relation with the pathogenic agents identified in the udder quarters

The study results suggest that interaction of the cow seasonal keeping and mastitis pathogens significantly ($p<0.05$) influence SCC in milk.

In **housing period,** the highest SCC in milk **817 000** (402.46) ml^{-1} was determined in the case of *S. aureus* infection, whereas during the grazing period SCC in milk in the case of this infection was 232.33 (397.22) ml^{-1}. A publication mentions that *S. aureus* most of all was isolated from milk samples in the winter and autumn period (Konošonoka, 2005) and risk to be infected increase particularly when animal resistance is lowered (Saran, Leitner, 2000).

In **grazing period**, the highest SCC **2 197 000** (930.55) ml^{-1} was observed in quarters infected with *Str. uberis*, whereas during the housing period SCC in milk in the case of this infection was 375 000 (812.52) ml^{-1}.

Str. uberis is an environmental microorganism unable to live in the udder for a long time and the most serious source of infection is the pasture surroundings for the herd under investigation. It is found out that *Str. uberis* not always causes acute disease signs, and the infection is possible to restrict by improving the cow keeping and surrounding hygiene conditions (Philpot, Nickerson, 1997).

Present research indicate that in grazing period SCC is significantly higher ($p<0.05$) in milk samples without pathogenic bacteria. Most probably it confirms the opinion on a non-infectious udder irritation as an important cause of SCC increase in cow milk. Unfortunately, here always is a high risk for non-infectious udder tissue irritation to be complicated by mastitis pathogens.

Conclusions

- Seasonal keeping of cows significantly affect somatic cell count in milk ($p<0.05$). In the grazing period, compared with the housing period, somatic cell count in milk increases significantly ($p<0.05$), including non-infected quarter milk samples ($p<0.05$).
- The interaction effect of cows seasonal keeping and pathogenic bacteria presence in the udder significantly affect somatic cell count ($p<0.05$) in milk.

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PREVALENCE AND OCCURRENCE OF ESBL ESCHERICHIA COLI IN EGYPTIAN BROILER FARMS

Marwa F. E. Ahmed

Department of Hygiene and Zoonoses, Faculty of Veterinary Medicine, Mansoura University, 35116, Mansoura, Egypt

Summary

ESBL *Escherichia coli* (*E.coli*) are one of the most important antibiotic resistant pathogens in poultry production. This study was aimed to longitudinal assessment of the prevalence and occurrence of ESBL *E.coli* isolated from broilers and their environment. Moreover, the potential risk factors of farm contamination with tested bacteria were also determined. Samples were taken from 3 broiler farms at four different times (-1d, 10d, 20d and 30d) within one fattening period. Cloacal swabs from the birds as well as from their environment in the barn were investigated for the occurrence of ESBL *E.coli*. Three air samples were obtained by filtration method. Environmental pooled samples from water, feed, dust and litter as well as walls and boot swabs were also obtained. A continuous increase in the prevalence of ESBL *E.coli* within time in cloacal swabs. The detection frequencies in housing environmental samples were relatively high depending on cleaning, disinfection and biosecurity level, and increase over time. The most significant risk factors in the tested farms were boot swabs, litter and wall swabs. ESBL *E.coli* was detected in the air of farms 2 and 3 only.

1. Introduction

In food producing animals, antimicrobial agents were used in veterinary medicine either to treat bacterial infections or as prophylactic treatment. Thus antimicrobial drug resistance is rapidly rising and of particular concern is *E.coli*. Nowadays, one of the most important antibiotics used is to prevent and control infections in poultry is cephalosporins 3rd and 4th generation. The use of this kind of antibiotic leading to production of Extended Spectrum Beta-Lactamase (ESBL) *E.coli* (Smet et al., 2008). Recently, a high prevalence of ESBL producing *E. coli* isolates in broilers farms was described in different literature (Dierikx, et al., 2013 and Laube, et al., 2013). However, there is scarcely of information regarding to ESBL *E.coli* distribution in broiler farms in Egypt. Therefore, this study was aimed to longitudinal assessment of the prevalence and occurrence of ESBL *E.coli* in broiler and their environment.

2. Materials and Methods

Study area and design. Longitudinal study was carried out in 3 conventional poultry farms (1, 2 and 3) located in different geographic areas in Dakhlia Governorate, Egypt, from Mai till July, 2014. The number of birds in examined farms ranged from 8,000 to 10,000 birds. Samples were collected 4 times during the production cycle starting with one day prior to chicks being placed and continued with 10 days intervals until day 30. At each farm, bird's samples were obtained at three occasions. However, environmental samples were taken from 4 sampling times to evaluate the efficiency of cleaning and disinfection before production cycle started.

Sample Collection

Thirty randomly selected birds were sampled by cloacal swabs using sterile cotton swabs. Air samples were taken by filtration using a personal air sampler SKC pump provided with an I.O.M. dust sampler (Institute of Occupational Medicine, Edinburgh, United Kingdom, and SKC Gulf Coast Inc., United States) and a polycarbonate filter (8-µm pore size) (Whatman, United States). In each barn for each sample, three pooled samples were collected from different locations inside the barn. 1-2 g of dust, 250 g or ml each of litter, feed from the feeder and water from drinkers in front of birds. Three wall swabs using 10 cm^2 size area sterile templates and three pairs of boot swabs.

Samples preparation for microbial analysis.

(a) Bird samples. Each swab was streaked onto Eosin Methylene Blue agar supplemented with 1 mg/liter cefotaxime (EMB+, Oxoid) and then inserted in 9 ml 0.1% Buffered Peptone Water supplemented with 1 mg/liter cefotaxime (BPW+, Oxoid)..

(b) Air sampling. Each filter was aseptically transferred to 9 ml PBW+, vortex for 10 min, then 100 µl was streaked on EMB+ in triplicate. Plates and tubed were incubated and handled as described above.

(c) Environmental samples. Each sample was mixed with BPW+ in a ratio 1:9. Thereafter, loopful was streaked onto EMB+ in triplicate. The plates and tubes were incubated aerobically at 37°C for 24 h. Identification of *E.coli*

For each sample, one suspect *E. coli* colonies displaying typical morphology were identified biochemically according to Quinn et al. (2002).

Antimicrobial testing for ESBL *E.coli* detection *E. coli* isolates were streaked on ESBL Chromo agar (Oxoid). Moreover, they were confirmed by the combination disc method following CLSI (2012) recommendations using Mueller Hinton agar (Oxoid).3. Results and Discussion

Based on Phenotypic characterization, ESBL *E. coli* bacteria were detected in cloacal swabs of three examined farms (Table 1). The detection frequencies of ESBL *E. coli* in birds swab were increased by increasing the time during the fattening periods in all examined farms. In which, collectively in 3 farms the prevalence were 43%, 61% and 82% for the first, second, and third sampling times, respectively. This result was in accordance with result obtained from Smet et al. (2008) and Laube et al. (2013). Moreover, the prevalence were higher in farm 3 (71%) than in farms 1 and 2 (49% and 67%, respectively) which may be explained depending on presence of such bacteria in one day old chicks due to parent infection or hatchery contamination as stated by Mevius et al. (2009).who describe that the risk of introducing ESBL *E. coli* in the broiler production chain occurs due to restocking of farms with positive chicks.

ESBL *E. coli* organisms were detected in examined environmental samples, with high frequency, in all investigated broiler farms at all examined times except for farm 1, there is no ESBL *E. coli* could be detected during the first sampling time (one day before chicken stocking) (Table 2). This may be explained on the bases of insufficient cleaning, disinfection and biosecurity level as well as the use of antibiotics considered as high incidences of ESBL *E. coli* in broiler farms as described in studies presented by Hiroi et al. (2012) and Laube et al. (2013).

Additionally, our results show that boot swabs, litter, and wall swabs are the main risk factors for ESBL *E. coli* transmission within or in between a broiler barn. Moreover, water, feed, dust and even air (detected only in farms 2 and 3with low percentage) may act all as a source of ESBL *E. coli* transmission.

Conclusion: our findings clearly show that ESBL E. coli is strongly prevalent in conventional poultry production systems at both bird and environmental levels.

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Table 1. Prevalence of ESBL *E. coli* from 30 cloacal swabs within examined apparently healthy chickens

Sampling time	Prevalence (%) of ESBL *E.coli* (positive samples) for tested farms			
	Farm 1	Farm 2	Farm 3	Total
10d	26.7 (8)	46.7 (14)	56.7 (17)	43.3 (13)
20d	46.7 (14)	66.7 (20)	70.0 (21)	61.1 (18.3)
30d	73.3 (22)	86.7 (26)	86.7 (26)	82.2 (24.7)
Total	48.9 (14.7)	66.7 (20)	71.1 (21.3)	62.2 (18.7)

Table 2. Distribution and percent of positive environmental samples of ESBL *E. coli* in investigated farms

	Number of positive (%) samples of ESBL *E.coli* for tested farms														
	Farm 1					Farm 2					Farm 3				
	Total % (Number)	-1d	10d	20d	30d	**Total % (Number)**	-1d	10d	20d	30d	**Total % (Number)**	-1d	10d	20d	30d
Air	**00.0 (0)**	0	0	0	0	**16.7 (2)**	0	0	0	67	**33.3 (4)**	0	0	67	67
Feed	**33.3 (4)**	0	0	33	100	**58.3 (7)**	0	67	67	100	**83.3 (10)**	33	100	100	100
Water	**41.7 (5)**	0	33	33	100	**66.7 (8)**	0	67	100	100	**91.7 (11)**	67	100	100	100
Litter	**58.3 (7)**	0	33	100	100	**75.5 (9)**	33	67	100	100	**91.7 (11)**	67	100	100	100
Dust	**16.7 (2)**	0	0	33	33	**58.3 (7)**	0	67	67	100	**66.7 (8)**	33	33	100	100
Wall swab	**58.3 (7)**	0	67	67	100	**75.0 (9)**	33	67	100	100	**91.7 (11)**	67	100	100	100
Boot swab	**66.7 (8)**	0	67	100	100	**75.0 (9)**	33	67	100	100	**91.7 (11)**	67	100	100	100

FACTORS ASSOCIATED WITH ACTINOBACILLUS PLEUROPNEUMONIAE SEROTYPE 2 INFECTION IN SLAUGHTER-AGED PIGS FROM 116 HERDS

C. Fablet[1], C. Marois-Créhan[2], V. Dorenlor[1], F. Eono[1], E. Eveno [1], V. Tocqueville[2], S. Gorin[3], S. Quéguiner[3], L. Bigault[4], B. Grasland[4], G. Simon[3], N. Rose[1]

[1] Anses, Unité Epidémiologie et Bien-Etre du Porc, B.P. 53, 22440 Ploufragan, France [2] Anses, Unité Mycoplasmologie et Bactériologie, B.P. 53, 22440 Ploufragan, France [3]Anses, Unité Virologie Immunologie Porcines, B.P. 53, 22440 Ploufragan, France [4] Anses, Unité Génétique Virale et Biosécurité, B.P. 53, 22440 Ploufragan, France

Summary: The aim was to identify the factors associated with *Actinobacillus pleuropneumoniae* serotype 2 (App2) infection in slaughter-aged pigs from 116 French farrow-to-finish herds. In each herd, a sample of 60 pigs from four different batches (aged 4, 10, 16 and ≥22 weeks) were sampled. Data related to biosecurity, management, housing and climatic conditions were collected during a farm visit. *Mycoplasma hyopneumoniae*, porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV-2) were detected by serological or PCR tests from samples taken in the four batches. Samples from the slaughter-aged pigs (≥ 22-weeks-old) were tested for App2 antibodies and swine influenza viruses (SIV) H1N1 and H1N2 antibodies by serological assays. A herd was deemed infected by App2 if at least one pig was seropositive for App2. Multiple correspondence analysis was used to identify factors associated with App2 infection at the herd level. Large herd size, low biosecurity measures and no SIV vaccination of the replacement gilts were factors associated with App2 infection. Herds infected by App2 were also characterized by insufficient warming of the nursery room before loading the pig, old nursery buildings, a low mean inside temperature in the finishing room, a small temperature range for the ventilation control rate and a temperature setpoint of the ventilation controller in the finishing room below or equal to 21°C. PRRSV and SIV infections and a high infection pressure towards PCV-2 before 16 weeks old were associated with App2 infection.

Introduction: *Actinobacillus pleuropneumoniae* (App) is among the most important bacterial pathogens affecting swine lungs. It is responsible for porcine pleuropneumonia and pleuritis. These diseases cause economic losses to the pig industry worldwide. App is divided in two biotypes also sub-divided in several serotypes. App2 infection has previously been found to be associated with extended pleuritis in slaughter-aged pigs [1]. To date, few studies investigated risk factors for App infections. The aim of the current study was therefore to identify the factors associated with App2 infection in slaughter-aged pigs.

Material and methods: Data and sera used were collected in 116 pig herds involved in a cross-sectional study on respiratory diseases in western France. Blood samples were collected from four different batches of pigs in each herd, aged 4, 10, 16 and at least 22 weeks (15 pigs/batch). Sera from all batches were tested for PRRSV antibodies (ELISA test 2XR-IDEXX). Sera from the oldest pigs were tested for *Mycoplasma hyopneumoniae* (Mhp) (DAKO ELISA-Kitvia) and App2 antibodies (Swinecheck App2-Biovet). Antibodies against European swine influenza viruses (SIV) H1N1 and H1N2 were searched in sera from the oldest pigs by a haemagglutination inhibition test [2]. The porcine circovirus type 2 (PCV-2) genome load in sera from batches of 4, 10 and 16 week-old pigs was quantified by real-time PCR [3]. Tracheo-bronchial swabs were taken from 10 of the 15 pigs selected from the batches of 4, 10 and 16 week-old animals and placed in 2 ml of buffered peptone water broth. Mhp DNA was identified in all swabs by modified nested-PCR [4]. Data related to herd characteristics, biosecurity, management and housing conditions were collected by a questionnaire. Temperature, relative humidity, CO_2 concentration, respirable dust level in the nursery and fattening rooms, where the oldest sampled pigs were housed, were measured over 20 hours. Ammonia concentrations were measured at pig nose level on the day of the visit. A herd was deemed infected by App2 if at least one pig was seropositive for App2. Multiple correspondence analysis was used to identify factors associated with App2 infection at the herd level.

Results: Out of the 116 herds, 14.7% (confidence interval at 95% [8.1%-21.2%]) were considered infected by App2. Large herd size (>250 sows), lack of footbath and no SIV vaccination of the replacement gilts were factors associated with App2 infection. Herds infected by App2 were also characterized by insufficient warming of the nursery room before loading the pigs, old nursery buildings (>25 years), a low mean inside temperature in the finishing room (≤23.5°C), a temperature range of less than 5°C for the ventilation control rate and a temperature set-point of the

ventilation controller in the finishing room below or equal to 21°C. PRRSV and SIV infections and a high infection pressure towards PCV-2 before 16 weeks old were associated with App2 infection.

Discussion: Eleven herd-level factors were singularly found to be associated with App 2 infection in our survey. Application of strict biosecurity measures is considered to be of paramount importance to prevent App infection [5]. Our results are in accordance with those of Maes *et al.* [6], who found that poor biosecurity measures significantly increased the risk of seropositivity for infections with serotype 2. The relationship between App infections and herd size has not been published yet. However, the effect of herd size on pleuritis has previously been found [7]. Housing the pigs in old nursery buildings was a particular feature of App2 infected herd. Old buildings are more likely to be poorly isolated than recent buildings. They may thus be less effective for the thermal control inside the room. Exposure to climatic stress is generally considered to be important in the development of porcine pleuropneumonia [5]. In the same line, four factors related to environmental temperatures and the control of climatic conditions were associated with App2 seropositive status of a herd. These results agree with the findings of a previous study where inappropriate thermal environment of the pigs were associated with App infection [8]. The lack of SIV vaccination of replacement stock was found to be a feature of App2 infected herds. The mechanisms underlying this effect have not been clearly defined. Infections by three viruses were found to be associated with herds infected by App2. However the study design did not allow to establish the time-sequence of events and the observed associations do not imply a causal relationships. Thus the results should be interpreted with caution. All those viral and bacterial pathogens are known to interact as a component of the porcine respiratory disease complex.

Conclusions: Risky herd profiles were identified as regard to App2 infection. Improvement of management, housing and climatic conditions at all rearing steps should significantly reduce the risk of App2 infection in slaughter-aged pigs.

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ISOLATION AND MOLECULAR DETECTION OF BRUCELLA MELITENSIS IN SHEEP AND B. ABORTUS IN CATTLE BY IS711 AND OMP2A GENE BASED PCR

[1]Habtamu Tassew, [2]Rajesh Rathore, [3]Kuldeep Dhama and [4]Karthik K

[1]Mekelle University, College of Veterinary Medicine, Mekelle, Ethiopia. P.O. Box: 231
[2,4]Division of Bacteriology and Mycology, IVRI, Izatnagar, Bareilly (U.P.) -243122, India
[3]Division of Pathology, IVRI, Izatnagar, Bareilly (U.P.)-243122, India

Summary

Brucellosis due to *Brucella melitensis* is of public health and economic importance in many developing countries including India and is the major cause of abortion in small ruminants. This study was conducted with the objective of cultural and molecular isolation of *Brucella* from a disease outbreak in sheep at Saharanpur District (U.P., India). Out of a total 56 clinical samples, 42 (75%) bacterial isolates of *Brucella* were recovered. On the basis of cultural characterization, the organisms were identified to be *B. melitensis*. Further confirmation was done by PCR amplification of *IS711* and *omp2a* target genes and resulted specific amplicons of 731 bp and 1104 bp fragment sizes, respectively for all 42 isolates obtained. In addition, 2 (33.33%) isolates of *B. abortus* were also identified from liver samples of aborted bovine foetus (n=6) in Bareilly (U.P.). Isolation and molecular detection of *B. melitensis* and *B. abortus* indicates a need for appropriate prevention and control strategies for this economically important pathogens having zoonotic significance.

1. Introduction

Brucellosis, caused by members of the genus *Brucella*, is an important re-emerging bacterial zoonosis and a significant cause of reproductive losses in animals. The disease is usually caused by *B. abortus* in cattle, *B. melitensis* or *B. ovis* in small ruminants, *B. suis* in pigs and *B. canis* in dogs (Osterman and Moriyon, 2006). Among the different species, *B. abortus* and *B. melitensis* are the most pathogenic and virulent, not only for cattle, sheep and goats, respectively, but also for other animal species including man. The conventional diagnostic methods that identify species of *Brucella* are time consuming, laborious, and costly, and thus are not suitable as routine diagnostics. Recently various molecular techniques have been developed for the rapid detection of *Brucella* in animals and human beings (Ignacio and Ignacio, 2004). The present study aimed at cultural isolation and molecular detection of *Brucella* spp. from a disease outbreak in sheep and from aborted bovine foetus by employing IS711 and *omp2a* gene based PCR assay.

2. Materials and Methods

Bacterial Strains: Reference strain of *B. melitensis* 16M and vaccine strain of *B. abortus* S19 (provided by National *Brucella* Laboratory and Division of Biological Product, IVRI, respectively) were used in the present study.

Sample Collection: During a disease investigation study of a suspected outbreak of ovine brucellosis at Nukad Tehsil, Saharanpur District of Uttar Pradesh, knee joint fluid samples from sheep (56) with clinical signs of joint swelling and abortion were collected aseptically for the study. In addition, liver samples of aborted bovine foetus (n=6) were collected from an organized Cattle farm of Bareilly (U.P.).

Cultural and Molecular Detection: All clinical samples were processed for cultural isolation of *Brucella* spp. as detailed by Corbel and Brinley- Morgan, 1984. Extraction of genomic DNA was performed following CTAB method described by Wilson (1990) with slight modifications. Primers specific for *IS711* and *omp2a* gene of *B. melitensis* and *B. abortus* were used to amplify a fragment size of 731 bp and 498 bp for *IS711* (Khosravi *et al.*, 2006), and 1104 bp (Vivekananda *et al.*, 2009) and 966 bp (Paquet *et al*., 2001) for *omp2a*, respectively.

3. Results

In the present study, out of a total of 56 clinical samples of sheep and 6 foetal samples of cattle subjected to cultural isolation, 42 (75%) and 2 (33.33%) bacterial isolates of *Brucella* spp. were recovered, respectively on the basis of cultural characteristics. The isolates were finally confirmed by PCR and a product of about 498 bp was obtained from two *B. abortus* isolates and about 731 bp from B. melitensis isolates using *IS711* as a target gene (Fig. 1). Similarly using *omp2a* gene, about 966 bp was obtained from *B. abortus* isolates and about 1104 bp from B. melitensis isolates (Fig. 2 and 3).

4. Discussion

The present investigation on a suspected brucellosis outbreak in sheep using both cultural and PCR assay indicated the causative agent to be *B. melitensis*. A PCR product of about 498 bp and 731 bp was obtained from two *B. abortus* and *B. melitensis* isolates, respectively using *IS711* as a target gene. Similarly with a specific primer to an outer membrane protein 2a (*omp2a*), a PCR product of about 966 bp and 1104 bp was obtained from *B. abortus* and *B. melitensis* isolates, respectively. Thus, all the isolates were identified and confirmed as *B. melitensis* and *B. abortus* based on cultural characteristics and the species specific PCR assay. These results were in accordance with the reports of Paquet *et al*. (2001), Khosravi *et al.* (2006), and Vivekananda et al. (2009) as they showed similar amplified products in different isolates of *Brucella*. In India, free roaming of animals and intermixing of livestock through grazing at common pastures and trading at local stock yards probably contribute to the spread and transmission of the infection (Henk *et al*., 2005).

5. Conclusions

In this study, B. melitensis was found to be the main etiological agent responsible for causing disease outbreak (brucellosis) in sheep. Along with this, *B. abortus* was also identified to be causing abortion in cattle. Being a contagious disease, the isolation of *B. melitensis* may indicate high prevalence of *B. melitensis* infection among sheep in Saharanpur region and due to that; the disease may pose threat to animal as well as human health. Rapid and reliable molecular tools are quite important to allow early diagnosis, epidemiological surveillance and adequate antibiotic therapy in time to decrease morbidity/mortality as well as prevent its public health implications.

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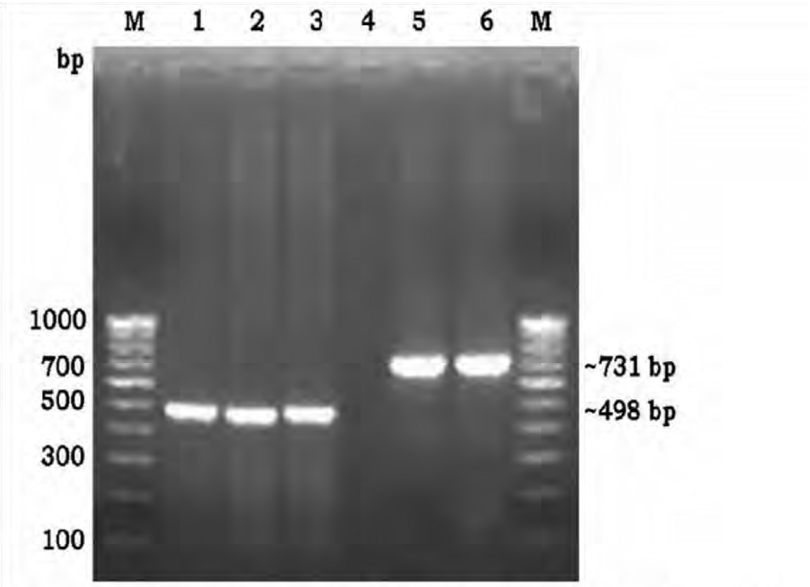

Fig. 1: PCR amplification of *Brucella IS711* gene: Lane M: 100bp DNA ladder; L1: *B. abortus* isolate 1 (498 bp); L2: *B. abortus* isolate 2 (498 bp); L3: *B. abortus* S 19 (Positive control); L4: Negative control; L5: *B. melitensis* (731bp); L6: *B. melitensis* 16M (Positive control); L = Lane

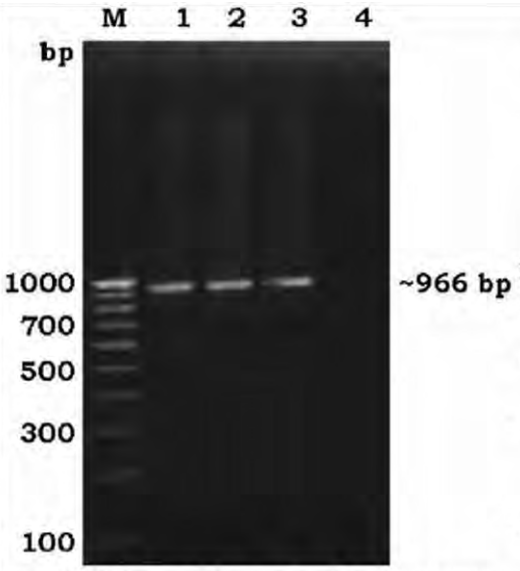

Fig.2: PCR amplification of *B. melitensis omp2a* gene: Lane M1: 100bp DNA ladder; L1&2: *B.melitensis* (1104 bp): L3: Negative control; L 4 & 5: *B. melitensis* 16M (Positive control): L6: Negative control; LM2: 1kb DNA ladder

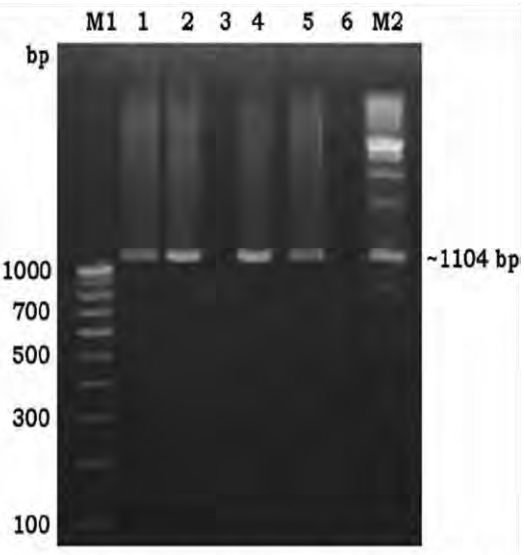

Fig.3: PCR amplification of *B. abortus omp2a* gene: Lane M: 100bp DNA ladder; L1: *B. abortus* isolate 1 (966 bp): L2: *B. abortus* isolate 2 (966 bp); L3: *B. abortus* S19 (Positive control): L4: Negative control

ESSENTIAL OILS – THE ANSWER TO ANTIBIOTIC RESISTANCE?

D. Pfeifer, B. Lohr, K. Hellmann

Klifovet AG, Regulatory Affairs, Munich, Germany

Summary

In light of increasing numbers of bacteria being resistant to antibiotics and the potential transmission from livestock to humans, researchers have recently focused on essential oils investigating their potential antimicrobial properties.

The antimicrobial activities of diverse essential oils and terpenoids have been investigated in several studies in a variety of bacterial strains. Results revealed that essential oils possess growth inhibition activities against a wide range of microorganisms, including foodborne pathogens, bacteria and yeasts *in vitro*.

Aiming on an alternative to conventional antimicrobial products, the antimicrobial activities of essential oils should target on specific pathogens relevant for animal infectious diseases. Furthermore, studies need to be conducted in a way allowing for quantitative estimation of antibacterial properties, thereby establishing a relationship between concentration and inhibition to define appropriate use conditions.

This review discusses the current knowledge on the antimicrobial potential of essential oils compared to conventional antibiotics. Further, the use of essential oils in feed additives is discussed.

Introduction

Essential oils are liquid, volatile compounds that are formed as secondary metabolites of edible, medicinal and herbal aromatic plants and are characterized by a strong odour [1, 2]. Essential oils occur in many parts of plants, including flowers, leaves, stems, roots and bark from which they can be extracted by steam or hydro-distillation, solvent extraction or cold pressing [1-3]. Chemically, essential oils are very complex mixtures of terpenoids. Their qualitative and quantitative composition depends on the source, part and age of the plant from which it is derived as well as on the extraction method used [1, 2, 4]. The oils usually contain two or three main components at high concentrations and further components in trace amounts. Due to the variable composition and with respect to a future use in animal health, the single component, i.e. the terpenoid, rather than the essential oil is thus be of central interest requiring precise characterization of the mixture.

Due to public concern on the use of antibiotics in livestock products, alternative methods of favorably altering animal productivity and improving feed efficiency have been explored. Aromatic plants have been used since ancient times for various purposes, such as medical treatments, food flavoring and food preservation [1, 3] thereby making their “active compounds”, the essential oils, a potential and natural alternative to common antibiotics.

Regarding new future uses of essential oils in animal health, it is important to develop a better understanding on the mode of action of the oils and their components.

Materials and Methods

This literature review summarizes the most relevant documentation searched in the public databases such as PubMed, XVET Veterinärmedizin, CAB Abstracts, ISTPB + ISTP/ISSHP, MEDLINE and Sci Search.

The existing studies on essential oils were evaluated with regard to a potential use in the therapy of infectious diseases in view of the data requirements as specified in the revised EMA Guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances [5]. According to this guideline, the minimum inhibitory concentration (MIC; defined as the lowest concentration of an antimicrobial substance that prevents the visible growth of bacteria) needs to be determined as a basis. MIC values should be derived using accepted standardized methodology, i.e. dilution methods.

A second focus of this review is the zootechnical use of essential oils in support of animal health (use as feed additives). Therefore, studies investigating the potential for improved feed efficiency and health effects and the potential mechanisms of action of the oils are discussed.

Results and Discussion

In several investigations, the antimicrobial activity of essential oils or their main components has been assessed using *in vitro* methods. A broad spectrum of pathogenic bacteria has been studied with the aim to discover therapeu-

tic alternatives for treatment of infectious diseases. The MIC values derived from these trials vary greatly between investigated oils and bacterial strains. For example, in *P. multocida*, causing respiratory infections in cattle, MIC values of 0.2 % v/v were derived for origanum oil and 0.04 % v/v for cinnamon oil [6]. Studies comparing MIC values for both essential oils and common antibiotics were also found in public literature. In *E.coli,* MIC values of 300 µg/ml were derived for both cinnamon oil and cinnamaldehyde, compared to 1.3 µg/ml for Pen/Strep [7]. Such studies show that essential oils have the potential for growth inhibition of bacterial strains but the effective concentration is much higher compared to common antibiotics.

The mechanism of action of essential oils has been attributed to terpenoid and phenolic compounds, as well as the chemical functional groups and the interactions between them [4, 8]: Comparison of MIC values of single terpenoids to the whole essential oil had shown additive or synergistic effects, i.e. lower MIC values for the oil compared to the single component [3]. The mechanistic processes behind the antimicrobial activity, however, are not yet fully understood. Potential modes of action that are discussed in the literature involve actions on the bacterial cell membrane and further influences on cytoplasmic elements [9].

Most of the few published *in vivo* studies on essential oils are related to their use as feed additive. In chickens, oregano oil (~70% thymol) was found to improve bodyweight and bodyweight gain when administered with the diet at 100 mg/kg diet from day 1 to 42 of age [10]. In pigs, supplementation of 300 mg menthol/kg diet improved feed efficiency, whereas cinnamaldehyde (300 mg/kg) failed to show an effect on feed intake and bodyweight gain [11]. In the same study, several segments of the pigs' gastrointestinal tract were examined for bacterial cell numbers, revealing only minor changes between supplemented and control diet. This might be due to the absorption of the oils in the stomach, thus making it impossible to reach the high concentrations of oil required for bacterial growth inhibition in the distant parts of the intestinal tract.

Conclusion

Many published studies on essential oils related to antimicrobial activity or regarding feed efficiency are *in vitro* investigations. The MIC values are, however, only the first step regarding a potential use in therapy (alternative antibiotic) or general animal health (feed additive). *In vivo* studies are required as a next step investigating antibacterial activity and/or feed efficiency of several livestock species using chemically defined terpenoids or terpenoid combinations. From these investigations a dosage that is suitable for growth inhibition or improving general animal performance needs to be defined. To demonstrate efficacy as a feed additive the relevant EFSA requirements on *in vivo* studies need to be followed.

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MIGRATORY BIRDS AND EMERGING INFECTIOUS DISEASES IN EUROPE

Ľ. Korytár, R. Hromada, D. Chvojka, J. Koščo, M. Ondrašovič, V. Petrovič

Department of Environment, Veterinary Legislation and Economics, The University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

Summary

Strong connections exist between human population, population of domesticated animals and migratory birds. It is necessary to understand certain aspects of life and biology of migrants. Wild birds can play an important role as reservoir hosts of pathogens.

Introduction

The aim of this article is to provide a reference text on infectious diseases which affect wild birds in Europe. The research on wildlife diseases has significantly increased across the Europe due to the growing concern of emerging and re-emerging pathogens. This interest has also concentrated on investigations into the risk to human and domesticated animals health.

Basic Types of Bird Movements

There are no species of birds, in which some part of the population does not udertake movement away from the breeding territory during some time of the year. Each bird species undertakes dispersion foraging movements, ore some form of migration. Migration is a form of dispersal involving regular movement and return between one locality and another (1).

Local seasonal movements include several different seasonal movements found in sedentary species – postbreeding dispersal, dostance foraging, single-species flocking and mixed-species flocking (2).

Facultative migration is typical for species in which most of the population migrates away from the breeding grounds every year, but settlement within the nonbreeding range varies according to variations in food supply and weather conditions (3).

Partial migration involves residency on the breeding area throughout the year by one portion of the population, whereas the other portion migrates.

Altitudinal migration involves movements from a higher-elevation to a lower elevation. Birds undertaking this type of migration usually descend from the seasonaly occupied breeding habitat to a lower located nonbreeding or wintering localities.

Many European bird species undertake *Obligate short-distance* and *long-distance migration.* It means that all individuals of a population leave the breeding ground for a nonbreeding range in a different geographic area (3).

The Palearctic-African Migration System

European territory is included in the Palearctic-Afrotropical migration system (4). The Palearctic-Afrotropical migration system consists of three migration corridors – East Atlantic, Black Sea/Mediterranean and West Asia/West African corridor (1). Many Eurasian species winter partly in Africa and partly in southern Asia. European breeding birds migrate mainly through Euro-African flyway. About 250 bird species, counting hundreds of millions of specimens, move through this migratory corridor two times per year (3). Among them short-distance and long-distance migrants can be distinguished. Some species move relatively short distances within Europe. Others species move longer distances to Africa or southern Asia.

Diseases of Avian migrants

Newcastle Disease - Avian Paramyxovirus Infections

Avian paramyxoviruses (APMV) are members of the Order *Mononegavirales*, Family Paramyxoviridae, Genus *Avulavirus* (5). Several related paramyxoviruses infect and cause disease in wild and domestic birds (6).

Avian Influenza

Avian influenza is caused by a group of influenza A viruses, members of the Family Orthomyxoviridae (7). Influenza viruses preferentially infect cells of avian intestinal tract. Whereas most bird species are susceptible to infection, the most commonly infected are waterbirds. Among them ducks (*Anseriformes*) and shorebirds (*Charadriiformes*) (8). Appearance of highly pathogenic avian influenza virus subtype H5N1 was first documented from samples collected from domestic geese (Anses anser) n Guandong Province of southern China in 1996 (9).

West Nile Virus

West Nile Virus is a flavivirus related to Japanese encephalitis virus and St. Louis encephalitis virus. It was first described in Uganda in 1973. The virus was first reported in Europe from Albania in 1958. By the 1990s, the virus was widespread in Africa and Eurasia (10).

Avian Pox

Avian pox is a common name for a mild-to severe slow-developing disease caused by viruses of the genus *Avipoxvirus* in the family Poxviridae. This widespread avian disease has been found in a large number of bird families. The first record from a Great Tit (*Parus major*) came from England in 2006 and in subsequent years the disease spread to other European countries (11).

Trichomonosis in Finches

Trichomonosis is disease caused by protozoan *Trichomonas Gallinae*. The disease was first reported as fatal to a finch in April 2005, after which it spread increasingly. Most mortality involved Greenfinches (*Chloris chloris*) and Chaffinches (*Fringilla montifringilla*). By 2007, only in Britain, the mortality reached 500,000 wild birds (12).

Conclusions

Along the bird flyways a great potential for origination of emerging and re-emerging diseases outbreaks exists. Birds are also known as long-range carriers of infectious agents. Vectors of infectious pathogens as ticks can be transported by birds too.Some of this diseases are of high importance. The function of wintering birds in the moderate zone of Europe is also significant. Not only birds on migration are important from epizootiological viewpoint in Europe. Thanks activity of wintering birds some arthropod vectors, especially winter ticks can be maintained on the endemic areas through winter months.

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IMPACT OF ZINC OXIDE ON THE IMMEDIATE POST WEANING COLONIZATION OF ENTEROBACTERIA IN PIGS

W. Vahjen[1], S. Durosoy[2], A. Romeo[2], J. Zentek[1]

[1]FUB Department of Veterinary Medicine, Institute of Animal Nutrition, Berlin, Germany ; [2]Animine, Sillingy, France

Summary

Dietary zinc oxide (ZnO) shows beneficial effect on *E. coli* induced diarrhea in pigs after weaning, but no data is available on the bacterial development directly after weaning. Twenty weaned piglets were fed diets containing 150 or 3000ppm Zn from standard ZnO sources and 150 or 300ppm Zn from a commercial ZnO preparation. Daily fecal samples were used to detect a range of relevant enterobacterial genes via PCR assays. The development of the *Escherichia* group (measured by 16S rDNA) showed an increase until 4 days after weaning in pigs fed the 150ppm Zn diets, followed by a drastic decline until day 10. However, the 300ppm as well as the 3000ppm diets showed an earlier decline already 2 days after weaning, which led to less colonization of the *Escherichia* group than in the other trial groups. The most prevalent toxin gene was the *E. coli* estIIb. Contrary to the colonization of enterobacteria, a severe decrease of the amount of estIIb genes was visible in animals fed the 3000ppm diet already after the first day after weaning. A decline was observed in all other trial groups. Generally, the impact of dietary Zn was lessened in all experimental groups 14 days after weaning. In conclusion, this study showed an immediate effect of ZnO ranging from 3 to 4 days after weaning until 8 to 10 days after weaning.

Introduction

Zinc oxide (ZnO) has been in use for a long time with beneficial effects in animal production (Pettigrew, 2006). The European legislation has limited the use of ZnO in animal nutrition to a maximum level of 150ppm in feed, because of suspected environmental pollution (Jondreville et al., 2003). However, in Asia and the Americas, supplementation of 3000ppm in weaned piglets feed is common.

It is generally believed that a direct effect of ZnO exists on enterobacteria induced post-weaning diarrhea; but it is unknown exactly when its beneficial effect starts and for how long it proceeds. Thus, the aim of this study was to monitor the impact of ZnO after weaning on the presence of *E. coli* pathogenic genes.

Material and Methods

Twenty weaned piglets were fed diets with 150 or 3000ppm Zn from standard ZnO sources and 150 or 300ppm Zn from a commercial ZnO preparation (HiZox®, Animine, France).

Daily fecal samples were taken on day 0, 1, 2, 3, 4, 6, 8, 10 and 14 after weaning. DNA was extracted from samples using a commercial extraction kit (Qiagen Stool kit, Qiagen, Hilden, Germany). To measure the development of enterobacteria, 180 fecal samples were analysed. Total enterobacteria and *Escherichia* spp. were analysed by qPCR (16S rRNA gene copy number). A multiplex-PCR targeting important *E. coli* toxins and fimbriae was used in samples from 0 and 1d of the trial. Prominent gene occurrences were further analysed by qPCR (eltIa estIb, estII, fedA, fae, fan).

Total Zn was analyzed in fecal samples via AAS.

Results

All animals finished the trial healthy. Fecal scoring showed significant differences between the trial groups. While the control group showed the lowest scoring values (3.38 for the entire period), the highest Zn dose showed significantly better scoring (3.90). In the groups with HiZox, intermediate values were observed. Interestingly, the 150ppm HiZox diet performed better than the 150ppm feed grade ZnO diet.

Concerning the total Zn content in fecal samples, during 2 days, no visible difference was observed. Starting on the third day of the trial, animals fed the highest Zn dose showed increasing fecal Zn concentrations, which seemed to plateau (14 g/kg Zn wet weight) after day 10. Fecal Zn levels between both 150ppm Zn trial groups and the 300ppm

HiZox trial group started to differ numerically on day 4, significantly so on day 10 of the trial; they remained below 2g/kg Zn wet weight.

The development of *Escherichia* spp. is shown in Fig 1. During the first three days of the trial, an increase was observed, followed by a decline starting on day 4. However, the differences for *Escherichia* spp. were more pronounced than for enterobacteria (data not shown). Significant differences for *Escherichia* spp. were observed between both 150ppm Zn groups and the 300ppm HiZox or 3000ppm ZnO groups. This trend continued on day 6, however without significance. No differences were observed after 10 days, where all trial groups showed similar concentrations of *Escherichia* spp.

The multiplex PCR assay located several *E. coli* associated pathogenic factors in samples from 0d and 1d. The most prominent pathogenic factors were the heat stable toxins estIb and estII, followed by the heat lable toxin eltIa and the fimbrium gene fedA. A qualitative Chi² test was done for all pathogenic factors. Although no significant differences were observed, based on the sum of all detected *E. coli* pathogenic factors, differences were visible between the control group and all other trial groups (Fig 2).

Quantitative results for estIb and estII showed that both toxin genes displayed the highest concentration in the 150ppm ZnO trial group (data not shown). For estII, the 3000ppm ZnO trial group showed the lowest concentrations over the whole trial period, followed by the 300ppm HiZox group. Differences between both 150ppm trial groups were marginal.

Discussion

The rearrangement of host intestinal physiology, immune system and bacterial composition after weaning was visible in the development of *Escherichia* spp. and total enterobacteria as well as *E. coli* pathogenic factors. Generally, the low dietary ZnO diets led to a later reduction (+1d) than the 300- and 3000ppm ZnO diets. The decline of estII copy numbers already appeared on the second day of the trial with 3000ppm feed grade ZnO, contrary to *Escherichia* spp., which showed reduced concentrations starting on the third day. This result may denote an increased sensitivity of pathogenic *E. coli* towards Zn, which has been noted in an in-vitro study on the growth of intestinal bacteria in the presence of ZnO (Liedtke and Vahjen, 2012). Quantitative differences of estII between the 300ppm HiZox and the 3000ppm ZnO were small. This may indicate an increased effectiveness of the HiZox preparation already at lower concentrations, as shown in growth performance trials (Morales et al., 2012).

Conclusion

This trial has shown an effect of ZnO on pathogenic bacteria until 8 to 10 days. The 3000ppm feed grade ZnO concentration and the 300ppm HiZox trial group were superior in reducing bacterial count as well as *E. coli* pathogenic factors.

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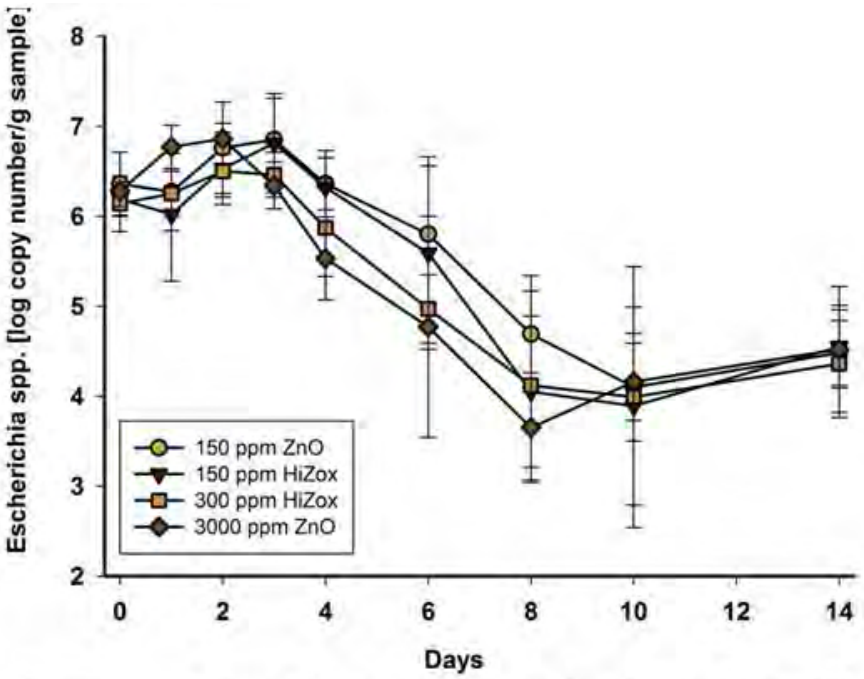

Figure 1: Development of *Escherichia* spp. in fecal samples of weaned pigs fed different amounts and preparations of dietary ZnO

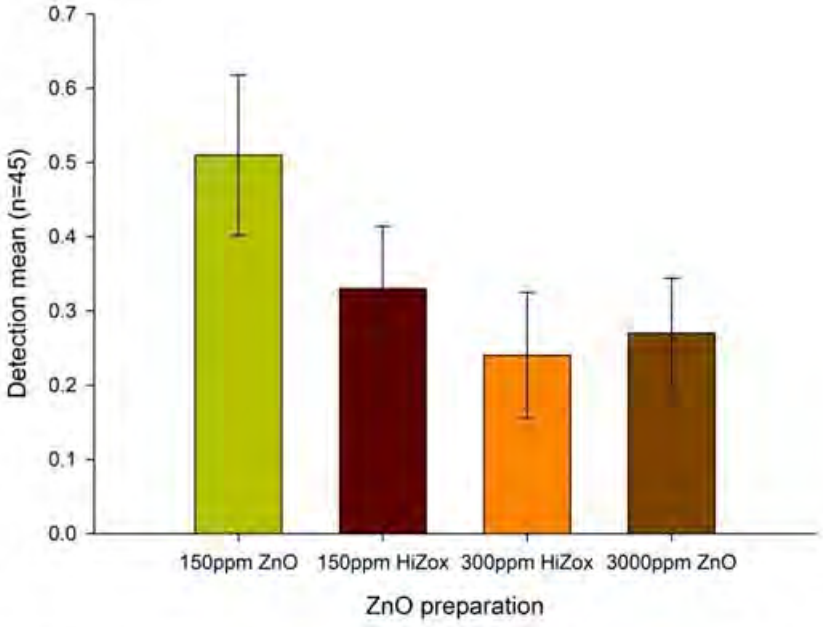

Figure 2: Sum of detected E. coli pathogen factors over the whole trial in fecal samples of weaned pigs fed different amounts and preparations of dietary ZnO (n=45 per trial group)

ASPERIGILLUS AWAMARI AND AFLATOXIN B1 IN FEED FOR LAYING HENS : 1- EFFECTS ON IMMUNE RESPONSE OF DOMESTIC FOWLS

M. H. El-Deep, M.H Assar, Kh. Attia, M. Shabaan, Y. Eide* and M. A. M. Sayed

Animal Prod. Inst. Agric. Res..Center, Ministry of Agric, Egypt
*Poultry Production Dept, Faculty of Agric, Kafr El-Sheikh Univ, Egypt**

The main object of this work was to investigate the effect of adding *Asperigillus Awamari* (*A. awamari*) to the diets contaminated by aflatoxin B1 (AFB1) 300ppb / kg diet of Inshas chickens (a local Egyptian chicken strain) on chickens immunity. The experimental design consisted of five experimental groups: control and 4 dietary treatments as follows; (T1) Basal diet (control), (T2) Basal diet + AF 300ppb / kg diet, (T3) Basal diet + AF 300ppb / kg diet + *A. awamari* 0.05%, (T4) Basal diet + AF 300ppb / kg diet+ *A. awamari* 0.1%, (T5) Basal diet + AF 300ppb / kg diet+ *A. awamari* 0.15%. Characteristics investigations included: Immune response (immune response against NBVD and Cell-mediated immunity (Cutaneous basophil hypersensitivity CBH). Results obtained can be summarized as following; It is clearly evidented that feed additives significantly improve the natural immunity of birds against viral invasions. An average antibody titer recorded in control diets was always significantly less than those found in all feed additives treatment (T3 to T5 diets) compared to AF group. Also, The addition of *A. awamari* with different levels to the chicks diet had significant increased in CMI response as compared with AF group, although in many cases *A. awamari* 0.05% seemed to be less effective than the other levels.

PREVALENCE OF BOVINE TRYPANOSOMOSIS IN BALI LOCAL GOVERNMENT OF TARABA STATE NIGERIA

A. A. Allahoki[1], I. O. Jeaga[2], M. I. Elijah[3], J. P. Tukura[4]

[1]Agricultural Technology, Federal polytechnic Bali, Bali, Nigeria; [2] Science and laboratory Technology, Federal polytechnic Bali, Bali, Nigeria;[3] Science and laboratory Technology, Federal polytechnic Bali, Bali, Nigeria; [4] Agricultural Technology, Federal polytechnic Bali, Bali, Nigeria;

SUMMARY

Trypanosomosis is a disease which is caused by a protozoan of the genus Trypanosome which affects all domestic animals and human beings. Prevalence was determined by sampling white Fulani breed of cattle from various wards in the local government. Thin and thick blood smear techniques were used in analysing the blood samples. The result shows that out of the two hundred (200) samples collected, 2 (1%) were found positive from Gang-Tiba and Takalafiya. Out of the one hundred and fifty 150 (75%) female analysed, 2 (1.33%) were positive. similarly out of 130(65%) adult cattle 2(1.5%) were positive with *Trypanosome vivax.* The low prevalence rate of Trypanosomosis in the study area could be due to low availability of the vector (Glossina). However I recommend that farmers and animal health personnel should pay more attention to examine and treat promptly any sign of disease similar to this condition to prevent it from being introduced into the area and the herds. I also wish to advice that modern diagnostic techniques should be used for future research and to involve higher numbers of animals from all the wards of the local government.

Keywords: prevalence, Bovine, Trypanosomosis, Bali.

INTRODUCTION

Trypanosomosis is caused by trypanosomes which live in the blood and tissue fluid of their host and are transmitted mainly by tsetse fly *(Glossina spp)* to man, cattle, pig, horses, rabbits, goat and sheep and other species.(Ikede,1983). The major pathogenic tsetse-transmitted trypanosome species *T congolense, T vivax* and *T brucei in* cattle sheep and goats and *T simae* in pigs (Nantulya, 1990). Clinical sign include intermittent fever, anorexia. Poor hair coat, emaciation, lethargy, anemia, enlarged- lymph nodes, abortion, infertility, decrease in milk yield, subcutaneous edema, ascites and ocular discharge(Ikede and Losos,1983). The disease is widely known as Nagana in southern Africa which is derived from a Zulu term meaning ‘’to be low or depressed in spirit’’- a very apt description of the disease. (Logan- hen Frey et al 1992).

Study area

The study area is Bali local government and is situated in the guinea savannah at latitude 7^0 12N to 9^0 00N of the equator and longitude 10^0 00E to 12^0 00 of the meridian (Atlas, 2006) the area is characterized by dry and rainy season. rainy season starts April and ends in October while the dry season begins by November and ends in March. Rainfall ranges from 7500mm to 1100mm/ annum and temperature ranges between $22^{0\,c}$ to 35^0 c.

MATERIALS AND METHOD

Blood samples were collected from white Fulani breed of cattle using EDTA bottles which were further submitted to the National Veterinary Research Institute field (north-east) parasitology laboratory in Adamawa state from March – May 2014 for diagnosis. Two hundreds blood samples were submitted, processed and examined for blood parasite (Trypanosomes) in a semi-intensive managed cattle in Bali local government area of Taraba state Nigeria.

A thin and thick blood smear was prepared from each blood sample, air- dried, fixed in methanol for 2-3 minutes, stain in a 5% giemsa stain and rinsed in buffered water, the smear were finally observed for the presence of trypanosome using x100 oil immersion objectives.

RESULT

Out of the 200 cattle from which blood samples were obtained and examined from two locations; (Gang-Tiba and Takalafiya) has prevalence rate of 1(0.5%) each with *T vivax* table 1. The result shows that out of the 150 (75%) female cattle analyzed, 2(1.33%) were positive with *T vivax.* Likewise out of 130 (65%) adult cattle analyzed for Trypanosome infection, 2(1.5%) were as well found positive as shown in table 3.

DISCUSSION

Trypanosome infection transmitted by glossina constitutes a major handicap to livestock production. W.H.O, 1992; F.M.A 1981) reported that the economic impact of the disease is mainly on cattle, although other domestics animal such as goat, sheep and pig are involved. The high infection rate observed among adult could be attributed to the fact that they are more exposed to the vector prone areas through grazing and migration compare to the calves that are usually restricted and kept in low concentration (Lima, 2000). Anemia was the major clinical sign in the affected animal, agreeing with Adejinmi et al, (2004) who reported that anemia as a reliable indicator for the severity of haemoparasitic infection.

CONCLUSION

The low prevalence rate of the infection in the study area could be due to the low availability of the vector (Glossina). It is concluded that further investigation or research involving large number of samples and from different breed of cattle be carried out in order to conclusively determine the presence of trypanosome parasite in the study area.I therefore recommend that much attention should be given to the control of the vector and also the conduct of more elaborate survey using specialized diagnostic tool to sample other breed in the study area.

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Table 1: the prevalence of bovine Trypanosomosis in Bali local government area of Taraba state.

Wards	No. examined	No. positive	% positive
Gang-Dole	20	0	0
Gang-Tiba	20	1	0
Bali A	20	0	0.5
Bali B	20	0	0
Suntai	20	0	0
Gang- Lari	20	0	0
Kaigama	20	0	0
Gang-Mata	20	0	0
Takalafiya	20	1	0.5
Maihula	20	0	0
Total	200	2	1

Table 2: Distribution of Bovine Trypanosomosis based on sex in Bali local government area.

	No.examined	no. infected	% infected
Males	50	0	0
Females	150	2	1.33
Total	**200**	**2**	**1**

Table 3: Distribution of Bovine trypanosomosis based on Age in Bali local government area.

Sex	No. examined	No. infected	% infected
Adult	130	2	1.5
Young	70	0	0
Total	**200**	**2**	**1.5**

RISK FACTORS ASSOCIATED WITH CRYPTOSPORIDIUM INFECTION IN DIARRHEIC PRE-WEANED CALVES

M. Imre[1], M.S. Ilie[1], K. Imre[2], G. Dărăbuş[1]

[1]Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timişoara, Romania
[2]Department of Animal Production and Veterinary Public Health, Faculty of Veterinary Medicine, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timişoara, Romania

Summary

Neonatal calf cryptosporidiosis is a gastrointestinal diarrheic disease with negative impact on animal welfare. The present study aimed to provide data's concerning the risk factors associated with the acquiring of *Cryptosporidium* infection in pre-weaned calves.

During the five years (2006-2011), diarrheic fecal samples from 428 pre-weaned calves from twenty cattle farms located in western Romania were collected and screened for the presence of *Cryptosporidium* spp. using ELISA technique. In order to identify risk factors a binary logistic regression model has been used enrolling the following data's: age (1-7, 8-14, 15-21 and 22-30 days), gender, breed (purebred-dairy or meat; and crossbreed), herd size (range 90-800), farming system (industrial and grazing), housing system (individual box and stable with 6-12 calves) and feeding regime (individual cow milk and milk replacer).

Overall, the prevalence of *Cryptosporidium* infection was 37.4%. The 8 – 14 days age category (odds ratio =2.06; 95%; confidence interval = 1.3 – 3.1; *p*=0.0007), purebred – dairy calves (OR=2.39; 95% CI = 1.6 – 3.6; *p*=0.0001), individual box housing (OR=1.59; 95% CI = 1.0 – 2.4; *p*=0.03) and industrial farming system (OR=1.59; 95% CI = 1.0 – 2.4; *p*=0.03) have been positively associated with the prevalence of *Cryptosporidium* infection in young calves. The findings of the survey indicate that both individual animal as well as management procedures can be considered risk factors in the acquiring of the disease. Also, the results offer useful insight for veterinarians, farmers and public health specialists in monitoring, preventing and controlling of pre-weaned calf cryptosporidiosis as important zoonosis in the screened area.

1. Introduction

Neonatal calf cryptosporidiosis is recognized as a gastrointestinal diarrheic disease with negative impact on animal welfare In terms of economic losses, beside mortality, the disease outbreak reflects the need of veterinary assistance and retarded growth rates after recovery (5). In practice, in the lack of the knowledge the risk factors little success can be achieved in the development of adequate control strategies (1).

In Romania, considering the lack of information concerning the possible risk factors associated with the acquiring of the *Cryptosporidium* infection, in the present study we aimed to provide data regarding this approach.

2. Materials and methods

The study was conducted between 2006-2011, in twenty cattle farms located in western Romania. Fecal samples from 428 pre-weaned diarrheic calves (1-30 days old) were collected directly from the rectum. Individual animal and herd management data were recorded including age, gender, breed, herd size, farming system, housing system and feeding regime. The herds enrolled in the study were visited several times and, every time, all available diarrheic calf per farms was included. Fecal samples were assayed for antigens to *Cryptosporidium* using a commercially available BIO K 151 ELISA kit, according to the manufacturer recommendation.

Statistical analysis of the obtained data was carried out using SPSS (ver. 16).

3. Results

Overall, the prevalence of the *Cryptosporidium* infection was 37.4%. The percent of the *Cryptosporidium* positive calves according to the sampled herds (farms) ranged from 10.0 % to 75.0%. The percentage of animals shedding *Cryptosporidium* oocyst according to age groups was 35.1% (1-7 days), 49.3% (8-14 days), 32.7% (15-21 days) and 28.3% (22-30 days), respectively. The *Cryptosporidium* infection prevalence according to the gender was 40.3% in male and 34.3% in female calves. A 28.9% of crossbreed, 27.3% of purebred - meat and 48.4% of purebred – dairy

calves were found to be positive to *C. parvum* ELISA. The percent of *Cryptosporidium* positive calves according to the size of the sampled herds was 33.3% in herds of 90-200 cows, 46.9% in herds of 200-500 cows and 34.7% in herds of 500-800 cows. The percent of *Cryptosporidium* oocysts shedding calves in the industrial systems was 41.1% compared to 30.4% in the grazing system. The prevalence of *Cryptosporidium* was 42.9% in calves raised in individual box and 33.2% in calves housed in stable with 6-12 specimens. The infection prevalence was 38.6% in calves fed with individual cow milk and 35.9% in calves fed with milk replacer.

The binary logistic regression model showed that the 8 – 14 days age, purebred – dairy calves, individual box housing and industrial farming system were positively associated with the prevalence of *Cryptosporidium* infection in young calves.

4. Discussion

The relatively high recorded prevalence value (37.4%) of *Cryptosporidium* infection confirmed the role of these protozoa in the etiology of neonatal calf diarrhea and its negative impact on animal welfare.

Concerning other studies aimed to detect *Cryptosporidium* infection in young calves using non-molecular tools, a lower infection values have been recorded in Southern Ontario (USA; 30%; 5) and higher in the Netherlands (44%; 3).

Similar to our findings the association between prevalence of the *Cryptosporidium* infection and 8-14 days age category has been previously reported by Dărăbuş et al. (2001) in the same region. In addition, Kváč et al. (2006) clearly demonstrated that the second week of life can be considered a highest risk factor for *C. parvum* infection. Also, significantly higher infection level of *C. parvum* was observed in pre-weaned dairy calves comparing with pre-weaned beef calves in South Bohemia (4). In a study conducted by Duranti et al. (2008) in central Italy the housing of calves separately from their dams has been proved to be a risk factor, whereas calves being nursed by their dams seem to be as protective factor (2). The significantly higher rate of calves shedding *Cryptosporidium* oocysts in industrial farming system comparing with grazing system can be related by continuous introduction of new susceptible calves resulting on continuous transmission process (4). The findings of the current survey indicate that both individual animal as well as management procedures can be considered risk factors in the acquiring of *Cryptosporidium* infection.

Acknowledgements

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STUDY ON PREVALENCE AND ASSESEMENT COMMUNITY AWARENESS OF BOVINE TUBERCULOSIS IN AND AROUND MIZAN TEFFERI, SOUTHERN ETHIOPIA

K. Tadesse[1], A.Girma[1], Z. Dange[2], E. Sollomon[1]

[1] College of veterinary medicine, Meklle University, Mekelle, Ethiopia
[2] Mizan Teferi Veterinary Regional laboratory, Mizan Teferi, Ethiopaia

Introduction: Bovine tuberculosis is economically important infectious disease of cattle caused by *Mycobacterium bovis* and it is one of the primary public health problems in developing countries. The objective of this was to determine the prevalence of bovine tuberculosis and asses the public perception about the disease.

Methods: A cross sectional study was conducted from November 2013 to April 2014 at Mizan Tefferi and its surroundings to determine the prevalence of bovine tuberculosis and associated risk factors. Both questionnaire survey and comparative intradermal tuberculin test (CIDT) were used for this study. A total of 220 cross and 164 local breed cattle were tested using comparative intradermal tuberculin and a total of 52 households were interviewed using semi structured questionnaire.

Result: The overall prevalence of bovine tuberculosis was 2.3%. Risk factors like; Herd size ($\chi 2 = 8.833$, $P = 0.001$), body condition ($\chi 2 = 10.31$, $P = 0.007$), presence of coughing animal in the herd ($\chi 2 = 139.36$, $P = 0.0001$) and farming system ($\chi 2 = 16.264$, $P = 0.0001$) were found significantly associated with the occurrence of tuberculosis in dairy cattle. The current study also revealed that 30.8% of the respondents have heard about tuberculosis in general, however only 42.3 % of the respondent knew and 69.2% of the respondent did not know that BTB was transmitted to human from livestock. Raw milk was consumed by 44.2% of the respondents.

Conclusion: The results of this study indicates the importance of bovine tuberculosis in the study area and signify the importance of collaboration between the smallholder farmers, medical and veterinary professional to evaluate control the disease.

Keywords: Comparative Intradermal tuberculin test, Mizan Teferi, Prevalence, risk factors, Tuberculosis, public perception

NEOSPOROSIS IN COWS FROM LARGE BREEDING FARMS IN SLOVAKIA

K. Reiterová, S. Špilovská

Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic

Summary

The occurrence of anti-*Neospora* antibodies in cattle post abortion (PA Group n=1,486) and free of reproductive problems (Control Group n=503) from different regions of Slovakia was determined by competitive ELISA. The mean seropositivity of PA Group (13.2%) was significantly higher ($p<0.0001$) than that of Control (3.8%), demonstrating the casual dependence of abortions via neosporosis. In eight different regions *Neospora*-positivity ranged from 2.9% to 25.8%. Decreasing seropositivity rate according to the years suggests spreading of neosporosis from eastern to western parts of the country. On the farm with the highest seropositivity (48%) the risk of abortion in seropositive animals was 3.8 times higher when compared with seronegative ones. However, 28.1% of the heifers born to seronegative cows were also seropositive, indicating postnatal horizontal transmission. Our results warrant the attention and requirement of surveillance in the Slovak herds. The examination of heifers prior to their serving would help to notice and interrupt the transmission and subsequent persistence of *N. caninum* in stock-rising.

Introduction

Intracellular Apicomplexan parasite, *Neospora caninum*, with a worldwide distribution, may infect many species of warm blooded animals. The most common intermediate hosts of *N. caninum* include cattle, less frequently sheep, goats, horses and deer. Abortions and stillbirth due to neosporosis, especially in dairy cattle, has been reported worldwide (Dubey, 2003).

The aims of the work were to evaluate the occurrence of neosporosis in dairy cattle in Slovakia and to determine the influence of *N. caninum* on the occurrence of abortions and dam-daughter serology in offspring from selected farm.

Materials and Methods

In the period of 2009–2013, a total of 1,486 sera from dairy cows (*Bos taurus*) post abortion (PA Group) from different regions of Slovakia were randomly collected. From a south-east region 503 sera of cows free of reproductive problems (Control Group) were tested. Sera were provided by the State Veterinary and Food Institutes in Bratislava, Dolný Kubín, Košice and Prešov. The selected farm with the highest seropositivity consisted of 150 heifers and 340 cattle, out of them 117 animals had a history of abortion.

The presence of *Neospora*-antibodies in sera was detected by competitive ELISA (VMRD, Inc., U.S.A.) according to the manufacturer's instructions. Inhibition percentage (%I) was calculated as follows: $\%I = 100 - (OD_{Sample}/OD_{Mean\ Negative\ Control} \times 100)$. %I values < 30% were negative and %I ≥ 30% were positive. Results were evaluated by Fisher exact test. Logistic linear regression analysis at 95% of confidence level, as well as relative risk of abortions due to neosporosis and odds ratio was performed using STATISTICA 6 Base (StatSoft, Inc., 2001).

Results

The overall 13.2% mean seropositivity in the PA Group was significantly higher ($p<0.0001$; 95% CI = 11.51 – 15.02) in comparison with the Control (3.8%; 95% CI = 2.29 **–** 5.84 (Table 1). On the farms from eight different regions *Neospora*-seropositivity varied between 2.9% **–** 25.8%. As much as 90.6% of seropositive dams had a positive history of abortion, while only five (9.4 %) did not abort previously. Thus, the relative risk of abortion was 3.8 higher in seropositive cows then in animals without antibodies to *N. caninum*. Similarly, a very high OR (30.3) suggests a very strong association between seropositivity and occurrence of abortions. The prevalence of neosporosis among heifers born to *N. caninum* seropositive cows (61.7%) was significantly higher ($P = 0.005$) than that in heifers born to seronegative mothers (28.1%).

Discussion

The high *Neospora*-seropositivity in cows remains mainly in the countries with an intensive farming (Dubey, 1996). Substantial differences have been detected in *N. caninum* seroprevalence in dairy cattle in Sweden (16%), Germany (49%), Spain (63%) and Netherlands (76%) (Barltes et al., 2006). In Hungary 10%; in Poland 15.6% and in the Czech Republic 3.9% of *Neospora*-seropositivity was detected in cows post abortion (Hornok et al, 1998; Cabaj et al., 2000; Václavek et al., 2003). These data signalise the occurrence of this infectious agent in surrounding countries a longer time ago.

The decreasing seropositivity rate according to the years suggests the potential spread of neosporosis from eastern to western parts of the country. This divergence may be caused by the various rates of environmental contamination that may play a key role during the grazing season.

Other important factor influencing the spread of neosporosis in herds is the breeding mode (Dubey et al., 2007). Our country, considering climate and geography, is a suitable model area for the understanding of relations and influences between the sylvatic and domestic cycles of *N. caninum*. With regards to the high number of pasture-bred cows on majority of the Slovak farms, the exogenous transmission may be regarded as the main source of the infection. On the other side, the vertical transmission within one farm is considered the main source of the infection. Our inspecting study on the farm with the highest seropositivity (48%) in north-eastern region revealed significant correlation between the presence of specific antibodies and the occurrence of abortions. The risk of abortion in seropositive animals was 3.8 times higher than in seronegative ones. However, 28.1% of heifers born to seronegative cows were seropositive, indicating postnatal horizontal transmission.

Conclusions

Our results suggest several points for the control. The examination of heifers prior to their mating would help to detect and interrupt the transmission and persistence of *N. caninum* in stock-raising. Improper farm management may contribute to spread and circulation of neosporosis in dairy herd, thus significantly damaging the reproduction and worsening economic indicators of breeding. The high seroprevalence rates in cows warrant the attention and supporting a need for surveillance in the Slovak herds.

Aknowledgement

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Table 1. Occurrence of anti-*Neospora* antibodies in cattle post abortion (PA) from different regions of Slovakia

Regions	2009		2011		2012		2013		Total	
			N/n	%	N/n	%	N/n	%	N/n/%	95% CI
Žilina					139/12	8,6	145/14	10,5	284/26/9.2	6.07–13.13
Trnava			136/4	2,9					136/4/2,9	0.81–7.36
Nitra			162/12	7,4					162/12/7.4	3.89–12.58
Bratislava			47/2	4,2					47/2/4.2	0.52–14.54
Trenčín					96/5	5,2	5/0	0	101/5/4.9	1.63–11.18
Banská Bystrica							28/2	12,5	28/2/7.1	0.88–23.50
Prešov	305/80	26,2					9/1	0	314/81/25.8	21.05–31.01
Košice	411/64	15,6					3/0	0	414/64/15.5	12,11–19.31
Total	**716/144**	**20,1**	**345/18**	**5,1**	**235/17**	**7,2**	**190/17**	**8,4**	**1486/196/13,2**	**11.51–15.02**

N – number of examined; n – number of positive; % - seropositivity

Table 2. Occurrence of anti-*Neospora* antibodies in Control Group of cows without reproduction problem from different regions of Slovakia

Districts	No. of examined	*Neospora caninum*	
		Positive/%	95% CI*
Košice- surroundings	50	1/2.0	0.05 – 10.65
Michalovce	47	1/2.1	0.05 – 11.29
Spišská Nová Ves	166	7/4.2	1.71 – 8.50
Rožňava	60	1/1.7	0.04 – 8.94
Trebišov	160	9/5.6	2.60 – 10.41
Rimavská Sobota	20	0/0	0.00 – 13.91
Total	**503**	**19/3.8**	**2.29 – 5.84**

*Confidence Interval

PATHOLOGICAL STUDIES ON THE EFFECT OF SODIUM LAURYL SULFATE ON SKIN OF RABBITS

Mohamed Kotob[1,2], Mahmoud A. Z.[1] and Allam Nafady[1]

[1] Department of pathology, Faculty of veterinary medicine, Assiut University, Assiut, Egypt
[2] Clinical Division of Fish Medicine, Department for Farm Animals & Veterinary Public Health, University of Veterinary Medicine Vienna, Austria
Email address of corresponding author: mohammedkotob@yahoo.com

SUMMARY

Sodium lauryl sulfate (SLS), is a detergent and a cleansing agent used by all population**,** Most of data reporting the damaging effects of SLS after cutaneous skin application. So this study was designed to confirm the toxic and damaging effect of Sodium lauryl sulfate on skin of rabbits. Twelve adult domestic rabbits were divided into two groups as treated group and control group. Treated group painted with sodium lauryl sulfate in a concentration of 7.5 % twice weekly for 6 months by means of a gentle brushing. The results were a wide area of alopecia, with thickening and wrinkling of the skin. Ulcerations and scabs with crust formation appeared in alopecic areas and leukocytosis. Morphometric studies revealed increased in the thickness of epidermis, dermis and keratinized layer of skin. Histopathological examination revealed hyperkeratosis, acanthosis with elongated rete ridges, necrosis with leucocytic cell infiltrations, sever dermatitis, necrotic folliculitis and perifolliculitis with destruction of the hair and hair follicles leads to alopecia and scales formation after SLS application as compared with control group. It was concluded that Sodium lauryl sulfate is a skin irritant, induce skin lesions and irritant contact dermatitis when applied to skin so strict control of its practical uses should be adopted.

Keywords: Sodium Lauryl Sulfate, Skin Lesions, Irritant Contact Dermatitis, Rabbits.

INTRODUCTION

Millions of populations all over the world (males and females) use cosmetics and other personal care products as soap and shampoo to improve their appearance. This fact give rise to question does the ingredients of this product is safe or not (1). Sodium lauryl sulfate (SLS), is one of these cosmetics and used as a detergent, a cleansing agent and found in a commercial soaps, shampoos and large number of personal Care products due to the fact that it is cheap and small amount generates a large amount of foam**,** Most of data reporting the damaging effects of SLS after cutaneous skin application where the results of these studies were wide skin areas of alopecia, skin erosions and crusts and this strongly indicative of SLS-induced eczema and contact dermatitis (2,3). Other study reported the histopathological changes after skin exposure to 7.5% SLS and the results were complete absence of the epidermis, necrosis involved the epidermis and papillary layer of dermis in some groups but other showed hyperkeratosis, acanthosis, with crust formation and dermis infiltrated with inflammatory cells(4). So the aim of this work was to study the toxic and damaging effects of SLS on Skin.

MATERIAL AND METHODS

12 white New Zealand rabbits (2 months old) ; divided into two groups:

-Group 1 (6 rabbits) painted with Sodium lauryl sulfate (was purchased from SIGMA-ALDRICH CO.,GERMANY) in a conc. of 7.5 % as found in shampoo, twice weekly for 6 months by mean of a gentle brushing.

-Group 2 (rabbits) used as a control.

During the Experiment rabbits were observed for skin lesions and clinical signs, after 6 months all rabbits were slaughtered and Skin samples were fixed in 10% neutral buffered formalin for histopathological examination using H&E stain. Anticoagulated blood samples were taken for determination of total leucocytic count and Differential leucocytic count. Sections of skin from all groups were measured for morphometrical and image analysis. And the data were statistically analyzed and expressed as the mean ± SE. by using one way analysis of variances (ANOVA).

RESULTS

-Rabbits treated with SLS developed clinical signs and gross lesions in about 100% of exposed rabbits, these gross lesions in the form of wide area of alopecia, thickening and scaling of the skin, with small few ulcers (Fig.2, 3, 4), sever hyperaemia, prominent dilatation of the subcutaneous blood vessels (Fig. 5) and decrease body weight, lying down in the corners and alone (Fig.6) as compared with control group (Fig.1).

-Histopathological results of examined skin sections showed acanthosis and hyperkeratosis with elongated rete ridges (Fig.8), thick layer of crusts covered massive area over the hyper keratinized epidermis (Fig.9), superficial area of necrosis with leucocytic cell infiltrations (Fig.10). Other showed hyaline degeneration and accumulated necrotic tissues in the hair follicles with necrotic folliculitis with accumulation of neutrophils, necrotic tissues and destroyed hair in the hair follicles (Fig.11 A,B,C) as compared with skin section from control rabbits (Fig.7,12).

-Total leucocytic count was increased in treated group (9962±199.35) as compared to control (7825±270.72) with a mild increase in both neutrophils and eosinophils in treated group.

-Morphometrical and image analysis results showed highly significant increase ($p>0.05$) in the thickness of epidermis, dermis and keratinized layer in SLS when compared with control group as shown in (table 1).

DISCUSSION

SLS induced cutaneous gross lesions in 100% of exposed rabbits. The gross lesions include wide areas of alopecia, thickening and wrinkling of the skin together with roughening and scaling of the area at the site of application of SLS. Congestion of the dermal blood vessels was also reported in slaughtered cases. Such lesions developed between 2-5 months of application of SLS. Toward the end of the experiment, ulceration and scab formation involved most of alopecic area. The skin of slaughtered animals at the end of the experimental period was thick, heavy and the blood vessels were prominently congested. Such result was in agreement with those reported in previous studies (2, 3, 4) Skin lesions indicate that SLS is skin irritant and strongly indicative of SLS to induce eczema and dermatitis.

Morphometric studies revealed that the thickness of layers of the skin include dermis, epidermis, keratinized layer was highly significant increased in SLS treated group when compared to control that agreed with results in (4).

CONCLUSIONS

1-Application of sodium lauryl sulfate on the skin of white new-Zealand rabbits was associated with contact dermatitis characterized by hyperkeratosis, acanthosis, folliculitis and perifolliculitis with destruction of the hair and hair follicles leads to alopecia and scales formation.

2-We recommend that the use of shampoo and soaps containing sodium lauryl sulfate is to be discouraged, so strict control of its practical uses should be adopted.

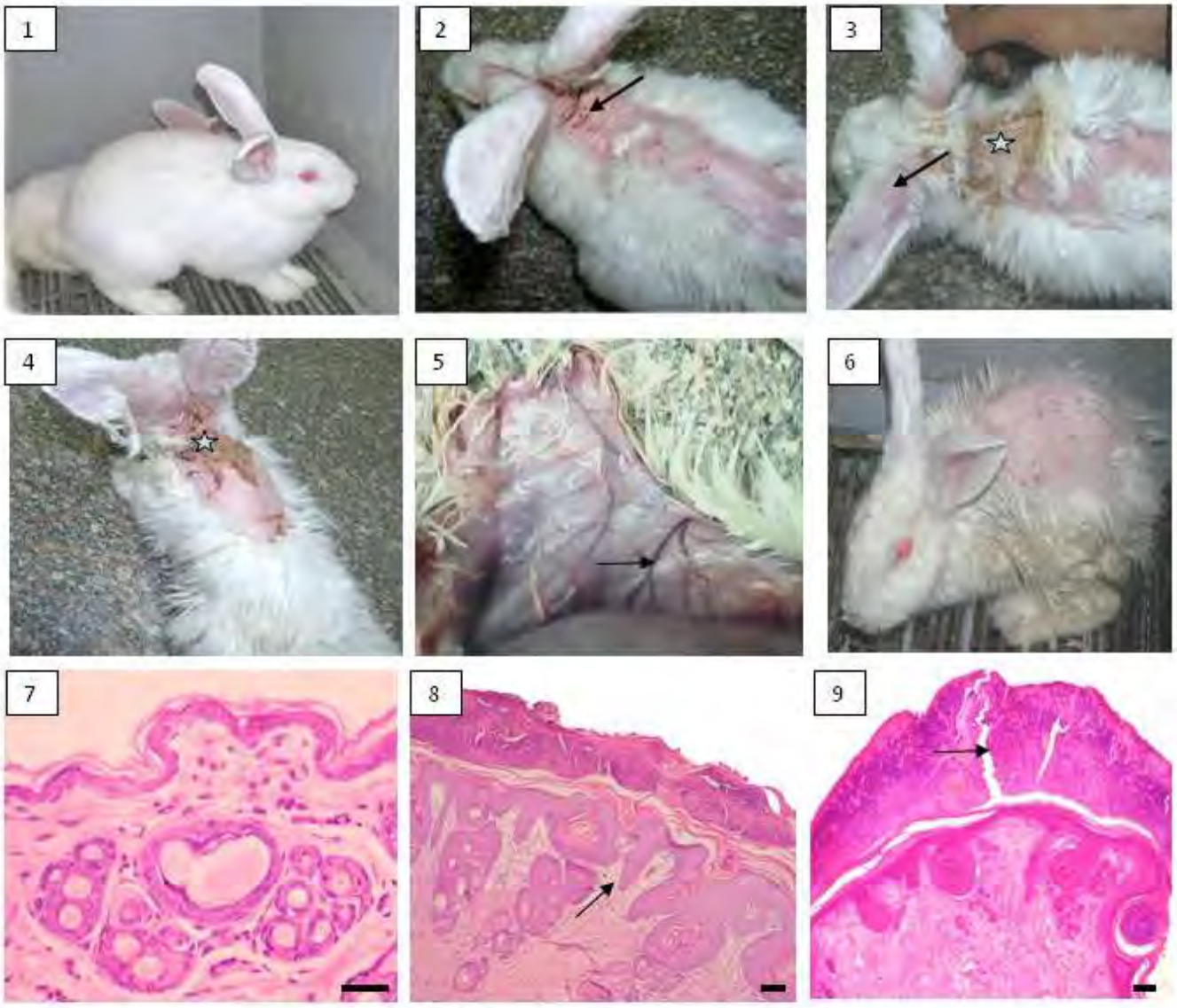
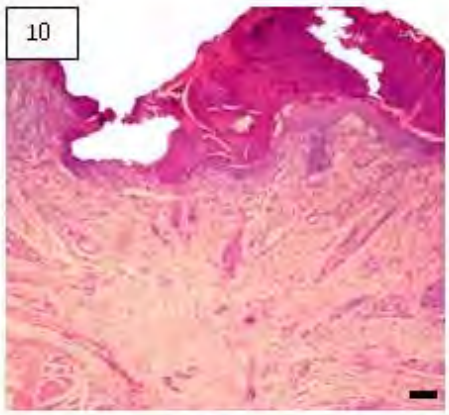
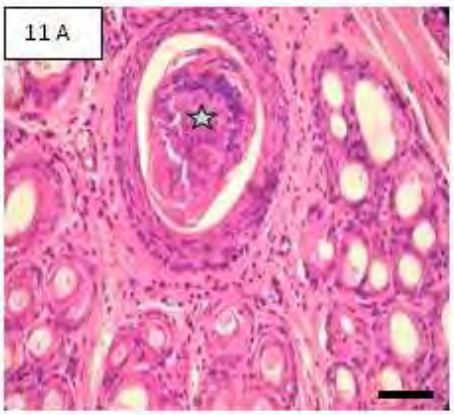
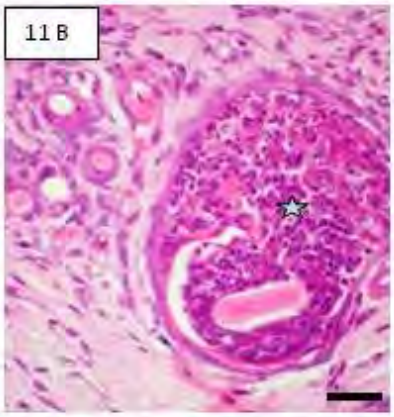
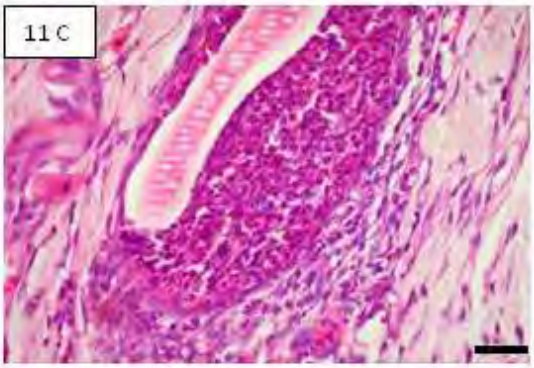


Fig.1: control rabbits showing signs of white healthy hair and body.
Fig.2: SLS group showing wide area of alopecia, thickening and scaling of the skin, note small few ulcers (arrow).
Fig.3: SLS group showing wide area of alopecia involve ears (arrow), with sever crust formation (star).
Fig.4: SLS group showing sever ulceration of skin with formation of prominent scales (star).
Fig.5: SLS group showing sever hyperaemia and prominent dilatation of the subcutaneous blood vessels (arrow).
Fig.6: SLS group showing wide area of alopecia, decrease body weight, lying down in the corners and alone.
Fig.7: Skin section from control group .H&E stain. Bar = 50µm.
Fig.8: Skin section from SLS group showing acanthosis and hyperkeratosis with elongated rete ridges (arrow). H&E stain. Bar = 100µm.
Fig.9: SLS group showing thick layer of crusts covered massive area over the hyper keratinized epidermis (arrow). H&E stain. Bar = 100µm.









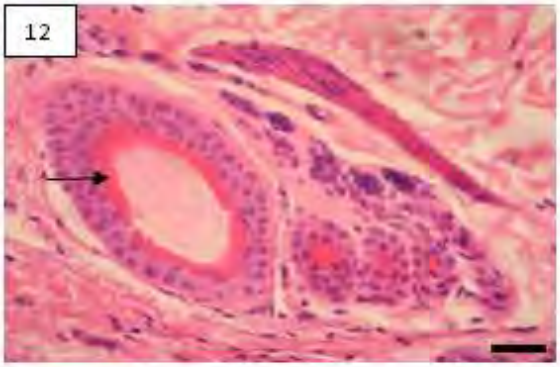


Fig.10: Skin section from SLS group showing erosions and ulceration with presence of thick necrotic masses on the skin. H&E stain. Bar = 100µm.
Fig.11: A, B Skin section from SLS group showing hyaline degeneration, accumulated necrotic tissues and inflammatory cells in the hair follicles (necrotic folliculitis) (star). C, same group showing perifolliclitis with accumulation of neutrophils, necrotic tissues in the hair follicles and the surrounding area. H&E stain. Bar = 50µm.
Fig.12: Skin section from control group showing normal hair follicle (arrow). Bar = 50µm.

Table (1): mean values of the thickness of epidermis, dermis and keratinized layer of skin in different groups (µm):

	SLS	control
Epidermis	94.80±	13.68±0.59 [b]
Dermis	1290.89±	717.61±39.47 [b]
Keratinized layer	58.28±	10.83±0.52 [b]

[a and b] means in the same row differ at ($p<0.05$).

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BACTERIAL MICROFLORA OF SKIN WOUNDS AND ABSCESSES ASSOCIATED WITH PNEUMONIA IN CAMELS

J. T. Bebawy[1], A. S. I. Abo-Zeid[2], A. H. Elyas[1] and A. A. Mottelib[3]

[1]Animal Health Res. Ist. Assiut, Egypt
[2]Sci. Office Manger, El-Nasr Chem. And Med. Com. Upper Egypt
[3]Dept. Anim. Med. Fat of Vet. Med., Assuit Univ. Assiut, Egypt

SUMMARY

A total of 60 camels showing clinical respiratory signs with skin wounds and abscesses were included. 65 samples were collected aseptically from various skin wounds and abscesses. Also, 39 samples were collected from nose, lung abscesses and lesions of dead and slaughtered cases. Samples were processed for isolation and identification of different aerobic bacterial agents based on primary and secondary tests using standard procedures. 127 bacterial isolates were identified. They constituted 8 genera with 62.2% gram positive and 37.8% gram negative. Staph aureus, non-coagulative staph and coryn pyogenes were dominants with frequencies of 17.5%, 16.5% and 15.8% respectively. Bacterial isolates were subjected to in-vitro sensitivity to antibiotics. In-vitro sensitivity tests against all tested isolates showed that Gentamycin was the drug of choice.

INTRODUCTION

Camel is frugal in habitual, but the use of unsuitable saddles, walking or recumbancy on harsh lands containing wires, broken glass, plant thorns, sharp tree roots, sharp pointed stones and others will cause skin lesions (4).

Various lower respiratory diseases has been reported in camels, but defentive etiology of most them is still not Determined. Pulmonary diseases are among emergency problems of camel diseases that causing death. Disease causes considerable losses in production and death.

Objectives of the present work was planned to record skin wounds and abscesses in camels showing clinical signs of pneumonia. Also, the work included isolation and identification of the causative pathogens and their in-vitro sensitivity to some antibiotics.

MATERIALS AND METHODS

60 camels were suffering from pneumonia with presence of wounds and abscesses on various parts of skin. 65 samples were collected from wounds and abscesses present on skin, Shoulder, Chest and abdomen (20), humps (20) and foot-pads (25). 39 samples were collected from respiratory system as nasal swabs from living diseased cases (20), lung abscess and tissues from dead (10) and slaughtered cases(9).

Aseptically collected samples were processed for isolation and identification of different aerobic bacterial pathogens based on primary and secondary tests characteristics standard prodcedures (5). All bacterial species were tested by using different antimicrobial discs supplied by Medical company diagnostic, Amman, Jordan.

RESULTS

Most of diseased camels showed elevated body temperature (range 38.5–40.2°c), hurried respiration (range 22–38/min) and increase pluse (45–75/min). Animals showed nasal discharges varied from mucoid to mucopurulent with distinct respiratory distress. Animals were emaciated, weak with decreased appetitie. Varieties of lacerated wounds, open and closed abscesses were observed on skin of head, neck, chest, abdomen, hump and foot-pads. 127 aerobic bacterial isolates were identified from all samples (104). Isolates constituted 8 genera, with 79 isolates (62.2%) gram positive and 48 isolates (37.8%) gram negative.

Details of bacterial isolates and their in-vitro sensitivity tests to antibiotics shown in tables 1 and 2.

DISCUSSION

The skin wounds at humps of diseased camels were due to loose or misfit saddles. Mostly, the wounds at the nostrils were of lacerated type and caused by nose-page. Wounds and abscesses of head, neck, chest abdomen were due to fights and bitting between animals, or contaminated physical injuries (4).

Respiratory tract infection in camels are caused by several agents among which aerobic bacteria is the most common. The reported clinical signs of pneumonia in the diseased camels were due to inflammation of the anterior respiratory passages that extends posteriorly to involve the bronchi and lungs. Haematogenous route of lung infection from infected skin wounds and abcesses may be considered. Staph aureus and non coagulative staph, constituted the highest number and per cent 43 (34%) as compare with other genera and species. Our findings were in agreement with (5). Corynbacterium pyogenes constitiuted the second highest gram positive isolates 20 (15.8%) among the eight bacterial genera and species. The organisms are also considered to be the most important agents that produce suppuration of skin affections and lungs. The reported microbial agents are present in the surrounding environment and the spread of infection within camel herds takes place either by direct contact between the infected and unifected animals or by mechanical transmission as flies (1). Saddle change from animal to another may cause spread of infection among the hard (4).

Streptococcus species constituted the lowest number and per cent 7 (5.6%) among the isolates. Strept. Spp. isolated from skin wounds and abscesses are considered as environmental contaminant (6), and those of lung lesions are principally living as commensals in m. m. of anterior respiratory passages. Diplococcus pneumoniae were not isolated from skin lesions, but found only in nostrils and lung lesions, 9 (7.3%).

Forty eight isolates are identified as members of family Enterobacteriaceae. Strains of E.Coli and proteus spp. are associated with skin wounds and abscesses as well as nostrils and lung lesions. Similar data of gram negative bacilli confirmed the presence of the organisms as anterior respiratory tract flora that may invade the lungs when the body immunity declines (2). The present work confirmed that the infective organisms can gain accesses the blood stream from any septic foci as wounds and abscesses in skin to the internal organs as lungs.

The antibiogram against the various isolates showed great variations (table, 2). Collectively 32 (40.5%), 40 (50.6%), 45 (56.9%) and 66 (83.5%) of the gram positive isolates were sensitive to penicillin, Kanamycin, Ampicillin and Gentamycin respectively. Also, 27 (56.2%), 29 (60.4%) and 40 (91.7%) of the isolated gram negative organisms were sensitive to streptomycin, Kanamycin and Gentamycin respectively.

CONCLUSIONS

For controlling respiratory affection of camels, due attention in alleviating stresses during different managemental practices including work, transportation, feeding, watering, crowdening etc. The antibiogram revealed that Gentamycin is the drug of choice beside the topical treatment of skin wounds and abscesses.

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Bacterial isolates	Skin wounds and abscesses (20)	Hump abscesses (20)	Foot pad lesions (25)	Nasal swabs (20)	Lung tissues and abscesses		Total isolates No.	%
					Dead (10)	Slaughtered (9)		
Staph aureus	4	2	2	5	5	4	22	17.5
Staph Non-coagulative	1	2	2	7	5	4	21	16.5
Strept spp	1	2	2	0	1	1	7	5.6
Diplococcus pneumonia	0	0	0	3	3	3	9	7.3
Cory pyogenes	2	3	1	4	6	4	20	15.8
E. Coli	6	4	3	3	2	1	19	14.7
Proteus spp.	4	5	2	2	1	1	15	11.6
Klepsiells spp.	0	0	1	3	5	5	14	11.0
Total (127)	18	18	13	27	28	23	127	100

Table (1): Aerobic bacterial isolates of various skin and respiratory lesions in camels

Bacteria spp. / Antibiotics		E.Coli (19)	Proteus spp. (15)	Klepsiell spp. (14)	Staph. Aureus (22)	Non-Coagulative staph (21)	C.Pyogenes (20)	Dipiococcus pheumoniae (9)	Strept spp. (7)
Ampicillin (25ug)	**S**	2	6	4	14	10	11	5	5
	%	10.5	40.0	28.5	63.6	47.5	55.0	55.0	71.4
Penicillin (10ug)	**S**	4	1	3	10	9	10	2	1
	%	21.0	6.1	21.4	42.4	42.7	50.0	22.2	14.2
Streptomycein (10ug)	**S**	16	5	6	8	7	5	1	1
	%	84.2	33.3	42.8	36.3	33.3	25.0	11.1	14.2
Chlorampheni. col (30ug)	**S**	2	2	5	6	4	3	1	1
	%	10.5	13.3	33.7	27.2	19.0	15.0	11.1	14.2
Gentamycin (10ug)	**S**	17	15	12	20	10	20	9	7
	%	89.5	100	85.7	90.9	47.6	100	100	100
Kanamycin (10ug)	**S**	10	11	8	18	6	10	4	2
	%	52.6	73.3	57.1	81.8	28.5	50.0	44.4	25.5

S = Sentitive, % = Per Cent

Table (2): The antibiotic sensitivity tests by the disc diffusion method

NYLON WASTE: A THREAT TO SEMI-INTENSIVE SMALL RUMINANT PRODUCTION

R. A. M. Adedokun[2], A. T. Jagun[1], L. O. Raji[3*], A. A. Adekola[1]

[1]*Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria*
[2]*Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria*
[3]*Department of Theriogenology and Production, University of Ilorin, Ilorin, Nigeria*

Summary

The increased usage of nylon in local and industrial food packaging and the poor waste management involved in its disposal has led to accumulation of nylon in the environment in Nigeria. Small ruminants are naturally scavengers, and are mainly reared by semi-intensive system of production in Nigeria. These animals are at high risk of the deleterious effect of poorly managed nylon wastes as they pick up these nylon wastes along with residues of food materials. This often results in serious cases of rumen impaction with its attendant health complications on livestock production. The content of the rumen of small ruminants (200) slaughtered at the Bodija Municipal Abattoir in Oyo State were collected and weighed at postmortem. The nylon content in the content rumen was also weighed along with the weight of the animals. The occurrence and ratio of nylon content to ruminant content was calculated. The species and sex differences were observed. There was 92% occurrence of small ruminant with nylon present in the ruminal content. 34% of these affected small ruminant had loose ruminal nylon contents while 66% had nylon contents that had adhered together to form a foreign body mass in the rumen with a significant body mass to ruminal foreign body ratio. This paper highlights the cases of rumen impaction caused by chronic accumulation of nylon waste in the rumen at post mortem. There is an urgent need for a multidisciplinary approach to correct this environmental pollution and poor waste management issue. The resultant effect could lead to increase in rumen impaction in small ruminant production and subsequent health effect.

Keywords: Nigeria, Nylon, Small ruminants, Rumen impaction, Waste management.

Introduction

Globally, there is a growing concern about waste management and its environmental impact. This has major influence on livestock production (Rotich *et al*., 2006). In Nigeria, most food products are packaged with nylon materials which are usually poorly disposed after use. This pre-disposes small ruminant scavengers commonly reared under semi-intensive system to the risk of consumption of these nylon wastes (Umunna *et al*., 2014). This exposes these animals to serious health risks. This paper highlights some cases of ruminal impaction caused by chronic accumulation of nylon waste in the rumen observed at post mortem.

Materials and Methods

The content of the rumen of two hundred small ruminants slaughtered at the Bodija Municipal Abattoir in Ibadan, Nigeria were collected, weighed and examined at post mortem. The nylon content in the content rumen was also weighed using a weighing balance along with the weight of the animals. The occurrence and ratio of nylon content to ruminant content was calculated. The species and sex differences were observed.

Results

There was 92% occurrence of small ruminant with nylon present in the ruminal content. 34% of these had loose ruminal nylon contents while 66% had nylon contents that had adhered to the ruminal content to form a foreign body mass in the rumen with a significant body mass to ruminal foreign body ratio of 5:1. 58% of these animals were goats while 52% were sheep; 62% were males while 38% were female animals. The were evidences of rumenitis, haemorrhage, and presence of foreign body in the rumen on gross examination which was mainly characterized by nylon materials and impaction. Gross lesions that were observed includes congestion, haemorrhages, thickening of the wall, erosion, ulceration and scar formation in the rumen. There were also congestion of the entire gastro-intestinal tract and sloughing off of the mucosa. Histo-pathological lesions include hydropic degeneration, cellular vacuolation, submucosal oedema and disruption of stratified epithelium. Focal hyperplasia of the ruminal epithelium in different regions.

Discussion

This study highlighted the cases of rumen impaction in small ruminants caused by chronic accumulation of nylon waste in the rumen at post mortem. The predisposing factors to the occurrence of these cases have been attributed largely to the semi-intensive husbandry system of livestock production; poor waste disposal and management practice; the scavenger nature of these small ruminants and nutritional deficiency syndrome resulting in pica or allotrophagia (Olafadehan *et al.,* 2010). At post mortem, there were accumulations of nylon waste in the rumen. In most of the cases, the accumulated nylons were adhered to the ruminal content forming large indigestible masses in the rumen resulting in ruminal impaction. Rumen impaction also causes loss of defecation, anorexia, depression and in chronic severe cases, death. resulting reduction in livestock production (Vanitha *et al*., 2010). Unfortunately, the prevailing Nigerian environment promotes these predisposing factors to ruminal impaction in small ruminants. Most farmers cannot cope with the cost implication of the nutritional requirements of these small ruminants under the intensive system. Also, most eateries package their products with nylon materials. Consumers of these products often poorly dispose these packaging materials after use. This results in accumulations of nylon waste materials often with food residues in the premises and refuse dumps. While grazing, these small ruminants ingest these food residues along with the accumulated nylon packages. Nutritional deficiency associated disease conditions like pica or allotrophagia also increases the scavengery feeding manner in these animals. The gross lesions and histopathologic lesions observed in this study were similar to earlier reports (Hailat *et al.,* 1998).

Conclusion

In conclusion, we recommend that government should put in place better waste disposal and management system. Polices on proper compliance with waste disposal should be formulated and enforced. Nylon and other synthetic packaging polymers can be recycled to prevent them from accumulating in the environment. Proper feed supplementation for livestock should also be provided.

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Waste management / Biogas production

DO RESIDUES FROM BIOGAS PLANTS POSE A BACTERIAL THREAT?

J. Pospiech, M. Ullrich, U. Truyen, S. Speck

Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Leipzig, Germany

Introduction: In Germany, biogas plants (BGP) play an important role in providing sustainable energy. Various substrates including manure and renewable primary products (RPP) are used to produce biogas. Their digested residues can be used as soil fertiliser. The role of BGP in the multiplication and spread of zoonotic agents is a controversial issue. We therefore investigated the occurrence of selected bacteria (enterococci, *E. coli*, ESBL-producing *Enterobacteriaceae*, *Salmonella*, *Clostridium perfringens*, *C. botulinum*) in digested residues.

Materials and Methods: Ten BGP (Saxony, Germany) volunteered for this study. They mainly apply pig slurry, cattle manure and RPP for biogas production at mesophilic temperature. Samples were taken from several sites of the facilities. Bacteria were grown and enumerated on selective media by the spread-plate-method. In addition, pre-enriched samples were investigated for *C. botulinum* by PCR. Furthermore, *C. perfringens* toxin types were determined by PCR.

Results: With the exception of *C. botulinum* and *Salmonella*, all other bacteria were detected. The initial substrates revealed 10^7-10^8 cfu/g aerobic bacteria. After anaerobic digestion (AD) the numbers decreased. *E. coli* was reduced by a factor of $\leq 10^4$. Enterococci were less affected with an average reduction of 10^1. There was no significant impact on *C. perfringens* numbers. The main toxin type detected in *C. perfringens* was type A but type C and D were also found. ESBL-producing bacteria, mainly *Escherichia* and *Klebsiella*, were found in samples from 6 of the 10 participating BGP. Their maximum reduction was 4 $\log_{10}$.

Conclusions: In our study, mesophilic AD achieved an upmost 4 $\log_{10}$ reduction indicating a 99.99% killing rate of aerobic bacteria. Hence, this technology helps to sanitise initial substrates and lowers the risk of pathogen dissemination.

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INFLUENCE OF THE THERMOPHILIC BIOGAS-PROCESS ON DIFFERENT PATHOGENS

T. Schilling, N. Hartmann, W. Philipp, L. E. Hoelzle

Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

Summary
When used in biogas-plants animal byproducts and biowaste should be pretreated before usage or the biogas-plant should be run at temperatures above 53 °C to avoid the risk of spreading pathogens and antibiotic resistant bacteria. Longtime storage is only an option if the storage times extend four month.

1. Introduction

Manure as well as other input materials for biogas plants can contain several pathogenic organisms that are associated with human and animal diseases including salmonella, mycobacteria or Coxiella burnetii and also viruses. Especially, ASF spreading across Europe and recurrence of bovine tuberculosis has attracted public attention to biogas fermentation. In addition, potential spreading of antibiotic resistances or resistant bacteria (MRSA, ESBL) due to biogas plants is controversially discussed.

2. Materials and Methods

In the present study, inactivation of MRSA, ESBL, Salmonella Typhimurium and Listeria monocytogenes using thermophilic treatment in four 3 liter laboratory fermenters (two operating at an average temperature of 51.4 °C and two at 57.4 °C) was examined. As substrates cattle slurry and biowaste mixed with maize silage were used. The fermenters were artificially contaminated with the aforementioned pathogens and samples were taken after 0, 4, 8, 18 and 24 hours.

For the examination of different storage conditions substrates from five different mesophilic biogas plants were used. Substrate A contained 40% pig slurry, 30% cattle slurry and 30% renewable resources. Substrate B 60% pig slurry and 40% renewable resources. Substrate C 60% cattle slurry, 40% horse, cattle and poultry manure mixed with renewable resources. Substrate D 60% poultry slurry and 40% poultry manure with renewable resources. Substrate E 60% cattle slurry and 40% cattle and horse manure with renewable resources. These substrates were inoculated with the aforementioned pathogens and were stored in four refrigerators at longtime monthly mean temperatures for six months and the reduction was examined every month. Four refrigerators were used to simulate the four seasons.

3. Results

All samples taken before contaminating the fermenters were negative for all pathogens used during the experiment. During the fermentation experiment two fermenters were run at an average temperature of 51.4 °C. The results within these fermenters showed a reduction of 4 to 5 log10 steps for ESBL after 18 hours and 5 to 6 log 10 steps after 24 hours. Salmonella were detectable as well after 18 and 24 hours and quantification showed that there was a reduction of 5 to 6 log10 steps during that time. For MRSA and Listeria monocytogenes the experiment showed that both were detectable after four and five hours but no longer. Whereas running the fermenters above 56 °C showed that no pathogens were detectable after 18 hours.

During the storage experiment the monthly results indicate that the reduction rate of pathogens in digested residues is very low and that there is an influence of the substrate on the reduction.

By comparing the results of substrates B and C to the substrates A, D and E a difference in the reduction after 5 month of 2 to 3 log 10 steps was observed.

4. Discussion

The results of the fermentation experiments showed that biogas plants run at temperatures below 53 °C and with an actual retention time less than 18 hours are not capable of reducing pathogens properly and the hygienic effect on the substrates is insufficient. In addition, ESBL and salmonella were not inactivated completely. So, the risk of spreading antibiotic resistant pathogens to the environment is still given after treatment in a biogas-plant.

The results of the storage experiments show that a hygienic effect by storing digested residues can only be achieved by storage times longer than four month.

5. Conclusions

Taking the results of the fermentation and the storage experiments, biogas-plants should be run at temperatures higher than 53 °C and the actual retention time of at least 18 hours or all input materials should be pre-treated (e.g. pasteurization) before used in the biogas plant to avoid the risk of spreading pathogens and resistant bacteria when bringing the digested residue to the field.

POSTERS

Animal hygiene, preventive veterinary medicine and herd health

ANTIMICROBIAL SUSCEPTIBILITY TESTING FOR COAGULASE POSITIVE STAPHYLOCOCCI ISOLATED FROM BOVINE MILK IN SMALL DAIRY FARMS IN BRAZIL

S. B. Lucheis[1,2], F. A. de Lemos[1]

[1]*Department of Veterinary Hygiene and Public Health, São Paulo State University, Botucatu, Brazil*
[2]*Paulista Agency of Agribusiness Technology – APTA, Bauru, Brazil*

SUMMARY

This study aimed to isolate and identify coagulase positive staphylococci (CPS) species isolated from bovine milk in seven small dairy farms in Brazil and establish the antimicrobial profile using ten commercially available antibiotic disks by Kirby-Bauer disk diffusion method. Identification of *Staphylococcus* spp. was performed using conventional microbiology.

INTRODUCTION

Chronic mastitis cases irresponsible to treatment are increasingly in dairy farms. The treatment of bovine mastitis is complicated by the overuse of antibiotics, and the generation of drug-resistent-bacteria. Many antibiotic treatments for mastitis are often not justified with a cost/benefit analysis.

Bovine mastitis is the disease that causes most economic damage to the dairy industry worldwide and also poses a potential health risk for consumers [1]. *Staphylococcus aureus* is one of the main microorganisms isolated from intramammary infections in dairy cows, and cases are usually subclinical and difficult to treat [2]. The indiscriminate use of antimicrobials to combat mastitis has led to the selection of resistant strains of *Staphylococcus* spp., undermining the efficacy of treatment [3].

MATERIAL AND METHODS

Thirty-eight (38) *Staphylococcus aureus* isolates were recovered from 102 milk samples, positive for the California Mastitis Test- CMT [4], from seven different small dairy farms in Brazil. The CMT-positive samples were plated on blood agar plates (Oxoid Brasil Ltda, Sao Paulo, Brazil) and incubated under aerobic conditions at 37°C, and readings were performed after 24, 48, and 72 h of incubation. Identification of *Staphylococcus aureus* was based on colony morphology, Gram staining, and catalase, coagulase and DNase activities. Differentiation from other coagulase positive staphylococci was performed by sugar fermentation tests maltose, trehalose, mannitol, polymyxin B resistance (300UI) and beta-galactosidase [5].

Antimicrobial susceptibility testing to vancomycin, cephalexin, cotrimoxazole, oxacillin, enrofloxacin, gentamicin, neomycin, tetracycline, penicillin and ampicillin was performed by the disk-diffusion agar method in accordance with the Clinical and Laboratory Standards Institute recommendations [6].

RESULTS

Of the 102 milk samples, 38 (37.2%) was CPS. Thirty-three (33) samples was identified as *Staphylococcus aureus (S. aureus)* (86.8%), four (4) samples as *Staphylococcus intermedius (S. intermedius)* (10.5%) and one single sample as *Staphylococcus hyicus (S. hyicus)* (2.6%). The distribution of CPS was different among herds: *S. aureus* was found in four herds, *S. intermedius* in two herds and *S. hyicus* was found in just one herd. The percentage susceptibility of *S. aureus* towards vancomycin was 100% while that for cephalexin was 97%; cotrimoxazole was 93.9%; oxacillin 90.9%; enrofloxacin and gentamicin were 87.8%; neomycin 81.8% and tetracycline was 69.7%. The percentage resistance of *S. aureus* was 93.9% for penicillin and ampicillin. For *S. intermedius*, the percentage susceptibility was 100% for vancomycin, enrofloxacyn, cephalexin, cotrimoxazole and tetracycline; 75% for oxacillin and 50% for gentamicin and neomycin; high rate of resistance to penicillin (100%) and ampicillin (75%) were demonstrated.

The antimicrobial susceptibility profile of *S. hyicus* was 100% for vancomycin, oxacillin, enrofloxacin, cephalexin, gentamicin, cotrimoxazole, neomycin and tetracycline, while 100% was resistant to ampicillin and penicillin.

DISCUSSION

The dominance of global resistance of Staphylococcus spp to the beta-lactam antibiotics is probably a consequence of the fact that these continue to be the most used in the treatment of bovine mastitis, especially in the case of small producers who, for lack of appropriate technical assistance, and difficulty acquiring animals to spare the flock eventually submit them to ineffective treatments. Frequent contact of the bacteria with a particular antibiotic may cause an increase in resistance and a decrease in effectiveness of treatment. The most common mechanism in this case is the production of beta-lactamase [7]. Dry cow therapy is adopted worldwide, especially with the principles β-lactams such as penicillins [8]. Penicillin remains as one of the drugs most widely used in veterinary medicine [9].

Monitoring of antibiotic resistance has been considered an essential part of mastitis control strategy and guide the selection of the most appropriate and effective therapy for breast staph infections [10].

CONCLUSIONS

The increasing resistance to antibiotics is a major concern to the treatment of mastitis; therefore, a detailed bacteriological diagnosis and susceptibility testing is required to overcome global problem of antibiotic resistance.

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A NEW APPROACH IN CONTROLLING FASCIOLIASIS IN FARM ANIMALS AND SOME STUDIES ON CONTROLLING EXPERIMENTAL FASCIOLIASIS IN WHITE BOSKAT RABBITS

M. Taha, M. Abdelzaher, S. Afifi, K. Hassanien

Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

OBJECTIVES: Fascioliasis is a widely distributed disease resulting in acute and chronic liver changes. As a result of drug resistance a mixture of two antifasciola drugs (Triclobendazole and Superivomec) was used as a therapeutic trial to overcome the drug resistance compared to other drugs. The histopathological and ultrastructural alterations of the liver were evaluated before and after treatment regimens. The degree of hepatic affection with different stages of fasciola was also recorded.

METHODS: Twenty-eight newly weaned white Boskat rabbit aging 1.5 month were divided into 7 groups, six of them were experimentally infected with metacercaria of Fasciola gigantic and one was used as control. The infected groups were treated with either with Flubendazole only, Flubendazole and superivomec, Superivomec only, Triclabendazole only, Triclabendazole and superivomec sequentially and one untreated group. All the animals were subjected to: A: Parasitological examination included examination of faecal samples according to Melvin and Brooke, (1982). Fluke samples were taken to study changes in morphology by carmine stain and scanning electron microscopy. B: Pathological examination: Tissue samples: Samples from liver were taken for gross, scanning electron microscopy and histopathological examination. C: Statistical analysis: The data were statistically analyzed using general linear model (G. L. M) procedure of SAS (1996). The significance differences between treatment means were tested by Duncan multiple range test (Steel and Torrie, 1982).

RESULTS: In the present study, the histopathological changes were in the form of vascular changes, bile duct lesions, portal lesions, migratory tracts and hepatocellular changes. The eggs of Fasciola gigantica appeared in the faeces at 40 days post infection. Statistical analysis of the egg counts from 7 to 11 weeks (PI) revealed significant increase in eggs in control +ve group when compared with other groups. In the present work, counting of the liver flukes and statistical analysis revealed significant increase of fluke's numbers in control +ve group (13.90±1.38) when compared with other groups. Scanning electron microscopy of the liver flukes in control +ve group revealed rough tegumental surface of the adult Fasciola gigantica. The surface covered with posterior directed spines and transverse folds. In superivomec treated group, there was significant decrease in the number and the length of flukes when compared with other groups. Also, it has a prominent effect on maturation of sexual organs and to lesser extend on the digestive organs. Hitopathologically, in superivomec and triclabendazole treated group, most of the hepatic lesions significantly decreased compared to other therapeutic trials.

CONCLUSIONS: It has been concluded that the mixture of superivomec and triclabendazole is the mixture of choice to treat different stages of Fasciola gigantic infection in the liver.

USE OF SUB-LETHAL DISINFECTANT CONCENTRATIONS TO TRIGGER ANTIBIOTIC RESISTANCE IN BACTERIA IN VITRO

F. Kirchner, C. Kopetz, M. Reinhardt, U. Truyen, S. Speck

Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Leipzig, Germany

Introduction: There is evidence that frequent exposure to minimum concentrations of selective biocides may trigger bacterial antibiotic resistance as has been shown for triclosan and chlorhexidine, both commonly used in cosmetics. In the veterinary field, the use of chlorhexidine and hexachlorophene was linked to antibiotic resistance in Gram-positive and Gram-negative bacterial strains. We therefore investigated the effect of biocides commonly used in animal husbandry and food production on antibiotic susceptibility in selected bacteria.

Materials and Methods: Sodium hypochlorite, peracetic acid, glutaraldehyde, and benzalkonium chloride were used in this study. For bacterial growth, sub-lethal disinfectant concentrations were chosen based on minimum inhibitory concentrations determined previously. Two experimental set-ups were chosen: In both experiments, a 50 µl-aliquot of the bacterial culture was transferred to a fresh tube with the same sub-lethal concentration. This was repeated nine times every 24 h (experiment 1) and every 72 h (experiment 2), respectively. The antibiotic susceptibility was tested prior and subsequently to these experiments using the Vitek®2 technology. Antibiotic susceptibility was evaluated according to CLSI.

Results: Two *Staphylococcus aureus* strains (DSM 799, DSM 2569) were tested using sodium hypochlorite. These strains were susceptible to all antibiotics tested except penicillin, ampicillin, and amoxicillin before sodium hypochlorite treatment. Subsequent to the treatment, a change in phenotype was observed in both strains. Strain DSM 799 remained susceptible to all antibiotics. Strain DSM 2569 developed colonies of distinct antibiotic susceptibility, including so-called “borderline oxacillin-resistance”.

Conclusions: Sodium hypochlorite at sub-lethal concentrations triggers oxacillin-resistance in certain *S. aureus* strains *in vitro*. Underlying mechanisms are currently under investigation.

This study was funded by the Saxon State Ministry of Social Affairs and Consumer Protection.

TREATMENT OF ACUTE MASTITIS BY STAPHYLOCOCCUS SPP DURING LACTATION

Helio Langoni[1], Anelise Salina[1], Marcela Z. Troncarelli[1], Benedito D. Menozzi[1]

[1]Department of Veterinary Hygiene and Public Health, São Paulo State University - UNESP, Botucatu, São Paulo, Brazil.

SUMMARY

Mastitis is the most frequent and expensive disease in a dairy farm. The treatment at beginning of infection shows better outcome. This study evaluated the efficacy of intramammary treatment with ceftiofur hydrocloride of acute mastitis in holstein cows by *Staphylococcus* spp. Immediately after diagnosis of mastitis by milk alterations was used one dose contained ceftiofur hydrocloride 125mg,every 24 hours for three consecutive days intramammary and if necessary for more two days. 150 cases of mastitis by *S. aureus* and 150 by coagulase-negative CNS had been considered. Two weeks after the end of treatment new milk sample was collected in an attempted to re-isolation the causative pathogen. As result, clinical and microbiological cure were obtained in 120 (80%) teats in case of CNS and in 85 (56.6%) of teats with *S. aureus* mastitis. The extended therapy was need only in 5 teats (3.3%) in case of CNS and 12 (8%) teats in case of mastitis by *S. aureus.*

INTRODUCTION

Mastitis is a complex disease and it is caused by approximately 140 pathogens, however some of them are more frequent as *Staphylococcus aureus*, CNS, *Corynebacterium bovis*, *Mycoplasma* spp, *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus dysgalactiae, Escherichia coli* and *Klebsiella pneumoniae*.

The treatment is very important for the mastitis control, and must be conducted at the begining of infection then the chances of cure are greater if the pathogen is responsive to the used medicament. There are a variety of medicaments recommended to the mastitis treatment. Cephalosporins are an important class of antibacterial agent in use for both humans and animals. There are four generations of this group, and all of them contains the beta-lactam substructure first found in penicillin. The range of cephalosporins available for the use in food-producing animals, is limited when compared to humans.

A few first and second generation cephalosporin are approved wordwide strictly for the treatment of mastitis infection in dairy catlle. Ceftiofur is a third-generation cephalosporin and it is effective against a wide range of contagious and environmental pathogens. Regarding to the food-animal residues, rapid metabolism and degradation and no persistence in the environment are important characteristics of this antibiotic class with respect to safety of use in animals.

It was verified the spectrum of susceptibilities of 76 samples of *Staphylococcus* coagulase positive-CPS, isolated from cows presenting clinical mastitis. The sensibility profile to ceftiofur was 94.0% for the CPS (1).

The minimum inhibitory concentrations of cephalosporin compounds was studied (2) in 488 mastitis pathogens from clinically and subclinicaly cases. All *S. aureus* isolates (n=98) and most CNS were susceptible to ceftiofur.

The efficacy of ceftiofur for the treatment of subclinical mastitis in lactating dairy cows and also if extendend therapy regimens enhanced efficacy of this product. It was administered 125 mg of ceftiofur via intramammary infusion and bacteriological cure was defined if a treated infected mammary quarter was bacteriologically negative for the presence of previously identified bacteria at 14 and 28 days after the last treatment. Efficacy was 38.8, 53.7 and 65.8% for the 2-, 5- and 8 days of treatment, respectively. In the control group 10.5% cured spontaneously without treatment. The 8 days extended group was significantly better than the 2-d treatment group. The cure rate for the 8-d extended treatment was 70% for *Corynebacterium bovis*, 86% for CNS, 36% for *S. aureus*, 80% for *Streptococcus dysgalactiae* and 67% for *Streptococcus uberis* (3).

The treatment of mastitis during the lactation or at drying off should belongs to the mastitis program control in one dairy farm to avoid the dissemination of the disease and also the die or culling the cows. So the aim of this study was to evaluate the efficacy of intramammary treatment with ceftiofur hydrochloride of acute mastitis in Holstein cows by *Staphylococcus* spp during the lactation.

MATERIAL AND METHODS

The study was realized in a dairy farm with mechanical milking system with an average of 120 cows on lactation, during the herd milk quality-monitoring period of 2011-2014. Immediately after the diagnose of mastitis by the milk alterations was used one dose containing ceftiofur hydrochloride 125 mg (Spectramast®) every 24 hours for three consecutive days intramammary. If necessary, according to the characteristics of the milk, the treatment could be extended for five days (extended treatment).

Milk samples were collected in sterile vials immediately after the diagnosis, after washing, drying and disinfection the cow teats with iodine alcohol 3% and frozen for subsequent forwarding to the laboratory for microbiological isolation. Were used bovine blood agar base 5% and MacConkey. 150 cases of mastitis by *Staphylococcus aureus* and 150 by CNS had been considered. Two weeks after the end of treatment, new milk sample was collected in an attempt to re-isolation the causative pathogen.

RESULTS AND DISCUSSION

Clinical and microbiological cure were obtained in 120 (80%) of teats in cases of CNS and in 85 (56.6%) of teats with *S. aureus* mastitis. Known therapeutic response in intramammary infections by *S. aureus* is always lower if compared to CNS. The results also demonstrated that there was need for extended therapy only in 5 teats (3.3%) in case of CNS and in 12 teats (8%) in cases of mastitis by *S. aureus.* According others authors both in vitro and in vivo answer to ceftiofur is favorable (1; 2; 4). For the intramammary treatment the results has shown better in comparision with others routes of administration.

CONCLUSIONS

We can confirm the importance of the early intramammary treatment of mastitis when evaluating the cure and the low percentage of cases of extended therapy obtained in the present study.

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HEMATALOGICAL VALUES AND STOMACH DEVELOPMENT IN TWO MONTH OLD GOAT KIDS

L. Otzule, A.Ilgaza

Preclinical institute, Faculty of Veterinary Medicine Latvian University of Agriculture, Jelgava, Latvia

Summary: In research we used 20 Saanen breed kids which were separated in groups with different feeding diets - first group (MMG) kids were fed with mother milk and lived with mothers, the second group (MRG) kids were fed with milk replacer and lived separate from mothers. Blood samples were collected in week 6 and 8. The stomach parts were collected after slaughter (at day 45 and 60) - weighed full and empty and obtained anatomical parameters. We confirm that the most important age of stomach development and kids' growth in postnatal period are the first 45 days. During this period the most significant differences can be observed. On day 60 there are no significant differences ($p>0.05$) between MMG and MRG stomach development.

INTRODUCTION

Latvian goat industry increases every year. Blood hematology is one of the indicators that shows if a kid gets a sufficient quantity of liquid, has a good supply of oxygen in the body, as well as an indication of inflammatory processes. In goats like in other ruminants, growth of the stomach parts and their functional development continues during the first months after birth. Studies have proved that different feeding factors and environmental conditions can influence the development of the ruminant stomach parts in kids (Church, 1979).

Kids in the transition from milk to roughage have changes not only the multi-part stomach size and volume ratio, but also in feed processing - from chemical to biological. Thereby kids become wholesome ruminant animals. Feed change definitely has an impact on blood parameters. Therefore, the aims of the present study was to determine if the feeding of milk replacer, which was intended for calves, changed both the functional activity of the abomasa in goats and the weight ratio of the ruminant stomach parts during the first month of postnatal development, hematological values and live weight gain during the second month of life.

MATERIAL AND METHODS

Research was performed in a farm of Latvia, Zemgale region, from February till April, 2014. In total 20 goat kids were used in the research. All kids were kept with mothers for two weeks and then separated. After age of two weeks kids were fed with foregoing calf milk replacer using nipple buckets and lived separately from mothers in cote. In the first group (MMG) were kids which were fed with dairy (mother's) milk *ad libitum* and lived with mothers (n=10), the second group (MRG) kids were fed with foregoing calf milk replacer using nipple buckets, and lived separately from mothers in cote (n=10). Drinking water and hay were easy accessible.

The animals were apparently healthy. At the age of two weeks blood analysis of all animals was examined and physiological parameters were measured. In week 6 and 8 we collected blood samples by jugular vena-puncture in EDTA vacutainer tubes. The blood samples were analyzed for leucocytes (WBC) and erythrocyte (RBC) number, hemoglobin (HGB) concentration, packed cell volume PCV (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrations (MCHC) and red cell distribution width (RDW) (Egbe-Nwiyi, 2000; Elitok 2012).

The stomach parts of the goat kids were collected after slaughter on day 45 and 60. After full stomach weighing we detected the length and width of rumen and length of abomasums (Church, 1979). The relative stomach weight was calculated by relating the body weight and concrete weight of stomach separately for each kid.

RESULTS

The results of hematological values are shown in Table 1. At age of 6 weeks there are no significantly differences between groups ($p<0.05$), but at age of two months RBC, HBG, HCT is significantly higher in MMG kids. The WBC in MRG kids is higher than in MMG kids. RDW results for each animals group remains constant, regardless of age. In research we also calculated the relative weight of the stomach (Tab.2.). Weight gain from day 45 till day 60 in MMG was 0.044 g/day, but in MRG it was 0.0073 g/day. The relative weight of stomach was significantly higher ($p<0.05$) in kids which from day 15 to day 45 were fed on mother's milk (MMG). On day 60 this difference is not significant.

DISCUSSION

Blood hematological values can vary depending on age, gender, goat breed, environmental temperature, feeding type and health status (Daramola 2005; Elitok, 2012). The results showed that the HCT (PCV), HGB, RBC, MCHC and WBC number was comparable ($p>0.05$) and this coincides with the studies of other authors (Daramola et.al. 2005; Shaikat et.al. 2013). Evaluating the above presented results it can be observed that hematological parameter values of the milk replacer group kids are lower than in MMG group. This indicates that unlimited access to mother's milk improves the digestibility of feed intake and absorption ability of the kid in the body, thus improving blood hematology.

Our data show that between 15 and 45 days of age feeding on milk replacer increases speed of rumen development compared to feeding with mother's milk. It is possible that kids' limited access to milk replacer contributed to reinforcement of the combined feed and hay intake, and feeding with roughage feed promotes rumen development (Mishra et al., 2013). It must be admitted, that the abomasum and rumen size on day 60 between groups did not show any differences.

In general, we can confirm that mother's milk at early age (up to 45 days of life) can significantly accelerate the growth of kid and multi-chamber stomach, where the animals are provided with sufficient amount of hay and roughage.

CONCLUSIONS

Hematological values in MMG group kids were more stable than in MRG group kids. Also we confirmed that the most important age of stomach development and kids' growth is approximately the 45th day of age when the most significant differences can be observed. Differences in weight gain were observed between groups – live weight gain in MMG was six times higher than in MRG.

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Table 2

Live weight and the weight of the stomach in kids fed with calves' milk replacer (MRG) or mother's milk (MMG) at 45 and 60 days of age

Group	Live weight (kg)				Weight of the stomach (g)				Relative stomach weight (%)			
	Day 45		Day 60		Day 45		Day 60		Day 45		Day 60	
	mean	Stdev	mean	Stdev	mean	Stdev	mean	Stdev	mean	Stdev	mean	Stdev
MMG	9.3	0.18	9.9	0.56	1978	94.3	2055	62.3	4.98	0.01	5.6	0.01
MRG	7.8	0.26	7.9	0.37	1339	54.7	1476	184.4	6.01	0.01	5.9	0.11

Table 1

Hematological values [mean ± S.D] of goats kids which were feed by milk replacer or mother milk.

Age	Groups	WBC x10^9/L	RBC x10^{12}/L	HGB g/dL	HCT %	MCV fl	MCH pg	MCHC g/dL	RDW %
6 weeks	**MRG**	11.6±3.35	13.4±1.50	8.4±0.90	25.7±3.40	19.2±1.99	6.2±0.39	32.8±1.33	24.9±1.51
	MMG	11.9±3.00	14.4±0.61	8.4±0.89	25.8±3.56	17.9±2.05	5.8±0.50	32.9±1.52	23.6±1.64
8 weeks	**MRG**	15.5±2.90	12.6±1.81	7.1±0.99	20.6±3.03	16.4±0.40	5.6±0.18	34.6±0.41	25±1.38
	MMG	14.2±2.56	15.5±1.32	9.0±0.99	26.3±3.28	16.9±1.44	5.7±0.36	34.3±1.21	23.5±1.80

STUDIES ON THE SUSCEPTIBILITY OF VARIOUS ENVELOPED AND NON-ENVELOPED VIRUSES AGAINST CHEMICAL DISINFECTANTS IN CARRIER TESTS

U. Truyen, K. Pirschel, E. Neblung, F. Schmidt, C. Köhler, S. Speck, C. Karnath

Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Leipzig, Germany

Introduction: Disinfection is an important tool in animal husbandry, minimizes the risk of infectious diseases and ensures animal health. A prerequisite for an efficient disinfection is the use of efficacious disinfectants which have been tested for their efficacy.

Efficacy testing in Germany has been done on a voluntary basis for several decades following test guidelines of the Deutsche Veterinärmedizinische Gesellschaft (DVG). The efficacy was basically defined using poplar wood carrier tests.

With the licensing of biocides according to the *REGULATION (EU) No 528/2012 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 May 2012 concerning the making available on the market and use of biocidal products* the efficacy testing using carrier tests is mandatory and test protocols have to be adopted. Of utmost importance is the selection of suitable test viruses.

Materials and Methods: Five active substances widely used for chemical disinfection in the Veterinary field were tested against various viruses in carrier tests using poplar wood or stainless steel carrier.

Ethanol, glutaraldehyde, sodium hypochlorite, sodium hydroxide, peracetic acid and formic acid were tested against parvoviruses (feline, murine and porcine parvovirus), bovine enterovirus 1 (ECBO virus), reovirus, feline calicivirus, feline herpesvirus, bovine viral diarrhea virus, equine arteritis virus, feline coronavirus, and Newcastle Disease virus.

Results: The susceptibilities of the various viruses for the respective substances and the two carriers will be presented at the meeting. Furthermore, recommendations for updating the DVG guidelines and for the new EN work item(s) will be given.

PHENOTYPIC AND GENOTYPIC ANALYSIS OF BIOFILM PRODUCTION BY STAPHYLOCOCCUS AUREUS AND COAGULASE NEGATIVE STAPHYLOCOCCUS ISOLATED FROM BOVINE MASTITIS

A. Salina[1], H. Langoni[1], F. F. Guimarães[1]

[1]*Department of Veterinary Hygiene and Public Health, São Paulo State University, Botucatu, São Paulo, Brazil*

SUMMARY

Staphylococcus aureus is common in intramammary infections, which often become chronic by the ability of these bacteria to produce biofilm. A total of 100 strains of *S. aureus* and 100 strains of coagulase negative *Staphylococcus* (CNS) were isolated from cases of subclinical and clinical mastitis. The presence of *ica*A, *ica*D and *bap* genes was performed by PCR and phenotypical characterization by the congo red agar (CRA). The frequency of *ica*A, *ica*D and *bap* in *S. aureus* were 82%, 83% and 58%, respectively. The phenotypical test on CRA showed that 25% of *S. aureus* strains and only 5% of CNS strains produced biofilm on plates. Two strains of *S. aureus* were negative for the genes researched, but produced biofilm on CRA. These results showed that the CRA was not suitable to assess the ability of *S. aureus* to produce biofilm on plates.

INTRODUCTION

S. aureus is frequently isolated from subclinical mastitis and chronic infections in worldwide. In Brazil, the frequency of isolation is estimated in 20% of cases of mastitis (1). The chronicity of mastitis by *S. aureus* can be attributed to different virulence factors, including biofilm formation within the mammary gland. Initially, *S. aureus* colonizes the teat canal and after this step, adheres in the ducts of the epithelium and alveoli, and starts the production of toxins. The biofilm production is mediated by *ica*ADBC cluster, an operon involved in the synthesis of a biofilm matrix polysaccharide (PIA). Studies has been demonstrated that the biofilm formation could be mediated by surface proteins. The expression of surface protein Bap (biofilm-associated protein) leads to biofilm formation even without the operon *ica*ADBC (2). CNS are historically reported in the literature as of minor importance in mastitis. However, more recent studies have showed that this group of micro-organism are responsible for producing enterotoxins (3) and biofilm formation, inducing cellular immune responses, as well as *S. aureus* (4). The aim of this present study was to investigate the presence of *ica*A, *ica*D and *bap* genes and verify the ability of biofilm formation by strains of *S. aureus* and CNS from bovine mastitis.

MATERIAL AND METHODS

We carried out a total of nine visits, at monthly intervals in order to achieve the proposed number of 200 strains of *Staphylococcus* spp. A total of 749 samples of bovine milk were analyzed. The characterization of bacterial strains was based in colonial morphology, gram staining, positive catalase test, and coagulase test. For confirmation of *S. aureus* the primers described according to Straub et al. (5), were used. For amplification of *ica*A and *ica*D genes the primers were used according to Vasudevan et al. (6). For the *bap* gene amplification were used the method described by Cucarella et al. (2). For confirmation of CNS were used the primer *coa,* described previously (7). The phenotipycal characterization was performed according to Vasudevan et al. (6). Reference strains were used as positive control (ATCC 25923) and negative control (ATCC 12228).

RESULTS

The frequencies of *ica*A, *ica*D and *bap* genes in *S. aureus* were 82%, 83% and 58%, respectively. The number of strains that presented the three genes researched were 56% and of these, only three strains were positive on CRA. The total of black colonies on CRA were 25% of strains of *S. aureus* and 5% for CNS. Two samples of *S. aureus* were able to form biofilm without the presence of the genes researched.

DISCUSSION

Two strains of *S. aureus* were able to produce biofilm without the presence of any of the genes studied. Chaieb et al. (8) found a strain that was negative for both genes (*ica*A and *ica*D) but produced some black colonies in CRA. It became apparent to the authors, that biofilm formation may be associated with other factors, not only the presence of *ica*A and *ica*D genes. It is also suggested that other regulatory genes or also other genes of biofilm formation may be present in these strains, stimulating the production of PIA (9).

The primers used for detection of *ica*A and *ica*D genes in this study described according to Vasudevan et al. (6) were also used by Simojoki et al. (10). These primers work well for recognition of *S. aureus* strains, but they fail to recognize *S. epidermidis* (ATCC 35984), known positive strain for these genes, considered therefore ineffective in the detection of these genes in CNS (10). Thus, it is necessary the use of primers that recognize these genes in several species of CNS (4).

CONCLUSION

This study showed the important of detecting the presence of biofilm formation genes in *S. aureus* strains that showed high positivity in genotypical test. We can conclude that the biofilm formation could be associated with other genes of biofilm formation than those researched.

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ASSESSMENT OF LUNAR CYCLE ASSOCIATIONS WITH ANIMAL REPRODUCTION

L. Könyves[1], P. Feczkó[1], B. Rózsa[1], T. Reibling[1], A. Horváth[2], C. Bajcsy[2], N. Solymosi[1]

[1]*Department of Animal Hygiene, Herd-health and Veterinary Ethology, Szent István University, Hungary*
[2]*Department and Clinic of Food Animal Medicine, Szent István University, Hungary*

Summary
In many cases the cyclic or non cyclic environmental changes have influences on the living beings. Among practitioners there is a belief, experience the lunar cycle affects on the farm animal reproduction. Statistical analyzes were performed on associations of dairy cow and swine reproduction data time series with lunar cycle phases. Half of the cattle population supports the practitioner experience of oestrus number increase around the full moon. Two quarters of moon cycle was identified when the inception rate of sows increases.

Introduction

Many of the environmental conditions having influence on the living beings have variation in time, some of them have irregular pattern, some show cyclic changes by the time (Refinetti 2006). In numerous animal and human physiological, behavioral phenomena there are empirical concepts of their dependency on lunar cycle (Cajochen et al. 2013, Cordi et al. 2014), even without any esoteric belief. One of these experiences is the relationship between the sexual cycle and the lunar phases. Nevertheless only a few quantitative studies were published analyzing such relationships. Numerous practitioners have a feeling the oestrus number in production animal populations is increased by full moon. In our study we analyzed associations of dairy cow and swine reproduction data time series with lunar cycle phases.

Material and methods

Data was gathered from four cattle and two swine large scale farms. The data sets covered the time period between 1995 and 2014 (except one of the pig farm having five year long data set only). The lunar cycle phase association with the daily oestrus number and the success rate of insemination was analyzed by Poisson and logistic regression, respectively. In the pig farms during the study period oestrus synchronization was used on both farms. Because in the involved dairy cow farms the synchronization became commonly used since 2008 the whole time series were divided by that year to two parts and analyzed separately. According to the methodology was proposed by Cajochen et al. (2013) the lunar cycle data was obtained from the Müncher Astro Archiv: http://www.maa.mhn.de/StarDate/moonphases.html. All statistical analyzes were performed using the R language and environment (R Core Team 2014).

Results

In the period without synchronization the daily oestrus number on two cattle farms (Farm1 and Farm 2 in Figure 1) showed positive (8.7%, 14.5%, $p<0.005$), on one farm (Farm 4 in Figure 1) negative (-6.3%, $p=0.04$) significant association with the full moon phase. During the synchronized period these relationships were lost. The results of the analysis of Farm 3 data did not show any statistically significant ($p<0.05$) association.

On the pig farms we found that success rate of insemination is significantly higher on certain periods of the lunar cycle comparing to other periods (Figure 2). On farm 1 the success rate is increased with insemination on day 7-8th (OR: 1.05, 95%CI: 1.02-1.08, $p=0.001$) and 21st (OR: 1.06, 95%CI: 1.02-1.10, $p=0.007$) of lunar cycle. On farm 2 the new moon (OR: 1.08, 95%CI: 1.03-1.14, $p=0.003$) and the 24-25th day (OR: 1.05, 95%CI: 1.01-1.10, $p=0.02$) of lunar cycle showed similar association.

Discussion

On two of the four cattle farms the results showed that there is a significant increase of the insemination numbers around the full moon. This may suggest that the oestrus synchronization might be adaptive to the moon cycle stages if the producer would need more homogenized insemination pattern in time. But these results have considerable limitation since one of the cattle farms showed the opposite phenomenon. Further data should be collected from the farm studied in this work and from others to clarify the possible reasons of the contradiction.

In the case of sow conception rate the results are more convergent, meaning that on both studied farm there are two significant conception rate peek. In the first and about the third quarter there was found a higher probability of conception of sows. These results are more concordant than in the case of cattle insemination clusters, but should be certified on more populations. If the conception rate peeks presented can be certified on further populations then the oestrus synchronization protocols might be adapted to this rule.

It is very important to mention as the study presented is a retrospective observational study it can't provide any causal relationship between reproductive features and the lunar cycle stages. Even the presented results can be generalized until the understanding of the dependency of physiological processes on lunar cycle will be far away.

Conclusions

We got contradictive results on the relationship of full moon and daily oestrus number increase of cows. But half of the study population is concordant with the empiria of farmers, inseminators. The results regarding the conception rate increase of sows in the first and the third quarter of lunar cycle are new in production animal literature. But, due to our current knowledge one can formulate very week biological relationship speculations on the statistical associations are presented only.

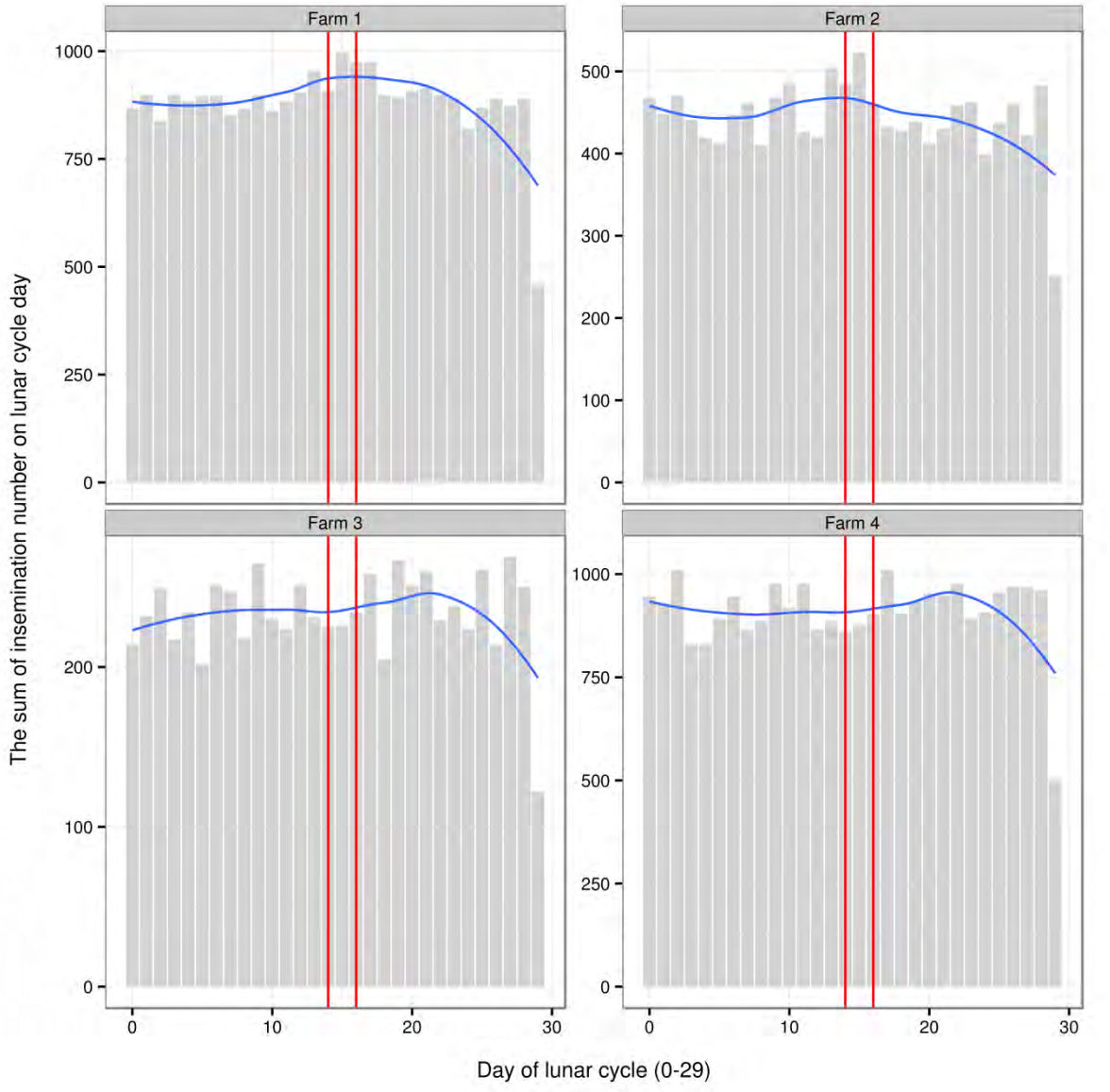

Figure 1. Sum of cattle insemination numbers on lunar cycle day in the study period.

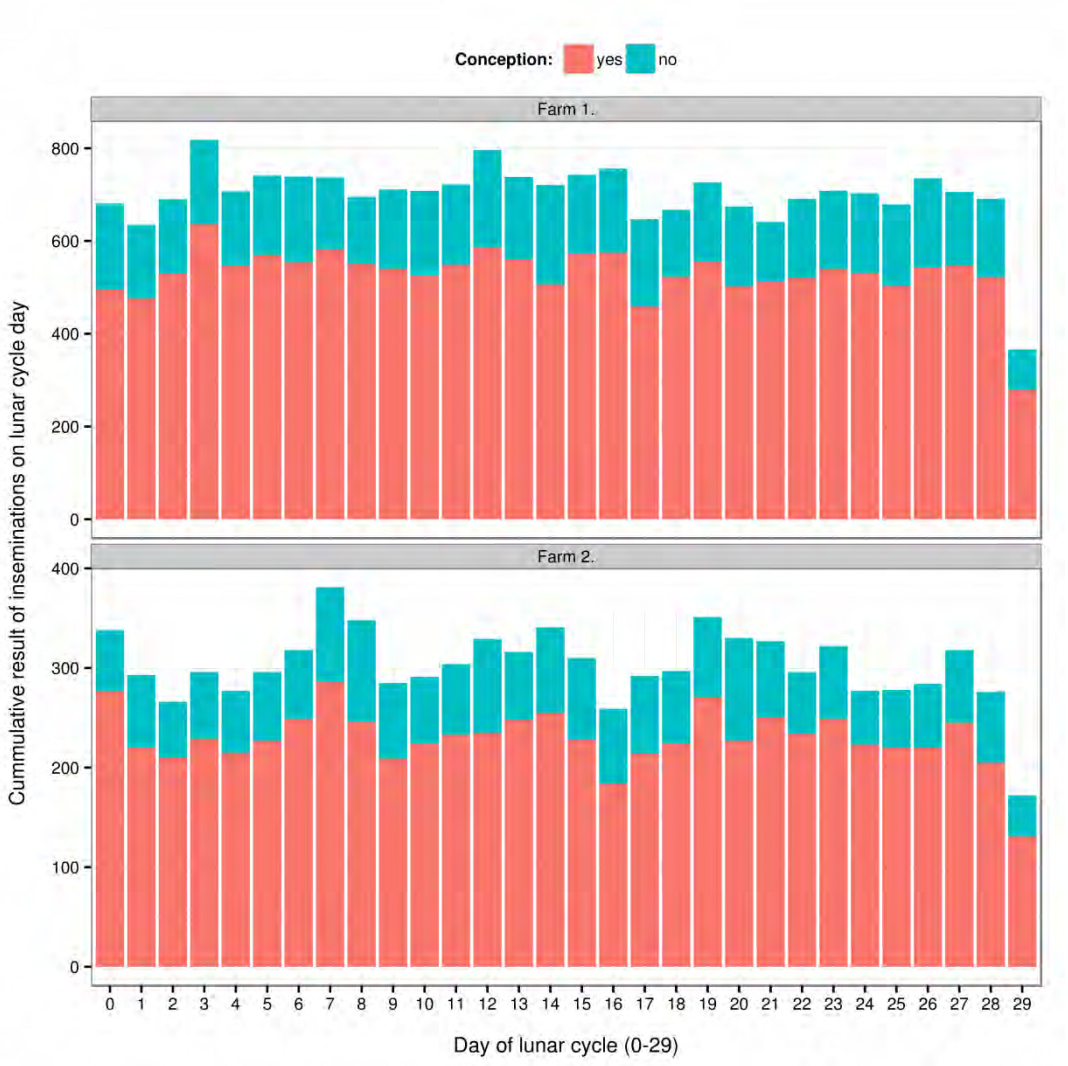

Figure 2. Conception success of insemination of sows in the study period.

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INFLUENCE OF A FEED ADDITIVE ON SUPPORT OF CALVES DURING PHASES OF DIARRHEA

J. A. Saltijeral O., L. Galicia L., C. Garcia D., Y. B. Lopez A., J. J. E. Guerra L.**, H. I. Rogge***

Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana, Mexico
***Facultad de Agronomía, Universidad Autónoma de Sinaloa, Mexico*
****Phytobiotics Futterzusatzstoffe GmbH, Germany*

Introduction

Diarrhea is one of the main reasons for economic losses (treatment costs and animal losses) in calves, is a multifactorial disease that is associated with inflammation in the gut. The active ingredients plant-derived quaternary Benzophenanthridine and Protopine alkaloids (QBA+PA) are known to have anti-inflammatory effects and a positive impact on intestinal integrity.

The incidences of infectious diseases among dairy calves are great, and recent reports indicate that 7.8% of heifer calves born alive die before weaning (NAHMS, 2007); the period of highest morbidity is from birth until weaning.

Other authors have considered additional factors associated with diarrhea were breed (Svensson et al., 2006), the placement of indoor calf pens against outdoor, keeping grouped calves on a slatted concrete floor versus other floors. these factors in the study were monitored.

The aim of the study was to analyze the effect of QBA+PA on growth performance, as well as on the course and intensity of diarrhea of calves.

Material and Methods

A total of 160 Holstein Friesians calves from 2 commercial farms of Tizayuca/Mexico were randomly assigned into 2 groups (control group; trial group with 5 g/meal of QBA+PA product added to the milk replacer from the 3rd until the 23rd day of life). The animals were fed 2 liters of milk replacer per meal (2 times/day) as well as a concentrate and alfalfa ad libitum. The meals should be given every day at the same time.

After completion of labor, the cow was milking in the first three hours postpartum. A plastic test tube with the sample of colostrum (300ml approx. temperature milk to 22°C.), which was deposited and the colostrometer specific density of each sample was determined, the colostrometer has been widely used to estimate IgG concentration in colostrum. That through a scale measured the concentration of immunoglobulins in mg / ml.

Bacteriological and parasitic analysis of feces (days 3 and 21), also analysis milk replacer were conducted.

Calves were weighed individually on a large veterinary animal platform scale (Arlyn Scales, East Rockaway, NY) at day 1, 7, 14 and 21, of the study.

Results

Data demonstrable that of the total calves, 96.8% took colostrum appropriately (immunoglobulins between 50 to 140 mg / ml colostrum). Ingested colostrum average in the experiment was 80.4 for both groups, whereas 3.1 % took an insufficient amount consequently. immunoglobulins between 20 to 50 mg / ml colostrum. In no case was there immunoglobulin concentration below 20 mg / ml of colostrum. Coincidentally the animals fed colostrum shoddy introduced in laboratory analysis hypoproteinemia.

Table No. 1

Analysis Milk Replacer

Farm	Fat	Protein	Lactose	Total Solid	Solid Non Fat
A	4.035	2.88	7.93	15.55	11.50
B	3.83	4.5	5.1	14.14	10.3

Table No. 2

AVERAGE WEIGHT GAIN. Kilograms

Group	Initial weight	Final weight	Gain
Control	38.794	42.840	4.046
Experimental	37.824	43.421	5.597

Discussion

General morbidity rate was 37.5 %. with no significant differences among the groups and the mortality rate was 2.5 % which is below the national parameters, however no significant differences between the study groups. In the control group occurred two deaths from diarrhea and the trial group no, the control group had a lethality rate for diarrhea of 15.38 %.

The average duration of diarrhea was lower in the experimental group 2.4 days vs. control group: 3.2 days SD 1.25 (P =0.07). ; But in the experimental group does not use any antibiotic, in the control group ranch routine is followed as administration of antibiotics (Flunixin Meglumine, Enrofloxacin or Oxitetraciclin) to treat diarrhea.

The most common bacteria found in the feces were Escherichia coli. and Bacillus spp. 38 cases of Entamoeba spp., 3 Eimeria spp., 1 Cryptosporidium parvum and 1 Giardia sp. were found. All cases of Eimeria and Giardia were in the control group. Cryptosporidium was the case in the experimental group. Cases of Entamoeba no significant differences 45.8 % of cases were in the trial group.

Conclusions

The QBA+PA had a positive influence on the well-being of the calves, weight gain and support to remedy intestinal disorders leading to lower persistence of diarrhea and no death due to diarrhea.

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THE EFFECT OF ANTIMASTITIS CONTROL PROGRAM ON THE AETIOLOGY AND INCIDENCE OF MASTITIS IN TWO COWS BREEDING IN SLOVAKIA

M. Vasiľ, J. Elečko, Z. Farkašová, F. Zigo

University of Veterinary Medicine and Pharmacy, Department of Animal Breeding, Košice, Slovak Republic

Summary

In the study are presents the results of applying of six-month antimastitis measures in two dairy herds with comparable conditions, and their effect on the changes in the aetiology as well as to reduce the incidence of mastitis. Application of concretely methods of prevention and therapy as hygiene during milking teat disinfection after milking, the sanitation of housing, retreatment 30, respectively 14 dairy cows during lactation and treating each cow udders before drying off, were at the end of the monitoring caused a reduction of the prevalence of mastitis in holding A from the original 34.8% to 11.8%; in holding B from 38.2% to 8.1%. In holding A were clinical mastitis 27.9%, (acute, subacute and subclinical) at the beginning of the monitoring period at while after half of the monitored year were already only 5.4%. In holding B were clinical mastitis (31.8%) and were reduced to 2.7%. Comparable conditions breeding technology, management levels and uniform mastitis control program are manifested in the relatively stable incidence of latent mastitis in each of the examinations (6.9% and 6.4% in holding A, respectively 6.4% and 5.4% in holding B).

Keywords: herd, dairy cows, mastitis control, reduction, pathogens

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Introduction

Mastitis is the most devastating disease of dairy animals despite of improved management practices and dry cow therapy, mastitis remains worldwide problem of major economic threat to the dairy farmers. For polyetiological and multifactorial character of secretory mammary gland there is no generally valid antimastitis program with a guaranteed effect (Mason, 2006). The methods applied in each antimastitis program must take account of diversity of bacterial agents causing mastitis in dairy herds and second trait technologies and breeding level (Barkema et al., 2009). The aim of the study was in comparable terms of two dairy holding during the six-month application of standard methods of antimastitis program to record the changes in the aetiology and reduction the incidence of mastitis.

Materials and Methods

The location and course of the experiment

The experiment was carried out in herd A (204 dairy cows, S. Ľubovna) and holding B (110 dairy cows, Gelnica). Slovak Pied cattle breed were situated in both holdings. Experiment consisted of an initial analysis of the incidence of mastitis in both holdings, subsequently from design and application of rational and damping antimastitis preventive measures while complex examination of the two herds following the six months to serve for the evaluation of the effectiveness of the measures.

Holdings characteristics

The holdings were comparable conditions: loose housing, feeding, clearing manure, the milking parlor. Milking hygiene program was conducted irregularly or incomplete.

Herd A has a problem with the required of medically innocuous water, removal of dung from housing is inadequate, cows are milked in the parlour Boumatic 2x10 (Madison, Wisconsin, USA), with SCC 800 x 103.

The holding B characterizes the milking parlour DeLaval 2x5 tandem (Tumba, Sweden). The herd is complemented by high pregnant heifers from own breeding. The level of housing in the house with litter and feeding operation chariot into the corridor can be evaluated as good. In contrast, the breed had to solve the problem of decreasing production for the occurrence of chronic mastitis, and high number of atrophic quarters.

Examination and analysis

Complex examination herds included: review of actual farming anamnesis, bacteriological investigation of mixed milk samples, clinical examination and evaluation of the mammary gland California mastitis test according to Jackson and Cockcroft (2002). Bacteriological examination of milk samples, and in monitoring the application of proposed procedures was carried out according to Vasiľ (2004).

Milk samples were inoculated onto blood agar (Oxoid LTD, UK), cultivated at 37°C for 24h and biochemical identified using the STAPHYtest, STREPTOtest, ENTEROtest (Erba-Lachema, Brno, Czech Republic) according to Vasiľ et al. (2004). All identified *Staphylococcus* spp. isolated from milk samples were in vitro tested on Mueller-Hinton agar (HiMedia, Mumbai, India) by disc method after 24 h incubation at 37°C, on resistance to 13 types of antibiotics. The bacteria were assessed as resistant or sensitive by reference zone according to manual instruction of producer. Based on the above examination results were each expressed: prevalence, incidence and proportion of the forms of mastitis. Bacteriological finding and results of susceptibility of bacteria isolated from clinical cases of mastitis to available antibiotics are taken into account in the selection of products for the treatment of mastitis in lactating or treatment udder at drying off.

Results and discussion

An analysis of the aetiology of initial tests showed that the main priority is elimination of the major pathogens of mammary gland and *S. agalactiae* in a holding A, and *S. aureus* in both holdings in the form of antibiotics therapy during lactation. By means of the antibiotic intramammary preparations with proven effect in holding A were treated with total 30 cows, that clinical cases and finding *S. uberis, S. dysgalactiae* and *Corynebacterium* spp. Among the 14 intramammary treatment in holding B, were cows with clinical mastitis caused by the bacteria *Corynebacterium* spp., coagulase-negative staphylococci (CNS) (S*. chromogenes, S. epidermidis* and *S. warneri*).

Along with continuously application the standard antimastitis measures was reached after six months the reduction of the mastitis in holding A from the original value 34.8% to 11.8% and holding B from 38.2% to 8.1%. The occurrence of *S. agalactiae, S. uberis, Bacillus* spp. and *Citrobacter* spp. in both holdings was eliminated.

Conclusion

In this work were presented the results of the effect of the six-month application antimastitis measures proposed conditions from each of the two holdings in form of the reduction of occurrence of mastitis and decrease of number of udder pathogens in milk of cows.

From the bacteriological analysis resulted in need of the mainly rapid treatment of cows with the finding of *S. aureus, S. agalactiae* and *Corynebacterium* spp. Other cases with findings CNS, *Aerococcus viridans*, and *Corynebacterium* spp. are long term recommended to register and at the last milking lactating treated with intramammary preparation.

After eliminating the major pathogen in organizational and material preparation were continuously applied all the measures proposed. Changes have been made mainly the involvement of workers on the final effect.

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EFFECT OF STRUCTURED AND ENRICHED WITH MINERALS WATER FOR MINK WELLNESS AND PRODUCTION

G. Gerulis, L. Kesaite, B. Bakutis, V. Baliukoniene, J. Jovaisiene, A. Vasiliauskas

Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

Summary: Aim of the study was to investigate effect of structured and enriched with minerals water for mink wellness and production.

Based on the collected results we determined that structured and mineral enriched water has no significant effect on mink weight. Results showed that mineralized and alkaline water have improved quality of mink fur approximately 30%, while the fur quality of control group has decline by 20%.

After studying blood samples we determine that blood indicators of both test and control group were in within normal limits except MCV, MCHC, RETIC, MPV, PLT. Control group MPV was 61.75 fL, in A group 3.26%, in B group 8.70%, in C group 8.90%, in D group 11.62% lower than in control group. MCHC in control group was 28.56 g/dL, while in A group was 5.23% lower, in B group 4.69% lower, in C group 3.90% higher, in D group 0.92% lower. Control group RETIC indicator was 84.09 K/µL, in A group 51.20%, in B group 57.06%, in C group 48.23%, in D group 48.56% higher. MPV in the control group was 4.76 fL, in D group 37.37% higher than control group. PLT rate was lower than normal pale only in control, B and C groups.

Keywords: mink, fur, production, blood indicators, structured, amber, quartz, flint, alkaline, water, effect

Introduction: Fur farms are facing with variety of viral, bacterial origin diseases, liver, kidney and other organ disorders. This adversely affects the well-being of animals also their production. This problem is particularly relevant to farmers that are why veterinary specialists keep working on researches for various preventive and health improving measures which does not contain any chemicals that can have a negative impact on animal health. Natural prevention and treatment measures have the better results are not harmful to the animal's health, and even enhances their constitution.

Material and methods: Research methodology: for investigation was used minks raised in farm. We chose 25 females and 25 males. They were divided in to 5 groups (A, B, C, D, K). Each group was given to drink different type of water: amber (A), quartz (B), flint (C) and alkaline (D). Water was made by soaking minerals in to it, except alkaline which was made using ionizer. Control group (K) was drinking ordinary farm water. Minks have been weighted three times- before, in the middle and in the end of the study. Also we assessed the quality of fur before and after research. Blood morphological test was performed as well before and after study.

Results: Based on the collected results we determined that structured and mineral enriched water has no significant effect on mink weight.

Results showed that mineralized and alkaline water have improved quality of mink fur approximately 30%, while the fur quality of control group has decline by20 %.

After studying blood samples we determine that blood indicators of both test and control group were in within normal limits except MCV, MCHC, RETIC, MPV, PLT. Control group MPV was 61.75 fL, in A group 8.70%, in B group 8.90%, in C group- 3.26%, in D group 11.62% lower than in control group. MCHC in control group was 28.56 g/dL, while in A group was 4.69 % lower, in B group 3.90% higher, in C group 5.23% lower, in D group 0.92% lower than the control group. Control group RETIC indicator was 84.09 K/µL, in A group 39.82%, in B group 27.45%, in C group 31.62%, in D group 27.90% higher than in control group. MPV in the control group was 4.76 fL, in A group 19.32%, in B group 2.08%, in C group 27.88%, in D group 37.37% higher than control group MPV. PLT rate was lower than normal pale only in control, A and B groups. PLT in control group was 129.8 K/µL, in A group 45.21%, in B group 19.58% higher.

Discussion: According to the study, the results of minks weighing showed that no significant difference between the control group and experimental groups, although during experiment feeding ration was not changed. Fur quality was assessed before and after the study. According to a study established that minks fur quality improved by 30%, when minks were given to drink flint and quartz water. When minks were given to drink amber and alkaline water has improved by 20%. Mink's blood morphological indicators test was performed before experiment and after drinking 40 days. Based on the results was detected that the blood indicators which were in the normal range before the experiment have changed slightly and remained in norm. A comparison of A, B, C, D groups and the control group indicators which were below or above the normal range, we found that the PLT, C (flint water) and D (alkaline water) groups have risen to norm and was significantly different from the control group PLT. A and B groups PLT also increased, but did not reach norm. PLT indicator shows the platelets in the blood. Platelets ensure blood clotting and show that there are viral diseases. RETIC blood indicator increased in A, B, C, D groups, in K group did not change. RETIC - shows a young, immature red blood cells (reticulocyte) count. Increased quantity shows the regeneration phase after anemia, tissue hypoxia, or hemolysis.

Conclusions

1. Structured and mineral enriched water has no significant effect on mink weight.
2. Structured water is enriched with minerals and improves the coat quality of mink.
3. These waters have an impact on blood characteristics.

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SEROPREVALANCE OF NEOSPORA CANINUM IN COWS IN BURDUR REGION: INVESTIGATION OF IT'S RELATIONSHIP WITH ABORTIONS AND INFERTILITY

Ramazan Adanir[1], Yunus Çetin[2], Mesih Kocamüftüoğlu[2], Onur Köse[3]

[1] *Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Parasitology, Burdur, Turkey*
[2] *Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Burdur, Turkey*
[3]*Adnan Menderes University, Faculty of Veterinary Medicine Department of Parasitology, Aydın, Turkey*

Summary: The purpose of this study is to determine the seroprevalence of *Neospora caninum* in the cows in Burdur region and to establish how seroprevalence varies in the cows having the problems of abortus and infertility in their history.The material of the study is composed of 400 cows. Sera samples were analyzed for antibodies against *N. caninum* using a commercially available competitive ELISA (c-ELISA) kit (VMRD, USA). According to the ELISA test results, seroprevalence of *N. caninum* in the region of Burdur was found to be 5.3%. Considering the results of this research, the presence of *N. caninum* in Burdur region has been revealed and the fact that *N. caninum* should not be ignored especially in abort cases has been emphasized.

Keywords: Abort, *Neospora caninum*, Infertility, Cow.

1. Introduction: *Neospora caninum*,an apicomplexan protozoan parasite closely related to *Toxoplasma gondii* was first described in dogs in Norway in 1984 and was described as a new genus Neospora, type species N. caninum, in 1988 and later in a wide range of other mammals including cattle, goats, horses, and sheep. (7,9). The life cycle of N. caninum is only partially known, but the dog has recently been established as its definitive host (12). Recently, enzyme-linked immunosorbent assays (ELISAs) with whole tachyzoite antigens also have been described for the identification of *Neospora*-infected hosts (6,8). Epidemiologic studies of bovine abortions in various regions of the world indicate that *Neospora* infections account for 3 to 42% of abortions in dairy herds whose causes have been diagnosed (3,4,5,11).Seroprevalence of N. caninum were reported different region of Turkey in previous studies. The seroprevalence rate of were as follows: Van (2) 4.88%, Kayseri (10) 7%, Malatya 4%, Muş 4.86%, Bingöl 4.69%, Elazıg 15% (1) and Sakarya (13) 9.2%.

This study was performed to investigate the status of *N. caninum* infection by cELISA among healthy and aborted dairy cattle in Burdur region and to establish how seroprevalence varies in the cows having the problems of abortus and infertility in their history.

2. Materials and Methods

2.1. Study area: The study was conducted in eight districts (Burdur city center, Bucak, Aglasun, Yesilova, Kemer, Çavdır, Karamanli, Gölhisar) of Burdur Province (Turkey), including Burdur city.

2.2. Experimental animals: The material of the study is composed of 400 cows from different ages and breeds, 49 of which aborted, 58 of which are infertil, 48 of which are pregnant and 245 of which are identified as healthy according to the findings of reproductive anamnesis.

2.3. Blood samples: Blood samples were collected from the jugular vein in sterile tubes. Sera were removed after centrifugation at 3000 rpm for 10 minutes and stored -20 ºC until serological tests were made.

2.4. Serological method: The antibodies to *N. caninum* in the sera were detected by a competitive cELISA using a commercially available test kit (VMRD, Pullman, USA).

2.5. Statistical analysis: The prevalences found in seropositive cows in the provinces were compared using the chi-square test.

3. Results: Seroprevalence of N. caninum in cattle in the region of Burdur in relation to age, and breed with regard to origin, breed,age and reproductive anamnesis are presented in Table 1.The highest prevalance of *N. caninum* infections were observed in 2-4 age group (%7.7) and this prevalance was followed by ≤2 age group (%6.4) and ≥4 age group (%4.2). Seroprevalence was not significantly different between age groups ($p>0.05$). Seropositivity rates obtained by c-ELISA were %5.7 in Holstein breed, %5.1 in Montofon breed, %4.5 in cross-breed and %3.6 in Simen-

tal breed. Seroprevalence was not significantly different between cattle breeds. (p>0.05).*Neospora caninum* infection according to reproductive anamnesis was evaluated, antibodies to *N. caninum* were found in 8 of 49 aborted animals (%16.3), in 4 of 58 infertil animals (%6.9) in 3 of 48 pregnant animals (%6.3) and in 6 of 245 healthy animals (%2.4). The prevalence of *N. caninum* was significantly higher in the aborted cows than in non-aborted cows. There was a significant difference (P < 0.01) in the seroprevalence of *N. caninum* between aborted cows and healty animals.

4. Conclusion: Considering the results of this research, the presence of *N. caninum* in Burdur region has been revealed and the fact that *N. caninum* should not be ignored especially in abort cases has been emphasized.

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		Examined (no)	Infected (no)	Prevalence (%)
Origin				
	Merkez	50	1	2
	Ağlasun	50	4	8
	Bucak	50	2	4
	Çavdır	50	2	4
	Karamanlı	50	1	2
	Kemer	50	2	4
	Gölhisar	50	4	8
	Yeşilova	50	5	10
Breed				
	Holstein	283	16	5.7
	Simmental	56	2	3.6
	Montofon	39	2	5.1
	Crossbreed	22	1	4.5
Age (years)				
	≤2	47	3	6.4
	2-4	91	7	7.7
	≥4	262	11	4.2
Reproductive anamnesis				
	Aborted	49	8	16.3
	Infertil	58	4	6.9
	Pregnant	48	3	6.3
	Healthy	245	6	2.4
Total		**400**	**21**	**5.3**

Table1. Seroprevalance of *Neospora caninum* in Cows in Burdur Region with regard to origin, breed, age and reproductive anamnesis.

EPIDEMIOLOGY OF MYCOPLASMA (M.) BOVIS AND M. ALKALESCENS INFECTIONS IN A SLOVAKIAN DAIRY HERD

M. Kargl[1], K. Schmidt[1], I. Prunner[2], M. Drillich[2], J. Spergser[1]

[1]Institute of Microbiology, University of Veterinary Medicine, Vienna, Austria; [2]Clinical Unit of Herd Health Management for Ruminants, University of Veterinary Medicine, Vienna, Austria

Summary

The present study aimed at evaluating the epidemiology of *M. bovis* and *M. alkalescens* infections in a Slovakian dairy herd displaying chronic mastitis. For this purpose, MLVA was applied to *M. bovis* and *M. alkalescens* isolates to elucidate their genetic relatedness in order to trace routes of infection and dynamics of dissemination. From the investigated dairy herd a total of 3705 milk samples were collected at different time points in 2012-2014. Mycoplasmas were isolated and identified using cultural procedures and a total of 61 *M. bovis* and 38 *M. alkalescens* isolates were selected for genotyping. Between 2012 and 2014, *M. bovis* and *M. alkalescens* were recovered from 216 and 199 milk samples, respectively. Isolation of both pathogens was reduced over the study period due to control measures. MLVA revealed a unique allelic profile for the majority of *M. alkalescens* isolates while genotyping of *M. bovis* recovered in 2012 generated heterogeneous profiles separating isolates into one main and 11 minor groups. In 2013 and 2014, only *M. bovis* isolates representing heterogeneous profiles were present. MLVA results indicate the introduction and spread of a single *M. alkalescens* strain throughout the herd. Genotyping of *M. bovis* suggests and a clonal emergence of a unique *M. bovis* subpopulation in 2012 and multiple sources of infection over time. Control measures reduced the prevalence of both mycoplasma species but were unable to eliminate the pathogens.

Introduction

Mycoplasma (*M.*) *bovis* is the most important etiological agent of bovine mycoplasmosis predominantly associated with therapy-resistant mastitis and calf pneumonia responsible for considerable economic losses in the cattle industries. In contrast, *M. alkalescens* has only been occasionally reported as cause of bovine mycoplasmosis but recent recurrent isolations in the absence of other pathogens suggest that it may constitute an emerging pathogen. In order to characterize the epidemiology of *M. bovis*, several genotyping methods have been applied for intra-species differentiation including Multiple Locus Variable Number of Tandem Repeat (VNTR) analysis (MLVA). By addressing genetic micro-variations between isolates, MLVA has shown to be a simple but reliable technique for the surveillance of *M. bovis* epidemiology. For *M. alkalescens*, no molecular typing methods have been developed so far. With the sequenced genome of a French isolate, *M. alkalescens* has recently turned amenable to MLVA for bacterial typing.

Materials and methods

From the investigated dairy herd (approx. 2500 cows) a total of 3705 milk samples were collected at different time points in 2012-2014. After the initial screening of milk samples for mycoplasmas in 2012 (n=1588), groups consisting of mycoplasma-positive individuals (Mpos) strictly separated from the remaining mycoplasma-fee herd (Mneg) were created and rigorous management including controlled animal movement was implemented to minimize the risk of disease transmission. Mycoplasmas-positive cows were culled if economic breakeven points were reached. In 2013, only milk samples from the Mneg herd were investigated (n=1654), followed by a re-examination of the Mpos group (n=108) and examination of selected animals from the Mneg herd (n=355) in 2014. Bovine mycoplasmas were isolated from samples and identified to the species level using cultural procedures and PCR-RFLP. A total of 61 *M. bovis* and 38 *M. alkalescens* isolates were selected for genotyping and the type strains of both pathogens were included as references. For *M. bovis*, a previously described MLVA typing scheme was employed (Spergser et al., 2013). Prior to the study, MLVA for *M. alkalescens* comprising 5 selected VNTR loci was established based on the genome sequence of a French *M. alkalescens* isolate. VNTR profiles of both *M. bovis* and *M. alkalescens* were recorded as character data using allelic profiles and dendrograms were constructed using neighbour-joining. In addition, median-joining networks for inferring *M. bovis* intra-species phylogeny were performed.

Results

Between 2012 and 2014, *M. bovis* and *M. alkalescens* were recovered from 216 (5.8%) and 199 (5.4%) milk samples, respectively. Co-infections were found in 47 milk samples (1.3%) solely present in 2012. Isolation of both pathogens from milk samples was reduced over the study period. MLVA revealed a unique allelic profile for all *M. alkalescens* except for two isolates exhibiting a single discrepancy in one VNTR locus. Genotyping of selected *M. bovis* recovered in 2012 (n=51) generated heterogeneous profiles (n=12) separating isolates into one main group comprising 34 isolates, two minor groups each containing 4 isolates, and 9 singletons. In 2013, only singletons were present (n=5) from which two exhibited profiles found in 2012. Genotyping of *M. bovis* recovered in 2014 (n=5) revealed 3 singletons (one isolated from the Mpos group) and two indistinguishable isolates, the latter descending from a singleton present in 2013.

Discussion

In the present study, the prevalence and epidemiology of *M. bovis* and *M. alkalescens* infection in a Slovakian dairy herd has been evaluated using cultivation procedures and molecular typing employing MLVA. Overall, intervention strategies including strict separation of reagents, controlled animal movements, and culling reduced the prevalence of both agents within the herd but failed to eradicate the pathogens. Using a newly established MLVA method for intra-species differentiation, a remarkable dissemination and persistence of a single *M. alkalescens* strain within the herd was evident. In contrast, genotyping of *M. bovis* isolates recovered at the initial examination in 2012 generated heterogeneous profiles suggesting multiple sources of infection. However, the majority of *M. bovis* isolates from 2012 clustered into one group exhibiting a unique and indistinguishable profile indicating a clonal emergence and spread of a unique *M. bovis* subpopulation within the chronically infected herd. Following strict control measures this unique *M. bovis* strain was successfully eliminated from the herd replaced by heterogeneous types recovered in 2013 and 2014 highly suggesting new introductions of *M. bovis* from several sources into the herd over time.

Conclusions

MLVA results indicate the introduction and spread of a single *M. alkalescens* strain throughout the Slovakian dairy herd. In contrast, genotyping of *M. bovis* suggests multiple sources of infection, the predominance of a single unique strain in 2012, and the introduction of new strains into the herd in 2013 and 2014. Control measures reduced the prevalence of both mycoplasma species including the predominant *M. bovis* strain but were unable to eliminate the pathogens from the herd.

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Animal hygiene and food quality and safety

NUTRITIVE VALUE OF EGYPTIAN SALTED FISH

H.Y. Ahmed[1], A.M. Abd- Ellah[1], R.S.Refaiey[2], M.H. Mohamed[2]

[1]*Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Egypt*
[2]*Animal Health Research Institute, Assiut, Egypt*

- **Summary**

This paper describes investigations the nutritive values (moisture, fat, protein, Ash, carbohydrate, cholesterol, energy and salt contents) as well as chemical indicators spoilage (total volatile base nitrogen content and thiobarbituric acid value) of commercially Egyptian salted *Hydrocynus forskalli* and *Alestes baremose*. Mean percentages of moisture, fat, protein, ash, carbohydrate & sodium chloride were 56.29 & 52.05, 5.57 & 8.29, 22.29 & 21.73, 15.39 & 17.39, 0.44 & 0.42, 14.54 & 17.57 in salted *Hydrocynus forskalli & Alestes baremose*, respectively. Mean content of cholesterol was 14.13 mg/100g & 23.42 mg/100g in the salted fish species, respectively. Energy values (kcal/100gm) were with a mean of 140.71& 164.29 in the two species, respectively. Indicators spoilage were detected with a mean values as 34.36 mg/100g &37 mg/100g for total Volatile Base Nitrogen (TVB-N) and 0.11 mg/ MDA/Kg & 0.11 mg/ MDA/Kg for thiobarbituric acid (TBA) value in the examined salted species, respectively. The statistical analytical results pointed that there was a significant difference ($p<0.01$) in moisture, fat, ash, sodium chloride between salted *Hydrocynus forskalli* and *Alestes baremose*. Moreover, there was a significant differences variation in protein content & energy values ($p< 0.05$). On the other hand, no significant difference ($p<0.05$) in carbohydrate, cholesterol, total volatile Base nitrogen & thiobarbituric acid value between the Egyptian salted *Hydrocynus forskalli* and *Alestes baremose*. The present study concluded that the Egyptian salted fish of *H. forskalli* and *A. baremose* are source of protein content. Species of *A. baremose* have high content of fat, cholesterol than species of *H. forskalli*. Cholesterol content as well as TVB-N content and TBA values of the examined species lies within the acceptable limit.

- **Introduction**

Ripening of fish by salting goes back to ancient time and is a common tradition in some Mediterranean countries. During processing and storage of salt-dried fish several changes occur in fish muscle texture and chemical composition. Several researchers (El-Sheshnagui, 2006; Egbal, *et al*. (2010), Abbas and Khogalie, (2012) investigated the nutritive value of commercial salted fish.

- **Material and Methods**

Forty two samples of salted fish *Hydrocynus forskalli* and *Alestes baremose* (21 of each species). The samples were collected randomly from salted–fish retail outlets in Assiut city, Upper Egypt.

Samples were prepared, and the nutritive values percentage (moisture, crude protein, ether extract, ash) were estimated according to AOAC (1995).

Total carbohydrate was calculated on dry weight basis according the following formula:

Total carbohydrate % = 100 - (protein % + fat % + ash %)

Determination of cholesterol, thiobarbituric acid (TBA) and total volatile base nitrogen "TVB-N" were estimated according to Pearson (1976), Ohkawa *et al*. (1979), Pasin *et al*. (1998).

Statistically analysis was done by using SPSS 20 (Scientific Package of Social Science) version and applied mean, standard deviation, correlation (Sperman correlation), student T-test and ANOVA test.

- **Results**

Table (1): Statistical analytical results of chemical composition%* of some Egyptian salted fish (n=21 for each species)

Constituents	Species	Min.	Max.	Mean ±SE	*P* value
Moisture	***H. forsaklli***	46.47	62.17	56.29 ± 0.79	0.001**
A.baremose	43.01	55.91	52.05 ± 0.61		
Protein	***H. forsaklli***	15.77	28.2	22.22±0.73	0.561***
A.baremose	18.92	26.77	21.73 ± 0.43		
Fat	***H. forsaklli***	1.86	8.37	5.57 ± 0.43	0.001**
A.baremose	3.92	15.45	8.29 ± 0.65		
Carbohydrate	***H. forsaklli***	0.30	0.77	0.44 ± 0.03	0.653***
A.baremose	0.32	0.63	0.42 ± 0.02		
Ash	***H. forsaklli***	12.80	18.60	15.39 ± 0.30	0.001**
A.baremose	14.2	19.95	17.39 ± 0.42		
Cholesterol	***H. forsaklli***	0.43	46.74	14.13 ± 2.79	0.139***
A.baremose	1.578	70.526	23.42 ± 5.49		
NaCl	***H. forsaklli***	12.29	18.42	14.54 ± 0.36	0.001**
A.baremose	14.04	23.11	17.56 ± 0.57		
TVB-N	***H. forsaklli***	233.8	499.8	343.36 ±17.87	0.219***
A.baremose	177.8	548.8	373±15.616		
TBA	***H. forsaklli***	0.007	0.228	0.108 ± 0.016	0.854***
A.baremose	0.005	0.432	0.114 ± 0.024		

*The results are average of duplicate analysis
** Significant difference ($p<0.01$)
*** No Significant difference ($p<0.01$)

- **Discussion**

Results recorded in table (1) pointed the chemical composition and spoilage indicators of *Hydrocynus forskalli* and *Alestes baremose*. The statistical analysis of moisture content, shown that there was a significant difference between *Hydrocynus forskalli* and *Alestes baremose* in moisture %, fat%, sodium chloride and ash% ($p<0.01$). This variation content may be attributed to species variation. Moreover, the two salted species showed no significant difference in protein and cholesterol contents ($p< 0.01$).

With respect to the chemical tests (TVB-N & TBA), they were indicated spoilage; it was found that there is no significant difference between *Hydrocynus forskalli* and *Alestes baremose* ($p<0.05$). The obtained data proved that 28.6% and 14.3% of the examined *Hydrocynus forskalli* and *Alestes baremose* were unacceptable, respectively, in which the TVB-N exceed 400 mg/100gm, and this may be attributed to longer storage period.

- **Conclusions**

Salted *Hydrocynus forskalli* and *Alestes baremose* are source of protein content. *Alestes baremose* have high content of fat, cholesterol and energy values than *Hydrocynus forskalli*. There are significant difference between *Hydrocynus forskalli* and *Alestes baremose* in moisture, fat, ash, values and sodium chloride. Cholesterol content of the examined species lies within the acceptable limit. Most of the examined fish have TVB-N content and TBA values within the acceptable limits.

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SENSITIVITY OF METHICILLIN-RESISTANCE AND METHICILLIN-SUSCEPTIBLE STAPHYLOCOCCUS AUREUS STRAINS TO DIFFERENT DISINFECTANTS

S. Kotb[1], M. Sayed[2]

[1]*Department of Animal Hygiene, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt*
[2]*Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt*

- **Summary**

This investigation was planned to evaluate the sensitivity of methicillin-resistance *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) strains to some different disinfectants. Eight common disinfectants were applied in concentrations as povidone-iodine 7.5%, Dettol 1%, quaternary ammonium compound 0.5%, MetriCide28 2.6%, AntisepticaCombi Surface 1%, formalin 0.5%, hydrogen peroxide 3% (10 v/v) and ethanol 70%. Minimal inhibitory concentrations (MICs) for the disinfectants against each microbe were determined. The obtained results of MICs% of the eight applied disinfectants against MRSA were 1.875, 0.6, 0.125, 0.325, 0.25, 0.0625, no sensitivity to hydrogen peroxide, 8.75, respectively. While, The results of MICs% against MSSA were 0.9375, 0.0375, 0.0156, 0.01015, 0.0625, 0.0156, 0.0937 and 1.0937, respectively.

- **Introduction**

The emergence of pathogenic microorganisms resistant to commonly used antibiotics is a worldwide concern of the 21st century. One of the most important bacteria in this regard is *Staph. aureus*, in particular its methicillin-resistant strains. Reemergence of diseases thought to be controlled or eradicated and the development of new drug-resistant organisms continue to be life and welfare threatening in all parts of the world **(Greene, 2006)**.

MRSA is now a huge burden in addition to MSSA for most healthcare institutions around the world and is by far the most significant antibiotic-resistant hospital acquired pathogen we have ever encountered **(EARSS, 2005)**.

- **Material and Methods**

Cultures: MRSA and MSSA strains were previously isolated and obtained from milk and its surrounding animal milking environment in the study of **Sayed and Kotb (2011)**, from 60 dairy cattle houses including 30 for cows and 30 for buffalos.

Refreshment of the cultured strains was done according to **Mazzola *et al*. (2009)**.

Inoculum preparation for MIC: The broth culture was incubated at 35-37°C until it achieved turbidity of the 0.5 McFarland standard as described in **NCCLS (1997)**.

MIC determination: This test consists of determination of disinfectants agent spectrum of action, according to resistance of the studied microorganisms. It was developed the determination of MIC for every disinfectants agent, through the classic method of 2 fold successive dilutions according to **Mazzola *et al*. (2009)**. The lowest concentration of test substance where there was no visible bacterial growth was defined as MIC.

Types of the used disinfectants

Disinfectant	Composition	Concentration
Povidone-iodine 7.5%	iodine 7.5% (equivalent to 0.75% available iodine)	7.5%
Dettol	chloroxylenol 4.8% (v/v)	1%
Quaternary ammonium compound	10% (w/v) benzalkonium chloride, mono quaternary mixture of alkylbenzyldimethylammonium chlorides	0.5%
MetriCide28	glutaraldehyde 2.6%	2.6%
AntisepticaCombi Surface	100g contained 8g glutaraldehyde, 5g benzalkonium chloride and 3g didecyl dimethyl ammonium chloride	1%
Formalin	formaldehyde 37%	0.5%
H_2O_2 (10 vol.)	H_2O_2 3%	3%
Ethanol 70%	ethanol 70% (v/v)	70%

- **Results**

MIC for disinfectants solutions to reduce MRSA and MSSA bacteria population over 6-$\log_{10}$

Disinfectant	MRSA (mg/l)	MRSA (%)	MSSA (mg/l)	MSSA (%)
Povidone-iodine 7.5%	18750	1.875	9375	0.9375
Dettol	6000	0.6	375	0.0375
Quaternary ammonium compound 0.5%	1250	0.125	156	0.0156
MetriCide 28	3250	0.325	101.5	0.01015
AntisepticaCombi Surface	2500	0.25	625	0.0625
Formalin	625	0.0625	156	0.0156
H_2O_2 (10 vol.)	-	-	937	0.0937
Ethanol 70%	87500	8.75	10937	1.0937

- **Discussion**

Although formaldehyde is a high-level disinfectant, the health-care uses of formaldehyde are limited by its irritating fumes and its pungent odor even at very low levels (<1 ppm) as well as its role as a suspected human carcinogen linked to nasal and lung cancer **(OSHA, 2002)**.

The obtained result of no effect of hydrogen peroxide against MRSA was nearly agreed with **VessoniPenna *et al.* (2001)** that may be attributed to presence of catalase or other peroxidases in these organisms can increase tolerance in presence of lower concentrations **(McDonnell and Russell, 1999)** or using lower concentration of hydrogen peroxide.

Suller and Russell (1999) reported that killing of MRSA strains was more delayed than killing of MSSA during exposure to some disinfectants. As well as, **Irizarry *et al*. (1996)** reported that overall, the MICs for some disinfectants were 5-10 times greater in the methicillin-resistant than in the methicillin-sensitive strains ($p < 0.001$).

- **Conclusions**

The examined MRSA and MSSA strains were sensitive to the different disinfectants applied except MRSA was not sensitive to hydrogen peroxide 3% (10 v/v), moreover, it was found that MSSA was sensitive than MRSA to all the applied disinfectants.

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INCIDENCE OF LEAD AND CADMIUM IN MARKET BUTTER AND CANNED GHEE

A. Abdel-Hameid[1], M. Sayed[1], Z. Zaky[2], W. Shaban[1]

[1]Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt
[2]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt

- **Summary**

This investigation was designed to detect and/or measure lead (Pb) & cadmium (Cd) in imported & locally butter and canned ghee products. A total of 90 random samples were collected from Assiut, Egypt; and examined using ZEEnit 700P Atomic Absorption Spectrophotometer with Graphite Furnace Unite (AASG). The obtained results revealed mean values of Pb were 0.2909±0.0397, 0.5869±0.0868, 0.4542±0.0902 and 0.7674±0.0864 ppm for the imported butter, locally made butter, imported canned ghee and locally canned ghee samples, respectively. While for Cd, mean values were 0.00348±0.002, 0, 0 and 0.0134±0.0091 ppm for the examined samples, respectively. The mean values of Pb in unpacked and packed imported butter samples were 0.3746±0.0521 and 0.1654±0.0417 ppm, respectively, while for Cd were 0.0053±0.0033 and 0.00068±0.00068 ppm, respectively.

- **Introduction**

Dairy cattle may be more susceptible to the accumulation of Cd & Pb than beef cattle **(Alonso *et al.,* 2003)**. While, post-milking contamination were from processing equipment, reagents, accidental contamination during storage and marketing and leaching from containers **(Ukhun *et al*., 1990)**.

Pb and Cd are among the most abundant heavy metals and are particularly toxic. The excessive content of these metals in food is associated with etiology of a number of diseases **(WHO, 1992 & 1995)**.

- **Material and methods**

Sampling: A total of 90 samples were divided as:

Butter samples: 60 random samples of imported and locally-made (30 samples each). The locally made (cooking butter) were collected from different villages of Assiut, Egypt. The imported samples were obtained from different groceries in Assiut as packed (12 samples) and unpacked (18 samples).

Canned ghee samples: 30 random samples of imported and locally (15 samples each). The samples were obtained from supermarkets in Assiut, Egypt.

Samples preparation: All glassware were washed (before use) with distilled water, soaked in nitric acid (30%), then rinsed in de-ionized water and air dried. Each sample (following weighing) was transferred into a clean digestion flask.

Digestion procedures: Each sample (0.5 g) was treated with 5 ml nitric:perchloric acid mixture (4:1 v/v) as described by **Kolmer *et al*. (1951)**. A blank (without sample) was prepared in the same manner.

Pb & Cd analysis using AASG (Perkin-Elmer Atomic Absorption Spectrophotometry model 2380, USA)**:** The analysis was carried out in the Central Laboratory of Faculty of Veterinary Medicine, Assiut University, Egypt.

Quantitative determination: Pb & Cd concentrations were calculated according to the equation described by **Horwitz (2000)** as C = R x D/W

C = concentration of heavy metal (mg/kg) wet weight (ppm)

R = reading of element concentration on digital scale of Atomic Absorption spectrophotometer

D = final volume of prepared sample in ml

W = weight of the wet sample

Statistical analysis: General linear model (G.L.M) of **S.A.S. (2001)** was used. The significance differences between the means treatment of procedure were tested by Duncan Multiple Range Test **(Steel and Torrie, 1982)**.

- **Results**

Pb & Cd concentrations (ppm) in the examined samples

sample (no.)	element	positive samples no. (%)	min. value	max. value	mean value	samples no. > permissible limit (%)
imported butter (30)	Pb	24 (80)	0.14	0.65	0.2909±0.0397	24 (80*)
imported butter (30)	Cd	4 (13.3)	0.0083	0.056	0.00348±0.002	1 (3.3**)
locally made butter (30)	Pb	29 (96.6)	0.074	1.86	0.5869±0.0868	29 (96.6*)
locally made butter (30)	Cd	0 (0)	0	0	0	0 (0**)
imported canned ghee (15)	Pb	12 (80)	0.093	0.9	0.4542±0.0902	12 (80*)
imported canned ghee (15)	Cd	0 (0)	0	0	0	0 (0**)
locally made canned ghee (15)	Pb	14 (93.3)	0.226	1.3	0.0864±0.7674	14 (93.3*)
locally made canned ghee (15)	Cd	2 (13.3)	0.099	0.102	0.0134±0.0091	2 (13.3**)

* according to **CE Regulation (2001)**, **Codex Standard for food grade Salt (2006)**
** according to **EOSQC (1993)**
0 represents non-detectable value (ND)

Mean value±SE (ppm) of Pb & Cd concentrations in the examined samples

Cd	Pb	sample (no.)
0.00068±0.00068	0.1654^{b}±0.0417	packed imported butter (12)
0.0053±0.0033	0.3746^{a}±0.0521	unpacked imported butter (18)
0.00348±0.002	0.2909^{c}±0.0397	imported butter (30)
0	0.5869^{d}±0.0868	locally made butter (30)
0	0.4542^{e}±0.0902	imported canned ghee (15)
0.0134±0.0091	0.7674^{f}±0.0864	local canned ghee (15)

$^{a, b}$ means significantly different (p< 0.05, ANOVA, Duncan's test)
$^{c, d}$ means significantly different (p< 0.05, ANOVA, Duncan's test)
$^{e, f}$ means significantly different (p< 0.05, ANOVA, Duncan's test)
0 represents non-detectable value (ND)

- **Discussion**

The acceptable permissible limit of Pb was 0.02 ppm by **CE Regulation (2001)**, **Codex Standard for food grade salt (2006)**, and for Cd was 0.05 ppm by **EOSQC (1993)**.

Presence of Pb & Cd in the imported butter samples may be due to the atmospheric condition during processing and packaging procedures; therefore, the mean value of Pb & Cd in the unpacked samples was higher than that in the packed ones. **Cabrera *et al.* (1995)** found that the ceramic or plastic containers used for dairy products can release Cd or metals**.**

- **Conclusions**

The examined imported butter and ghee samples were better than the local ones in its Pb & Cd contents.

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FATE OF LEAD, CADMIUM AND ALUMINUM RESIDUES DURING PROCESSING OF SOME MILK PRODUCTS

A. Abdel-Hameid[1], M. Sayed[1], Z. Zaky[2], W. Shaban[1]

[1]Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt
[2]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt

- **Summary**

This investigation was done to study the fate of some heavy metal residues during manufacture of some milk products. Buffalo's milk and its manufactured milk products were analyzed for estimation of lead (Pb), cadmium (Cd) & aluminum (Al) using ZEEnit 700P Atomic Absorption Spectrophotometer with Graphite Furnace Unite (AASG). The results showed 82.86, 90.3 & 89.55% of Pb, Cd & Al, respectively, were removed in skim milk during cream manufacture; 0, 20.38 & 78.86% of Pb, Cd & Al, respectively, were drawn into butter milk during butter manufacture; ghee produced from such butter caused reduction in Pb, Cd & Al levels by 17.03, 3.07 & 28.15%, respectively, than found in that butter; Kareish cheese produced from such skim milk contained 45.26, 18.64 & 71.36% of Pb, Cd & Al, respectively, and comparatively lower than that skim milk.

- **Introduction**

Pb, Cd & Al in cow's milk are of interest because of their toxic nature **(Munoz and Palmero, 2004)**, and they are readily transferred through food chains and are not known to serve any essential biological function **(Liu, 2003)**.

The principle source of Al daily intake is milk and dairy products as 36% **(Biego e*t al.,* 1998)**. Al cooking utensils are widely used and in the food industry, hence the intake of Al from such utensils is of great concern **(Lopez *et al*., 2000)**.

- **Material and methods**

Samples

Bulk milk: A total of 20 kg of market buffalo's milk was obtained from Assiut, Egypt; and then divided equally as 10 kg for test milk and another 10 kg as control milk.

Test milk: After thoroughly mixing of the 10 kg test milk, 1 ml was taken to determine the existing heavy metals under investigation.

Test milk products: The rest of 10 kg test milk was subjected to manufacture milk products. From each of the manufactured milk products and by-products, 1 g and/or ml was taken as a test sample.

Control milk & milk products: After thoroughly mixing of the 10 kg control milk, 10 ml (1000 mg/L) of each of standard stock solutions of heavy metals (Merck, K GaA, 64271 Darmstadt, Germany) of lead (Pb), cadmium (Cd) and aluminum (Al) was added. That is means 1 mg/kg of each metal was added to the milk (1 ppm), then, 1 ml was taken as a standard milk sample (control) and the rest was subjected to manufacture milk products.

Manufacturing of milk products: Milk products were manufactured in the milk products laboratory of Faculty of Agriculture, Assiut University, Egypt.

Samples preparation: All glassware were washed (before use) with distilled water, soaked in nitric acid (30%), then rinsed in de-ionized water and air dried. Each sample (following weighing) was transferred into a clean digestion flask.

Digestion procedures: Each sample (0.5 g) was treated with 5 ml nitric:perchloric acid mixture (4:1 v/v) as described by **Kolmer *et al*. (1951)**. A blank (without sample) was prepared in the same manner.

Metal analysis using AASG (Perkin-Elmer Atomic Absorption Spectrophotometry model 2380, USA)**:** The analysis was carried out in the Central Laboratory of Faculty of Veterinary Medicine, Assiut University, Egypt.

Quantitative determination: Metal concentrations were calculated according to the equation described by **Horwitz (2000)** as C = R x D/W

C = concentration of heavy metal (mg/kg) wet weight (ppm)

R = reading of element concentration on digital scale of Atomic Absorption spectrophotometer

D = final volume of prepared sample in ml

W = weight of the wet sample

- **Results**

Fate of elements% during milk products manufacture in relation to initial milk (100%)

Al% control	Al% test	Cd% control	Cd% test	Pb% control	Pb% test	milk product
1.64	1.52	8.86	8.76	4.08	3.65	cream
98.03	89.55	88.53	90.30	92.78	82.86	skim Milk
0.14	0.15	6.35	6.08	3.07	2.89	butter
1.44	1.19	1.89	1.78	0.89	0.00	butter milk
0.00	0.00	5.39	5.23	2.01	1.43	ghee
0.12	0.04	0.63	0.19	0.87	0.49	morta
71.52	63.90	17.33	16.84	42.91	37.50	Kareish cheese
14.97	5.30	64.94	66.58	47.45	33.21	whey

- **Discussion**

Curdling of cheese increase Cd concentration compared to initial raw milk and this may be due to its preference for binding to casein and fat **(Coni *et al*., 1999)**.

Concentration of Al in the test milk was 5 ppm which was above the reported by **WHO (2007)** (Coni *et al*. (1996) observed an increase in Al level after cheese salting.

- **Conclusions**

Metals are released by more than 80% into skim milk, with different distribution of metals between whey and Kareish cheese; and there was a reduction in heavy metals in fat milk products especially ghee and most of heavy metals escaped to Kareish cheese. So, it advisable during manufacturing a contaminated milk, to manufacture fat milk products especially ghee as it showed a lower concentration of heavy metals than other milk products like cheese.

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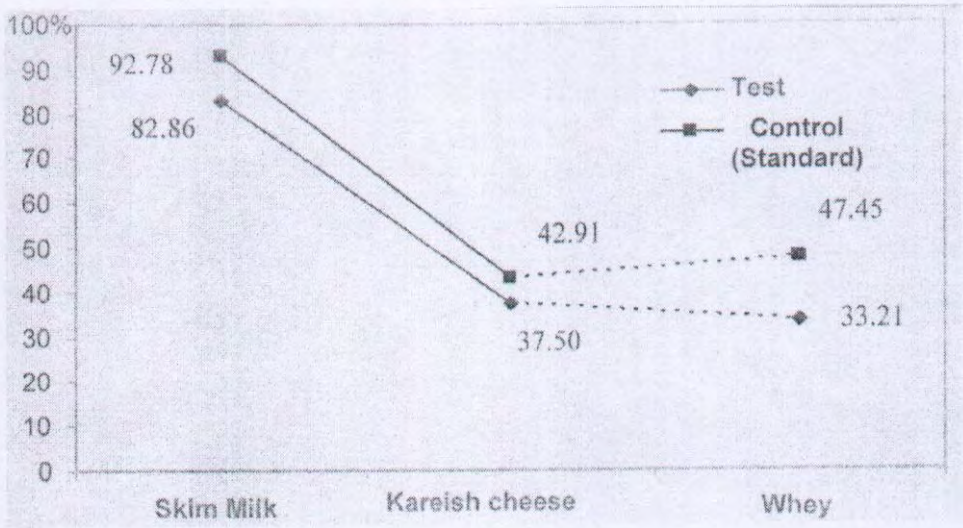
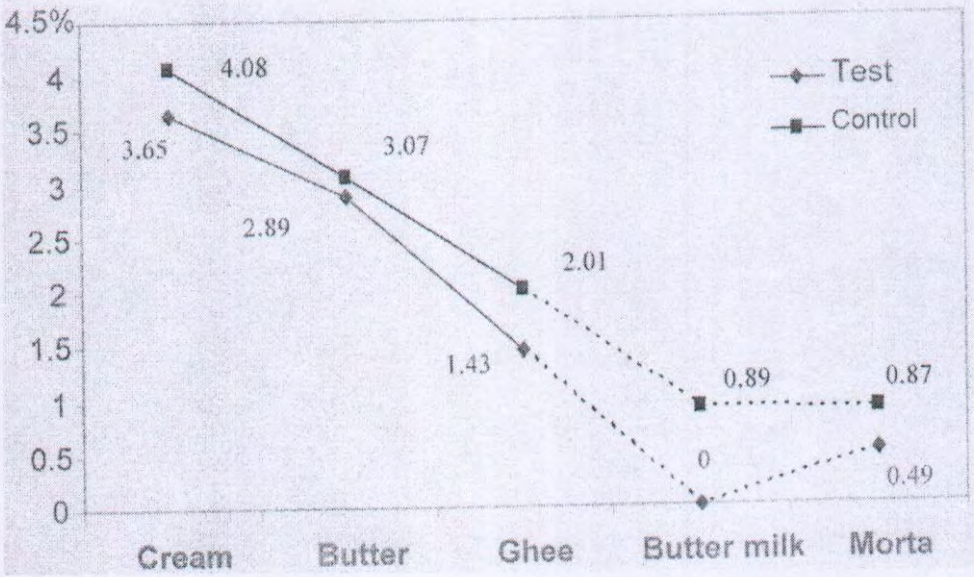
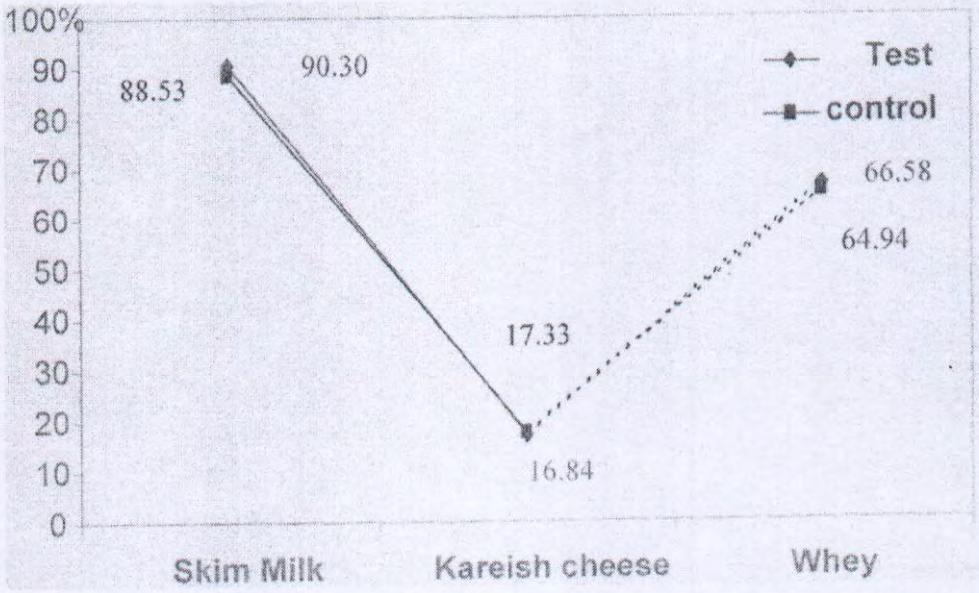


Fate of Pb% during kareish cheese manufacture in relation to initial milk



Fate of Pb% during butter manufacture in relation to initial milk



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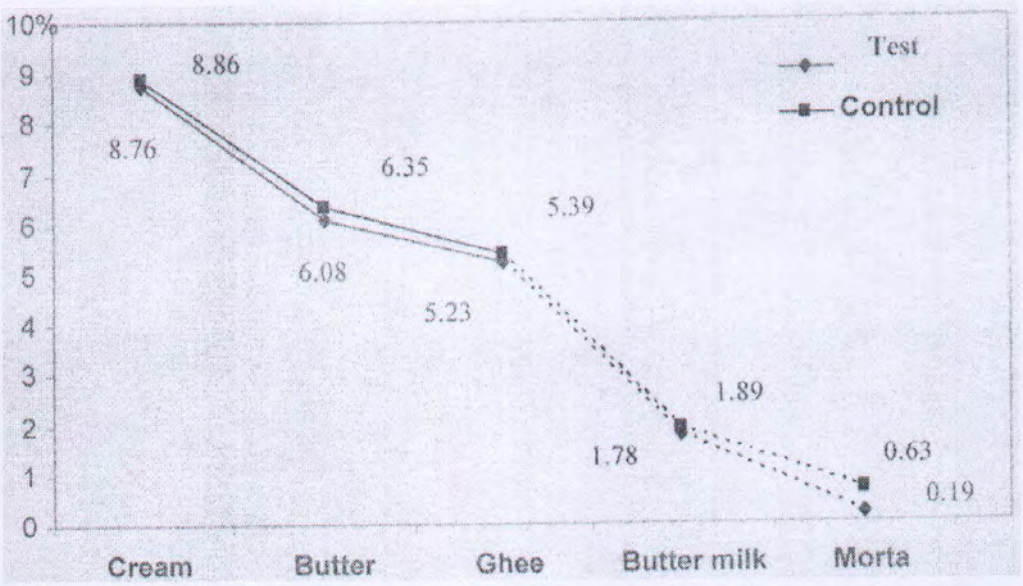

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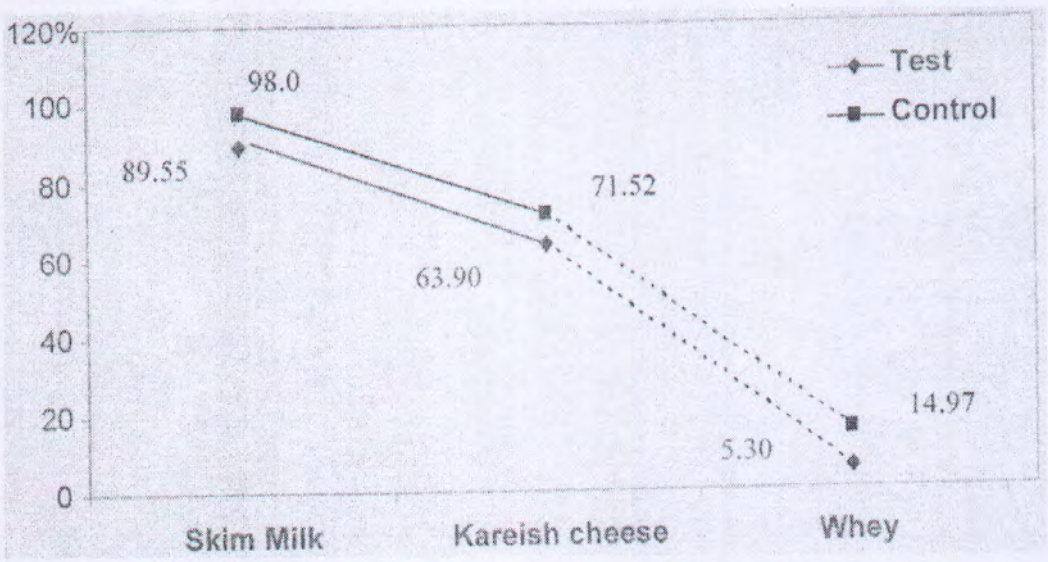
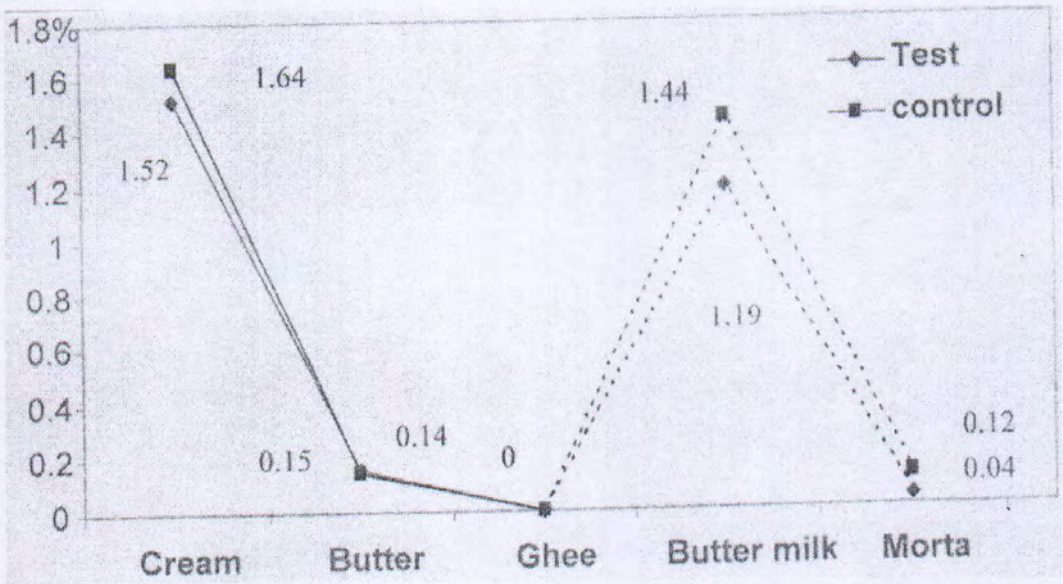


Fate of Al% during Kareish cheese manufacture in relation to initial milk



Fate of Al% during butter manufacture in relation to initial milk

ACTIVITY AND COOPERATION OF THE NATIONAL FOCAL POINT WITH EFSA

M. Kuzmiak Theiszova, Z. Birosova

National Focal point for scientific and technical matters for EFSA, Food Safety and Nutrition Department, Ministry of Agriculture and Rural Development of the SR, Bratislava, Slovak Republic

Summary

Focal points in MSs play an important role of EFSA's ambassadors. In general the Focal Points support their Advisory Forum Members in the practical implementation of activities related to networking and scientific cooperation, including ensuring the exchange of scientific information between national authorities and EFSA, supporting competent organisations under Article 36 of EFSA's Founding Regulation and EFSA's scientific networks, promoting the Expert Database and cooperation at national level and raising EFSA's scientific visibility and outreach in MSs. Activities of Focal point in the Slovak Republic, which is appointed at the Ministry of Agriculture and Rural Development of the SR, are focused mainly on the coordination of national risk assessment including the data collection, strengthening of scientific cooperation with scientific research organisations, organising of scientific conferences, workshops and trainings in issues of the food safety, publishing in the field of EFSA's remit and risk communication.

Introduction

General food law of EU Regulation (EC) No. 178/2002 of the European Parliament and of the Council appoints European Food Safety Authority (EFSA) as the competent authority for the scientific risk assessment and the risk communication in the whole food chain at the EU level. To accomplish its mission, EFSA strengthens the cooperation with the Member States (MSs) through the national networking of EFSA's Focal points. In the Slovak Republic the EFSA's Focal point is appointed at the Food Safety and Nutrition Department of the Ministry of Agriculture and Rural Development.

Material and methods

To fullfill all Focal point's tasks the cooperation with experts from research, control and practice is needed. Therefore, Focal point established 25 national scientific expert groups in which more than 340 experts are involved. These groups partly copy EFSA's scientific panels and they partly copy Codex Alimentarius working groups as well. Having the experts from different areas concentrated in one place, it gives us a uniform view on the issues of the food chain safety.

The main activities of Focal point are focused on the national risk assessments coordination, including the data collection and risk communication. Moreover the Focal point ensures the exchange of scientific information within national authorities, national authorities in the MSs and EFSA, promotes and raises EFSA's visibility at the national level, educates in the field of the risk assessment, as well as coordinates EFSA's scientific networks and publishes in the field of EFSA's remit.

Results

The main Focal point activities are the coordination of the national risk assessments and the risk communication. National risk assessment`s mandates are approved by the independent Committee of Food Safety and Nutrition and shared among Focal points in the MSs and EFSA, in order to avoid duplicity in this area. Risk assessment outputs, prepared by the external experts are publicly available for professionals, as well as for the consumers and the all interested parties at the Focal point website (http://www.mpsr.sk/index.php?navID=47&sID=111&navID2=494). Additionaly, risk assessment outputs are shared within FPs in MSs and EFSA. The most interesting outputs have been identified in the area of animal health and contaminants in the food chain (more than 2.000 clicks on published documents). From 2007 Focal point has coordinated the preparation of 65 national risk assessments. In 2015, the Committee of Food Safety and Nutrition approved 4 risk assessments that are going to be prepared in the areas of Biological hazards, Animal health, Plant health and Contaminants. To raise the level of the national risk assessments, Focal point has organised the national trainings for the experts and scientists. The lecturers have been chosen from the experts nominated for the trainings organised in the framework of the Commissions` Better Training for Safer Food (BTSF) programme. A successful set of five risk assessment courses (microbiological, chemical, pest risk

assessment, nutrition and GMO) has been delivered. Over 2015 – 2018, these five courses are going to continue and are going to be completed by three other courses (animal health, animal welfare and environmental risk assessment).

To be prepared for possible risks in the food safety area, the permanent exchange of scientific information within national authorities, national authorities in MSs and EFSA is necessary. Focal point in cooperation with the experts of 25 national scientific expert groups has processed more than 20 requests for information annualy. Most of them are focused on the issues regarding contaminants and nutrition.

EFSA's cooperation with partners in the Member States has been important since EFSA's inception. There is the interaction with many different organisations at national level. Strengthening of the national networking is fostered by the coordination of Scientific EFSA's Networks and Article 36 organisations. It is the task of the Focal Point to help to coordinate this interaction and to support scientific cooperation. EFSA's Focal point, that is appointed at the Ministry of Agriculture and Rural Development of the SR, Food Safety and Nutrition Department, coordinates nine Article 36 organisations and 20 experts in the 15 EFSA's Scientific Networks.

How to interpret the main conclusions of the risk assessment to all interested parties including consumers? That is the challenge for the risk communicators. Risk communication should be based on transparency, openness and independency and by this way to strengthen the consumers' confidence in the food safety. Therefore, Slovak Focal point cooperates with the national consumer associations in the area of the current food safety information, joint consumer surveys, education projects on the food safety for children and teenagers etc. Focal point has published opinions, recommendation, as well as informative leaflets for consumers.

Conclusions

Focal Points in Member States play important role for the strenghtening of scientific cooperation with EFSA. Only working together ensure that Europe's food is safe and strenghten the consumer confidence.

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THE ALTERNATIVE HOUSING SYSTEM FOR PIGS GROWING IN ORGANIC FARMS

V. Baliukoniene[1], B. Bakutis[1], J. Cerniauskiene[2], G. Zaborskiene[1], J. Jovaisiene[1], G. Gerulis[1]

[1]Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Department of Animal Nutrition, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

Summary: The aim of this study was to investigate cheap alternative housing system for pigs growing in organic farms in Lithuania and to analyze organically grown Lithuanian White breed pig's carcasses and meat quality traits.

Was established that an alternative housing system is fitted for regional Lithuanian Native and Lithuanian White pig breeds. The studies proved the exclusive quality of organically grown pork carcasses, higher pH level, however the way of growing had less influence on content and quantity properties of meat.

Keywords: organic pigs, keeping system, meat quality

Introduction: Based on national histories of organic pig production, diverse climatic conditions and national organic farming regulations, different housing systems are used for keeping pregnant and lactating sows in organic farms in European countries (Barton, 2002). The aim of organic pig production is to ensure high animal welfare and natural products. In Lithuania, pork quality is based on mixing and hybridisation of various breeds. Industrial pork producers are aimed at growing such pigs that grow fast and require less feed. However, old basic pig's breeds, such as Lithuanian White and Lithuanian Native, are best adapted to the environment and feeds of organic farms.However organic meat actually does not exist in the market, its traits are not researched in detail.

Material and methods: In 2011-2012 the organically growing pig technology was investigated in D. Vaitelio farm (Lithuania). Sudy was carried out with 25 pigs (one and two years of age) of Lithuanian White (org. LW) and Lithuanian Native (org. LN) breeds. Was evaluated pigs keeping technology, feeding and watering technological parameters, litter size, piglets viability, daily gain, health.

Were analyzed quality indicators of meat from org. LW breed pigs.In meat samples, moisture, general fat, hydroxyproline, nitrogen, and pH levels were analyzed. Protein, collagen, connective tissue amount, cholesterol, fatty acids were measured. Meat colour was determined in linear contrast colour space. Water holding capacity of samples was investigated evaluating their boiling and defrosting loss. Texture properties of samples were evaluated.

Results: Sows were mated in January and were kept in pig-house to April. From April to November pigs were kept on perennial grassland. Sows farrowed in May-June. In 2011 the number of live born piglets was 9±1.2 per litter, in 2012 - 8±2.0. Born piglet weight was about 1.5 kg. 2-months age piglet weight was average 15 kg. During study period fell 2.0% piglets due to injuries. In August 70% piglets have been selling. The remaining piglets were kept together with sows. The piglets (weight 50.0±10.0 kg) were transferred to pig-house for fattening to 100-120 kg. The average daily weight gain was about 500-600 g. In December when pigs were 95.0 ± 5.0 kg, were selected 30 breeding gilts and in January gilts was mated. Fatting pigs were slaughtered.

Before farrowing sows were fed grain mixture (50% triticale, peas and 25% to 25% of oats) and perennial grass haylage. Lactating sows were watered and fed individually grain mixture and pasture grass and weaned piglets were fed grain mixture.

One month after farrowing org. LW sows significantly lost weight than the org. LN sows. LN sow's constitution was stronger.

Org. LW breed pig's meatiness parameters and their comparison with LW and their crossbred pigs with 25% of blood of English Large White (ELW) breed pig's meatiness parameters according Jukna et al., 2010 are given in Table 1.

Studies of muscle chemical composition, pH, color, texture characteristics, technological characteristics (Table 2) have shown organic meat qualitative difference.

Meat indicators of org. LW significantly differed from traditionally grown ones (Jukna et al., 2010). General protein content was not significantly different in traditionally and organically grown meat of LW.

Organically grown pork samples contained biologically valuable isomers of conjugated linoleic acid and omega-3 alfa-linolenic acid. In organically grown meat, polyunsaturated fatty acids and saturated fatty acids were balanced respectively 0.19 in loin and 0.15 in ham. Organically grown meat of LW contained higher pH by 0.1-0.2 than traditionally grown pigs. Organically grown meat was less light (i.e. darker), colour coordinates differed only slightly. Instrumental texture analysis showed significant differences in texture. Average boiling loss was 35.00, while defrosting loss was about 2%; differences were minor.

Discussion: In Europe have developed different housing systems for pigs. In some countries sows are at pasture throughout all stages of pregnancy and lactation. In other countries most lactating sows are housed indoors during this time. Mixed indoor and outdoor housing systems can also be found. These systems allow combine the advantages of both housing systems. According to our observations, old basic pig's breeds, such as LW and LN, are best adapted to the environment and feeds of organic farms. However, according to many researchers, their meat quality is lower. Studies show that hybrid pigs containing various proportions of the English Large White breed (25%, 50% and 75%) have better feeding and meat quality properties than purebred LW (Jukna et al., 2010).

Fat at 10th rib of LW that had 25% of blood of English Large Whites was thinner by 6.4 mm, while that of org. LW was thinner by 6.45 mm than traditionally grown LW. The area of the longest back muscle, weigh of ham, and carcass muscularity were higher in organically grown pigs than that of traditionally grown Lithuanian Whites, when $P<0.001$.

In samples of organically grown ham, the average quantity of proteins was 21.8±0.15%, while in loin it was 22.2±0.9%. According to the data of G. Garmiene et al. (2010), loin of Lithuanian Whites contained 22.1% of proteins. Meat of org. LW contained higher pH level, which reached 5.65±0.060, than traditionally grown LW and hybrids with English Large Whites - respectively - 5.55 and 5.54 (Jukna et al., 2010). Organically grown loin was harder, more adhesive and more resilient than traditional loin (Garmiene et al., 2010).

Conclusions: Lithuanian White and Lithuanian Native breed's pigs growing in alternative housing system meet with requirements of organic animal husbandry and environmental diversity permitted better expression natural animal behavior with a positive influence on health. The studies proved the exclusive quality of organically grown pork.

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Table 1. **Organically grown Lithuanian White (org. LW) breed pig's meatiness parameters and their comparison with Lithuanian White (LW) and their crossbred pigs with 25% of blood of English Large White (ELW) breed pig's meatiness parameters according Jukna et al., 2010**

Parameter	100% LW	75% LW x 25% ELW	Org. LW
Longest back muscle area, cm^2	30.79±0.483	37.19±0.664***	37.29±0.341***
Ham weight, kg	10.87±0.087	11.28±0.055***	11.10±0.065***
Thickness of fat on ribs (at 10th rib), mm	23.25±0.658	16.83±0.464***	17.80±0.363***
Muscularity, %	48.37±0.488	52.96±0.582***	56.00±1.000***

***- P<0.001 (100% LW group compared with 75% LW x 25% ELW group and org. LW (old genotype) group.

Table 2. Pork ham and loin general fat amount, cholesterol, proteins, moisture, water holding capacity and pH analysis results

Parameter	Ham		Loin	
	X	*S*	*X*	*S*
General fat amount, %	4.40	2.17	4.60	1.70
Cholesterol amount, g/100g	85.60	4.67	73.95	3.32
Moisture amount, %	73.80	2.37	73.20	1.80
Proteins amount, %	21.80	1.45	22.20*	0.90
Collagen amount, %	0.75	0.09	0.72*	0.08
Collagen free meat proteins amount, %	20.20	2.55	21.30***	3.89
Connective tissue amount, %	3.70	0.35	3.00**	0.28
Water holding capacity, bound water, g / protein, g	2.30	0.07	1.70*	0.45
pH	5.65	0.06	5.60	0.05

*- P<0.05; **- P<0.01; ***- P<0.001

USING BIOLUMINESCENCE METHOD FOR THE MONITORING OF SAFE PRODUCTION OF MEAT PRODUCTS

K. Veszelits Laktičová, N. Sasáková, J. Venglovský, M. Ondrašovič, R. Hromada, G. Gregová, T. Čornejová

University of Veterinary Medicine and Pharmacy in Kosice, Department of Environment, Veterinary Legislation and Economics, Komenského 73, 041 81 Košice, Slovak Republic

Summary

In terms of rapidity of delivering results, examinations using classical microbiological methods fail to meet the requirements of practice which requires quick assessment of sanitation effectiveness or condition of production hygiene. The combination of permanent monitoring of hygiene standards and provided results of laboratory tests is optimal for the production within the time when it is possible, in case of negative findings, to make correction in order to prevent deterioration of a product. Nowadays, bioluminescence methods are becoming more and more popular. Bioluminescence methods are new and modern methods distinguished by their speed, simplicity, little time consumption, few personnel needed, easy handling, detection of present contaminants, and in particular, by possibility of immediate correction.

In practical conditions, good disinfecting effects of Topax 66 have been reported. Disinfection of particular surfaces was effective in all monitored rooms except for the swabs taken in the section room where we observed an increased total microbial and coliform germs counts present on a saw and a work desk after disinfection. These increased numbers of germs did not change even after the time until the start of production elapsed. The results obtained by determination of ATP also indicate contamination during and at the beginning of production. On the basis of the findings obtained by the use of bioluminescence method remedial actions were taken retrospectively.

Introduction

Sanitation is one of the most important functions in the meat industry. Production of meat products must be realised in clean, hygienically safe conditions. Insufficient cleaning and disinfection in the meat production premises has a negative impact on manufactured products.

Material and methods

The control of sanitation effectiveness in the meat plant was assessed and compared using two methods: classical microbiological and bioluminescence method. Swab samples were taken from various surfaces before disinfection, during production, and after disinfection, in three different rooms of meat plants: slaughter, section, and production. By using microbiological swabs taken from the surface of 10 cm² we determined the total microbial counts, coliform germs, and moulds. After 24 hours, we assessed the total microbial counts (TMC) after the their cultivation on MPA agar at 37°C. Coliform germs were assessed after 24 hours of cultivation on endo agar in thermostat at 37°C. Moulds were cultivated at 22°C during 5 days. To measure bioluminescence we used HY – LITE NG system from Biotrace company. Examination of air was conducted by the use of sedimentation method.

Results

Microbiological swabs and ATP swabs taken from the monitored technological devices in the slaughter room indicate that in the given room, disinfection was performed sufficiently (Table No.1). Hygienic level of surfaces before production was good. Depending on production, microbial counts increased during production process.

In the section room, we observed an increased TMC and coliform microorganisms present on the saw after disinfection. These values did not change even after the time until the start of production elapsed. The total microbial counts increased during production. The results obtained by determination of ATP indicate the contamination during production.

Table No.1 also shows results of microbiological and ATP swabs taken from technological devices in the meat production room. Hygienic level of surfaces before production was good. During production, we observed an increased TMC. ATP results correspond with contamination during production.

Place of swabs taking	Before production				During production				After disinfection			
	TMC	*Coliform germs*	Moulds	ATP	TMC	*Coliform germs*	Moulds	ATP	TMC	*Coliform germs*	Moulds	ATP
Slaughter room												
Desk	2	0	0	0	115	5	5	over	1	0	0	0
Saw	0	0	2	0	100	2	2	1700	5	0	1	0
Cleaning machine	0	0	2	0	22	1	0	5	10	0	0	0
Setae remover	6	0	0	0	240	3	1	1200	2	0	0	0
Section room												
Desk	150	5	0	over	152	7	0	over	0	2	0	900
Scales	2	0	0	0	34	1	1	700	1	1	0	700
Saw	300	5	0	over	300	10	8	over	75	2	1	900
Bolster	1	0	0	0	25	2	2	900	0	0	0	0
Production room												
Desk	0	0	0	0	15	0	2	0	1	0	1	0
Cutting machine No.1	0	0	0	0	250	2	1	900	0	0	0	0
Cutting machine No. 2	1	0	1	0	12	1	0	700	0	0	1	0
Stirring machine No.1	0	0	0	0	22	0	2	0	0	0	0	0
Stirring machine No.2	1	0	0	0	13	0	0	0	0	0	0	0

Table No.1, Average numbers of microorganisms present on particular surfaces and technological devices (CFU.10 cm^{-2}) and average numbers of ATP swabs (RLU.100cm^{-2}) in the slaughter room, section room, production room

Table No. 2 shows results of air examination in the observed rooms of the plant. Before production, the rare incidence of moulds and TMC was observed. During production, the total microbial counts present in the air increased in direct proportion to production.

Place of swabs taking	Before production			During production			After disinfection		
	TCM	*Coliform germs*	Moulds	TCM	*Coliform germs*	Moulds	TCM	*Coliform germs*	Moulds
Slaughter	2	0	1	15	47	14	3	0	1
Section	3	0	2	13	8	23	0	0	0
Meat production	5	0	2	10	9	20	0	0	2

Table No.2, Average numbers of air microorganisms in slaughter, section and meat production rooms (CFU in 1 m³ of air)

Discussion

Cleaning and disinfection as a part of everyday production practice are inevitable for proper food production (VOJTAŠŠÁK, 2003). Food production hygiene is one of the crucial factors in ensuring production of high quality and hygienically perfect products (STANGA, 2010).

In this study we focused on hygiene of particular premises in the meat processing plant by using classical microbiological swab method and ATP method. On the basis of the obtained results we can say that disinfection was effective in all monitored rooms of the plant except for swabs taken in the section room where we observed an increased TMC and coliform germs on the desk and saw after disinfection. These values did not change even after the time until the start of production elapsed. The results obtained by determination of ATP indicate contamination on the abovementioned surfaces. ATP shows the presence of any organic contamination which represents a substrate for multiplying of microorganisms. Higher values of ATP indicate either the presence of microorganisms or their potential culture medium (DOSTALEK and BRANYIK, 2005; LOPAŠOVSKÝ and POPELKA, 2006; Suková, 2002; CERESA and BALL, 2006; RAMSA, 2002; DOSTALEK and BRANYIK, 2005).

For ensuring a good quality of products, it is also needed to minimize the risk of possible contamination by germs present in the air of production premises. The results show that disinfection of air in the monitored meat processing plant was effective and it does not have any impact on production process.

Conclusion

The combination of permanent monitoring of hygiene standards and provided results of laboratory tests is optimal for the production within the time when it is possible, in case of negative findings, to make correction in order to prevent deterioration of the product, contamination of food processing premises or emergence of food borne diseases.

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GRAPE POMACE AS A FEED ADDITIVE

S. Sobolewska[1], J. Orda[1], M. Serowik[2], M. Rychlik[3], M. Świniarska[4]

[1]Department of Animal Nutrition and Feed Management, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland
[2]Institute of Agricultural Engineering, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland
[3]The Faculty of Apparatus and Process Engineering, Wroclaw University of Economics, Wrocław, Poland
[4]Department of Environment Hygiene and Animal Welfare, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland

Summary

Grape pomace has a high antioxidative activity. The dried wine grape pomace can be used for long-term storage. Grape pomace should be dried before adding to feed. The aim of this study was to investigate the influence of various drying methods on antioxidant potential of grape pomace. The potential antioxidative activity and concentration of polyphenols of grape pomace were investigated in the samples of Pinot Noir from Poland The concentration of polyphenols and antioxidative activity was measured in samples dried at 60°C with 6´100W. The antioxidant capacity of grape pomace detected by ABTS decreased with the increased of microwave power.

Eggs enriched in ω-3 fatty acids belong to functional foods. Feed additives such as linseed oil, which contains a high proportion of ω-3 fatty acids, causes changes in the profile of fatty acids in egg yolk (Foster et al 2009). However, the problem is a susceptibility to oxidation process due to the presence of double bonds in ω-3 fatty acids. The oxidation process induce the formation of peroxides and affects the worse quality of the eggs resulting in: unpleasant odor, deterioration in taste, texture and color. Toxic products such as free radicals and their degradation products are formed during the oxidation reaction (Drużyńska and Klepacka 2005.) Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole, which cause concern owing to possible carcinogenic effects, are widely used in animal feeds. Therefore, alternative methods are currently beeing investigated (Botterweck et al. 2000).

Grape (*Vitis vinifera*) is one of the largest fruit crops in the world. The estimated annual world production occurs more than 60 million tons (Abdelraheem et al. 2013). Grape pomace is the residue left after juice extraction by pressing grapes in the wine industry. It consists of peels, stems and seeds, and accounts approximately 20% of the weight of the grape processed into wine (Laufenberg at al. 2003). Recent investigations have stressed the importance of this by-product from wine processing as plant material particularly rich in a wide range of polyphenols (Yilmaz and Toledo 2006, Guendez at al. 2005). Grapes are rich in flavonoids, including monomeric phenolic compounds and dimeric, trimeric and tetrameric procyanidins (De Freitas at al. 1998). The concentration of polyphenols determines the antioxidant capacity to scavenge free radicals and terminate oxidative reactions (Yildirim at al. 2005, Rockenbach, et al. 2011). The dried wine grape pomace can be used for long-term storage.

The aim of this study was to investigate the influence of various drying methods on antioxidant capacity of grape pomace.

The potential antioxidative activity and concentration of polyphenols of grape pomaces were investigated in the samples of Pinot Noir from Poland. Samples were dried by convective and vacuum–microwave method. In this study, radical cation (ABTS - 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) were used for the measurement of total antioxidative activity of pomace. The differences between treatment means were evaluated using the Tukey test. Two levels of significance were presented ($P \leq 0.05$ and $P \leq 0.01$). All statistical analyses were performed with commercial software: Statistica 10 (2011).

The highest antioxidant capacity was measured in samples dried at 60°C with 6´100W. The concentration of polyphenols and the antioxidant capacity of grape pomace detected by ABTS decreased by 15% and 20%, respectively, with the increased of microwave power.

Polyphenolics are sensitive to heat and oxygen. We compared different temperatures of drying and different initial microwave power. Grape pomace with highest antioxidative properties was dried at 60°C with 6´100W. Larrauri at all (1997) observed also that drying at 60°C did not affect antioxidative properties of grape seeds.

Grape pomace is a valuable source of natural polyphenols and a powerful antioxidant. Polish grape pomace after properly drying can be used as a natural antioxidant to inhibit the lipid oxidation in feed.

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THE EFFECT OF Ω-3 FATTY ACIDS AND GRAPE POMACE SUPPLEMENTATION ON BLOOD PARAMETERS OF JAPANESE QUAILS

S. Sobolewska[1], J. Orda[1], M. Świniarska[2]

[1]*Department of Animal Nutrition and Feed Management, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland*
[2]*Department of Environment Hygiene and Animal Welfare, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland*

Summary

Diet enriched in ω-3 fatty acids reduce the risk of thrombosis, myocardial infarction and atherosclerosis. Nevertheless, ω-3 fatty acids are susceptible to oxidation processes which can be prevented by addition of antioxidants, like grape pomace, to the feed. The aim of our experiment was to investigate the effect of ω-3 fatty acids and grape pomace in quails diet on bird's blood parameters. Laying quails were divided into 4 groups: control (1), grape pomace (2), linseed oil (3), linseed oil and grape pomace (4). Blood samples were collected three months after experiment started. The addition of ω-3 fatty acids as well as natural antioxidants decreased ($P \leq 0.05$) the blood level of triglycerides (more than 40%). In group 4 was observed the higher level ($P \leq 0.01$) of alpha-2 globulins.

In human diet, the ratio of ω-6 to ω-3 fatty acids amounts about 15:1 but should range between 1:1 to 4:1 (Marciniak-Łukasiak 2011, Wongcharoen and Chattipakorn 2005). Many chronic diseases such as atherosclerosis, hypertension, and cardiovascular disease are caused by lowered intake of polyunsaturated fatty acids (PUFA) and an incorrect ratio of ω-6 to ω-3 fatty acids in human diet (Kubiński 2012). Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are essential for cell membrans and proper brain function (Achremowicz and Szary-Sworst 2005). Diet enriched in ω-3 fatty acids decrease blood pressure and degree of platelet aggregation. Furthermore reduce the level of triglycerides and low-density lipoprotein (LDL) in the blood. Increased amount of ω-3 fatty acids in human diet reduce the risk of thrombosis, myocardial infarction and atherosclerosis (Oh et al. 1991, Jiang and Sim 1993, Lewis et al. 2000, Kassis at al. 2012, Reiffel and McDonald 2006).

Nevertheless, ω-3 fatty acids are susceptible to oxidation processes because of the double bonds presence. Toxic products such as free radicals and their degradation products are formed during the oxidation reaction. Free radicals increase the aging process and cause cells and tissues damage (Drużyńska and Klepacka 2005). Oxidation process can be prevented by antioxidants addition to feed. Addition natural antioxidants to food reduce the level of triglycerides and LDL in the blood (Lien et al. 2008, Jung et al 2011, Ali et al 2012). In this experiment, we used grape pomace as a natural antioxidant and linseed oil as a ω-3 fatty acids source to enhance health properties of the animal quail model (Coturnix coturnix japonica).

The aim of this experiment was to investigate the effect of ω-3 fatty acids and grape pomace in quails diet on bird's blood parameters.

180 quails were divided into 4 groups: control (1), grape pomace (2), linseed oil (3), linseed oil and grape pomace (4). Blood samples were collected after three months from experiment beginning. Blood samples were used to determine a lipid profile, liver enzymes: aspartate aminotransferase (AST) and alanine aminotransferase (ALT), protein electrophoresis. The differences between treatment means were evaluated using the Tukey test. Two levels of significance were presented ($P \leq 0.05$ and $P \leq 0.01$). All statistical analyses were performed with commercial software - Statistica 10 (2011).

Supplementation of ω-3 fatty acids and grape pomace decreased ($p \leq 0.05$) the blood level of triglycerides in: 2 group: 13%, 3 group: 22% and 4 group more than 40% in comparison to control group. ALT and AST levels were normal in each group. No significant differences of total protein, albumins, alpha-1 and gamma-globulins blood level were observed between all groups. The level of alpha-2 globulins was higher ($p \leq 0.01$) in group 4. Albumin/Globulin ratio was similar in all groups.

The addition of ω-3 fatty acids as well as natural antioxidants had significant influence on blood lipid profile. Supplementation of ω-3 fatty acids and grape pomace in group 4 result the lowest blood level of triglycerides. ALT and AST

levels were proper which is the sign of healthy liver. The higher level of alpha-2 globulins in group 4 provides more effective immune defense in future infections.

Feed additives containing ω-3 fatty acids and antioxidants improve the health and the welfare of the quails, therefore, birds can be kept for a longer period of time.

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EFFECT OF A NEW ALGAL BIOPREPARATION ON EGG QUALITY PARAMETERS

M. Świniarska[1], M. Korczyński[1], S. Opaliński[1], S. Sobolewska[2], Z. Dobrzański[1]

[1] Department of Environment, Animal Hygiene and Animal Welfare, Wrocław University of Environmental and Life Sciences, Wrocław, Poland
[2] Department of Animal Nutrition and Feed Management, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

Recently, a need to search for innovative products containing naturally derived, animal-safe ingredients has been observed. Algae, being a rich source of bioactive compounds, may play the role of fodder additive. The aim of the present work is to investigate the effect of microalgae, microalgae extract and post-extraction residue supplementation on egg quality parameters.

The biomass of *Spirulina platensis* was subjected to innovative extraction with CO_2 in a supercritical state. The applicability of the preparations was tested on laying hens (Lohman Brown, 36 weeks of life), housing in the deep litter system. A total of 120 laying hens was divided into 4 groups (6 replicates in group): group I – diet supplemented with microalgae (powdered form), II – microalgae extract (water additive), III –post extraction residue (powdered form) and control group (C) - fed with the basal diet. Feeding experiment was conducted for 6 weeks and was divided into three series. The effect of the preparations on egg traits was examined. Also, a consumer questionnaire (regarding general appearance, texture of yolk and albumen, smell and taste of eggs) was undertaken to investigate the sensory characteristics of the eggs.

Obtained results demonstrated that microalgae extract had a favorable effect on eggshell strength (20, 10,5 and 5% higher than in the C, group I and III, respectively), egg weight (12% higher than in the C) and yolk color (increase in the yolk color intensity by 13% comparing with the C). Consumers indicated that eggs from microalgae extract group looked more appetizing, smelled more pleasant and were highest rated based on their texture but were less tasty than eggs from the other groups. Also, consumers indicated that microalgae positively affects the taste of eggs.

Summarizing, microalgae extract could be potentially used as an alternative for currently used feed additives in poultry feeding.

ANALYSIS OF BACTERIAL CONTAMINATION OF THE ENVIRONMENT ON DAIRY FARM

G. Gregová[1], J. Venglovský[1], T. Čornejová[1], N. Sasáková[1], K. Veszelits Laktičová[1]

[1]University of Veterinary Medicine and Pharmacy in Kosice, Komenského 73, SK-041 81 Košice, Slovakia

SUMMARY

The aim of this study was to investigate the bacterial contamination of dairy farm and rates of antibiotic resistance among *Escherichia coli* isolates.

We focused on monitoring of airborne microbial concentration on cattle farm which may present potential risk to the health of workers and affect the surrounding environment.

We measured also some microclimatic factors (physical, chemical), which can affect health, productivity and welfare of the housed animals. These factors affect the survival of micro-organisms on surfaces and in the air in animal houses.

Air samples were collected by means of MAS 100 Eco. After microbial examination of air in order to investigate the total count of bacteria, coliform bacteria and moulds, we evaluated the results in comparison with relevant physical (temperature, relative humidity) and chemical parameters and dustiness, which influence the aerial microbial concentration.

The air in animal facilities can be a reservoir of primary and potentially pathogenic or resistant micro-organisms involved in the etiology of infectious and allergic diseases. The highest risk results from resistant *Escherichia coli* with production of ESBL (extended spectrum beta lactamases), including cephotaximases (CTX-M), AmpC beta lactamases, carbapenemases (CPC) and plasmid chinolone resistance (PMQR).

INTRODUCTION

Micro-organisms and endotoxins belong to the prominent aerial pollutants in farm animal housings which have been linked with several production diseases and are assumed to pose a risk to the health of farmers and workers on the farms and to the neighbouring residential areas around intensive livestock enterprises (WATHES a CHARLES, 1994).

Bacterial antibiotic resistance is transferable by dust particles. If the resistant bacteria are allowed to distribute in the environment, they will most likely transfer resistance gene to other bacteria of the same or different species. Dissemination of these resistant bacteria will not be restricted to a particular geographical area; drug resistance can be expected to spread steadily to all parts of the Word (NIHAD ADNAN et al., 2013).

Hygiene of housing and good ventilation systems are important factors to be considered in intensive animal production. Ventilation systems in livestock housing serve an important function, maintaining a comfortable animal environment. Ventilation continuously removes the heat, moisture, and odours produced by livestock and replenishes oxygen by bringing in drier, cooler outside air. Adequate air exchange also removes gases such as ammonia (NH_4), hydrogen sulfide (H_2S) and methane (CH_4) which can be harmful to both animal and stockman health (MESCHER and VEENHUIZEN, 2003).

MATERIAL AND METHODS

The experiment was conducted at a commercial feedlot in Slovakia, holding an average of 200 dairy cows. The study was conducted over 4 weeks in April and May, 2014.

From animal houses were collected samples of organic materials (dust, excrements) and air. Samples of aerosols were collected by an air sampler MAS-100 Eco directly to Petri dishes with respective nutrient agars. In animal houses we also measured physical microclimate parameters and aerial dusiness. Minimal inhibitory concentrations (MIC) were determined according to CLSI 2013: VET01-S2. ESBL genes (CTX-M and CMY-2) were detected by PCR.

RESULTS

Average concentration of dust particles and airborne bacterias are pressented in Table 1. Dust concentrations were the highest during manipulation with straw beding and feeding operations (22.6-48 mg/m^3).

Total counts of airborne bacteria were high at all sampling sites even if the the dust concentrations were not very high ($8 \cdot 10^4$ KTJ·m^{-3} - $>10^5$ KTJ·m^{-3}). Coliform bacteria in the air ranged from $3 \cdot 10^3$ KTJ·m^{-3} to $3.3 \cdot 10^3$ KTJ·m^{-3} and moulds were present mostly at levels higher than 10^5 KTJ·m^{-3}.

Physical and chemical factors of microclimate can influence not only welfare, health and productivity of animals but also survival of micro-organisms in the air and persistance of dust particles in the air.

From the point of view of welfare the housing microclimate in the housing was suitable for the animals. Air velocity in animal houses was very variable due to the problems with ventilation system and abrupt temporary door ventilation. Concentrations of gases did not exceed the acceptable level (Table 2).

DISCUSSION

Dairy cows are comfortable at lower temperatures and show very small declines in milk production if properly fed, protected from wind, precipitation, and provided a comfortable, dry place to rest. If these buildings are closed too tightly during cold weather, moisture and gasses will accumulate, condensation can occur, and poor air quality will result. During hot weather conditions, a properly constructed dairy barn should act as a sunshade. Ventilation openings provide air movement past the animals to remove excess heat and reduce typical drops in milk production during extremely high temperatures (MESCHER and VEENHUIZEN, 2003).

Air microbial contamination is relating to dust concentration. Remaining dustiness increases amount germs in air. Dust supports agglomeration microorganisms, which are thus protects for adverse environmental conditions (THORNE a kol., 1992). In poultry houses the highest respirable dust concentrations (up to 10 mg/m^3 resp. 1.2 mg/m^3) were found, followed by pig houses (5.5 mg/m^3 resp. 0.46 mg/m^3) and cattle barns (1.22 mg/m^3 resp. 0.17 mg/m^3) (SEEDORFA and HARTUNGA, 2009).

Important role in environmental hygiene act especially cleaning, disinfection and effective air condition, which reduce microflora and decrease air recontamination (WATHES a CHARLES, 1994).

CONCLUSSION

Resistant microorganisms that survive in air in animal houses pose sometimes risk to animals and people particularly through exposure to infection resistant pathogens and related potential mortality and failure of therapy.

Place of sampling	Calves in milk nutrition	Dairy cows	Delivery room	Calves in vegetable nutrition
Microbial contamination				
TCB	$>10^5$	$7.3 \cdot 10^4$	$>10^5$	$>10^5$
CB	$1.5 \cdot 10^4$	$0.4 \cdot 10^3$	$1.75 \cdot 10^4$	$1.7 \cdot 10^4$
Moulds	$>10^5$	$1.64 \cdot 10^4$	$3.1 \cdot 10^4$	$5.2 \cdot 10^4$
Dust contamination				
Average	3.28 mg/m^3	0.068 mg/m^3	0.09 mg/m^3	0.37 mg/m^3
Maximum	48.7 mg/m^3	13.59 mg/m^3	17.04 mg/m^3	19.58 mg/m^3

Table 1 Average concentration of airborne micro-organisms and dust particles

Place of sampling	Calves in milk production	Dairy cows	Delivery room	Calves in vegetable nutrition
RH (%)	59 %-71 %	63,3%-82%	68.1 %-68,8 %	65 %-71,6 %
Temperature	16.3°C-19.5°C	16,8°C- 20.1°C	15.9°C-19.6°C	17°C-19,8°C
Dew point	8,3°C-13,1°C	10.0°C-14.2°C	1.1°C-13.4°C	10.6°C-13.5°C
Air velocity	0.3 $m \cdot s^{-1}$	0.5 $m \cdot s^{-1}$	0.8 $m \cdot s^{-1}$	0.4 $m \cdot s^{-1}$
Oxygen	20.9 %	20.9 %	23.2 %	22.7 %
NH_3	1 ppm	2 ppm	1 ppm	3 ppm
H_2S	0 ppm	0 ppm	0 ppm	0 ppm
CO_2	300 ppm	800 ppm	500 ppm	500 ppm

Table 2 Average values of physical and chemical microclimate parameters

In the environmental samples (including air) were detected high numbers of methicillin resistant *Staphylococcus aureus* strains and *E.coli* with the presence of ESBL genes (CTX-M and CMY-2).

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HONEYSUCKLE BERRIES AS A SOURCE OF BIOACTIVE COMPOUNDS IN POULTRY FEEDING – PRELIMINARY ASSESSMENT OF PRODUCTION PROCESS

M. Świniarska[1], A. Wojdyło[2], S. Opaliński[1], M. Korczyński[1], Ł. Bobak[3], S. Sobolewska[4], Z. Dobrzański[1]

[1]Department of Environment, Animal Hygiene and Animal Welfare, Wrocław University of Environmental and Life Sciences, Wrocław, Poland
[2]Department of Fruit and Vegetable Processing, Wrocław University of Environmental and Life Sciences, Wrocław, Poland
[3]Department of Animal Products Technology and Quality Management, Wrocław University of Environmental and Life Sciences, Wrocław, Poland
[4]Department of Animal Nutrition and Feed Management, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

Lonicera caerulea L. (blueberry honeysuckle, *Caprifoliaceae* is a rich source of health supporting biological active compounds that exhibit beneficial activities such as, antioxidant, anti-inflammatory and chemoprotective, thus they may be useful in the prevention of many chronic diseases.

The aim of the present work is to investigate the composition of nutritional properties in honeysuckle berries. It may vary depending on the production process. The obtained extract will be applied to laying hens feeding to enrich animal origin products in phenolic compounds.

Honeysuckle berries were hand-harvested at optimum ripeness in June from a private farming near Wrocław. Samples of fruits were dried using two methods: convection and lyophilization. Freeze-dried fruits were subjected to extraction with ethanol, to form an extract containing the valuable biologically active compounds. Polyphenols of blue honeysuckle in fresh fruits (FF), extract of freeze-dried fruits (E), post-extraction residue (PER), fruits after convection (CDF) and freeze-drying (FDF) were identified by LC-PDA-QTOF/MS and quantified by UPLC-PDA and UPLC-FL.

The obtained results showed that the main groups of phenolic compounds in honeysuckle were: anthocyanins, phenolic acids, flavonols and flavanols. Lyophilization is more appropriate method of drying fruits. Total amount of polyphenols in FDF was higher by approximately 68% compared with the CDF. Berries-based extract is the concentrate of biologically active compounds, especially anthocyanins. Phenolic content (mg/100g d.m.) in extract was remarkably higher than in others honeysuckle products (47,2, 81, 80,2, 93,6 % higher than in the FF, PER, FDF and CDF, respectively)

Honeysuckle extract may play the role of functional fodder additive in poultry feeding. This preparation could be potentially applied to enrich eggs in polyphenols to improve antioxidant activity and thus to produce functional food.

IMPROVEMENT OF KEEPING QUALITY OF RAW COW'S AND BUFFALO'S MILK BY ACTIVATION OF LACTOPEROXIDASE SYSTEM

A. Abdel-Hameid, M. Sayed, A.M. Fareid

Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt

- **Abstract**

This study was conducted to evaluate the effect of activation of lactoperoxidase system (LPS) on the keeping quality of raw cow's and buffalo's milk stored at ambient temperature. 50 random samples of freshly drawn evening cow's and buffalo's milk (each 25) were obtained from Assiut, Egypt. Each sample was aseptically divided equally into control and LP-activated (by adding 10:10 ppm SCN^-:$H_2O_2^+$), then all samples were kept at ambient temperature with periodical evaluation of keeping quality at 0 h, 12 h and every followed 3 hours till milk spoilage occurred. The obtained results showed pronounced difference as the average extension of shelf life of LP-activated milk was 5.52 h in cow's milk and 5.88 h in buffalo's milk.

- **INTRODUCTION**

It has been proved that small addition of naturally occurring substance in the lactoperoxidase system (LPS) would stimulate the indigenous antibacterial system in milk and considerably extend its shelf life time **(FAO/WHO, 1991)**.

- **Material and methods**

Activation of lactoperoxidase system (LPS): The 50 random samples of freshly drawn evening cow's and buffalo's milk (each 25) were aseptically divided equally into control and LP-activated according to **FAO/WHO (2005)**.

Keeping quality (K.Q.)

Sensory: Off flavor was tested according to **ADSA (2005)**. The sensory attributes including overall acceptability (OAA) were evaluated using age point hedonic rating scale (0-9).

Sanitary: Clot on boiling (COB) & alcohol precipitation test (APT): **FAO/WHO (1999)**

Bacteriological: Aerobic plate count: **APHA (1985),** coliforms, fecal coliforms & *E. coli* count (MPN/g): **AOAC (1990)**

- **Results**

Analysis of raw cow's milk samples milked at evening and then stored at ambient temperature (n=25)

test	treatment	accepted samples	0 h	12 h	15 h	18 h	21 h	24 h	27 h	30 h	33 h	36 h	39 h
COB	LP-activated	no.	25	24	24	24	23	21	18	15	6	1	0
COB	LP-activated	%	100	96	96	96	92	84	72	60	24	4	0
COB	control	no.	25	24	23	23	20	14	6	0			
COB	control	%	100	96	92	92	80	56	24	0			
APT	LP-activated	no.	25	24	24	24	21	21	16	10	1	0	
APT	LP-activated	%	100	96	96	96	84	84	64	40	4	0	
APT	control	no.	25	24	22	21	19	11	4	0			
APT	control	%	100	96	88	84	76	44	16	0			

Analysis of raw buffalo's milk samples milked at evening and then stored at ambient temperature (n=25)

test	treatment	accepted samples	0 h	12 h	15 h	18 h	21 h	24 h	27 h	30 h	33 h	36 h	39 h
COB	LP-activated	no.	25	25	25	24	22	20	14	12	7	2	0
COB	LP-activated	%	100	100	100	96	88	80	76	48	28	8	0
COB	control	no.	25	24	24	22	20	10	5	2	0		
COB	control	%	100	96	96	88	80	40	20	8	0		
APT	LP-activated	no.	25	25	25	24	21	20	11	8	1	0	
APT	LP-activated	%	100	100	100	96	84	80	44	32	4	0	
APT	control	no.	25	24	23	21	14	7	3	0	0		
APT	control	%	100	96	92	84	56	28	12	0	0		

Average reading of the examined cow's milk samples

36 h	33 h	30 h	27 h	24 h	21 h	18 h	15 h	12 h	0 h	treatment	test
	1	2	2	3	4	5	6	7	8	LP-activated	sensory
			2	3	4	5	5	6	8	control	sensory
5.6×10^6	4×10^6	4.7×10^6	4.4×10^6	4.5×10^6	4.3×10^6	4.1×10^6	4×10^6	3×10^6	6.5×10^5	LP-activated	TBC
			4.5×10^6	5.1×10^6	4.3×10^6	4.9×10^6	4.8×10^6	4×10^6	6.5×10^5	control	TBC
	8×10^2	8.62×10^2	7.42×10^2	5.62×10^2	3.76×10^2	2.62×10^2	2.36×10^2	2.78×10^2	3.5×10	LP-activated	coliforms
			7.20×10^2	8.72×10^2	6.39×10^2	4.43×10^2	2.53×10^2	2.96×10^2	3.5×10	control	coliforms
0.1×10	0.1×10	0.1×10	0.1×10	0.1×10	1×10	6×10	3.5×10	1.53×10^2	1×10	LP-activated	fecal coli-forms
			1.1×10	0.1×10	1.1×10	1.8×10	1×10	0.7×10	1×10	control	fecal coli-forms
0.1×10	0.1×10	0.1×10	0.1×10	0.1×10	1×10	1×10	3.3×10	1.57×10^2	0.1×10	LP-activated	*E. coli*
			1.1×10	0.1×10	1.1×10	1.8×10	1×10	0.5×10	0.1×10	control	*E. coli*

Average reading of the examined buffalo's milk samples

36 h	33 h	30 h	27 h	24 h	21 h	18 h	15 h	12 h	0 h	treatment	test
1	2	2	3	4	5	6	6	7	8	LP-activated	sensory
			2	2	3	5	6	7	8	control	sensory
4.8×10^6	3.5×10^6	3.5×10^6	3.4×10^6	2.8×10^6	2.2×10^6	2.4×10^6	1.9×10^6	1.6×10^6	1.2×10^5	LP-activated	TBC
		4.8×10^6	4×10^6	3.2×10^6	2.7×10^6	2.6×10^6	2.6×10^6	1.9×10^6	1.2×10^5	control	TBC
9×10^2	6.57×10^2	6.66×10^2	7.19×10^2	2.58×10^2	2.99×10^2	4.04×10^2	4.97×10^2	3.82×10^2	2×10	LP-activated	coliforms
			4.41×10^2	1.49×10^2	3.89×10^2	4.37×10^2	4.80×10^2	3.76×10^2	2×10	control	coliforms
0.1×10	0.1×10	0.1×10	0.6×10	.4×10	1.7×10	2.9×10	0.3×10	5.6×10	0.1×10	LP-activated	fecal coli-forms
		0.1×10	0.1×10	0.1×10	6.6×10	5.8×10	5.2×10	2.2×10	0.1×10	control	fecal coli-forms
0.1×10	0.1×10	0.1×10	0.6×10	0.4×10	1.7×10	2.9×10	0.3×10	5.6×10	0.1×10	LP-activated	*E. coli*
		0.1×10	0.1×10	0.1×10	6.6×10	5.8×10	5.2×10	2.2×10	0.1×10	control	*E. coli*

Summarized shelf life time of the control and LP-activated samples

shelf-life time (h) at ambient temperature	buffalo's milk control (h)	buffalo's milk LP-activated (h)	cow's milk control (h)	cow's milk LP-activated (h)
Min.	12	18	12	12
Max.	33	39	30	39
average	24.84	30.72	25.2	30.72
extension		5.88		5.52

- **Discussion**

It was noticed that the control samples spoiled earlier than the LP-activated; and that agreed with **FAO/WHO (2005)**.

- **Conclusions**

Thiocynate / peroxide added to milk have an effect on improving the keeping quality of milk kept at room temperature.

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Animal health, welfare and behaviour

PRODUCTIVITY ASPECTS OF THE HEN'S WELFARE UNDER SEMI-OPEN REARING DURING COLD PERIOD WITH ZN- AND VITAMIN C SUPPLEMENTATION

N.A.Bozakova

Faculty of Veterinary Medicine, Trakia University - Stara Zagora, Bulgaria

Summary

One way for poultry welfare improving in the moderate continental regions is the dietary supplementation of Zn or vitamin C. The aim of the present study was to monitor the effect of dietary 35 mg/kg zinc (Zn-group) and combination 35 mg/kg zinc and 250 mg/kg vitamin C (Zn+Vit.C-group) on some productive traits (egg production and live body weight) in DeKalb Brown hens during the cold winter period. Low ambient temperatures caused a significant stress to the hens, and influenced negatively the daily egg production(%) : Control -84.81±0.68; Zn-group - 86.39±0.76; Zn+Vit.C-group -87.42±0.74, and live body weight in hens (kg): Control -2.040±0.020; Zn-group - 2.13±0.010; Zn+Vit.C-group - 2.15±0.020.

The dietary supplementation of 35 mg/kg zinc and 35 mg/kg zinc + 250 mg/kg vit. C to experimental groups contributed to higher egg production and preservation of the live weight during the cold winter period. The supplementation with the combination had a superior effect on egg production and body weight because of the synergic stress-reducing effect of both compounds.

Keywords: laying hens, cold stress, productivity, zinc, vitamin C.

Introduction

The semi-open rearing of hens during the winter in subtropical and moderate continental regions is characterized with low ambient temperatures, which have a adverse influence on birds. The cold winter temperatures provoke thermal stress in hens and worsen their welfare. According to Lin et al. (2006), the response of birds to stress is mediated by activation of the hypothalamo-pituitary-adrenal system and is accompanied by a series of physiological and metabolic changes, which result in reduction of daily egg production and live weight of birds (Sahin et al. 2002; Sahin et al. 2005).

The use of dietary supplements in these regions during the cold months is economically profitable. For reduction of the adverse effect of cold stress, Nollet et al. (2008) recommend feed supplementation with zinc due to its antioxidant and anti-stress effect.

Others included vitamin C in the diet to alleviate cold stress (McDowell, 1989). Sahin et al. (2002) reported that the addition of 30 mg Zn/kg feed in laying hens submitted to cold stress (6.8°C) decreased blood corticosterone an improved egg laying performance.

The aim of the present study was to monitor the effect of supplementation of 35 mg/kg zinc and combination 35 mg/kg zinc and 250 mg/kg vitamin C on daily egg production and live body weight in hens in DeKalb Brown hens during the cold winter period.

Material and Methods

The experiments were performed with 885 female *DeKalb Brown* laying hens at the age of 38 weeks from 1 December 2011 to March 30, 2012 at a private poultry farm near Stara Zagora, Bulgaria.

The birds were reared under semi-open farming system and divided in 3 groups (n=295, ♀). Theywere placed in a semi-opening building 21x7 m. On the south side of the building there was a solid one-meter high wall, and protective metal mesh up to the roof of the building. The each group was located in a 7x7 compartment (49 m^2), 6.02 birds/m^2 (norm 8 birds/m^2 as per Regulation 44/2006). Each compartment was covered with 20 cm soft bedding of chopped straw and cobs and provided with 7 round feeders and 5 drinkers ensuring feeding - 4 cm and drinking – 2.8 cm widths (Regulation 44/2006).

DeKalb Brown hens from the first group were used as control (Control group). They were fed a commercial diet (2 842 kJ/kg metabolizable energy, protein 171 g/kg, crude fat 40 g/kg). The diet of the second group was supplemented with 100 mg/kg Zinteral 35 (Lohmann animal health, Germany) containing 35 mg/kg zinc oxide (Zn-group). The diet of third group was supplemented with the same amount of Zinteral 35 and 250 mg/kg vitamin C (L-acidum ascorbicum, CSPC Weisheng Pharmaceutical, China) (Zn + Vit. C-group).

Microclimatic conditions were determined by routine methods : ambient temperatures and humidity - 2 weekly thermohygrographs; air velocity - by a catathermometer, the light intensity - by a digital luxmeter; the concentration of ammonia – by indicator tubes.

Laying capacity was determined as: L egg = N egg / N hens=100%. **Live body weight** was determined with precision up to 0.001 kg.

The statistical processing of the results was performed by one-way ANOVA (GraphPad InStat 3.06) at level of significance $P<0.05$.

Results and Discussion

The average ambient temperature in the hen's living area was 5.67±0.50, i.e. substantially lower that the allowances of 18-25 ^{0}C for this category birds.

The evaluation of daily egg laying performance (**Fig. 1**) showed statistically significantly lower percentages in non-supplemented birds. Under the influence of Zn and Zn+Vit C, the average daily egg laying performance increased considerably in treated groups compared to controls.

The live body weight of birds (**Fig. 2**) was statistically significantly lower in control hens. Under the influence of Zn and Zn+Vit C, treated hens maintained their live weight with significant differences vs controls.

Birds supplemented with both zinc and vitamin C exhibited significantly higher daily egg laying performance ($P<0.05$; $P<0.001$), and higher live weight ($P<0.05$) during the cold period. Thus, the supplementation of 35 mg/kg zinc and the combination 35 mg/kg zinc and 250 mg/kg vitamin C improved daily egg production and live body weight in *DeKalb Brown* hens during the cold winter period, because of the synergic stress reducing effect of the both compounds.

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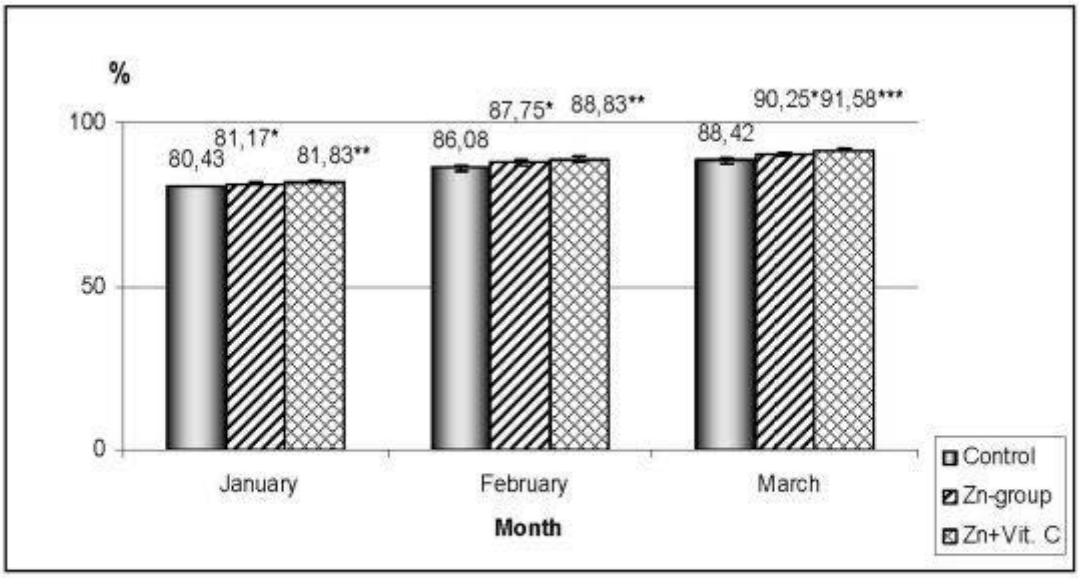

Fig. 1. Changes in daily egg laying performance of DeKalb Brown hens treated with Zn and Vit. C supplements. *P<0.05, **P<0.01, statistically significant difference between controls and experimental groups (Zn and Zn+vit. C).

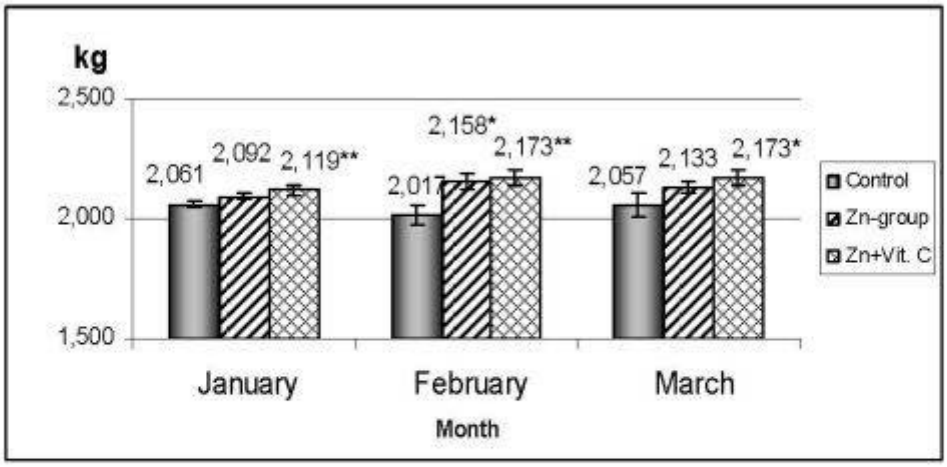

Fig. 2. Changes in live body weight of DeKalb Brown hens treated with Zn and Vit. C supplements. *P<0.05, **P<0.01, statistically significant difference between controls and experimental groups (Zn and Zn+vit. C).

THE USING OF ANIMAL NEEDS INDEX SYSTEM FOR ASSESSMENT THE WELFARE OF COWS IN DIFFERENT HOUSING CONDITIONS

J. Kottferová, E. Tracíková, L. Lešková, J. Kachnič, I. Miňo, R. Matos, A. Demeová, J. Mareková, L. Kottferová, J. Kišová

Department of Animal Hygiene and Environmental Protection, University of Veterinary Medicine and Pharmacy in Kosice, Košice, Slovakia

Summary

The aim of the study was to compare and evaluate the welfare of cows kept on two conventional and two organic farms using Animal Needs Index (ANI) system. Our total evaluation showed that two farms (No 1 and No 4) were included in the highest welfare category – one organic and one conventional, both with free housing of cows. The two farms (No 2 and No 3) that kept the cows tethered were rated as little and fairly suitable. Our experience with evaluation of farms by the system ANI 35L was very positive as the system is rapid and easy to use in practice.

Introduction

In Austria an „Animal Needs Index" – ANI („Tiergerechttheitsinindex" – TGI) has been in development since 1985 (Bartussek, 1999). In Germany a similar system with similar name was developed (Sudrum et al., 1994). The purpose of ANI was to assess welfare of cattle, pigs and laying hens focusing particularly on organic farms. ANI concentrates on housing conditions and on their influence on animal welfare. It includes several animal-based parameters also.

The aim of the study was to evaluate and compare welfare of cows on selected conventional and organic farms using the ANI system and to verify suitability of this way of welfare evaluation under practical conditions.

Material and methods

Animal welfare was evaluated on 4 farms in Slovakia, 2 conventional and 2 organic. The evaluated category were dairy cows and nursing cows without production of milk.

Farm No. 1 was a conventional farm. The mean number of dairy cows on the farm was 363. An open herd system was practised purchasing heifers in calf. Insemination was artificial. Evaluation included a house for dairy cows with free-housing system. The cows had access to a cattle-run but not to pasture.

Farm No. 2 was a conventional farm. The number of cows on this farm reached 220 on average. The cows were tethered and could graze on pasture approx. 200 m away from the house. The cows were inseminated artificially. There was a closed herd system on the farm with heifers kept in the herd and bulls transferred for fattening to another section of the farm.

Farm No. 3 was an organic farm with approx. 120 dairy cows in the herd. The cows were housed in two houses, they were tethered and no dehorning was practised on the farm. The animals grazed on pasture next to the farm. The insemination was artificial. There was a closed herd system on the farm with irregular replacement of culled animals.

Farm No. 4 was an organic farm. The were nursing cows without production of milk on the farm. T There was a natural mating system using bulls housed together with dairy cows. Approximately from half of May till half of November the animals were kept in pen-folds on pasture and for the remaining period they were housed using a free housing system.

Evaluation by the ANI focuses on five fields of influence, namely movement, social contact, quality of flooring, climatization and care of stockman (human factor).

The final ANI evaluation consisted of assigning points for relevant criteria which allowed us to classify the farms using a 6-category system of welfare: < 11 = not suitable with respect to welfare;11 – < 16 = scarcely suitable with respect to welfare;16 – < 21 = little suitable with respect to welfare; 21 – 24 = fairly suitable with respect to welfare; 24 – 28 = suitable with respect to welfare; > 28 = very suitable with respect to welfare.

Results and discussion

The results obtained allowed us to assess animal welfare on the respective farms as follows:

Farm No. 1:

Of the 4 farms evaluated by the ANI system this farm obtained the highest number of points – 35.0 from 45.5 points (76.92%) which corresponds to very suitable welfare. From the point of view of animal hygiene the housing facilities were the best. There was an ample housing space with sufficiently clean deep bedding. Animals did not graze on pasture but had access to a small (as evaluated by ANI) as well as large cattle run. The access to run was limited – depending on weather - and they had to share it with animals from the neighbouring object. In the small run there were heaps of hay which were are used by animals for resting and a non-typical form of comfort-behaviour as the animals tossed the hay up on themselves using either muzzle or head movements.

Farm No. 2:

This farm rated as the worst from among all investigated farms when using the ANI system - 18.5 points (40.66%) – and was included in the category little suitable with respect to animal welfare. Serious shortcomings were observed regarding the housing: cleanliness, slipperiness, insufficient lighting, draft. In 2007 the milk yield on this farm decreased down to less than 2000 l/year/cow. According to information that we were able to obtain this was caused by unsuitable and foul feed. This resulted in dramatic deterioration of health and subsequently also productive parameters. At the time of the experiment the milk yield varied around 3900 l/year/cow.

Farm No. 3:

The system ANI ascribed to this farm 21.5 points (47.25 %) which corresponded to the category fairly suitable. This was a surprising result as this was an organic farm. However, contrary to the requirements on housing on organic farms, the animals were tethered. This was, however, permitted by an exception according to Council Regulation No. 2092/91 on organic production. With regard to high incidence of agonistic behaviour (manifested by skin injuries) at second observation we assumed that hierarchy in this herd was not stabilised. This could result from shorter stay of animals in the cattle run than declared by stockmen or higher number of replacement animals related to occurrence of BSE and subsequent compulsory slaughter of 52 animals.

Farm No. 4:

Using the ANI system of welfare evaluation this farm reached 33 points (72.53%) and was included in the very suitable category. In comparison with the Farm 1 it lost some points for external cattle run as it did not comply with the criteria for area and the area of run was added to the internal area. Another shortcoming was the absence of shelter in pen-folds. Despite that the animals seemed composed and no pathological changes were observed. This was the only one of the four

Conclusions

Our total evaluation showed that two farms (No 1 and No 4) were included in the highest welfare category – one organic and one conventional, both with free housing of cows. The two farms (No 2 and No 3) that kept the cows tethered were rated as little and fairly suitable.

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COMPARISON OF FARM ANIMAL WELFARE LEGISLATION IN DIFFERENT COUNTRIES

I.Miňo, R. Matos, L. Lešková, E. Tracíková, J. Kachnič, J. Kottferová

Department of Animal Hygiene and Environment, University of Veterinary Medicine in Kosice, Komenského 74, Košice 041 81, Slovakia

Animal welfare is the well-being of animals. Concern for animal welfare is often based on the belief that non-human animals are sentient and that consideration should be given to their well-being or suffering, especially when they are under the care of humans. These concerns can include how animals are slaughtered for food, how they are used in scientific research, how they are kept (as pets, in zoos, farms, circuses, etc.) and how human activities affect the welfare and survival of wild species.

Animal Welfare Legislation in Europe

As regards to united Europe law, EU legislation on farm animals welfare has different levels and focuses. Some legislation is developed for all farm species, while other legislation is species specific. The Council of Europe produces Conventions, European Commission – Regulations and Directives. The role of the first type of document is to sketch policy guide for members countries, while second has to set minimum standards for animals. The main themes of Conventions are: protection of animals during international transports, stunning of animals before slaughter and the protection of animals kept for farming purposes. Most common are directives which laying down minimum standards for the protection of: Laying hens – Council Directive 1999/74/EC; Conventionally reared meat chickens – Council Directive 2007/43; Calves - Council Directive 97/2/EC amending Directive 91/629/EEC; Pigs - Council Directive 2001/88/EC amending Directive 91/630/EEC. EU. Community legislation concerning the welfare conditions of farm animals in Europe lays down minimum standards. National governments of European Union may adopt more stringent rules provided they are compatible with the provisions of the Treaty.

Animal welfare Legislation in USA

The USA welfare law is more complicated taking into account double legislation, state and federal adopted in this country.

Federal Laws

As regards to federal laws dealing with farm animals, they may be seen as insufficient. Federal Human Slaughter Act (1958) is one of a few. American Animal Welfare Act (1966) also does not regulate use of farm animals. In addition the U.S. has no federal laws protecting farm animals while they're actually on the farms where they are raised. Two federal laws cover farm animals during transport /1/ and slaughter /2/, but tragically, all poultry species are excluded, making these protections inapplicable to 95% of land animals killed for food.

1/ Transport: The 28-Hour Law requires that animals transported across state lines for slaughter, by means other than water or air, be unloaded every 28 hours for rest, food and water. In addition to excluding poultry, this law is riddled with loopholes.

2/ Slaughter: The Humane Methods of Livestock Slaughter Act (HMLSA) requires that livestock be quickly rendered insensible to pain before being slaughtered. In addition to excluding poultry, the law exempts certain forms of religious slaughter such as Kosher and Halal.

State Laws

The majority of U.S. states expressly exempt farm animals, or certain farming practices, from their anti-cruelty provisions, making it nearly impossible to provide even meager protections. Exemptions usually include common agricultural practices that, while common, are often shockingly cruel. Some states include farm animals in at least some of their anti-cruelty laws, such laws are rarely enforced in favor of farm animals. In state legislation there is visible tendency in recent yaers to amend earlier adopted anti-cruel legislation. This evolution leads to more elastic definition what is ´´accepted´´, ´´common´´,´´customary´´ and ´´normal´´ farm practice. It seems that there is strong opposition to animal welfare in various circles of American politics which resulted in limited legal farm animal protection.

Animal welfare organizations

World Organisation for Animal Health (OIE): The intergovernmental organisation responsible for improving animal health worldwide. The OIE has been established "for the purpose of projects of international public utility relating to the control of animal diseases, including those affecting humans and the promotion of animal welfare and animal production food safety."

World Animal Protection: Protects animals across the globe. World Animal Protection's objectives include helping people understand the critical importance of good animal welfare, encouraging nations to commit to animal-friendly practices, and building the scientific case for the better treatment of animals. World Animal Protection was founded in 1981. They are global in a sense that they have consultative status at the Council of Europe and collaborate with national governments, the United Nations, the Food and Agriculture Organization and the World Organization for Animal Health.

Non-government organizations

Canadian Council on Animal Care: The national organization responsible for overseeing the care and use of animals involved in Canadian Science.

Canadian Federation of Humane Societies (CFHS): The only national organization representing human societies and SPCAs in Canada. They provide leadership on animal welfare issues and spread the message across Canada.

The Canadian Veterinary Medical Association: Brings in veterinary involvement to animal welfare. Their objective is to share this concern of animals with all members of the profession, with the general public, with government at all levels, and with other organizations such as the CFHS, which have similar concerns.

National Animal Interest Alliance: An animal welfare organization in the United States founded in 1991 promotes the welfare of animals, strengthens the human-animal bond, and safeguards the rights of responsible animal owners, enthusiasts and professionals through research, public information and sound public policy. They host an online library of information about various animal-related subjects serving as a resource for groups and individuals dedicated to responsible animal care and well-being.

National Farm Animal Care Council: Their objectives are to facilitate collaboration among members with respect to farm animal care issues in Canada.

Conclusion

Farm animals have been and are an integrated and important part of human society. Animal husbandry is not of great economic interest, but also forms the basis of significant part of human culture and traditions. The quality of farm animal´s life depends on human care. This has become a matter of increasing concern in society, and is discussed in terms of animal welfare. Therefore we consider it necessary to continue in research in this area.

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MOVEMENT ACTIVITY OF COWS AND THEIR CALVES ON PASTURE

M. Šoch, J. Uherová, L. Zábranský, A. Šimková, K. Švejdová, B. Čermák, L. Smutný, Š. Smutná, I. Novotná

University of South Bohemia in České Budějovice, Faculty of Agriculture, Department of Animal Science, Studentská 13, 370 05, České Budějovice, Czech Republic

Abstract

Monitoring of physical activity of mothers and their calves in the herd of breed Czech Red took place was conducted in May and June in the Czech Budejovice.

Monitoring confirmed the knowledge about the natural behaviour of animals, such as maternal behaviour, grazing periods, playful behaviour of calves. It was verified that the movement of calves replicates the motion of mothers. With the increasing age of calves decreased gradually their physical activity, playful behaviour subsided and with gradual transition to self-herding increased the independence on mothers and the periods of rest and rumination became longer.

Keywords: *cattle grazing, cattle movement, cow, calf, pasture, vitalimeter*

1. Introduction

As TOUSOVA and STADNIK (2004) stated, behavioral research of wild animals confirmed that each species and breed of animal has certain regularities of daily routine, which is governed by the course of a season, by the daytime or by the developmental stage of each individual.

Cattle in the vast majority of situations move slowly step by step, which can reach the speed at most 5 $km.h^{-1}$. Younger and therefore lighter animals move more easily (SPINKA *et al.*, 2009).

KILGOUR *et al.* (2012) describes in five monitored herds a period of increased grazing in the early morning and late evening, and in one of these five herds he observed also an increased grazing in the middle of the day. This theory corresponds to claim VORISKOVA *et al.* (2001) that grazing during a day is divided into 3-4 periods. The first major period begins just before dawn and lasts 2-3 hours. The second major period starts after noon and ends at sunset. In the meantime, there are shorter periods of grazing, both in the morning and in the afternoon but this depends on temperature and the abundance of grazing.

In order to monitor movement activities of animals on pasture during the day various methods can be used, e.g. ethological monitoring, pedometers or vitalimeters (ALSAAOD *et al*., 2012; NIELSEN *et al*., 2010).

2. Animals, Materials and Methods

Monitoring of physical activity of mothers and their calves in the herd of breed Czech Red took place at the School agricultural farm of University of South Bohemia in the Czech Budejovice. A herd of about 30 cows without milk market production was reared on a pasture area of approximately 22 ha and cows with their sucklers were on the pasture all the year round. During the experiment specifically eight cows with calves were studied. Monitored calves were born in the months of April (3 heifers and 3 bulls) and May (2 bulls). Mothers of calves were cows in the first lactation (3 of them), 4 cows in the third lactation and one cow in the sixth lactation. To monitor physical activity of cows vitalimeters connected via receiving antenna on a notebook with the necessary software programs were used. Monitoring was conducted in May and June.

3. Results and Discussion

It was found that physical activity of the individual calves ran in approximately equal periods. Calves began to be active in the morning (between approximately 7 and 8 pm), another significant activity was evident around noon and in the evening. It can therefore agree with the statement of SPINKA *et al*. (2009), that a frequency of playful behavior in calves of cattle in the pasture reaches its peak in the morning before breastfeeding and in the afternoon before the start of grazing. There were no differences between physical activity of calves in terms of gender.

The oldest calf showed a lower physical activity during the day than the youngest calf.

There is a regular alternation of vital signs of mothers throughout a day. Animals showed the greatest activity at dawn and dusk, the smallest activity occurred in the middle of the night and also in the middle of the day (graf 1). This is confirmed by KILGOUR *et al*. (2012) and corresponds to VORISKOVA *et al.* (2001).

We confirmed the existing dependency between physical activity of mothers and their calves. The calculated value of the Pearson correlation coefficient r = 0.928 indicates that there is very tight weave between the number of movements of mothers and the number of movements of their calves in the different hourly intervals. From the course curves of both mothers and calves can be concluded that the movement of individuals in the herd corresponds with SPINKA *et al*. (2009).

Mothers moved occasionally also at night, while in the calves almost any movement was not recorded. In herd we could observe grazing periods followed by periods of low activity - rumination or resting. In calves playful behavior was manifested, especially in the afternoon. The curve of physical activity of calves roughly followed by their course the curve of the mother, only the number of movements reached in the calf lower values in comparison with the number of movements of its mother. Therefore, in this case no difference in the dependence of calves´ movement activities on the activity of their mothers was confirmed relative to the age of calf, which is proved also by two almost identical correlation coefficients 0.799 and 0.794. Presumption that the dependence of the calves on their mothers decreases with their increasing age would likely manifested itself only in case of a higher age gap of calves (graf 2).

It was also found that in the mother in the 6th lactation dependence of movements of the calf was in a closer relationship than in the mother - heifer.

4. Conclusion

Monitoring of physical activity of Czech Red cattle herd confirmed the knowledge about the natural behaviour of animals, such as maternal behaviour, grazing periods, playful behaviour of calves. It was verified that the movement of calves replicates the motion of mothers. With the increasing age of calves decreased gradually their physical activity, playful behaviour subsided and with gradual transition to self-herding increased the independence on mothers and the periods of rest and rumination became longer.

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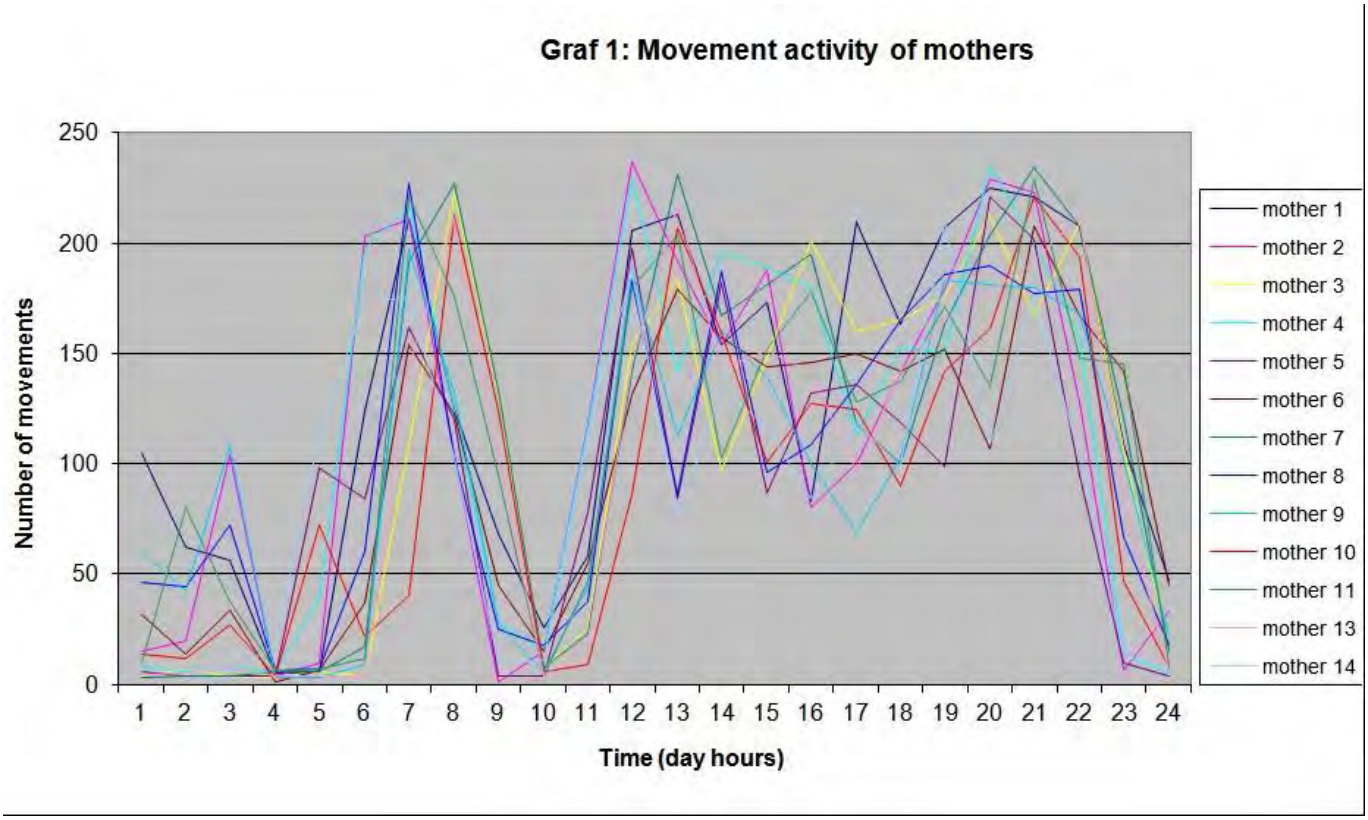
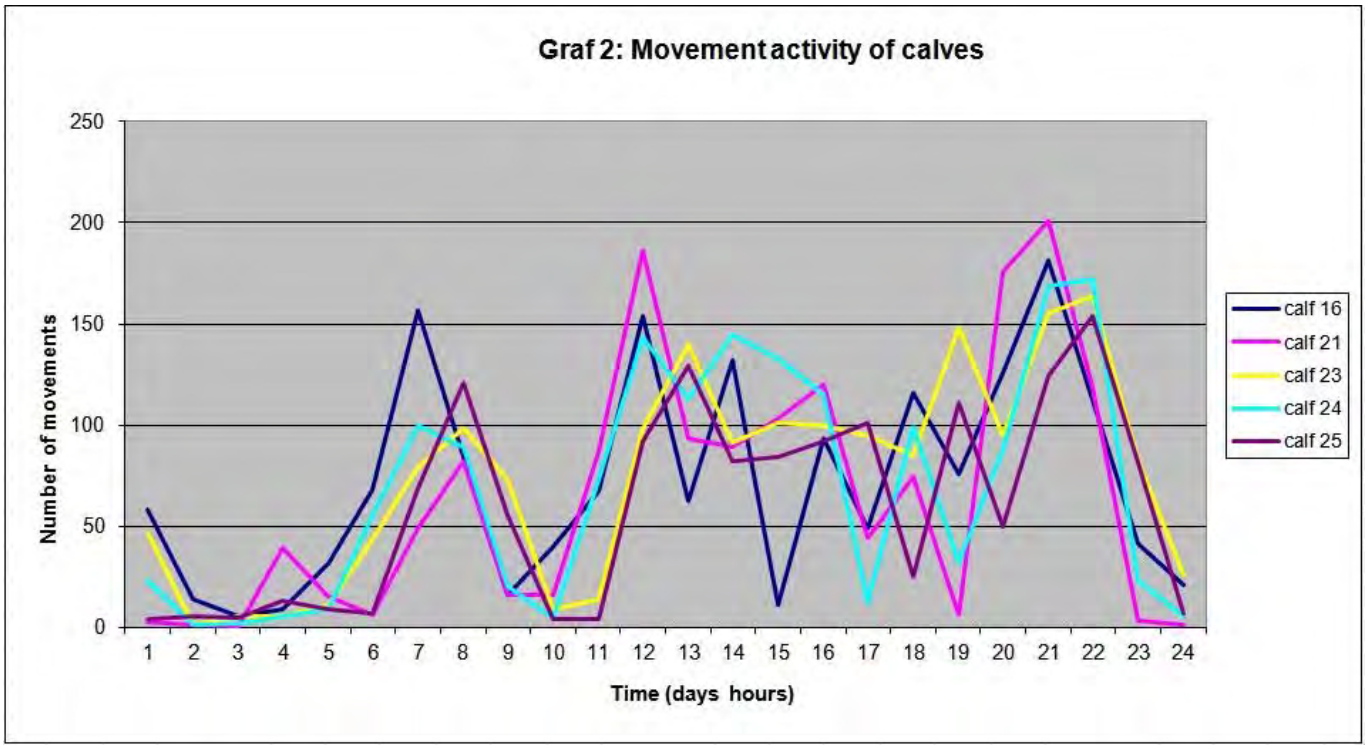

Graf 1: Movement activity of mothers
Number of movements
Time (day hours)
mother 1
mother 2
mother 3
mother 4
mother 5
mother 6
mother 7
mother 8
mother 9
mother 10
mother 11
mother 13
mother 14


Graf 2: Movement activity of calves
Number of movements
Time (days hours)
calf 16
calf 21
calf 23
calf 24
calf 25

EFFECT OF FEED SUPPLEMENTS ON THE HEALTH CONDITION OF CALVES

L. Zábranský[1]*, M. Šoch[1], A. Švarcová[1], P. Novák[1], J. Brouček[1], T. Frejlach[1], Z. Křížová[1], M. Staňková[1]

University of South Bohemia in České Budějovice, Faculty of Agriculture, Department of Animal Science, Studentská 13, 370 05, České Budějovice, Czech Republic

Introduction

Homeopathic remedies are based on plants, animals or mineral substances (Hektoen, 2001). Probiotics have been defined as live microbial supplements that beneficially influence the host's microbial equilibrium (Oelschlaeger, 2010). Prebiotic oligosaccharides stimulate the growth and colonization of probiotic bacteria beneficial for health (Rastall *et al*., 2002). Prebiotics are selectively fermented components facilitating specific changes in the large intestine, both in the composition and growth and in the activity of bacteria in the digestive tract (WANG, 2009).

Material a methods

An experiment was conducted on 45 experimental and 15 control calves that were divided into 4 groups – first *Lactobacillus sporogenes*, second *Ascophyllum nodosum*, third PVB homeopathic drugs for verminous conditions and fourth control. The first blood samplings were taken from day 3 to day 5 of age and the second samplings were done 21 days later. In *Lactobacillus sporogenes* group the calves were orally administered 1 pill/head/day that was dissolved in 2.5 litres of colostrum, later in milk replacer. *Ascophyllum nodosum* was applied orallywith 2.5 litres of colostrum and later with milk replacer at an amount of 5 ml/head/day. The third experimental group received "homeopathics" *per os* every day with 2.5 litres of colostrum and later with milk replacer when 20 ml of homeopathics per head/day that were mixed in water were added to milk replacer. The control group was administered a feed ration without any supplements.

Results and Discussion

In the table no. 1 is the obtained values in the blood of calves. In both samplings of *Lactobacillus* group and in the first samplings of control group and *Ascophyllum* group the haematocrit value was lower than the reference values reported by Bouda *et al*. (1984). Erythrocytes were low in all groups. According to Vrzgula *et al.* (1990) a decrease in the erythrocyte count can be caused by anaemia, haemoglobinaemia, dietary deficit of Fe, Cu, Co and proteins. The urea level was low in all measurements. Kraft *et al.* (1999) and Jazbec (1990) stated that the low values may be related with a decreased intake of proteins in milk. Cholesterol values were low in all measurements. Racek *et al.* (2006) stated that such a decreased level indicates malabsorption, which is an absorption disorder that may apply only to the process of absorption e.g. at a disorder of the intestinal mucosa, but it may also arise as a result of insufficient digestion. The values of zinc were low in the second sampling of all groups measurements. ENGLE *et al.* (1997) and JELÍNEK *et al.* (2003) concluded that the low level of zinc causes worse growth, loss of appetite, lesions on skin and mucous membranes, skin formations, weakened immune response, and for these reasons calves can be more susceptible to infectious diseases. SUCHÝ *et al.* (2011) reported that copper deficiency may cause the loss of hair pigmentation around the eyes, anaemia, diarrhoea and immunity disorder. Čermák *et al.* (2000) stated that phosphorus deficiency increases urinary calcium excretion and causes bone decalcification. Jelínek *et al.* (2003) reported that the low calcium values could cause many disorders of the health status of calves, especially disorders of skeleton growth and development, first of all rachitis. According to Slanina (1991) and Racek *et al.* (2006) calcium deficiency in calves is a result of the insufficient exogenous supply of calcium to the organism, resorption disorderin the small intestine, oxidized fat in milk replacers, incorrect Ca:P ratio (increased P), excessive magnesium intake, which was also found out in this experiment, and vitamin D deficiency in the calf organism.

Conclusion

The investigation of the efficiency of probiotic, prebiotic and homeopathic substances showed positive trends in some studied values of blood parameters in the blood of calves. But no statistically significant difference between control and experimental groups was revealed in any studied parameter, and so it is to conclude that in this experiment these substances did not have a significant influence on the dynamics of haematological and biochemical parameters in the blood of calves from 3 to 26 days of age.

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	Units	1st sampling L gr.	1st sampling A gr.	1st sampling H gr.	1st sampling C gr.	2nd sampling L gr.	2nd sampling A gr.	2nd sampling H gr.	2cn sampling C gr.
Haemoglobin	$g.l^{-1}$	↓ 89.52	101.63	100.11	103.30	105.65	109.09	109.53	111.18
Haematocrit	%	↓ 0.22	↓ 0.25	↓ 0.25	↓ 0.26	↓ 0.25	0.26	0.26	0.27
Erythrocytes	$T.l^{-1}$	↓ 4.46	↓ 4.95	↓ 4.78	↓ 5.05	↓ 5.36	↓ 5.79	↓ 5.56	↓ 5.68
Leukocytes	$L.l^{-1}$	7.37	7.11	6.84	7.31	9.54	7.98	8.46	8.20
Glycaemia	$mmol.l^{-1}$	↑ 6.52	↑ 6.17	↑ 6.08	↑ 6.50	↑ 6.38	5.35	6.03	5.65
Urea	$mmol.l^{-1}$	↓ 3.56	↓ 3.62	↓ 3.19	↓ 3.66	↓ 2.56	↓ 4.21	↓ 3.24	↓ 3.40
Alkalinephosphatase	$\mu kat.l^{-1}$	↑ 5.58	↑ 4.27	↑ 5.55	↑ 5.49	↑ 3.67	↑ 3.75	↑ 5.08	↑ 4.76
GMT	$\mu kat.l.1^{-1}$	5.70	4.00	4.57	4.18	0.53	↑ 4.88	0.66	0.75
Total protein	$g.l^{-1}$	↑ 70.65	↑ 69.30	↑ 68.97	↑ 73.37	↑ 69.13	↑ 66.46	↑ 68.15	↑ 68.80
Cholesterol	$mmol.^{-1}$	↓ 1.34	↓ 1.21	↓ 1.27	↓ 1.58	↓ 1.67	↓ 1.76	↓ 1.60	↓ 1.73
Zinc	$mmol.^{-1}$	22.46	20.01	26.33	21.50	↓ 20.87	↓ 14.23	↓ 20.43	↓ 21.19
Copper	$mmol.^{-1}$	13.17	16.64	13.79	12.83	↓ 14.23	↓ 12.86	↓ 14.59	↓ 13.24
Phosphorus	$mmol.l^{-1}$	↓ 2.26	↓ 2.24	↓ 2.1	↓ 2.22	↓ 2.33	2.49	2.53	↑ 3.67
Calcium	$mmol.l^{-1}$	2.56	2.48	↓ 2.42	2.64	↓ 2.46	↓ 2.45	↓ 2.27	↓ 2.45
Magnesium	$mmol.l^{-1}$	↑ 1.04	↑ 1.03	↑ 1.1	↑ 1.07	↑ 1.02	↑ 0.98	↑ 0.99	↑ 0.94

L –*Lactobacillus*group, A –*Ascophyllum*group, H – homeopathics group, C – control group

SELECTED BEHAVIOURAL INDICATORS FOR PAIN ASSESSMENT IN FARM ANIMALS

Lenka Lešková[1], Igor Miňo[1], Eva Tracíková[1], Lucia Kottferová[2], Alena Demeová[1], Ján Kachnič[1], Jana Kottferová[1]

[1]Department of Environment, Veterinary Legislation and Economics, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia
[2]Small Animal Clinics, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia

Recognition and assessment of pain and/or discomfort have played a crucial role in effective prevention and treatment of various ailments. In contrast with human medicine professions dealing with animal health and welfare must take into account much broader field of interest, which may lead to misunderstanding of animal needs, and resulting in frequent cases of unnecessary suffering.

Compared to human pain management recognizing pain in animals cannot be carried out by simple interview or subject's self-rating. Measuring of various responses to painful stimuli is carried out by parameters that may generally fall into three main categories: measuring of selected biochemical indices in blood serum/plasma, and saliva, such as e. g. corticoid hormones (however those may refer also to distress caused by another discomfort except of painful stimuli), monitoring of physiological parameters used for basic clinical examination (heart rate, enlarged pupils, etc.), and assessment of behavioural responses. The latter belongs to the least-researched issue that needs further investigation for further application in stable management. Behavioural signs of pain in most cases include changes in mood (communication, reactivity), changes in locomotion and comfort behaviour, as well as changes in digestive/elimination behaviour. Changes in vocalisation are frequently observed. All the given behavioural patterns may be impaired in the way of increased/decreased level.

According to Bracke *et al.* (2001) presence of goal-directed behaviour in animals may be noticed. It is characterised by motivational systems (or so called biological needs) that respond to the certain endogenous or external stimuli by sets of behavioural responses. Welfare has been defined as a state when primary, and secondary needs are met in accordance with normal behaviour. When subject is unable to satisfy those needs, we may question whether its welfare is sufficient or not. As stated by Chrousos (2000) such external stimuli as acute stressors (including noxious stimuli) stimulate the amygdala-sympathoadrenal and hypothalamic-pituitary-adrenal (HPA) axes to elevate plasma and brain levels of catecholamines and glucocorticoids resulting in enhanced arousal, appraisal, cardiovascular and cognitive performance in the situation of imminent danger. Painful stimuli thus influence behavioural patterns in different ways depending on their duration and intensity.

Behavioural signs that should serve as criteria for pain assessment are included in Tab. 1.

Advantage in monitoring of behavioural responses is little or no need of direct interaction with animals. However when assessing different types of pain in different species one has to consider it separately before generalisations are made about the value of particular sets of indices for assessment (Molony and Kent, 1997).

Assessment for pain or distress can be affected by many complicating factors including the age of the animal, degree of apprehension, the nature and frequency of human contacts, and control of visual, auditory, olfactory and tactile stimuli. As such, it is imperative that personnel involved in the care and use of animals are knowledgeable of normal behaviour patterns of the species with which they are working and are able to recognize changes from such normal patterns (PACUC Guideline, 2013).

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Tab. 1 Behavioural signs of pain

Altered behaviour	Sign	Note
Locomotion	Unwilling to stand up/lay down Reluctant to move Lameness/abnormal gait Shifting weight Rolling/jumping Pacing, circling Stomping Kicking stomach area Tail flagging	Avoiding contact with some types of beddings/flooring Limited/guarded use of affected limb Effort to contact with painful and/or itchy area to relieve pain
Posture	Rigid standing/stiffness Arched back Weight transfer off the affected limb (partial/total) Tucked abdomen Changed body profile Recumbency	Trying to avoid moving/making contact with a body part Freezing/trying to escape/attack when touched Abdominal pain During chronic painful processes change in muscle volume of affected body parts
Facial expression	Eyes rolling Lips curling	
Sweating		Excessive sweating is often associated with some types of pain but also another stressful stimuli
Comfort/resting	Rough/dull coat Dirty coat (mud, dust) Hair loss (local patches) Self-mutilation Altered sleeping patterns	Decreased/lack of self-grooming Intensive licking/biting/scratching of altered body part/area to relieve pain
Vocalisation	Panting Grunting Teeth grinding Shrieking Growling Bellowing Neighing	Changed vocalisation patterns expressing pain Vocalisation during examination of altered body part Vocalise when moving to avoid being handled
Altered behaviour	Depression Low reactivity High reactivity Restlessness More frequent agonistic interactions Increased aggression Flight distance increased Change in personality Cognitive dysfunction	Head down; half-closed eyes Altered social interactions with another members in a group Isolation from a group Attempt to kick/bite/hunt another individuals Altered reaction to human staff Try to escape when handled When restraint and altered body part touched - effort to bite/kick
Foraging feeding/drinking	Decreased appetite Anorexia Weight loss	May be spotted during painful processes however this sign of impaired foraging/feeding behaviour point to the non-specific reaction to numerous stressors which account e.g. change of diet, housing, or social environment Weight losses point to the chronic painful processes

COMPARISON OF THE OCCURRENCE OF CANNIBALISM IN LAYERS WITH INTACT AND TRIMMED BEAKS IN COMMERCIAL FARMS

S. Sepeur[1], B. Spindler[1], R. Andersson[2], N. Kemper[1]

[1]Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour, University of Veterinary Medicine Hannover (Foundation), Bischofsholer Damm 15, 30173 Hannover, Germany [2] Department of Agricultural Sciences and Landscape Architecture, University Osnabrück, Emsweg 3, 49090 Osnabrück, Germany

Summary

In Germany, beak trimming of laying hens in alternative housing systems is a usual method to prevent or to reduce damages due to feather pecking and cannibalism. This practice is a major welfare issue. Therefore, the aim of the study was to investigate the effect of intact and trimmed beaks on the frequency of injuries in conventionally kept layers. In total, 16 flocks of 11 farms were monitored during the rearing and laying period. Every farm kept one flock with intact beaks and one flock with trimmed beaks under same management conditions at the same time. At 7 different points in time, layers were examined for skin injuries. Cannibalism outbreaks (at least 10% of examined birds show a whole body score ≥ 0.5 cm) were detected in 5 flocks with intact beaks and in 4 flocks with trimmed beaks. On average, 41.5 % of layers with untrimmed beaks were affected by cannibalism, while 20 % of layers with trimmed beaks showed skin injuries ≥ 0.5 cm. Birds with intact beaks were 17.5 weeks younger than birds with trimmed beaks at the time of cannibalism outbreak. The results indicate that there seems to be an effect of the beak condition by laying hens in the extent of skin injuries and in the age when cannibalism occurred. However, cannibalism was detected in flocks with untrimmed beaks as well as in flocks with trimmed beaks.

Introduction

In Germany, beak trimming of laying hens in conventional housing systems is a usual method to prevent or to reduce damages due to feather pecking and cannibalism. Economic and animal welfare problems are caused by feather pecking and cannibalism, as they may lead to feather damage, injuries and even increased mortality (Wechsler, 1998). On the other hand the proved acute and chronic pain during and after the beak amputation (Gentle, 1990) is a major welfare issue. The aim of the present study was to investigate the effect of intact and trimmed beaks on the frequency of injuries in conventionally kept layers.

Animals, Material and Methods

In total, 16 flocks of 11 farms were monitored during the rearing and laying period. Every farm kept one flock with intact beaks and one flock with trimmed beaks under same management conditions at the same time (14 flocks of the layer line Lohmann brown, 2 flocks of the breeding line Dekalb white). All flocks were housed in floor pens in combination with an aviary system, with the exception of two flocks that were kept in a classical litter and wire system. All of the flocks comprising hens with an intact beak were provided with several manipulable materials (sand baths, pecking blocks, straw and hay bales as well as colourful plastic objects) to keep the laying hens occupied. The farms were visited at regular intervals during the rearing and laying period. The first visit was carried out between the 14thand 17th week of age at the end of the rearing period. Flocks were visited after being rehoused on the laying farm (17th to 23rd week of age) and during their peak laying period (26th to 29th week of age). Following visits were carried out in flocks aged 40 to 41, 52 to 55 and 64 to 65 weeks, respectively. Last examinations of flocks took place at the end of the laying period in week of age 71 to 77. During the visits, a total of 50 laying hens per flock were randomly chosen for a scoring of skin injuries. The scoring was performed using a modified scoring system based on that of Gunnarsson (2000). According to the extent of largest skin injury of the body, scores 0 to 3 were assigned per bird. Score 0 described a bird without an injury of skin. Score 1 were assigned if the maximal injury of the skin was smaller than 0.5cm. Score 2 was defined as a maximal skin injury per bird of 0.5cm or larger. Score 3 described a bird with a massive skin injury. According to our definition of a cannibalism outbreak, the flock was affected by cannibalism if at least 10% of the examined hens showed a whole body score of 2 or higher. In such cases, additional manipulable material was given, light intensity reduced and salt or magnesium provided via the drinking system to calm the situation in affected flocks.

Results

During the rearing period, none of the ten visited flocks showed an outbreak of cannibalism. A total of nine (75%) out of the twelve examined flocks were affected by cannibalism during the laying period. Cannibalism outbreaks were detected in 5 flocks with intact beaks (62.5%) and in 4 flocks with trimmed beaks (50%). With the exception of one flock with untrimmed beaks, both flocks of the same farm were affected by cannibalism in all cases.

Birds with untrimmed beaks showed significant more massive injuries than their beak-trimmed conspecifics in case of a cannibalism outbreak (Figure 1). On average, 41.5 % of layers with untrimmed beaks were affected by cannibalism, while 20 % of layers with trimmed beaks showed skin injuries ≥0.5cm. On average, birds with intact beaks showed cannibalism in week of age 40, while birds with trimmed beaks showed cannibalism in week of age 57.5. At the following visits (3- 17 weeks after cannibalism outbreak), an effect of the emergency procedures was detected (Table 1). In four of six flocks, the number of massive injuries decreased.

Discussion

According to Niebuhr (2006), the present study detected no indications for cannibalism during the rearing period. In agreement with the current investigation, Staack et al. (2006) detected wide differences in the extent of injuries between birds with intact and trimmed beaks during the laying period. Because of the different time of the skin examination of birds, Staack et al. (2006) showed a lower extent of skin injuries (20% affected layers with untrimmed beaks and 12% affected layers with trimmed beaks) compared to our study (70.5% affected birds with untrimmed beaks and 48.67% affected birds with trimmed beaks).

Conclusion

In summary, flocks with intact and trimmed beaks were affected by cannibalism. However, birds with intact beaks showed a higher extent of skin injuries and a younger age at the time of a cannibalism outbreak than their beak-trimmed conspecifics. The emergency procedures effected partially a calming of cannibalism.

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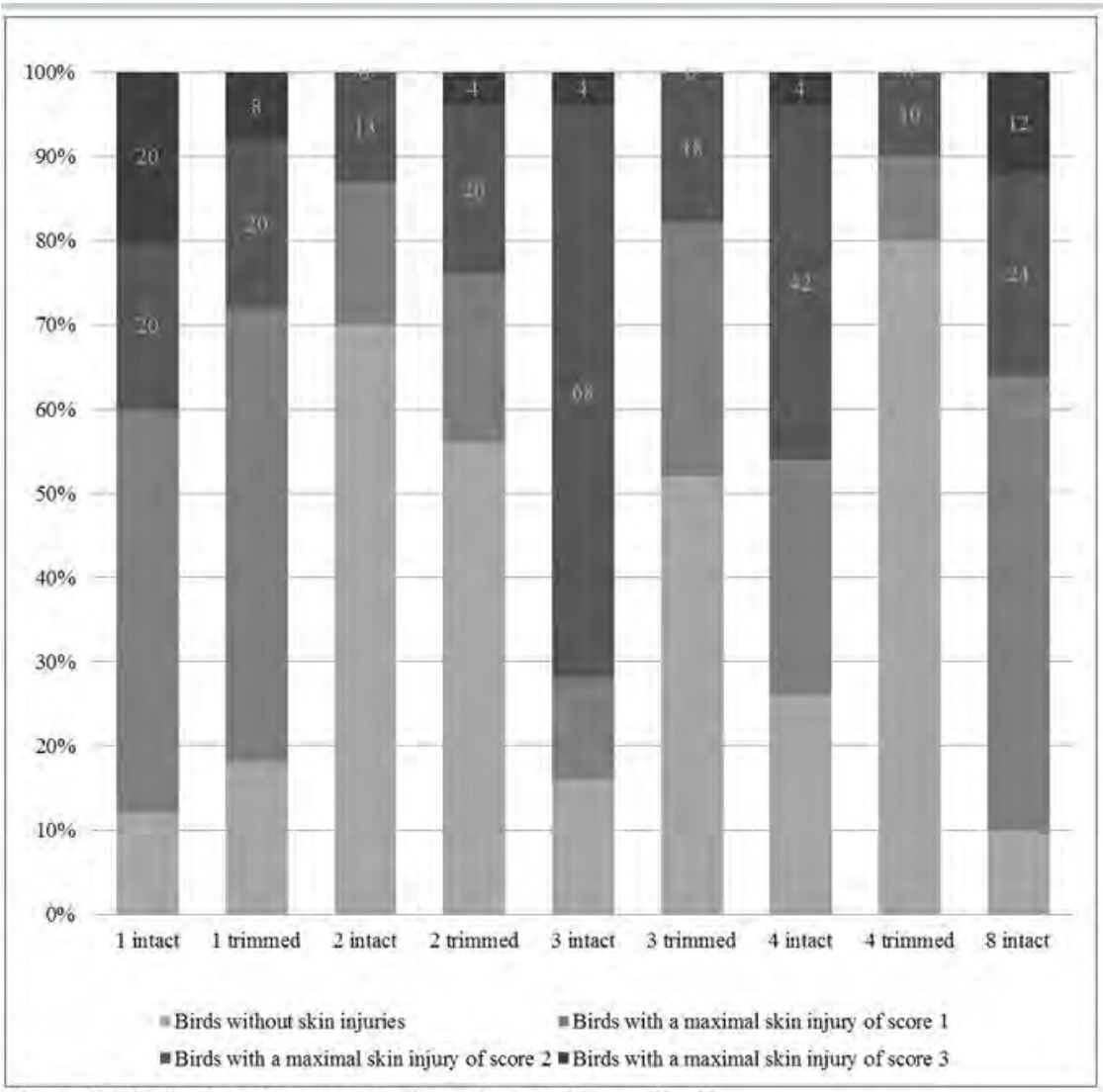

Figure 1: Distribution of scores at the time of cannibalism outbreak.

Table 1: Number of maximal skin injuries of score 3 at the time of cannibalism outbreak and the following visit of control.

Flock	Beak condition	Birds with a maximal skin injury of score 3 (%)	
		At the time of cannibalism outbreak	At the visit of control
1	Intact	20	8
2	Intact	0	4
2	Trimmed	4	2
3	Intact	4	0
4	Intact	4	2
4	Trimmed	0	0

AIR FILTRATION SYSTEMS TO PREVENT AIR-BORNE INFECTIONS IN PIG FACILITIES

S. Speck[1], J. Pospiech[1], D. Rüster[1], U. Truyen[1], T. Reutter[2]

[1]*Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Germany*
[2]*Reventa GmbH & Co. KG, Horstmar, Germany*

Introduction: Air filtration systems have already been shown to decrease the risk of porcine reproductive and respiratory syndrome virus (PRRSV) outbreaks in large sow herds. Own studies on different air filter types using equine arteritis virus (EAV, as surrogate for PRRSV) revealed filtration efficiencies of ≥98% for certain filters. Based on this result further experiments are currently performed to assess filtration efficiencies regarding PRRSV, *Mycoplasma hyorhinis*, *Staphylococcus hyicus*, *Streptococcus suis*, *Actinobacillus pleuropneumoniae*, and *Aspergillus brasiliensis* (as surrogate for other moulds) using different filter media.

Materials and Methods: Two filtration systems for incoming air and one system for recirculating air have been designed. Currently, prototypes for the respective filter technology are tested using EAV and *Mycoplasma hyorhinis* in a laboratory test facility. Filter media tested are polyester, glass fibre, and synthetic organic fibre. The specified filter efficiency varies between 100% for particles >5 µm and ≥95% for particles of 0.4 µm. Artificial aerosols are directed through the different filter media. The difference in pathogen content measured ahead of the filter compared to the amount measured behind the filter is used to calculate the deposition rate. Virus detection is performed by cell culture and quantitative real-time RT-PCR. Bacteria are grown and quantified by the spread-plate-method. Based on these initial experiments, the most effective filter medium will be selected and further evaluated using the above mentioned pathogens.

In a second part of the project, the three respective filtration systems will be implemented in a pig production plant consisting of four identical husbandry facilities. The objective is to determine potential differences in performance parameters between the filtered and the non-filtered animal facilities.

Results: The results of our investigations will be presented at the meeting.

The project was funded by the Landwirtschaftliche Rentenbank.

ANIMAL HYGIENE – AN ESSENTIAL FACTOR IN POULTRY FARMING

D. Chvojka, R. Hromada, J. Koščo, Ľ. Korytár, M. Ondrašovič, K. Veszelits Laktičová, N. Sasáková, V. Petrovič

Department of Environment, Veterinary Legislation and Economics, The University of veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

Summary

A flock of broilers can consist of several thousands birds. It is the reason why are the conditions in grow out houses provided by the farmer very important.

The optimal farming environment is determined by temperature and relative humidity, fresh air circulation, low dust concentration and illumination intensity and by effective sanitation of breeding spaces.

Introduction

Animal health care, comprising both non-infectious and infectious diseases, can be focused on prevention and eradication (epidemic diseases), on vaccination strategies, as well as on disease reduction and control of (often endemic) diseases by either a curative approach or a risk identification and risk management approach.

In each animal rearing a huge amount of interactions exists between animals and environmental factors. Animal diet, nursing, microclimate and macroclimate belong to the most important exogenic factors in the breeding.

This complex of interactions influences numerous physiological and biological processes in animal organisms and a performance of breeding animals.

This influence may be positive (higher performance, better immunological status) or negative (physiological burden, immunosupression), long-lasting or short-lasting, direct or indirect.

EFSA specialists postulated that the most important problems in breedings of broilers are related with their rapid growth. During the second half of the 20th century, the growth rate of broilers increased 4-fold due to a genetic selection. Under modern farming methods, broilers bred indoors normally reach slaughter weight at 5 to 9 weeks of age.

During the first week of life, broilers can multiply their weight on average three times. Therefore the genetic selection of broilers has to be targeted except of the performance also on higher resistance against the environmental factors.

Material and methods

On the studied farm an inadequate sanitation protocol has been used by a private sanitation company previously. It resulted to a high mortality rate of reared chickens.

Aspergillosis has been identified as the main factor of morbidity and mortality. At the request of a farmer a disinfection of buildings was conducted on the farm of broilers.

Therefore, the aim of disinfection was to devitalisate *Aspergillus sp*., the group of fungal organisms causing aspergillosis in birds.

Disinfection was carried out using Pedox-PAA/50 (Polychem s.r.o., Prievidza, the Slovak republic). Pedox-PAA/50 contains paracetic acid (30%) and hydrogen peroxide (20%). This combination of chemical substances has shown highly destructive effect on mold.

Two buildings were treated (Hall No. 1 and Hall No. 2). Disinfectant was applied to a surface of 2172 m^2.

The application has been made using the High Pressure Cleaner Kärcher. The applied dose of Pedox was 0,5 litre per 1 square meter.

The samples for microbiological examination were collected before the disinfection and two hours after the application of Pedox. Also 40 surface swabs and 20 aerial samples were examined using microbiological cultivation

Results

Before the appropriate disinfection (until the 12th July 2012), the highest losses of reared broilers ranged from 38,98 % in the hall No. 1 to 27,23 % in the hall No. 2 (Table 1 and Table 2).

In september 2012 the mortality increased quickly. It was caused by the failure of air conditioning.

Conclusions

A flock of broilers can consist of several thousands birds. It is why are the conditions provided by the farmer very important. The optimal farming environment is determined by temperature and relative humidity, fresh air circulation, low dust concentration and illumination intensity and by effective sanitation of breeding spaces.

After changing the disinfection process and the disinfectant a significant improvement in breeding results has been recorded.

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HUMIC ACID AND THE CONTENT OF SOME TRACE ELEMENTS IN MITOCHONDRIA UNDER NORMAL AND STRESS CONDITIONS

J. Vašková[1], L. Vaško[1], D. Žatko[1], P. Patlevič[2]

[1]*Department of Medical and Clinical Biochemistry, Faculty of Medicine, P.J. Šafárik University in Košice, Košice, Slovak Republic*
[2]*Department of Ecology, Faculty of Humanities and Natural Science, University of Prešov, Prešov, Slovak Republic*

Summary We compared status in chickens without any transport to those who underwent transportation to slaughterhause. An addition of 0.6% humic acid into the diet of farm chicken for 42 days was evaluated. The administration of humic acids (Humac®) had a positive effect on the antioxidant status of plasma and mitochondria of the liver and kidney. Our finding incline to an expectation that the administration of humic acid does not affect the binding abilities to metals but rather competition, leading to a decline in selenium use and compensatory responses which should be considered especially when administered over 42 days. In the induction of sudden stress, according to the levels of elements detected, still a better response of the body can be expected even humic acid administred for 42 days.

Introduction Chickens slaughtered and processed in the meat processing industry are often transported from a farm a few hundred kilometers immediately before slaughter. Animals react to stress by changes in behavior, and biochemical parameters. These changes have a negative effect on the final quality of chicken meat by increase of oxidative stress conditions. Humic acids (HAs) are known for their antidiarrheal, analgesic, immunostimulant and antimicrobial properties. Other studies indicate that HAs can modulate the toxicity of pollutants, xenobiotics, bioavailability of metals, alter pH, ionic concentration and enzymatic activity [7,9]. *In vitro* studies of antioxidant properties of HAs [10] pointed out that the HAs markedly balance the mitochondria redox status. We investigated mitochondria from liver, kidney and plasma from chickens, having received HAs (0.6%) for 42 days and compared to the status of chickens transported for slaughter, which creates a uniquely stressful conditions that may show higher oxidative stress of the organism

Materials and Methods The experiment was carried out on broilers COBB 500 from poultry farm Vinica in Veľký Krtíš region (Slovakia). The control group (15700 pcs) was fed conventional feed mixtures. The experimental group (20000 pcs) was fed feed mixtures enriched by 0.6% HA (Humac® Natur, Humac Ltd, Slovakia) from the first day of fattening. Feed and water was provided *ad libitum* for 42 days. Chickens were killed by cervical dislocation. Mitochondria were isolated by Fernández-Vizarra *et al*. [3]. The activity of glutathione reductase (GR) was measured according to Carlberg and Mannervik [2], glutathione peroxidase (GPx) by Flohé and Gunzler [4]. Superoxide Dismutase (SOD) activity was measured by means of the SOD-Assay Kit-WST (Sigma-Aldrich, Switzerland) following the user manual provided. Reduced glutathione (GSH) levels in mitochondria and plasma were measured by Floreani *et al*. [5]. All the measured parameters were calculated per mg or g of mitochondrial protein (mg_{Prot}, g_{prot}) determined using the bicinchoninic acid assay. Determination of total content of Fe, Zn by flame and that of Cu, Mn, Se by graphite furnance atomic absorbtion spectrometry were detected (Shimadzu AA7000).

Results and Discussion Administration of HAs resulted in the stabilizing activity of SOD in the liver under the stress conditions, and the activity of GPx was reduced (Table 1). Increase peroxide formation together with NO production under stress conditions are essential for catalase activity. What seems to be as the preventive mechanism against the peroxynitrite formation. We observed increased activity of SOD in the kidney, which is explained by the need of superoxide to metabolize catecholamines by renalase, exclusively produced in the kidney. The mechanism of GSH regeneration was boosted in normal and also stress conditions. The levels of metals detected by atomic absorption spectrometry pointed to particularly significant changes in the amounts of metal present (Table 2). The results obtained by Zralý and Písaříková [11] confirmed that feeding sodium humate to animals had no significant adverse effect on the Cu or Zn content in the investigated organs and tissues and cited many other authors with the same findings. The highest content of trace elements, except Se, was detected in the liver which is a depot organ. The kidneys, where the highest concentrations of selenium were detected in the present study, are the most important organ involved in selenium disposition [6]. Owing to sodium humate feeding, the levels of Mn and, above all, Se were significantly decreased in blood serum [11]. The observations in the bodies and mitochondria share a lot of similarities. The increased amount of Fe may contribute to oxidative stress alone, but also to increased activity of iron-

dependent enzymes for the synthesis of antioxidant enzymes, thus directly relating to the use of Cu, Zn and Mn in the mitochondria. Finally, GSH levels were offset in the plasma. Despite the increased levels of Se in the kidney the total level of Se is significantly lower than in the control group, as the circulating isoforms of glutathione are formed only in the kidney. Stress conditions caused significant changes in the levels of detected elements. There was a marked, in order of ten-fold decrease in Mn, Se and Fe, when compared to unstressed groups. The levels of these elements, however, in the HA groups were significantly higher when compared to control under stress conditions. In the group without HA was re-distribution of these elements with higher values in the plasma. Also of interest are higher levels of Se in HA group under stress conditions in the plasma, which are probably the effect of defense mechanisms against oxidative stress. In the same manner may be explained increased levels of Cu in stressed groups, due to the mechanism of maintaining the SOD activity. The hypothesis is additionally supported by the fact that only the Cu/Zn containing SOD of eukaryotes can exist as an apo enzyme that is readily activated by Cu without new protein synthesis [1].

Conclusion Antioxidant enzyme activities suggest that the addition of humic acids marked alleviation of oxidative stress induced by transport of chickens. There is an expectation that the administration of HAs does not affect the binding ability to metals but rather competition, leading to a decline in Se use, producing a sequence of compensatory responses. In the induction of sudden stress, according to the levels of elements detected, still a better response of the body can be expected when HA administred even for 42 days.

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Table 1: Antioxidant enzyme activities and levels of non-enzymatic antioxidant in liver, kidney mitochondria and in plasma of broiler chickens (*p<0.05; **p<0.01).

		organ	SOD (μkat/mg p)	GPx (μkat/mg p)	GR (nkat/g p)	GSH (nmol/mg p)
normal conditions	Control	liver	4.14 ± 0.87	7.379 ± 2.292	20.25 ± 4.93	49.31 ± 5.91
		kidney	4.85 ± 0.93	4.721 ± 0.959	12.07 ± 1.87	15.86 ± 6.56
		plasma	2.51 ± 0.85	4.283 ± 1.386	6.19 ± 1.74	31.61 ± 8.3
	HA	liver	4.41 ± 1.03	2.538 ± 0.726*	39.13 ± 9.71	25.86 ± 6.74**
		kidney	3.84 ± 0.87	2.592 ± 0.408*	21.06 ± 1.57	27.88 ±10.86
		plasma	2.67 ± 0.85	1.158 ± 0.294*	3.96 ± 0.25	38.03 ±9.31
stress conditions	Control	liver	2.59 ± 0.44	5.735 ± 0.629	8.64 ± 1.78	14.55 ±8.62
		kidney	3.01 ± 0.44	3.401 ± 1.248	17.18 ± 5.91	14.74 ±7.37
		plasma	0.9 ± 0.32	1.758 ± 0.222	7.75 ± 0.40	64.86 ±17.54
	HA	liver	4.98 ± 0.48**	3.227 ± 1.062*	20.25 ± 4.45*	15.11 ±5.24
		kidney	3.11 ± 0.58	2.963 ± 0.648	18.57 ± 6.59*	23.53 ±7.3
		plasma	1.24 ± 0.43	1.139 ± 0.479	4.01 ± 1.12	11.4 ±6.38**

Table 2. Distribution of mean concentration of Zn, Cu, Mn, Fe and Se in plasma and mitochondria isolated from liver and kidney (* p<0.05; ***p<0.001).

	Organ	Normal conditions without HA	Normal conditions HA	Stress Conditions without HA	Stress Conditions HA
Zn (μg/g)	plasma	59.62 ± 2.97	60.16 ± 4.36	90.03 ± 2.08	9.65 ± 0.48***
	kidney	49.67 ± 3.10	29.188 ± 2.01***	15.89 ± 0.43	14.12 ± 2.51
Det. lim. 0.001 mg/l	liver	63.86 ± 4.50	50.35 ± 3.33*	10.28 ± 0.62	45.55 ± 3.64***
Cu (ng/g)	plasma	50.99 ± 0.01	56.27 ± 0.03***	470.00 ± 100.0	392.00 ± 2.00
	kidney	73.16 ± 0.04	55.21 ± 0.08***	430.00 ± 6.00	508.00 ± 6.00***
Det. lim 0.0004 mg/l	liver	33.34 ± 0.03	112.58 ± 0.04***	508.00 ± 8.00	626.00 ± 2.00***
Mn (ng/g)	plasma	44.14 ± 0.27	71.03 ± 0.33***	0.24 ± 0.002	0.15 ± 0.006***
	kidney	127.28 ± 0.65	46.54 ± 0.56***	0.12 ± 0.002	0.33 ± 0.004***
Det. lim 0.0002 mg/l	liver	124.79 ± 0.11	50.04 ± 0.31***	0.18 ± 0.004	0.36 ± 0.006***
Fe (μg/g)	plasma	1.52 ± 0.03	3.84 ± 0.05***	0.148 ± 0.004	0.162 ± 0.007***
	kidney	0.79 ± 0.01	2.98 ± 0,02***	0.111 ± 0.008	0.171 ± 0.007***
Det. lim 0.004 mg/l	liver	2.12 ± 0.08	3.51 ± 0,02***	0.172 ± 0.008	0.171 ± 0.004
Se (ng/g)	plasma	535.13 ± 0.04	199.24 ± 0.03***	Under limit	72.65 ± 0.028
	kidney	107.17 ± 0.05	1163.27 ± 0.04***	Under limit	65.75 ± 0.002
Det. lim 0.0009 mg/l	liver	Under limit	51.95 ± 0.01	Under limit	23.92 ± 0.014

DOES THE USE OF FLOOR GRATINGS IN CALF HUTCHES IMPACT ON THE LEVEL OF WELFARE DURING MILKING PERIOD?

G. Mala[1], P. Novak[2], P. Jiroutova[1], J. Knizek[1], S. Stanek[1], D. Prochazka[1], M. Slavikova[1]

[1]Institute of Animal Science, Prague Uhrineves, Czech Republic; [2]Tekro Ltd, Prague, Czech Republic

Summary

The aim of the study was to determine the effect of using the floor gratings in calf hutches on improvement the welfare of calves during the period of milk nutrition. The seven calf hutches with floor gratings (experimental group) and seven hutches without the floor gratings (control group) were observed for 17 months. We monitored air temperature, temperature and dry matter of bedding, average daily gain, health and behaviour of calves during the experiment. The experimental data were analysed by the Statistica package (StatSoft). The use of floor gratings in the individual hutches during mild winter and transition of macroclimate period led to increase of bedding temperature but there was just a minor reduction of bedding moisture. All these changes caused, that the calves spent lying down about one hour longer.

Introduction

Opportunity for a proper rest and sleep is essential welfare factor. Calves spend lying down about 70 to 80 % of the day [1,2]. To ensure the welfare of calves, it is necessary to maintain dry bedding that reduces a heat loss through conduction and thus helps the animals to cope with cold environments [3]. Deep straw bedding has better thermal insulating properties than other bedding materials [4] and moreover can provide a high "score nesting" which has a preventive effect against calf respiratory disease in naturally ventilated calf housing [5].

The quality of bedding material has crucial influence on the amount of heat loss from surface body of calves lying down via conduction [4]. Wet bedding does not insulate the calf from the cold and contributes to a wet hair coat which, in turn, allows increased heat loss from the body of the calf (60%) compared with the dry bedding.

The aim of the study was to determine the effect of using the floor gratings in calf hutches on improvement the welfare of calves during the period of milk nutrition.

Material and methods

The fourteen of outdoor individual hutches for calves rearing during the milk period [from birth to 60 days] were observed for 17 months. The floor gratings were placed into seven hutches and covered up by straw before calf housing (experimental group). The second half of calf hutches without the floor gratings were control group. The quantity and quality of bedding, feeds and water were similar in both groups.

Digital data loggers were installed to all calf hutches. They recorded the temperature of bedding at hourly intervals during four different macroclimatic periods of the year (mild winter – MW - from -9.9 to 0 ^{o}C, transition period – TP - from 0.1 to 10.0 ^{o}C, mild summers – MS - from 10.1 to 20.0 ^{o}C and hot summer – HS - over 20.0 ^{o}C).

The health status and average daily gain (ADG) were monitored in 67 Holstein calves and in 30 Czech Simmental calves.

Dry matter of bedding was evaluated twice during of calf rearing (22 and 50 days). At the same time, were recorded two ethological observation of each calf by IR camera (time of standing, lying down, eating, drinking) during 24-hour period.

The obtained values were analysed by general linear model (Statistica software package, StatSoft). The qualitative parameters were evaluated by non-parametric Kruskal-Wallis ANOVA.

Results

Temperature of bedding fluctuated from 0.6 to 45.6 °C. Significantly higher temperatures of bedding were measured in the experimental group in compared with the control group during mild winter ($p<0.01$) and the transition macroclimate period ($p<0.01$). In contrast, during hot summer were found significantly higher temperature of bedding ($p<0.01$) in the control group (Table 1).

Higher value of dry matter bedding was determined in the experimental group. Calves from experimental group had significantly drier bedding during mild ($p<0.05$) and hot summer ($p<0.01$) than calves from control group (Table 1).

At the same time has not been shown the influence of using the floor gratings in individual hutches on average daily gain and health status of calves.

There was no significant difference in ADG and health state between both groups of calves (Table 2).

The average resting times, which calves spend in hutches, were from 18 to 20 hours per a day. Only during the mild winters and transition macroclimatic period calves from experimental group lay 19 hours, while calves from control group only 18 hours.

Discussion

Dry bedding is for calves and their thermoregulation very important because it significantly reduces heat loss through conduction from the body and thus helping the animals to cope with cold environments [5,6].

The results from this study are consistent with earlier works where average daily gain of calves were from 0.560 kg to 1.100 kg [7,8].

Dairy calves spend approximately 17-18 hours/day lying down [1,3,9]. Total lying times of calves depends not only on the depth of the bedding, but also its moisture [2]. We found out just as Camiloti et al. [3] that calves preferred to lie down on the dry compared with wet bedding.

Conclusions

The use of floor gratings in the calf hutches during mild winter and transition macroclimate period led to increase of bedding temperature but there was just a minor reduction of bedding moisture. All these changes caused, that the calves spent lying down by one hour longer. That increased the level of welfare of calves during the milk period.

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Table 1. Values of temperature and dry matter of bedding depending on macroclimate period

Macroclimate period	Group of calf hutches	Temperature of bedding [°C]		Dry matter of bedding [%]	
		n	mean±S.D.	n	mean±S.D.
MW	experimental	136	11.1±2.5 ^{A}	24	61.07±11.07
	control		7.5±1.9 ^{A}		47.50±16.25
TP	experimental	609	19.2±4.2 ^{B}	50	66.07±18.90
	control		13.6±3.9 ^{B}		54.93±18.64
MS	experimental	986	21.3±5.8	50	68.39±18.77 ^{a}
	control		22.0±4.6		51.14±18.89 ^{a}
HS	experimental	265	24.6±8.2 ^{C}	24	72.63±14.25 ^{D}
	control		27.8±2.3 ^{C}		49.56±18.72 ^{D}

Statistical significance: ^{A,B,C,D} ($p<0.01$); ^{a} ($p<0.05$)

Table 2. Average daily weight gain, health and behaviour of calves depending on macroclimate period

Macroclimate period	Group of calves	Average daily weight of calves [kg.den^{-1}]		Relative frequency of prevalence of signs of disease [%]		Time spent on different activities [%]			
		n	mean±S.D.	without signs of disease	with signs of disease	standing	lying	eating	drinking
MW - TP	experimental	22	0.661±0.126	18	10	16.0	80.0	2.0	1.4
	control	19	0.689±0.111	14	8	21.0	75.0	2.2	1.8
MS - HS	experimental	22	0.683±0.138	16	6	16.1	79.9	1.7	2.3
	control	24	0.686±0.154	15	10	16.1	80.3	1.4	2.3

THE EFFECT OF HUMIC ACIDS ON THE ANTIOXIDANT STATUS IN CHICKENS FATTENED IN EXPERIMENTAL AND FARM CONDITIONS

L. Vaško[1], J. Vaškova[1], S. Marcinčák[2], J. Nagy[2]

[1]*Department of Medical and Clinical Biochemistry, Faculty of Medicine, P.J.Šafárik University in Košice, Košice, Slovak Republic*
[2]*University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic*

Summary

Humic acids (HAs) are actively used in prophylaxis and as therapeutic drugs in veterinary practice. In some studies were found to have a positive effects on feed conversion, meat quality, growth and other considered important parameters in poultry. Many studies, however, are performed within the experimental conditions rather than in large-scale farming. Therefore, we compared effectiveness of conventional feed mixtures enriched by humic acid (Humac®) for 42 days on the antioxidant status of liver, kidney and plasma in chickens reared under experimental conditions and farmed. By comparing the values obtained we confirmed that administration of humic acids have a positive effect on antioxidant status also in large-scale farming.

Introduction

Prohibition on the use of antibiotic growth promoters in feed is reason to investigate alternative strategies for improving the health of broiler chickens [1]. HAs are known for their antidiarrheal, analgesic, immunostimulant and antimicrobial properties. There are not antibiotics but if used properly, along with nutritional measures can be a useful instrument for maintaining and improving the health of poultry and poultry performance [6]. In a number of previous experiments carried out on chickens, it was found that the beneficial effect is observed after 3 weeks. Therefore, two cases with HA administration for 42 days will be covered, one of which was conducted as an experiment and the other within a poultry fattening farm.

Materials and Methods

The first experiment (experimental) was carried out on 80 one-day old broilers COBB500. Chicks were randomly divided into 2 groups (n=40). The control group (C) fed conventional feed mixtures, the second group (HA) was fed with the 0.6% addition of humic acic preparation, HUMAC® Natur (Humac, Ltd., Košice, Slovakia). The second experiment (farm) was carried out on broilers COBB 500 from poultry farm Vinica in Veľký Krtíš region (Slovakia). The control group (15700 pcs) was fed conventional feed mixtures. The experimental group (20000 pcs) was fed feed mixtures enriched by 0.6% HA from the first day of fattening. Feed and water was provided *ad libitum* during the whole experimental period (42 days) in both experiments. Animals were killed by cervical dislocation, followed by tissue harvesting and collection of blood plasma. Mitochondria were isolated by Fernández-Vizarra *et al.* [3]. The activity of glutathione reductase (GR) was measured according to Carlberg and Mannervik [2], glutathione peroxidase (GPx) by Flohé and Gunzler [4]. Superoxide Dismutase (SOD) activity was measured by means of the SOD-Assay Kit-WST (Sigma-Aldrich, Switzerland) following the user manual provided. Reduced glutathione (GSH) levels in mitochondria and plasma were measured by Floreani *et al.* [5]. All the measured parameters were calculated per mg or g of mitochondrial protein (mg_{Prot}, g_{prot}) determined using the bicinchoninic acid assay.

Results and Discussion

In the first case (Figure 1), we found lower GPx activity and an unchanged GSH level in liver. Applied HAs in the diet of broiler chickens demonstrated the ability to participate in the antioxidant defense of the organism. It is likely that their application effect decline in superoxide anion, which acts as generators of other reactive particles in the body. In the latter case there were reduced levels GSH. As shown in Figure 2, there was no demonstrated change in the activity of SOD compared to the control, but the activity of GPx was significantly lower in the group with HAs. One aspect to consider is the fact that, once taken up, humic substances are able to migrate to organs or organelles and may provoke stress response reactions [7]. They have both non-specific and specific effects. The non-specific effects are physical and chemical membrane irritation, induction and modulation of biotransformation activity, induction of chemical defense proteins, the development of internal oxidative stress, and the induction of ROS defense enzymes.

All organisms have the means to rid themselves of chemical burdens (exotic food chemicals, xenobiotics etc.), i.e. they have developed so-called biotransformation pathways. Also humic substances behave like chemical clues in the biotransformation pathway. Since HA possess a variety of functional groups, we assume that the Phase II enzymes of the biotransformation system (conjugation reactions with glutathione), in particular, are subject to modulation upon HA exposure [7,8]. It is interesting to observe the antioxidant enzyme activity in the mitochondria, as they are the second most important organelle involved in the metabolism of xenobiotics and circulating antioxidants. We found increased demands for peroxide decomposition probably as the result of the metabolism of xenobiotics in the kidney (Figure 1). In the case of HA administration increased requirements do not lead to conditions of oxidative stress and the level of GSH in plasma increased. The activity of GR in farmed chickens (Figure 2) was significantly higher in the liver and kidneys and level of GSH significantly decreased in liver in group supplemented with HAs. Taking into account the interdependencies between the activities of enzymes and levels of GSH in comparison between the three bodies, the results are favorable. The redox potential of GSH is not lost in either kidney or circulating plasma, demonstrating the antioxidant effect of HAs.

Conclusion

We can summarize that the addition of humic acids into the diet of chickens affected antioxidant status of the liver and kidney mitochondria of chickens. We observed specific changes in antioxidant parameters in particular organs. Overall, however, we can conclude that the administration of humic acids have a positive effect on antioxidant status in both cases.

Acknowledgment

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Figure 1: Antioxidant enzymes activities and levels of reduced glutathione detected in chickens bred under the experimental conditions.

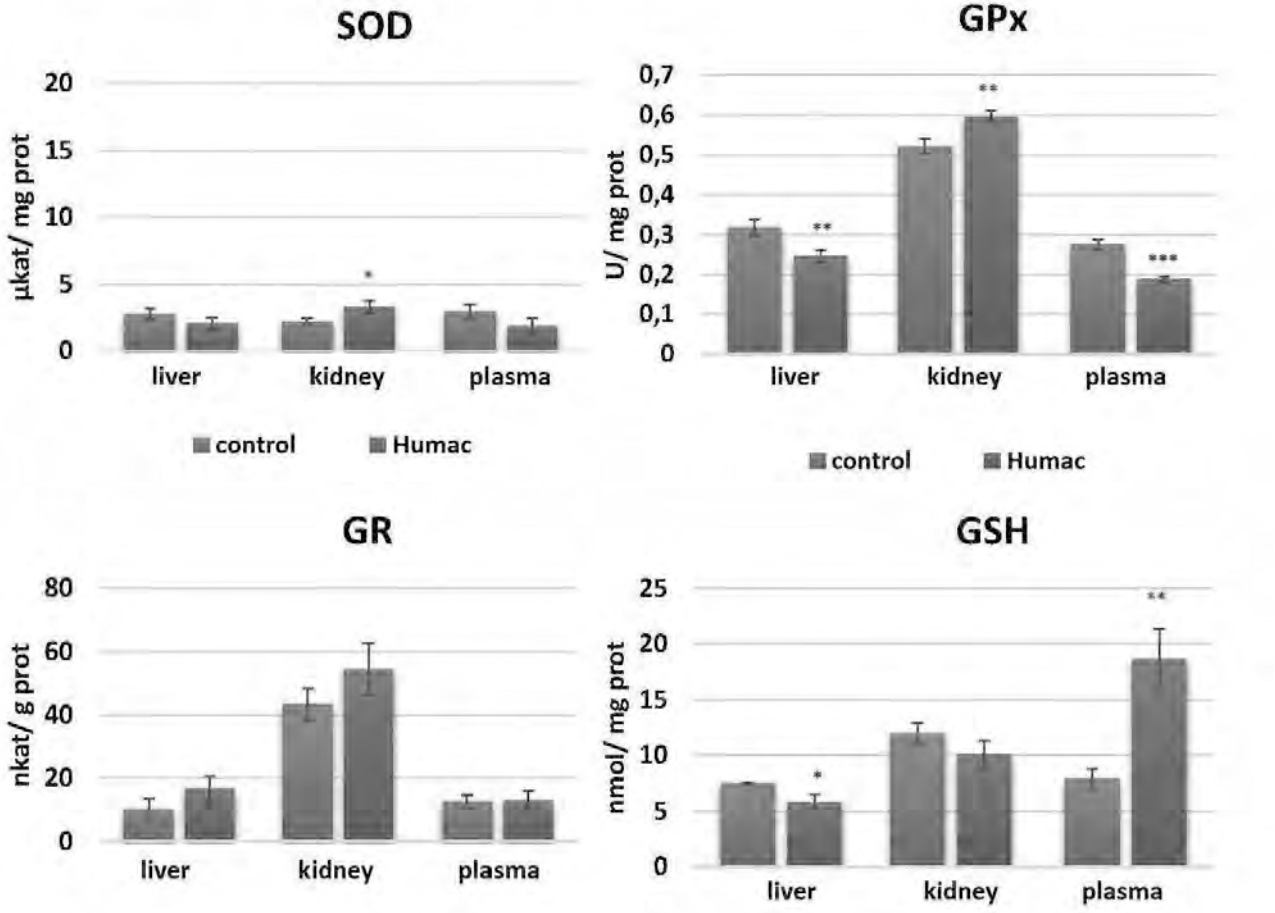
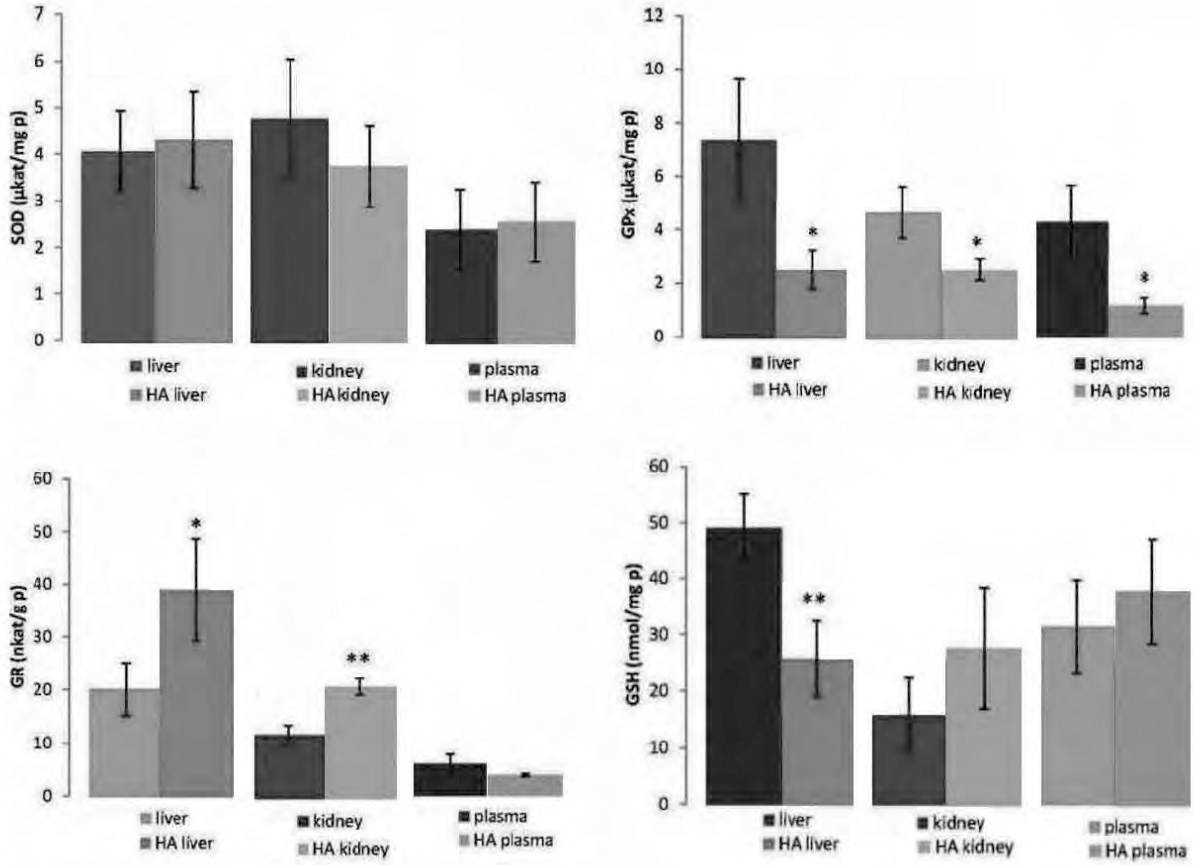


Figure 2: Antioxidant enzymes activities and levels of reduced glutathione detected in farmed chickens.



EFFECT OF CATTLE SLAUGHTER METHODS ON CONCENTRATION CORTISOL AND ACUTE PHASE PROTEINS IN THE BLOOD

R. Kupczyński[1], E. Tracz[1], K. Śpitalniak[1], M. Dzięcioł[2], A. Zwyrzykowska[1]

[1]Department of Environment Hygiene and Animal Welfare, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland
[2]Department of Reproduction and Clinic of Farm Animals, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

SUMMARY

The aim of this study was to evaluate the concentration of cortisol, haptoglobin, serum amyloid A (SAA) and blood biochemical parameters in adult cattle subjected to various methods of slaughter. The different treatment groups of 10 head each were have been submitted to slaughter with stunning (group I), ritual slaughter Shechita (II), and control (III). It has been found that ante-mortem pre-handling, irrespective of the type of slaughter method used in the studies, is associated with a significant increase in the concentration of cortisol in the blood. The SAA concentration in blood before slaughter was higher in group I than in group II what proves the impact of pre-handling of animals before slaughter. In cattle slaughtered with stunning and Shechita slaughter is followed by a similar increase in cortisol levels after slaughter.

INTRODUCTION

Animals waiting for slaughter can be stressed by factors such as restraint, handling, novelty environment, hunger, desire, thirst and fatigue (Muchenje et al. 2009). Activation of the HPA axis is manifested by the release of glucocorticoids (e.g. cortisol) from the adrenal cortex. Catecholamines, clearly invoke significant changes in lactate metabolism affecting post-mortem pH and muscle color (Ferguson and Warner 2008).

Beef cattle experience some level of stress prior to slaughter and this in turn, may have additional effects to meat quality (Ferguson and Warner 2008). In legal terms, the EU Council Regulation 1099/2009 (Dz. U. L. 18.11.2009 EU) on the protection of animals at slaughter. These rules assume limit of pain and suffering of farm animals. Food choice normally reflects aspects of lifestyle, culture, religion, diet and health concerns. Religious slaughter is carried out legally in the UE. Welfare for slaughter without stunning can be improved by restraining an animal in a less stressful manner, but there are still serious welfare questions about pain or distress from the throat cut (Grandin 2014).

The aim of this study was to evaluate the concentration of cortisol, haptoglobin, SAA and blood biochemical parameters in adult cattle subjected to various methods of slaughter.

MATERIAL AND METHODS

Animals were divided into three groups by the analogue method. Experimental groups: I - slaughtered with stunning (n = 10), II - ritual slaughter Shechita (n=10) and III - control group animals kept in the holding (n =10). The stunning procedure involved pneumatic captive bolt gun (group I), in II group stunning was not used before the slaughter. In the control group were performed simulations of transport of introducing the animals into the transporter then placed on the individual quarters. Groups assigned for slaughter were transported for 5h to slaughter house and pre-handled for 2h before slaughter. The animal age was approx. 2 years, body weight 700+/-50 kg. Blood samples were collected from the *vena jugularis externa* about 10-15 minutes before slaughter, and immediately after slaughter to approx. 2 minutes after the start of bleeding (*arterio-venous* mixed blood). Blood samples from control group were collected in the beginning in normal conditions of animal farm handling and after the performed transport simulation. Determination of blood biochemical parameters was performed using Pentra 400 (HORIBA ABX, France) determine total protein, albumine, Na, K, Cl. Cortisol and SAA level was detected with an enzyme-linked immunosorbent assay (ELISA) with using commercial kit (Cortisol kit EIAab Wuhan Science, AAS Tridelta kit). The obtained results were analyzed statistically using Statistica ver. 10.

RESULTS and DISCUSSION

Short-term transport and the stress of the proceedings before slaughter (unloading, moving in lairage, movement of the slaughter line) had little impact on blood biochemical parameters (Tab. 1). As it is known long-term transportation and stress is associated with dehydration, however, in the case of our study parameters associated with dehydration.

Research shows highly significant decrease ($P<0.01$) in content of TP and albumin in blood after kosher slaughter. Large differences in cortisol concentrations were found before and after slaughter with stunning (increase from 14.18 to 28.12 ng/mL), at the kosher slaughter that increase was from 16.56 to 27.37 ng/mL. The concentration of cortisol in the control group was low. Relevant ($p<0.01$) decrease of SAA after the slaughter was observed after the kosher slaughter. In case of control group SAA concentration had not significantly increased. The concentration of haptoglobin was not subject to any remarkable changes.

Acute phase proteins play a potential role as an indicator of stress response in cattle (Murata et al. 2007). The findings reported in the literature concerning the effect of transportation on APPs in cattle vary. In particular, Pre-slaughter stress can stimulate the release of cortisol hormone. Cortisol is released whether in acute or chronic stress and functioned to supply energy reserves for each individual through the conversion of glycogen into energy (Bayazit 2009).

CONCLUSIONS

Research shows that pre-handling of the animals before slaughter is always connected with stress as evidenced by the concentration of cortisol in blood. Therefore, it is recommended that best management handling practices should be used at all times during the pre-slaughter period to minimize the effect of acute stressors. It was found similar levels of cortisol in the blood of the slaughtered cattle despite various slaughter methods, which does not give a clear assessment of the better slaughter method in terms of decreased stress

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Tab. 1. Selected biochemical blood parameters and concentration cortisol and serum amyloid A (SAA)

Group		TP g/L	Albumin g/L	Na mmol/L	K mmol/L	Cl mmol/L	Cortisol ng/mL	SAA µg/mL
Sampling before slaughter								
I	x	75.45	30.91	133.77	4.50	98.01	14.18^{A}	94.59
	SD	4.74	1.92	2.73	0.31	2.62	6.48	21.75
II	x	74.46^{A}	31.00^{a}	135.81	4.11^{a}	99.15	16.56^{A}	83.49^{A}
	SD	5.66	1.58	3.09	0.35	2.68	10.46	24.23
III	x	86.80	29.58	136.37	3.96	98.24	6.77	14.87
	SD	13.47	3.03	0.96	0.31	2.02	1.36	4.87
Sampling after slaughter								
I	x	76.32	31.58	136.52	4.74	97.89	28.12^{B}	89.78
	SD	6.28	1.96	3.18	0.45	2.52	5.50	11.93
II	x	55.95^{B}	26.34^{b}	131.65	5.18^{b}	98.96	27.37^{B}	69.40^{B}
	SD	26.97	9.60	11.43	1.73	7.66	20.10	20.07
III	x	81.59	30.07	136.77	3.90	97.96	10.87	17.23
	SD	6.93	1.40	1.26	0.21	2.15	1.50	9.87

A, B- significant differences in group before and after slaughter ($p \leq 0.01$)
a, b - significant differences in group before and after slaughter ($p \leq 0.05$)

INVESTIGATION OF A NOVEL GROUP HOUSING SYSTEM FOR SOWS DURING LACTATION: HEALTH STATUS OF THE SOWS

Lilith Schrey, Michaela Fels, Christin Habig and Nicole Kemper

Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour
University of Veterinary Medicine Hannover (Foundation), Germany

Summary

Keeping sows in single housing systems with farrowing crates can affect animal health and welfare in conventional pig production. Therefore, there is a need for alternative housing systems providing more space for sows and piglets. For this reason, we investigated a group housing system for sows concerning health and welfare aspects during lactation. The group housing system had five single pens for farrowing and one shared area between the pens. Data of 23 sows in group housing and 16 sows in conventional pens with farrowing crates were collected in five batches. We analysed the occurrence of skin injuries and shoulder lesions as well as weight development of the sows. During the lactation period, the skin lesion score decreased in group housing and in conventional pens. The increase of shoulder lesions during lactation was obviously lower in group housing (left shoulder 4.3% and right shoulder 0%) compared to the pens with farrowing crates (right and left shoulder 31.2%). Sows in farrowing crates did lose more weight than sows in group housing (13.9% versus 4.7%) during lactation.

Introduction

In conventional pig production, it is common practice to keep sows in single housing systems with farrowing crates from parturition until the end of lactation. The restriction of free body movement can affect health and welfare of the sows; for instance stereotyped behaviour (Arellano et al. 1992) or skin lesions (Verhovsek 2007) can occur. The aim of this study was to investigate a novel group housing system concerning health and welfare of the sows during lactation.

Materials and methods

The study was carried out at the research farm of University of Veterinary Medicine Hannover, Germany. The group housing system had five single pens for farrowing and a shared area between the pens including a sow feeder (ad libitum). Instead of farrowing crates, the pens were equipped with flexible iron bars and rubber bollards to prevent crushing of piglets by the sow. The sows were able to move freely, they could turn around inside the pens, leave them and meet other sows in the shared area. A flexible step at the entrance of the pens prevented the piglets from leaving the pens during the first days of life. The group housing system was compared to conventional single housing system with farrowing crates located in the same piggery. In both systems, the lactation period was 35 days. In five batches, data of 23 sows and their litters in group housing system and 16 sows in conventional system were collected. Skin lesions were detected when the sows entered the farrowing system, three days later and when the sows left the system in order to analyse the occurrence of aggressive interactions. Several parts of the body, such as head, ears, shoulder/neck, side, ham, belly, back, vulva and tail were scored by a scoring-system from 0 to 3 (modified by Parratt et al. 2006). Score 0 described a body part with no lesions. Score 1 was assigned if less than five superficial scratches were observed, and score 2 was given if five to 10 superficial scratches or less than five deep scratches were detected. Score 3 described a body area with more than 10 superficial scratches or more than 5 deep scratches. A cumulated scoring index (min.:0/ max.:51) was calculated for each sow. Furthermore the occurrence of shoulder lesions was analysed and sows were weighted when entering and leaving the farrowing system. Data were analysed by using IBM SPSS Statistics (version 22). After testing the data for normal distribution, ANOVA analysis was conducted followed by post-hoc-tests of Student-Newman-Keuls.

Results

The average cumulated scoring index of all sows is shown in Figure 1 for group housing system and conventional farrowing pens. When the sows entered the group housing system, their scoring index was 19, on average. Three days later, the scoring index was significantly lower (16.1) and until the end of lactation the index declined to 11.9 ($p< 0.05$). In conventional pens with farrowing crates, the same tendency was found. The scoring index decreased with increasing lactation period. In contrast, the occurrence of shoulder lesions increased during the lactation period which was more obvious in conventional pens (31.2% for the right and left shoulder) than in group housing system (4.3% for the left shoulder and 0% for the right shoulder, Figure 2). During the lactation period, weight-losses of the sows differed between both systems. When the sows entered the systems, the weight of sows in group housing was about 247.1 kg vs. 258.9 kg in conventional pens. Until the end of lactation, weight-losses in group housing were lower (4.7%) compared to the conventional pens (13.9%).

Discussion

Sows used in this study, which were all kept in a dynamic group housing system during pregnancy, showed a decrease of skin lesions when entering the farrowing system either in group housing or in conventional farrowing pens. During lactation, lesion scores in both systems declined further, and there was no significant difference between group and single housing system at the end of the study. Thus, it seems that group housed sows did not carry out intensive aggressive behaviour. Sows in the group housing system showed less shoulder lesions compared to sows in conventional farrowing crates. Verhovsek (2007) detected less shoulder lesions in alternative farrowing pens compared to farrowing crates, as well. However, the occurrence of shoulder lesions could also be influenced by parity, body condition score and body weight at weaning (Zurbrigg 2006). Thus, an influence of the different weight-losses of sows in group housing system and conventional pens during lactation could be possible. According to Kamphues et al. (2014), weight-loss should be fewer than 10% during lactation. The low weight-loss in group hosing (4.7%) leads to the conclusion, that the feeding system (ad libitum, 5 sows per feeder) was more effective than the restrictive feeding system in single housing.

Conclusions

Despite the possibility to interact, sows in the group housing system did not show an increase in skin injuries caused by aggressive interactions. At the end of lactation, shoulder lesions were found less often in sows in the group housing system compared to conventional farrowing crates. Sows in group housing system did lose less weight during lactation than sows in conventional pens.

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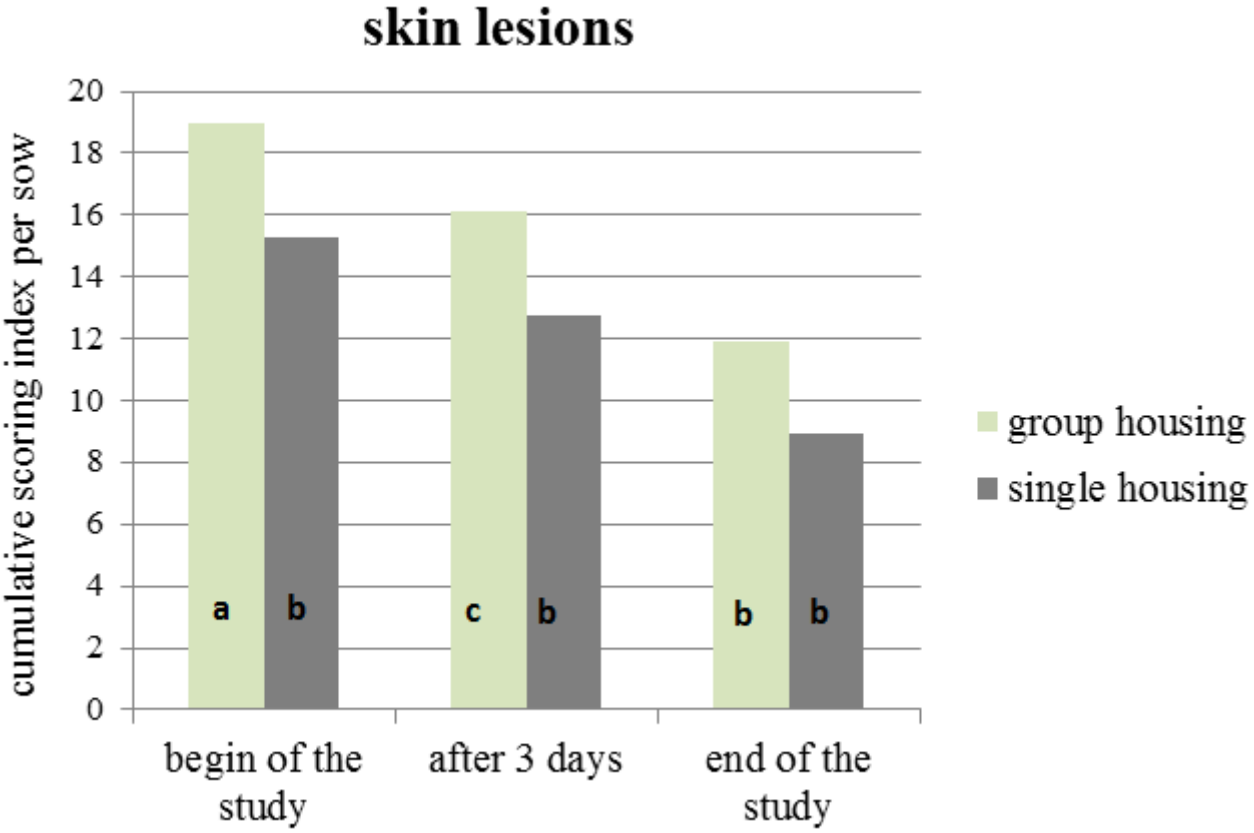
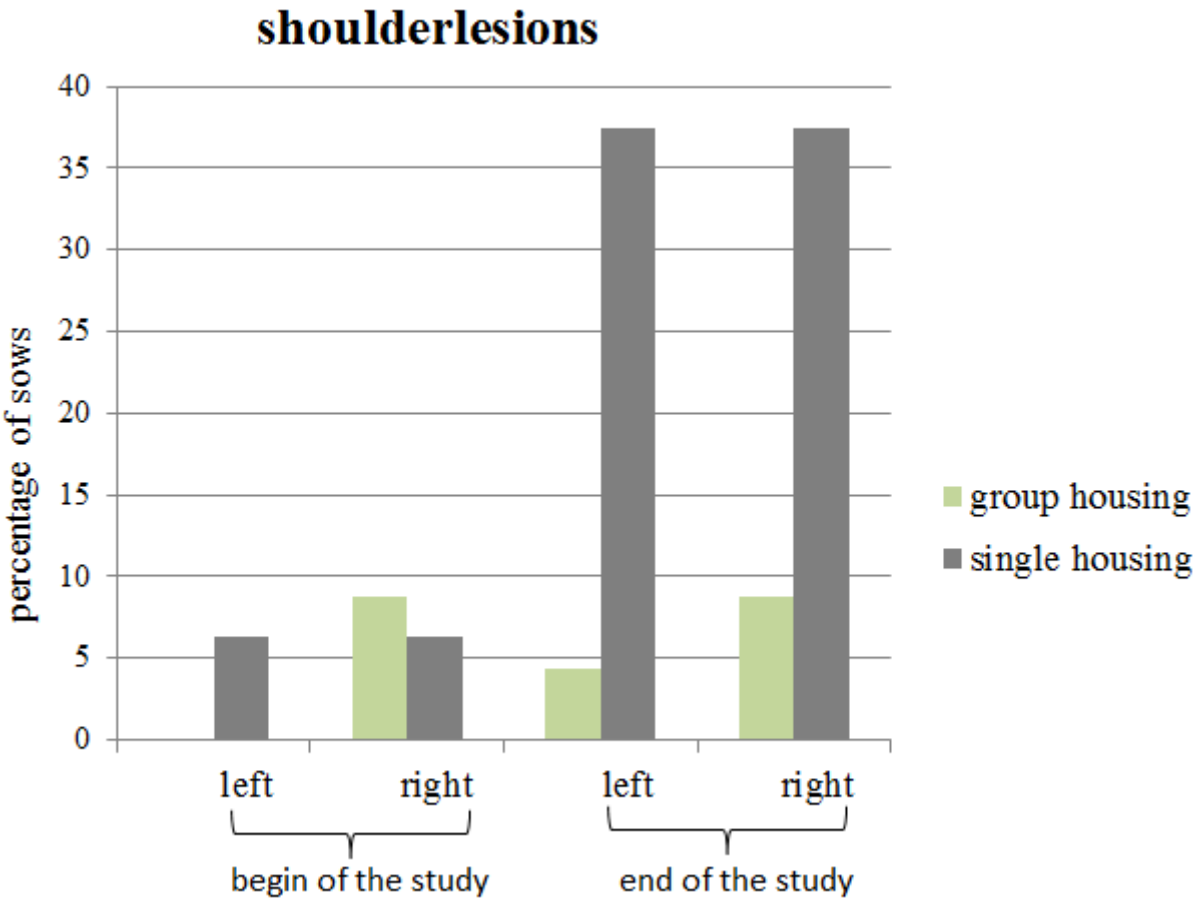

skin lesions
cumulative scoring index per sow
20
18
16
14
12
10
8
6
4
2
0
a
b
c
b
b
b
begin of the study
after 3 days
end of the study
group housing
single housing


shoulderlesions
percentage of sows
40
35
30
25
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5
0
left
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right
begin of the study
end of the study
group housing
single housing

COMPANION ANIMALS WELFARE IN THE SLOVAK REPUBLIC

D. Takáčová, G. Vargová, L. Bodnárová, R. Balajty

Institution of Forensic and Public Veterinary Medicine and Economics, University of Veterinary Medicine and Pharmacy in Košice, the Slovak Republic

Summary

Welfare of companion animals is not covered in the legislation in the European Union. Some countries passed bills regulating animals' protection. Animal welfare is included in legal norms, that governing e.g. animal transportation or welfare of farm animals. The article discusses the main problems related to the welfare of companion animals governed (or not) in the legal rules in the Slovak Republic.

Introduction

Companion animals covers the whole spectrum of species which might otherwise be considered as 'pets' such as rabbits, rodents, cage birds, non-indigenous species, and in particular ornamental fish. It is important to take care of companion animals and protect them from harm. There's no EU legislation on the welfare of pets. However, the legislation on details on the protection of pet animals and requirements for quarantine stations and animal shelters was passed in the Slovak Republic as well as the Law on Veterinary Care that regulates animals' protection in general. Specific rules are regulated in the Law on some requirements for dogs' breeding. Companion animals' transport is not included in the welfare rules, there are only specific schedules when transporting animals in cars, wagons or aircraft. The biggest problems are related to the trade in companion animals, especially to puppy mills.

Material and methods

There are processed data of abstract character, so the adequate scientific tools were used in respect thereof (methods of analysis and analogy). The modified method of comparing functions was used when comparing the current legal acts in the SR. Information was researched and collected from the Internet, as well as from published articles and books.

Results

When analysing the rules regulating the welfare of companion animals, we can see provisions that regulate only "animals' protection" in Slovak legal regulations. The law on veterinary care *inter alia* provides veterinary requirements in terms of animal health and animal welfare. Till now, there were not defined the details of zoohygienic and hygienic conditions of pet animals, by generally binding regulation. Problems are related to dogs' keeping and breeding, especially to those, which are "labelled" as dangerous [Law on some requirements for dogs' breeding (N 282/2002 Coll.)]. There were attempts of Parliament members to prepare the list of breeds presumed to be dangerous. As to us, it is not possible to consider as a solution a simple ban on breeding some dog breeds without a previous serious risk analysis. There was also an attempt to regulate the number of companion animals kept in block of flats in Bratislava city. Rearing bigger number of dogs and cats was conditioned by the consent of competent authorities. As the municipal ordinance is not a normative act, the Constitutional Court expressed that the concerned municipal ordinance is not in conformity with the Constitution of the SR. Very similar unlawful restraint was published in some municipal ordinances, where the size of animal (dogs) kept in dwelling was limited. These ordinances were contrary to law, too. Animals' transportation is governed by laws within the European Union. But companion animals do not fall within those regulations. Pet relocation is serious business, only left in the care of trustworthy pet carriers and animal courier services. Such services have their own conditions that must be complied with during transport. Illegal puppy mills are currently the biggest problem. Puppy mills are operated with an emphasis on profits over the welfare of the dogs bred, with substandard conditions of care often the norm. Because the cross-border migration is out of control within the Schengen, illegal transporters are revealed only occasionally. Only half of the puppies survive during exhausting travel only to make it to the pet shop until they are sold.

Discussion

A good start to ensuring the welfare of an animal is to respect its five fundamental freedoms. But there is more to animal welfare than this. It is necessary to consider less obvious sources of harm, such as breeding practices that result in genetic disorders, puppy farms, or the long-distance transport of prematurely weaned puppies (VAARTEN, 2013). ALESSANDRINI (2013) mentioned, that providing companion animals with housing, feeding and vaccination is a good start but responsible pet ownership should include animal health care and social interaction. ORR (2013) stated that the biggest problem is that prospective owners don't consider animal welfare before acquiring a pet. They receive information from the breeder, but it is of variable quality. The big issue is how to reach people who intend to buy a pet before they get to a "puppy farm" or a breeder. The biggest problem is related to trade in companion animals, where trade practices fall short of animal welfare standards. Legally animals are things (or property). People pay for the fashionable breed and the younger one. For this purpose the best way is to buy a pet from companies, (from puppy mills) that show marvellous images of breed dogs. After selling animals suffer before, during and after the transportation, before finding an owner. Everybody knows, that such trading is illegal and unethical. Lack of control leads to endogamous mixes and genetic disorders (INFANTE, 2014). This problem must be solved, too. As it was stated, there is no legislation on the welfare of pet animals in the EU. So it arises a task for the EU to pass a new directives about companion animals in 2015 (The EU strategy for the protection and the welfare of animals 2012 – 2015). There is an increasing need to pass laws on cross-border legislation aimed at eliminating the worst abuses at puppy mills.

Conclusion

We are familiar with provisions that protect animals, but the legislation on welfare of companion animals is missing. The new, effective legislation must therefore include a clear and concise definition of the welfare of companion animal and the penalties dealing with animal welfare must be included, both monetarily and punitively, too. The legislation must contain also legal standards on prohibition of puppy mills as well as details on the welfare of companion animals during transportation.

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CAN THE PRESENCE OF OLDER CONSPECIFICS REDUCE THE STRESS OF WEANED PIGLETS?

B. Morrone Lima[1], T. B. de Almeida[1], F. A. M. L. Rodrigues[1], P. Tatemoto[1], J. Hartung[2], A. J. Zanella[1]

[1] Department of Preventive Veterinary Medicine and Animal Health, Faculty of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga Brazil
[2] Institute of Animal Hygiene, Welfare and Farm Animal Behaviour, University of Veterinary Medicine Hanover, Foundation, Hannover, Germany

Summary
In commercial farms worldwide, piglets are weaned abruptly, between 21 to 28 days of age, often compromising their welfare and causing high levels of aggression. The aim of this study was to evaluate if the presence of an older animal among newly weaned pigs can reduce aggression levels assessed by the occurrence of skin lesions. A photographic record was conducted on days D 0 (before weaning), D 1, D 2, D 3 and D 4 post weaning. The number of lesions was lower in the pens with the presence of the older pigs, compared with the control pens, in the first 48 hours after weaning, when more aggressive behaviours among piglets happens. We have shown that the older animal acts as a social buffer on the stress situation of newly weaned piglets thus, improving their welfare.

Introduction

It is estimated that approximately 1.16 billion pigs will be raised on commercial farms worldwide, in 2015 (USDA, 2014). As a routine management procedure, piglets are weaned abruptly, between 21 and 28 days of age, and they are exposed to a variety of stressful events (Merlot *et al.*, 2004). Piglets learn how to feed and how to behave socially by watching older animals of the group (Andersson *et al*., 2011).

When raised in a semi natural environment, sows and piglets began to leave their nests around 7 to 10 days postpartum, when piglets are introduced to the others members of the group. This social strategy can result in little or no aggression as piglets are exposed to complex social interaction in the presence of sows (Dellmeier and Friend, 1991). When there is a mixture of piglets from different litters, it is has been reported the occurrence of aggressive behaviours, among the newly weaned animals, especially in the first 24 hours, lasting up to 48 hours, when a new hierarchy is established (Meese and Ewbank, 1973). The number of skin lesions is often used as an indicator of aggression that occurs after mixing litters (Turner *et al*, 2006).

Our specific objective was to evaluate if the presence of an older pig in nursery pens, immediately after weaning can reduce agonistic interactions among weaned piglets.

Material and methods

A total of 96 piglets (Large White x Landrace: LDxLW) were studied in two experimental blocks, with 48 animals each. Four animals per litter were selected, two males and two female piglets, and weaned at 21 days of age (D 0). The animals were distributed in 4 pens with 12 piglets each, and each pen housed piglets from 3 different litters. Two pens (Treatment: T) in each study block housed, together with the weaned piglets, one 4 months old LDXLW castrated male pig in each pen. In the two other pens (Control) only the newly weaned pigs were housed together. Half of the pen had concrete floor while the other half was covered with dried sugar cane bagasse. Water and food were offered *ad libitum* to all animals. Photographic record of the whole body of the animals was carried out, to count lesions on post-weaning days D 0, D 1, D 2, D 3 and D 4. Lesions were counted, independently by two observers for all the assessed days.

The experiment was approved by the Ethics Committee on Animal Use - CEUA at the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo under protocol number 6240230114.

Data Analysis

We analysed data normality using the Kolmogorov-Smirnov test; homoscedasticity by the Levene test (Statistica 7). Visually evaluating the data and graphics we identified animals that appeared to be outliers. After confirming outliers identity using the formula: mean ± (SD * 2), we replaced the data of such animals by the group average. The tests used for analysis are specified in the respective figures. For all analyses a *P* value

Results

The number of lesions was higher in the control piglets (C) when compared with the animals housed with the older pigs (T) on the first and second day after weaning (Fig. 1; repeated measures ANOVA, F = 4.43; p = 0.001). We speculate that older pig worked as a social buffer for the newly weaned piglets.

Discussion

Our work is in agreement with previous studies that demonstrated that the presence of older animals reduced weaning stress. Henry *et al.* (2012) used the presence of an older horse in the pasture when weaning foals and noted a reduction in stress indicators such as vocalization and salivary cortisol levels. The same authors also reported a decrease in abnormal behaviours and aggression among foals weaned in the company of an older animal. Paula Vieira *et al*. (2012) placed an older and experienced calf with newly weaned calves and showed that the animals had higher ingestion of solid food and therefore better growth rate in the presence of the older calf, when compared to conventionally housed calves The first two days have higher incidences of agonistic interactions, as reported in previous research (Meese and Ewbank, 1973).

Conclusion

The presence of the older pig decreased total lesions, indicating a reduction in aggression among newly weaned piglets during the two days after weaning. In this work, we showed that the presence of older individual reduced aggression among piglets, measured by the number lesions. The reduction of aggressive interactions may indicate an improvement in piglet welfare, with possible reduction of stress.

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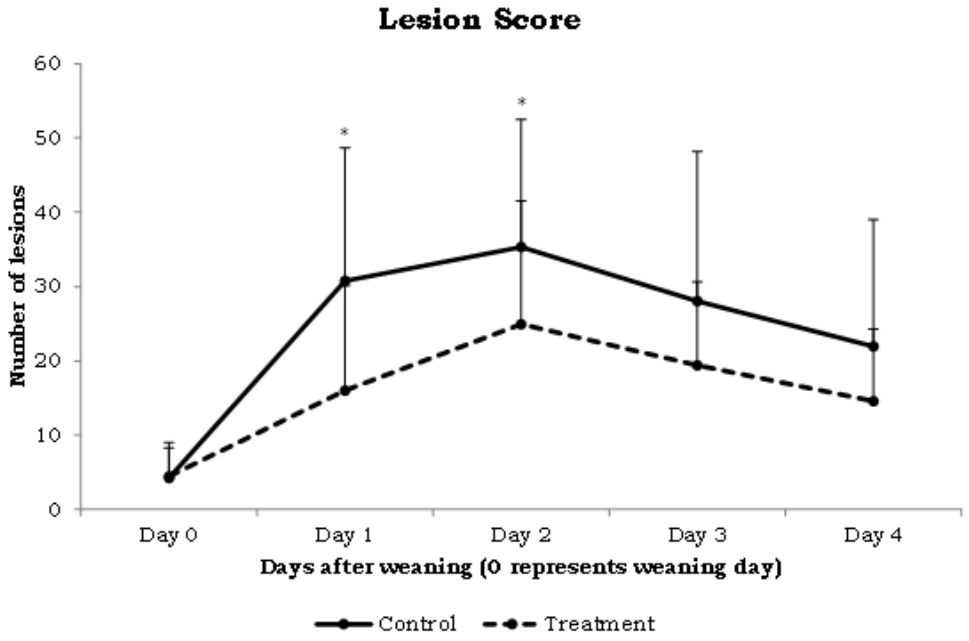

Figure 1. Total number of lesions. Mean values of 2 different observers (± SD) of 48 piglets in each independent treatment. * Indicates difference between treatments compared on the same day (repeated measures ANOVA, F = 4.43; p = 0,001; post hoc Tukey).

WELFARE STATUS IN HUNGARIAN PIG FARMS

Á. Végh[1], B. Hoemsnes[2], L. Könyves[2], V. Jurkovich[2]

[1]Alpha-Vet Animal Health Ltd, Székesfehérvár, Hungary
[2]Department of Animal Hygiene, Herd Health and Veterinary Ethology, Faculty of Veterinary Science, Budapest, Hungary

In the study we assessed animal welfare quality in eight Hungarian pig farms using the Welfare Quality (WQ) Protocol. The farms housed Hungarian Large White pigs, with population ranging from 500 to 25,000. The conventional housing system was applied on all farms, seven with partly slatted floors and one with concrete floors and straw bedding. The WQ Protocol is based on four welfare principles ("Good feeding", "Good housing", "Good health", and "Appropriate behaviour") further divided into sub-areas based on more than 30 measures. The overall welfare score is converted into one of four welfare categories "Excellent", "Enhanced", "Acceptable" and "Not classified".

In the overall scoring of principle "Good feeding", six farms were rated "Excellent" and two "Enhanced" due to the presence of lean pigs (6.7 % and 3.3%, respectively).

The scores of the principle "Good housing" were generally low with all farms rated only "Acceptable", due to low scores in the criteria "Thermal comfort".

The principle "Good health" was only "Acceptable" on all farms, due to the practice of castration and taildocking without any kinds of pain relief.

The "Appropriate behaviour" principle was rated "Excellent" on two farms, "Enhanced" on another two and "Acceptable" on the rest. On these latter farms, a high percentage of pigs showed aggression or panic.

In the overall assessment, four of the farms received the category of “Enhanced”, four farms received the category of “Accepted”.

It can be stated that the Welfare Quality protocol is a reliable method to assess the welfare status of Hungarian pig farms. There is definitely room for improvement, especially in the fields of castration, thermal comfort, animal and stock hygiene, and human-animal relationship.

PERCHING BEHAVIOUR AND NIGHT-TIME ROOSTING IN LAYING HENS REGARDING DIFFERENT TYPES OF PERCHES

M. F. Giersberg, B. Spindler, N. Kemper

Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

Summary

There are numerous studies dealing with perching behaviour in laying hens. However, most of them were carried out under experimental conditions regarding small groups of hens. In contrast we assessed the use of nine differently located perches and three cross-braces in a commercial layer farm (aviary system). Monthly data recording using scouting cameras started at week 20 for five consecutive days (5:00-22:00h). We observed a sharp increase in the number of hens perching at night-time. Furthermore, the hens showed a high preference for certain locations of perches, whereas age had only a minor impact on perching behaviour.

Introduction

Access to perches during day-time allows laying hens to escape from threatening pen mates (Cordiner and Savory, 2001) and thus gives them the opportunity to avoid being feather-pecked or cannibalized (Heikkilä et al., 2006). Furthermore Olsson and Keeling (2000) showed that domestic hens are still highly motivated to use elevated perches for night-time roosting and that thwarting access results in frustration. Accordingly, the EU requires perches in all housing systems for laying hens (99/74/EC). But how frequently are these perches used by hens in commercial aviaries? Although Newberry et al. (2001) found changes in the perching behaviour of young hens with increasing group size, there are no studies dealing with the use of perches in intensive housing systems where several thousands of laying hens are kept together. The aim of this study was to determine the impact of perch location, diurnal rhythms and age on the perching behaviour of laying hens kept under intensive housing conditions.

Material and methods

The use of nine metal perches (diameter: 3.5cm) was observed in four identically equipped compartments in a commercial layer farm (aviary system, Big Dutchman Natura-nova twin, about 4,600 hens/compartment). Four perches (height: 38cm) were placed on an intermediate ceiling above the nests (on the balcony). The remaining perches were located next to the feeding trough and the drinkers (in the system; height: 38cm, 54cm and 62cm above an intermediate ceiling). Photo-based data recording using scouting cameras with invisible infrared flash started at week 20 for five consecutive days and was repeated monthly. The number of hens (Lohmann Brown) residing on the perches was detected in a 15 minute interval between 5:00 and 22:00h via time sampling method. The data analysis was performed separately for the twilight phase in the evening as well as for the day- and night-time. In addition, the number of hens roosting on three cross-braces (height: 38cm, balcony and 62cm, system) which were structural elements of the aviary was recorded in exactly the same way. The total height of the aviary reached 2.62m (balcony) and 2.50m (system) above ground level.

Results

Throughout the observation period (20th to 38th week) the perches on the balcony were less frequented than those in the system (Fig. 1). During the day 1.13±1.03 hens/m were observed on the perches (balcony), respectively 1.51±1.02 hens/m (system). The mean use of perches in the twilight phase was 1.90±0.92 hens/m (balcony) and 3.43±1.20 hens/m (system). At night, 4.43±1.10 hens/m rested on perches on the balcony, whereas 6.21±0.92 hens/m perched in the system. Except for the perches on the balcony in the 20th week, the average number of hens/m perch increased both in the twilight phase and at night (Fig. 2). The mean use of perches during the day ranged from 0.72±0.83 hens/m (24th week, balcony) to 1.92±1.25 hens/m (20th week, balcony). In the twilight phase an average of 1.40±0.76 hens/m (24th week, balcony) up to 4.01±1.44 hens/m (20th week, system) was observed on the perches. The mean use of perches at night-time increased from 2.46±1.10 hens/m (20th week, balcony) to 6.49±1.14 hens/m (38th week, system). Regarding the different types of perches on the balcony, it is obvious that the maximum number of hens was observed on the cross-braces at any time of day, i.e. 11.43 hens/m (20th week, daytime), 8.57 hens/m (20th week, twilight phase) and 14.29 hens/m (33rd week, night-time). Similar results apply to the perches located in the system. Again, the maximum number of hens rested on the cross-brace during the day and at

night (7.14 hens/m, 33rd week and 11.43 hens/m, 28th week). Merely in the twilight phase most hens (9.17/m, 33rd week) rested on the 38cm high perch. However, the corresponding number of hens on the cross-brace was 8.57/m.

Discussion

The aim of this study was to assess the perching behaviour of laying hens kept under commercial conditions focussing on perch location, diurnal rhythms and age of the animals. At night time there was a sharp increase in the number of hens using the perches. These findings are in accordance with former investigations (e.g. Olsson and Keeling, 2000) showing that diurnal rhythms still influence the perching behaviour in the domestic fowl. At any time of the day the perches located in the system were used more frequently than those on the balcony. This may be due to the fact that perching in the system allowed easy access to the feeding through and the drinkers. Height effects are unlikely, since neither the total height nor the height of the individual perches actually differed between the system and the balcony. During the whole observation period the frequency of perching did not change considerably, except for the 20th week. However, an increase in perching with age was mostly observed in 3 to 18 week old chickens (e.g. Newberry et al. 2001; Heikillä et al. 2006). On top of that, hens showed a preference to rest on the cross-braces (structural elements of the aviary). Again, this preference cannot be explained by the height, since proper perches at the same height were present on both the balcony and in the system. Therefore, the frequent use of the cross-braces may be due to their rectangular arrangement to the longest side of the barn, since all proper perches were arranged in parallel. Whether the hens' perching behaviour was affected by drafts or similar disturbances due to this arrangement has yet to be studied in detail.

Conclusions

First results indicate that laying hens kept under commercial conditions show a preference for certain perches and also use structural elements of the aviary for perching. Moreover, the time of day had a strong impact on perching behaviour, whereas the effects of age were less important.

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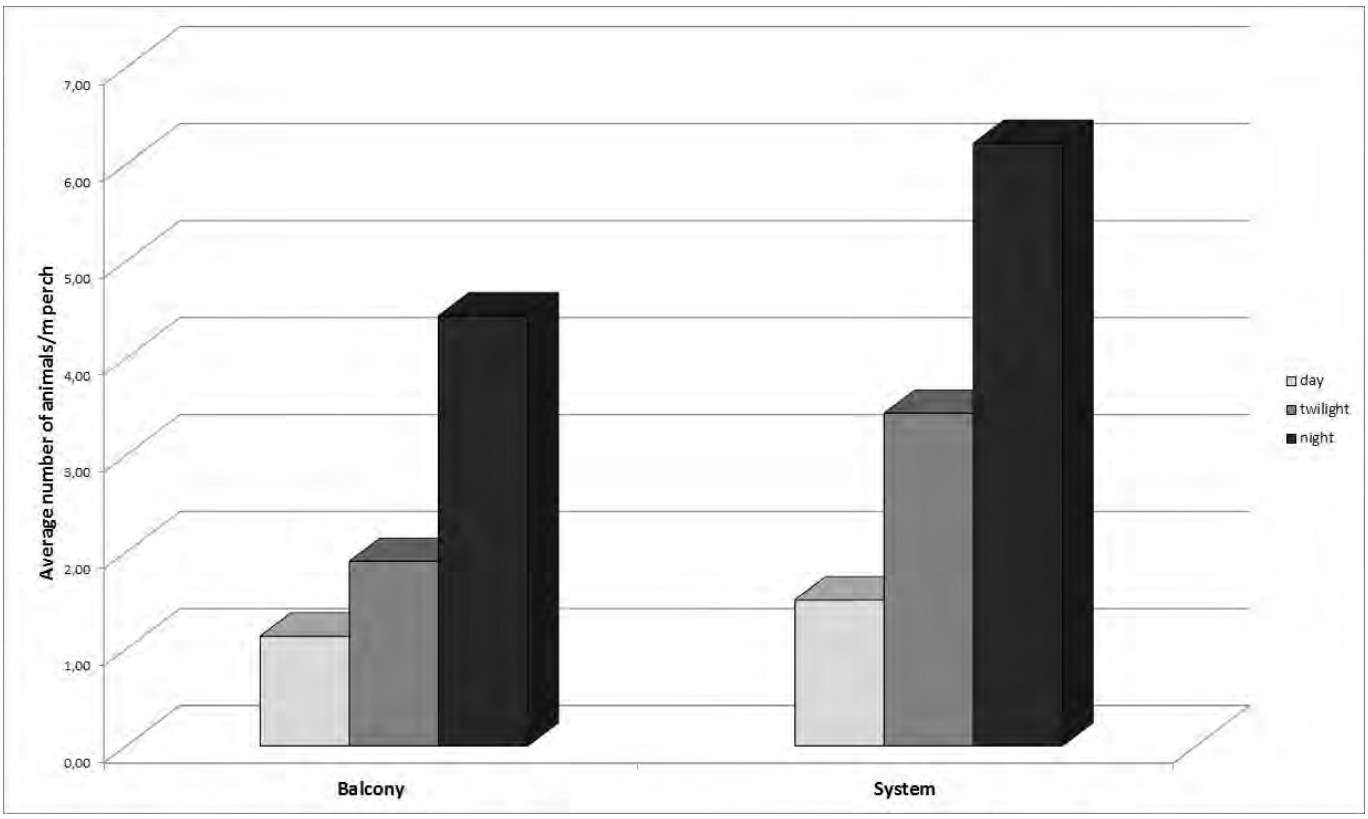
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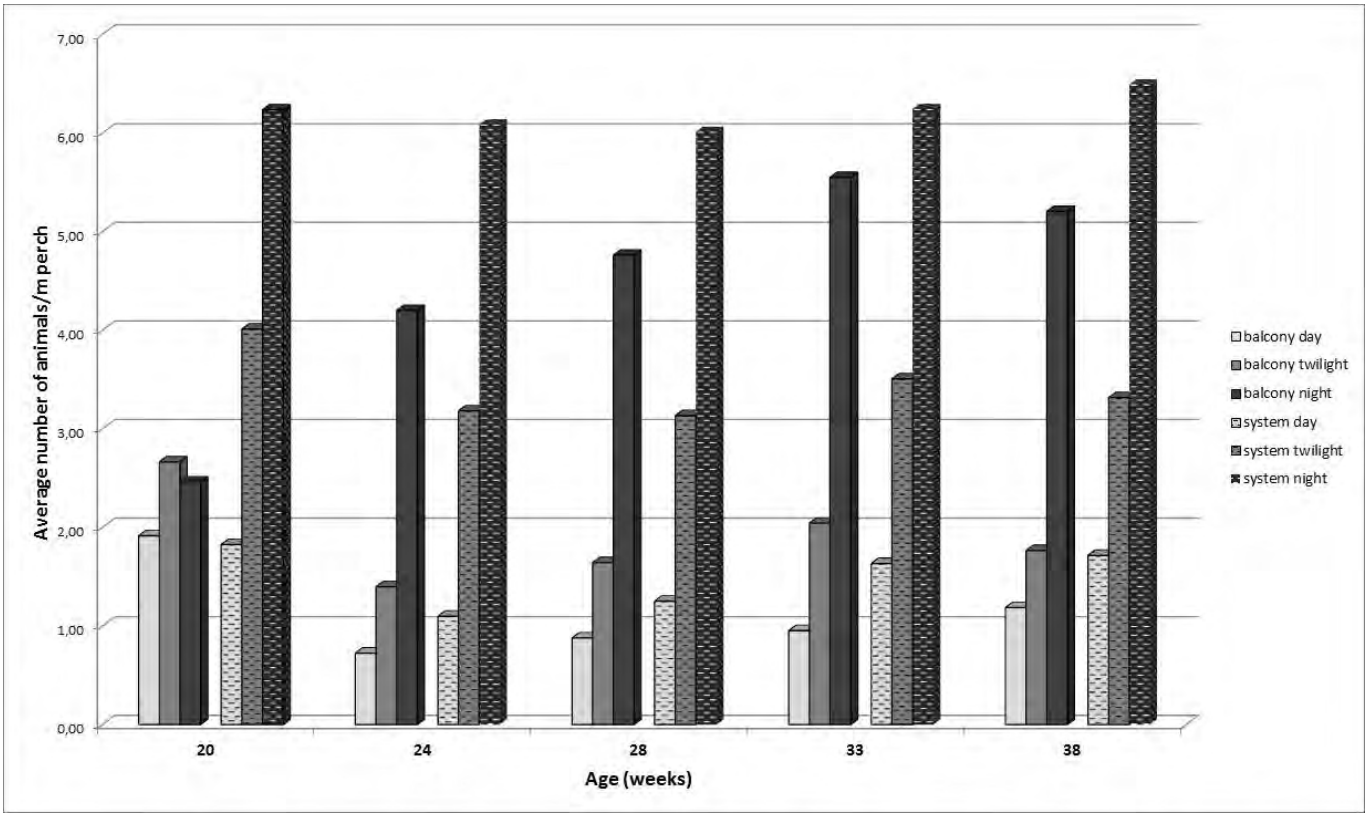
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Environmental pollution, emissions, abatement

REPRODUCTIVE TOXICITY ASSOCIATED WITH LEAD TOXICATION IN SMALL RUMINANT ANIMALS

Mahmoud Abdel Zaher[1], Mohamed Salah El-Din Youssef[1], Sohair Rashed Ali[2], Nabawia Mahmoud Shehata[2]

[1]*Department of Pathology and Clinical Pathology, Faculty of Veterinary medicine, Assiut University, Assiut, Egypt*
[2]*Animal Health Research Institute, Assiut, Egypt*

Summary: Two mature females and one mature male goats were used for these studies. Animals administered lead in a dose of (85mg / kg body weight) daily for seven weeks. The two mature females were kept with the male seven weeks for natural breeding and reproduction. Clinical studies revealed that lead toxicity had a deleterious effect on the reproductive behavior of the experimental animals. In male complete loss of libido was reported. The male was unable to serve females, although they were kept together for seven weeks. Attempt to obtain semen samples using artificial vagina were failed. In females pregnancy test and uterine sonography were negative. Histopathological studies of the testes demonstrated severe necrobiotic changes in the epithelium of seminiferous tubules. Some tubules were completely denuded of its epithelium. Disappearance of mature sperms from the lumen of seminiferous tubules was a constant finding. Prominent decrease in the amount of sertoli cells, in between the germinal epithelium was reported. The interstitial tissue were edematus or showed proliferation and hyalinization and fatty infiltration. A decrease in the number of the interstitial lyedig cells was also demonstrated. Immature spermatogonia forms were demonstrated in between the degenerated sperms in the lumen of the epididymis. Histopathological changes reported in the ovary include wide spread proliferation of C.T, sclerosis of the small sized arterioles with intimal hyperplasia, hypertrophy of the muscular layer of the medium sized arteries. Most of the ovarian follicles showed degenerated ova, or the ova were completely disappeared. IT had been concluded that lead toxicity was associated with clinical and pathological reproductive disturbances in goats, manifested by complete secondary infertility in both males and females.

Keywords: Goat, Lead, Reproductive toxicity

INTRODUCTION:

Lead is a ubiquitous environmental toxin that induces a broad range of physiological, biochemical, and behavioral dysfunctions.

Analysis of computer literature s revealed that the effect of lead toxicity on the ovary and testes during cyclic changes was not sufficiently studied both clinically and histopathologicaly (Foster et al.1993).

Materials and methods:

Two mature females and mature uncastrated male were used; they administered lead acetate in a dose of 85 mg/Kg BW daily in gelatin capsule for 7 weeks (Sigma chemical company & Sant Luis USA).

Results:

Clinical studies:

After seven weeks week of the experimental period, one male and two females were kept together for another 7 weeks for purpose of breeding and reproduction. Attempts for collection of a semen sample from that male using artificial vagina were failed. Male was unable to serve females. Weekly ultra sonography examination on the two females for pregnancy diagnosis and pregnancy test were negative.

Histopathological examination of the testis

Changes in the seminiferous s tubules were very prominent. Most of the tubules were completely empty of sperms (Fig.1).The germinal epithelium lining the seminiferous tubule either degenerated or completely disappeared (Fig.2). Only basement membrane and remnant of degenerated epithelium were observed. Sometimes the germinal epithelium was dissociated from each other showing decrease cellular density (Fig.3). In some tubules both germinal epithelium and sperms were hyalinized with a reminant of pyknotic nucleus (Fig.4, 5). Hyalinization and degeneration of

all layers in the seminiferous tubule were frequently observed (Fig.6, 7). Sometimes mass of sperms in the seminiferous tubule undergo hyalinization and form plugs in the lumen of seminiferous tubule. In most cases, there was prominent decrease in the Sertoli cells in between the germinal epithelium. The main changes in the interstitial tissue were edema, increase amount of fibrous connective tissue (Fig.8) hyalinization and deposition of fat cells in the interstitial tissue (Fig.9, 10). Prominent decrease in the interstitial Lyedig cells was also observed (Fig.11). Histopathological changes in the epididymis include hyalinization of the basement membrane of tubules, which were completely empty of sperms (Fig.12). Sometimes hyalinization of epithelium of the epididymal wall was associated with degeneration and hyalinization of sperms in the lumen. Immature spermatogonia form appeared in between the degenerated sperms in the lumen of the epididymis (Fig.13).

Histopathological examination of the ovary

A wide spread proliferation of the connective tissue in the ovary was demonstrated (Fig.14). Most of small arterioles were sclerosed showing intimal proliferation (Fig.15). Medium sized arteries showing hypertrophy of the media intimal endothelium proliferation and subendothelial vacuolation(Fig.16,18). Most of the ovarian follicles showing degenerated ova (Fig.17).

Discussion and conclusion:

Complete loss of libido was observed in male. Attempts to obtain semen sample from male goat using artificial vagina were also failed.

In females, pregnancy didn't occur although pregnancy test and ultra sonography were carried out periodically.

Severe prominent histopathologial changes, of degenerative nature were reported in all anatomical parts of the testis, including seminiferous tubules interstitial tissue and epididymis (Wadi et Ahmed.1999).

A deleterious pathomophological changes were observed in the ovary, including wide spread proliferation of connective tissue sclerosis of its small sized arterioles intimal hyperplasia and medial hypertrophy of medium sized artery. Most of the ovarian follicles showed degenerated ova.

From our investigation we can concluded that: -

-Lead toxicity in goats resulted in pathomophological changes of degenerative nature.

-Reproductive toxicity was evident in both male and female goats clinically and histopathologicaly with a prominent sever infertility.

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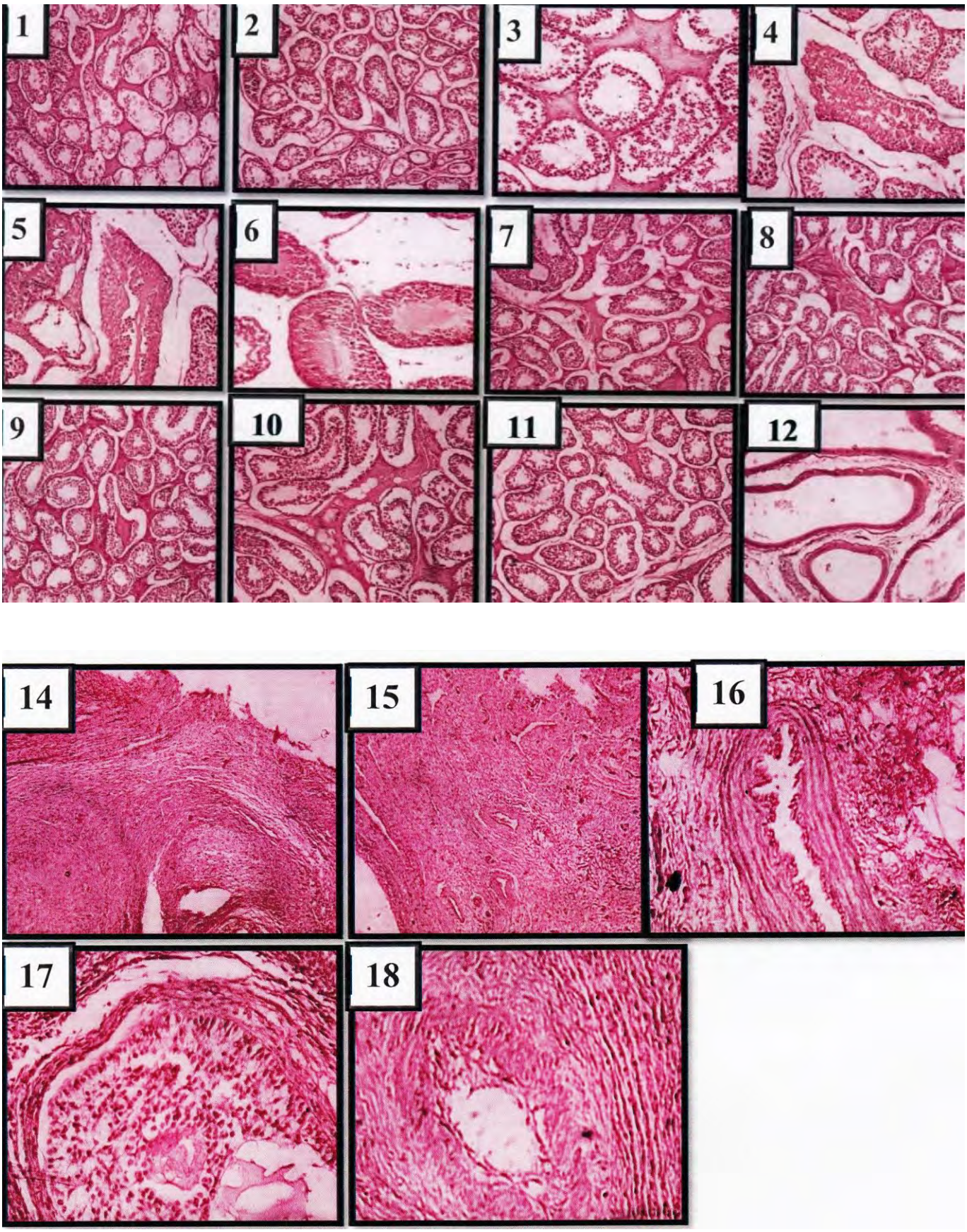
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ASSESSMENT OF HEAVY METALS IN MUSCULAR TISSUES OF TILAPIA NILOTICA IN SOHAG GOVERNORATE, UPPER EGYPT

Thabet Abdel Moniem Ibraheim*; Hussein Youssef Ahmed**; Hesham Abdel Moez Ahmed** and Abeara M.***

** Dept. of Forensic Medicine & Toxicology, Assiut, Egypt*
*** Department of Food Hygiene; Faculty of Veterinary Medicine, Assiut, Egypt*
**** Veterinarian in Sohag Hospital, Egypt*

- **Abstract**

This study was performed to estimate some heavy metals pollutants (cadmium, chrmomium, copper, mercury, manganese, lead and zinc) in *Tilapia nilotica* tissues from Soahg governorate – Egypt. 101 Fresh fish samples were caught from five different districts namely Sohag city, Issawiya, Gerga, Maragha and Dar Elsalam which located on River Nile,. Fish samples average weight 200-500 gm. Collected samples were analyzed for the content of the mentioned heavy metals using ICAP 600 Series ICP (Inductively Coupled Plasma apparatus).The obtained results of cadmium levels revealed that the minimum, maximum and mean ± SE from Sohag city,were 0.5, 0.13, 0.09 ± 0.005. While chromium from Sohag city, were 11, 96, 43.77±5.53; respectively. In case of copper from Sohage city, were 0.01,3.94,1.55±0.26. of mercury values from Sohag city, Issawiya, were 0.02, 0.18, 0.071±0.011. For manganese, from Sohag city, were 0.06, 1.58, 0.46±0.08.Lead values from Sohag city, were 0.14, 2.6, 0.48±0.12. For zinc, the minimum, maximum and mean ± SE from Sohag city. Comparing our result with the permissible limit of EOSQC, 1993, for each one of the estimated metals 45.5. 10.9, 42.6, 62.4, 99.01 and 2.97% of the examined samples exceeded the permissible limits(PL) for Cd, Cu, Hg, Mn, Pb and Zn. While comparing with PL of FAO/WHO, 1985 and 1992, 81.2, 29.7, 15.84, 42.6, 24.7 and 63.33% of the examined samples exceeded PL for Cd, Cr, Cu, Hg, Mn and Pb respectively.The analysis of variance of the results indicated the presence of highly significant difference between the five examined districts in values of estimated heavy metals.

- **Introduction**

The pollution of the aquatic environment with heavy metals has become a serious health concern because of their toxicity and accumulation by organisms **(Mendil *et al*., 2010)**.

Non essential metals (e.g., Pb, Cd) are held to be the most dangerous, since continuous exposure of organisms to their low concentrations may result in bioaccumulation and subsequent transfer to man by the food chain **(Chale, 2002)**.

Assessment of heavy metals residues in fish have been a major environmental focus of many researches in different countries: Egypt (Ibrahim, 2014); and Saudi Arabia (Mohammed, 2009).

The pollution of the aquatic environment with heavy metals has become a serious health concern because of their toxicity and accumulation by organisms (Mendil et al., 2010).

The present study was designed to estimate the levels of some heavy metals (cadmium, chromium, copper, mercury, manganese, lead and zinc) in Tilapia nilotica fish as a biomarker to pollution. and to compare these levels with the permissible limits recommended by Egyptian and international organizations.

MATERIALS AND METHODS

101 *T. nilotica* samples (200-500 gram) were collected from 5 districts in Sohag (winter, 2012) (Sohag city, Issawiya, Gerga, Maragha and Dar El-Salam). These five districts lie on River Nile. The edible part of fish was kept deeply frozen at -20°C until the samples were prepared for digestion and analysis. Determination of Cd, Cr, Cu, Hg, Mn, Pb and Zn were carried out in Soil and Water Department, Faculty of Agriculture, Sohag University, by ICAP 6000 Series ICP (Inductively Coupled Plasma apparatus).

- **Results**

Statistical analytical results of mean±SE (Min-Max) of metals levels (ppm)*

in *T. nilotica* fish

District	Sohag	Issawiya	Gerga	Maragha	DarEl-Salam
Cd	0.091±0.005 (0.5-0.13)	0.109±0.030 (0.01-0.385)	0.080±0.004 (0.045-0.125)	0.209±0.042 (0.01-0.645)	6.55±1.01 (0.25-13.45)
Cr	43.775±5.529 (11-96)	59.275±9.998 (23.5-232.5)	37.298±6.022 (3.85-96)	39.825±7.56 (7.5-121.5)	1.48±0.104 (0.25-2)
Cu	1.55±0.256 (0.01-3.94)	2.987±0.456 (0.96-6.27)	1.89±0.344 (0.05-6.455)	2.097±0.379 (0.195-5.105)	21.99±3.33 (1.25-53.75)
Hg	0.071±0.011 (0.02-0.18)	0.739±0.079 (0.1-1.25)	0.165±0.071 (0.01-1.55)	0.184±0.024 (0.02-0.325)	1.81±0.51 (0-11.25)
Mn	0.459±0.079 (0.06-1.58)	5.639±4.889 (0.265-98.5)	0.424±0.055 (0.02-0.855)	7.395±1.492 (0.535-19.715)	8.987±0.78 (2.25-12.25)
Pb	0.479±0.116 (0.14-2.6)	1.362±0.475 (0.15-7.31)	0.641±0.094 (0.14-2.35)	3.377±1.239 (0.06-24.29)	48.75±3.90 (8-76.25)
Zn	0.315±0.005 (0.26-0.34)	9.237±2.44 (0.55-42.45)	0.309±0.01 (0.155-0.395)	40.412±2.416 (19.64-58.44)	4.25±1.20 (0-19)

- Samples% of T. nilotica according to PL of certain heavy metal levels

elements	Permissible limits(PL) (ppm)	Samples No. within PL	Samples% within PL	Samples No. over PL	Samples% over PL
Cd	0.1 #	55	54.5%	46	45.5
Cd	0.05 ##	19	18.8%	82	81.2
Cr	50**	71	70.3%	30	29.7
Cu	20 #	90	89.1%	11	10.9
Cu	10*	85	84.16%	16	15.84
Hg	0.5 #	58	57.4%	43	42.6
Hg	0.5 ##	58	57.4%	43	42.6
Mn	0.5 #	38	37.6%	63	62.4
Mn	5.4*	73	72.3%	28	24.7
Pb	0.1 #	1	0.99%	100	99.01
Pb	0.5 ##	37	36.67%	64	63.33
Zn	50 #	98	97.03%	3	2.97
Zn	150*	101	100%	0	0%

- # **EOSQC (1993)**, ## **FAO/WHO (1992)**, ** **WHO (1985)**, * **FAO (1985)**

- **DISCUSSION**

When comparing the obtained results with the permissible limit of **EOSQC (1993)** for each one of the estimated metals, it was found that 45.5, 10.9, 42.6, 62.4, 99.01 and 2.97% of the examined samples exceeded the permissible limits (PL) for Cd, Cu, Hg, Mn, Pb and Zn, respectively. While comparing with PL of **FAO/WHO (1992)**, it was found 81.2, 29.7, 15.84, 42.6, 24.7 and 63.33% of the examined samples exceeded the PL for Cd, Cr, Cu, Hg, Mn and Pb, respectively.

- **Conclusions**

It was found a presence of highly significant difference between the five examined districts in values of the estimated heavy metals.

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THE CONTENT OF TOXIC ELEMENTS IN THE EGGS FROM HENS KEPT IN A FREE RANGE SYSTEM IN THE AREA OF COPPER INDUSTRY

P. Cwynar[1], Z. Dobrzański[1], R. Kołacz[1], R. Kupczyński[1], S. Opaliński[1], F. Bubel[2]

[1]Department of Environmental Hygiene and Animal Welfare, Faculty of Biology and Animal Science, Wrocław University of Environmental and Life Sciences, J. Chełmońskiego 38C, 51-630 Wrocław, Poland
[2]Laboratory of Biotechnology "Poltegor- Institute" Institute of Opencast Mining, Parkowa Str. 25, 51-616 Wroclaw, Poland

Laying hens kept in extensive, free range systems in rural areas are exposed to environmental pollutions when the farm location is close to urbanized and industrialized areas.

The aim of the study is to evaluate the content of toxic elements (Cd, Cu, Hg, Pb and Zn) in the eggs from hens kept in a free range system in the cooper industry area "Żelazny Most" (Low Silesia, Poland). This is one of the largest tailing pond in Europe, situated on the area of approx. 1,400 ha, and the volume of wastes stored in the pond is approx. 500 million m^3. "Żelazny Most" is a source of metal pollution for all of the surrounding villages.

The eggs were collected in the autumn 2012 and 2013. The tailing pond area was divided for two zones: closer villages (zone I 2 – 4 km from the pond) and further villages (zone II – over 5 km from the pond). Eggs in the total number of 46 were cooked and homogenized after removal the shell, whereupon the wet mineralized concentrated nitric acid HNO_3 analysis of concentration of Zn and Cu was performed by atomic absorption spectroscopy with excitation flame (F-AAS), using a spectrometer SpecrAA 220FS. The determination of total mercury (Hg) was made using atomic absorption spectrometer AMA 254 (Altec). The concentration of Cd and Pb were determined using plasma spectrometer with mass detection ICP-MS.

There was no correlation between the I and II zone in the concentrations of heavy metals, with the exception of Hg and Pb in 2012. Taking into account the mean contents of the elements in 2012, there was a significant increase of cooper (Cu) in eggs as well as the lead (Pb) and mercury (Hg) in correlation to 2013.

ECOTOXICOLOGICAL RISKS ASSESSMENT RELATED TO RODENT CONTROL BASED ON ANTICOAGULANT RODENTICIDES

Rudolf Hromada, Vladimír Petrovič, Ľuboš Korytár, Dušan Chvojka, Ján Koščo, Naďa Sasáková, Miloslav Ondrašovič, Katarína Veszelits Laktičová, Slavomír Horňák[2], Ján Čurlík[3], Ján Pošivák[4]

[1]Department of Environment, Veterinary Legislation and Economics, The University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia
[2]Clinic of small animals, UVLF, Košice, Slovakia
[3]Department of nutrition,dietetics and animal husbandry, UVLF, Košice, Slovakia
[4]Clinic of horses, UVLF, Košice, Slovakia

Summary

The study investigated residues of brodifacoum rodenticide that was applied to soil in containers at the time of wheat sowing in the form of preparation Broder G containing 75 g.m^{-2} bait of active ingredient brodifacoum. At harvest time we collected samples of wheat grains and determined brodifacoum residues by HPLC chromatography. The residues were determined every year and the obtained values ranged between 0.009 and 0.016 mg.kg^{-1}. The results obtained were used to calculate bioaccumulation factor (BAF) and toxicity to exposure ratio (TER). The calculations showed that the ecotoxicological risk was considered acceptable. However, when taking into consideration the basic toxicological properties of brodifacoum and biological properties of the common vole (*Microtus arvalis*) such conclusion is disputable as we try to point out in this study.

Introduction

The use of pesticides is a part of environmental protection and management in terms of control of harmful plants and noxious animals. Their use must be target-oriented because the risk of development of residues may disturb biological balance within an ecosystem and endanger food chain and thus also health of humans and animals (Ondrašovič et al., 1996).

On agricultural land we face periodically the problem of excessive infestation with noxious rodents, particularly the common vole (*Microtus arvalis*). Its control is based on rodenticidal preparations. With regard to preparations used for protection of agricultural plants there is a List of permitted plant protection preparations which does not include anticoagulant rodenticides. As there is no ban on the use of these preparations, they are commonly available in stores, are economically advantageous, and their use in practice is common and not controlled.

On the basis of above mentioned, we conducted this study in order to determine residues of brodifacoum, accumulated after application of Brother G to soil used for growing of wheat, and to carry out ecotoxicological risk assessment.

Material and Methods

We investigated accumulation of residues after application of preparation BRODER G with active ingredient brodifacoum, the anticoagulant rodenticide of IInd generation. The preparation was worked evenly into soil (75 g bait per m^2) placed in containers of dimensions 1x1x0.5 m before sowing wheat. Another container of the same dimensions but without rodenticide served as control. During the vegetation stage the containers were exposed to climatic conditions and no other agrotechnical measures were taken. At the beginning of August, at harvest time, we collected samples of grains from wheat grown on the soil treated with brodifacoum and on control soil. From the area of 1 m^2 we collected 100 – 150 g wheat grains from which we prepared 5 samples weighing 10g and used them for analysis together with one control sample.

The chromatographic analysis was carried out using column LiChrospher^R 100 RP-18 (5µm) with mobile phase acetonitril (A) + acetate buffer, pH 4.6 (B) (60 : 40), flow rate l ml.min^{-1}, injected analyte 10µl, UV detection at 265 and 310 nm.

The ecotoxicological risk was calculated using the respective residual levels by the procedure according to Manual SANCO/4145/2000 (2002).

Results

There were not big differences in the levels of residues between individual years which is supported by standard deviations that are illustrated together with the mean values of residues in Fig. 1. Individual maximum and minimum values measured in samples and mean values for individual years are shown in Table 1. The lowest mean value was recorded in 2005, namely 0.009 mg.kg^{-1}. In this year we recorded also the lowest level found throughout the investigation, namely 0.0083 mg.kg^{-1}. The highest mean value (0.016 mg.kg^{-1}) was determined in 2006 when the lowest level in the samples reached 0.0150 mg.kg^{-1} and the highest 0.0180 mg.kg^{-1}. During the experiment we recorded the highest residuum 0.0180 mg.kg^{-1} in the years 2006 and 2008. The mean level was the same in the years 2007 and 2008 and reached 0.015 mg.kg^{-1}.

For the purposes of statistical evaluation we used as the control level the sensitivity of the respective probe used for determination of brodifacoum residues in wheat (0.000001 mg.kg^{-1}) at zero values in control samples. When using T test for comparison of residues in wheat in comparison with control samples we observed that the differences were significant. Results shows the ecotoxicological risk assessment related to consumption of wheat with residues of rodenticides based on calculation of BAF, FIR and TER. The bioaccumulation factor, which is directly related to residues in wheat, reached the highest level in 2005, i.e. the year with the lowest content of residues. In 2007 and 2008 the levels of this factor were the same and the lowest level was calculated for 2004. With regard to FIR the results are presented in two columns in dependence on the data of daily consumption of food by the vole. When calculating FIR and TER according to Jagosz et. al. (1979), who reported consumption of 7.5g food per day the results differed from those calculated according to Škuciová (2011), who reported consumption of 25g food per day. In dependence on the value used there is a 3-fold difference between values of FIR and TER in the respective columns.

Discussion

Pesticides are currently an inevitable part of modern agrotechnical procedures and owing to intensification of agriculture they reach the entire ecosystem including the food chain. Residues of pesticides following their application have been detected in both the abiotic and biotic environment. These chemical contaminants present serious risk with regard to their residues in the food chain (Ondrašovič et al., 1996). Maximum limits were set for residues of selected types of most frequently used pesticides (Codex Alimentarius of SR, 2012).

According to Hayes and Laws (1991) brodifacoum is not resorbed by plants from contaminated soil which is in contradiction with our results.

In our experiments performed in the period of 2004−2008, brodifacoum residues in wheat grains ranged between 0.0083 and 0.0180 mg.kg^{-1}.

Conclusion

When investigating these aspects in detail we believe that the mentioned Manual does not respect sufficiently neither the toxicological properties of brodifacoum, such as the way of its elimination, which occurs in two phases with elimination half-time of 130 days (WHO, 1995) nor biological properties of the concerned species, in our case the life span of the common vole which is 120 days for spring populations and 520 days for autumn populations. When considering these aspects and making the relevant recalculations we find out that the common vole is capable of consuming LD_{50} for brodifacoum (0.22 mg.kg^{-1}) within 16 to 54 days, which subsequently points to the risk of contamination of the food chain and development of secondary poisonings.

DETECTION OF DNA FRAGMENTATION IN LEAD EXPOSED GOATS FROM THE BAGEGA DISTRICT OF NIGERIA

A. J Jubril[1], A.A. Adekola[1], O.A Fagbohun[2]

[1]*Department of Veterinary Pathology, University of Ibadan. Nigeria*
[2]*Department of Veterinary Microbiology, University of Ibadan. Nigeria*

Summary

The lead toxicity clinicopathological effect has been overwhelming in the Bagega District of Zamfara State, Nigeria where due to artisanal gold mining, tons of lead contaminants are being released from the extracted ore.

For the purpose of this study, the liver lead levels from exposed group (goats sourced from the Bagega lead contaminated areas) was evaluated using Atomic Absorption Spectrophotometry. The liver tissue were then analysed using ethidium bromide stained agarose gel electrophoresis for detection of Apoptotic DNA Ladder.

The degree of DNA fragmentation detected in the exposed goats group was found to correspond with the high tissue lead level found in this group.

This study points to the relevance of the Caspase activated DNase (CAD) mediated oligonucleosomal fragmentation of DNA as major biochemical hallmark of apoptosis. It also shows the important role of apoptosis in the clinicopathological features associated with lead toxicity.

Introduction

Lead is a non-biodegradable heavy metal pollutant in the environment. It is ubiquitous due to its relative abundance in nature and has found widespread use due to its desirable malleability and relatively high resistance to corrosion. The continuous release of lead due to anthropogenic activities has made lead pollution a big environmental risk (Needleman 2004).

Lead has been associated with a wide range of toxicity effect on the body affecting the central nervous system, haemopoietic system, gastrointestinal tract, respiratory tract, liver and bone, kidneys and the male and female reproductive organs (Valko *et al.,* 2005).

Some of the alteration associated with lead toxicity include lead interaction with enzymatic processes resulting in inactivation of important biochemical activities and the induction of oxidative stress in animals and humans (Sharma *et al.,* 2014). The generated oxidative stress have genotoxic effect and results in oxidative DNA damages which along with the direct impact of lead on the DNA repair enzyme also increases the potential for DNA damage accumulation. This damage also predisposes to potential oncogenic transformation and may trigger the activation of the apoptotic mechanism (Nagata, 1997).

Apoptosis is a programmed cell death mechanism characterized by specific biochemical and morphological hallmarks. The apoptotic oligonucleosomal DNA fragmentation forms an important molecular hallmark and can be detected using techniques such as DNA ladder assay and TUNEL assay (Otsuki 2000).

Materials and Methods:

A total of 12 goats selected from the goats slaughtered at the Bagega Community Slaughter slab constituted the Exposed Goat group population while goats from an unexposed population were used as control.

The liver tissue samples were taken from the slaughtered animals. The liver samples were used for the evaluation of the liver lead level using atomic absorption spectrophotometry as described by Julshman (1983).

The liver tissue samples of the goats were divided based on the lead level and subjected to the agarose gel electrophoresis detection of apoptotic DNA ladder using the Abcam® Apoptotic DNA Ladder Detection

Results:

The tissue lead levels in the exposed group animals as shown in Table 1. were found to be very high and above the WHO acceptable limits. The animals were categorized into 3 groups based on the severity of the exposure according

to the WHO standard using tissue lead levels viz: Mild Exposure, Moderate Exposure and Severe Exposure with each category having a limit of <100 µg/g, <200 µg/g and >200 µg/g respectively.

Table 1: Mean levels of lead in the liver of exposed goats (µg/g)

Category	WHO Standards (µg/g)	Number (n) of animals in category	Values (µg/g)
Mild exposure	<100	3	78.64±20.24
Moderate exposure	<200	4	158±62.24
Severe exposure	>200	5	586.54±144.59
Total		12	324.25±138.54

In Figure 1., there was a level of exposure dependent difference in the degree of fragmentation with the goats in the severe exposure Category (lane 3) showing more prominent ladder compared to lane 1 and 2 which represent the goats in the mild and moderate exposure goat category respectively.

Discussions

The liver serves as an important organ for the monitoring of heavy metal exposure. The liver lead levels obtained in the exposed goats was similar to the findings of Akoto *et al.,* 2014 although the lead liver level in the Bagaega District goats was relatively higher.

In this study, the detection of apoptotic DNA ladder points to a caspase mediated mechanism in lead induced apoptosis. This can be adduced to the findings by Wolf *et al.,* 1999 in which the manifestation of oligonucleosomal apoptotic DNA fragmentation starts with effector caspase (caspase 3) mediated activation of CAD. This then facilitates the sequential cleavage of the DNA to yield oligonucleosomes in multiples of 180 to 200 bps.

Furthermore, the difference in the extent of DNA fragmentation seen in the different agarose gel electrophoresis lane in corresponds to the level of exposure of the animals to lead. This is similar to studies by Ahmed *et al.,* 2013 in which dose dependent increase in the levels of apoptotic markers such as caspase 8, caspase 9 and Bax in the liver, kidney and brain tissues was discovered in experimentally lead exposed rats.

Conclusions:

The detection of DNA ladder in the lead exposed animal tissues is of unique importance as it indicates the role of caspase mediated effector mechanism in the apoptosis associated with the clinicopathological features of lead toxicity. The study also highlights the potential for lead induced DNA damage as the primary apoptotic mechanism trigger. This DNA damage also serves as a potential source of the gentoxic and clastogenic effect associated with lead toxicity and the potential oncogenic risk that could arise due to long term exposure.

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OCCURRENCE OF ESBL IN ESCHERICHIA COLI ISOLATED FROM MUNICIPAL WASTEWATER TREATMENT PLANT

J. Venglovský, T. Čornejová, G. Gregová, N. Sasáková, K. Veszelits Laktičová

University of Veterinary Medicine and Pharmacy in Kosice, Department of Environment, Veterinary Legislation and Economics, Komenského 73, 041 81 Košice, Slovak Republic

Summary

The aim of this study was to detect and assess the production and the occurrence of ESBL - extended spectrum beta-lactamases in investigated *E. coli* strains. The samples of *E. coli* were obtained from influent and effluent water samples from the municipal wastewater treatment plant. The ESBL-positive strains were confirmed by phenotypic testing, and also by PCR analysis. ESBL phenotype and also ESBL genes of resistance were detected in *E. coli* strains.

Introduction

Municipal wastewater treatment plant is one of the main sources of antibiotics released into the environment. During the last years, the prevalence of bacteria producing extended-spectrum beta-lactamases (ESBL) have had an increasing tendency (GREGOVA et al., 2010). This increasing of the antibiotic resistance significantly limits the selection and possibilities of the treatment for infection disease caused by these bacteria and also present high health risk for animal and human population (NEMEC, 2006).

The aim of our study was to detect an antibiotic resistance and ESBLs production in *E.coli* isolated from wastewater. ESBL in the samples of *E. coli* strains was confirmed by a presence of ESBL phenotype and also by ESBL genes resistance.

Materials and methods

The samples were collected from the influent and effluent of the wastewater treatment plant. Each sample was inoculated and multiplied in buffered peptone water (Oxoid, Basingstoke, United Kingdom). Identification of bacteria was carried out by a cultivation on Mac Conkey agar overnight at 37 °C and Uriselect medium. The second way of the identification of bacteria was also performed by Maldi Tof biotyper (KMET et al., 2013).

Minimal inhibitory concentrations (MIC) were determined according to CLSI 2013: VET01-S2. MIC testing results were read by means of a scanner and digital analysis software MIDITECH (GREGOVA et al., 2012). By means of analysis software the values resistance mechanisms were also evaluated percentually. These findings were also confirmed by PCR analysis for detection of ESBL genes (CTX-M group genes) in selected strains.

Results

The occurrence of antibiotic resistance and ESBL production in *E.coli* strains were revealed. Our results showed that in a considerable amount of examined samples ESBL-confirmed *E. coli* strains with ESBL phenotype were observed. By digital software analysis MIDITECH the values of resistance mechanisms were found out. ESBL CTX – M type was detected in 17 % of all samples.

On the basis of PCR analysis in the investigated samples also the occurrence of ESBL genes was determined. From the environmental isolates *E. coli* the presence of CTX-M group genes almost half of the examined samples – 47 % was identified. This fact indicates an increased prevalence of the ESBL genes in the wastewater and in the environment, what can be probably explained by an excessive use of antibiotics in human population of the studied area.

Discussion

In the last years, the antibiotic resistance and incidence of ESBL in *E. coli* strains had increasing tendency. People and animals may obtain ESBL-producing bacteria through different exposure routes, including contact with human or animal carriers or consumption of contaminated food. However, contact with faeces contaminated surface water may also represent a possible exposure route. There is the evidence that antibiotic resistance can also occur in marine environments without the addition of antibiotic contamination. For instance, the same resistance genes found in clini-

cal human pathogens have been reported among pristine ecosystems without a history of antibiotic contamination (SCHMIEDER and EDWARDS, 2012).

ESBL-producing *E. coli* were detected in all four recreational waters in The Netherlands, with an average concentration in 62% of all samples (BLAAK, 2014). In our study, ESBL phenotype was detected in 25% of all investigated samples. A present of ESBL CTX – M type was confirmed almost in a half of the selected examined samples, what constituted 47,05%. In the air of municipal wastewater treatment plant a high concentration of antibiotic resistant *E.coli* with CTX-M1 betalactamase were also detected (GREGOVA et al., 2010).

The ESBL variants (CTX-M groups 1 and 9) detected in the outflow of the wastewater treatment plants generally correspond to the most common variant detected in association with clinical infection in our population (MORRIS et al., 2009).

Conclusions

Municipal wastewater treatment plants belong among the main sources of antibiotics released into the environment. The occurrence of antibiotics may promote the selection of antibiotic resistance genes and antibiotic resistant bacteria. In *E. coli* strains ESBL phenotype and ESBL genes of resistance were also detected. These genes with virulence factors represent a potential environmental health risk and their spreading to the population can cause horizontal transfer to other organisms.

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Climate change and livestock production

EFFECTS OF SUMMER HEAT STRESS ON ESTROUS BEHAVIOR, OVULATION AND CORPUS LUTEUM FUNCTIONALITY OF PELIBUEY EWES UNDER NATURAL OUTDOOR CONDITIONS IN AN ARID REGION

MA. Gastelum-Delgado[1], JE. Guerra-Liera[1], U. Macías-Cruz[2], Francisco D. Álvarez[2], A. Correa-Calderon[2], CA. Meza-Herrera[3], L. Avendaño-Reyes[2].

[1]Facultad de Agronomia, Universidad Autonoma de Sinaloa, Mexico
[2]Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California, Valle de Mexicali, B.C., México
[3] Unidad Regional Universitaria de Zonas Áridas, Universidad Autónoma Chapingo, Bermejillo, Durango, México

SUMMARY

Pelibuey ewes were housed in a corral to evaluate the effects of summer thermal stress on physiologic variables, estrous behavior, ovulation and *corpus luteum* functionality under conditions of an arid region. An environment of heat stress was detected in summer and thermoneutral in autumn. RT and RF were greater ($P < 0.01$) in summer than in autumn. Season did not affect ($P > 0.05$) live weight (LW), body condition (BCS), length of estrous cycle, and percentage of ewes in estrous and ovulating. Serum progesterone concentrations in summer decreased ($P < 0.05$) between days 8 and 14 of the estrous cycle. It is concluded that under outdoor conditions of arid regions, while estrous and ovulatory activities of Pelibuey ewes were not affected by summer thermal stress, the corpus luteum functionality was decreased.

INTRODUCTION

Pelibuey sheep is distributed in arid and semiarid regions due to its rusticity and adaptability to environmental conditions of heat stress (HS) (Macías-Cruz *et al*. 2010), but available information on their reproductive and productive ability under these natural environmental conditions is scarce.

It has been observed that HS causes the activation of compensatory mechanisms to regulate body temperature in sheep (Marai *et al*. 2007). Increased respiration rate and a redistribution of blood flow toward the periphery are thermoregulatory mechanisms generally used by sheep to dissipate the excess of body heat load (Cain *et al*. 2006). However, when these physiological mechanisms fail to dissipate the excessive heat load, the rectal temperature increases and a series of changes in biological functions are evoked, such as reduced feed intake, increased water intake, and disturbances in hormone secretions and reproductive processes (Marai *et al*. 2008).

MATERIALS AND METHODS

This study was conducted in the Mexicali Valley, northwestern Mexico (32°24´ N and 115°22´ W). The highest and the lowest temperatures recorded during summer and winter seasons are 50 and -5° C, respectively. Treatments, summer was considered as of HS while in autumn as thermoneutral. The sampling period in summer was from July 3 to August 18, and in autumn from November 1 to December 12. Physiological variables were measured twice per week 800 h and 1600 h on each experimental period. Ewes were weighted at the beginning and end of the experimental period on each season; BCS was simultaneously measured. Likewise, estrous behavior, ovulation and CLF were measured in two consecutive natural estrous cycles on each season. The estrous behavior in ewes was determined using two rams fitted with an apron, while ovulation and CLF were determined by blood progesterone (P_4) concentration. Blood samples were collected on days 2, 5, 8, 11, 14 and 17 after presentation of estrous signs (d 0). It was considered that an ewe ovulated when P_4 levels were >1.0 ng/ml in 2 consecutive sampling (Arroyo *et al*. 2007). LW, BCS and length of estrous cycle were analyzed under a CRD. Physiological variables and serum P_4 concentrations were analyzed with BCS repeated measures over time; the model included effects of season, time (week or day of the estrous cycle) and the interaction between main factors. Mean comparisons were performed with the option PDIFF when significant differences were detected at $\alpha=0.05$. Percentages were analyzed with X^2 test. Analyses of variance were performed with the MIXED procedure and X^2 tests with the FREQ SAS (2004).

RESULTS

Temperature and THI averages in the summer experimental season were 36.1º C and 82.7 units, while in autumn season were 19.1º C and 63.4 units, respectively. RT and RR at 800 and 1700 h were higher ($P < 0.01$) in summer. Percentages of ewes in estrous and ovulating were similar ($P > 0.05$) between summer and autumn. The average length of the estrous cycle was of 17.5 ± 0.5 d, with no season effect ($P > 0.05$). There was a significant interaction effect ($P < 0.05$) between season and days of the estrous cycle for P_4 concentration. At days 2, 5 and 17 of the estrous cycle, P_4 levels did not differ ($P > 0.05$) between seasons, but between days 8 and 14 of the cycle were higher ($P < 0.05$) in autumn. The highest ($P < 0.05$) serum P_4 concentration was detected at d 11 of the cycle, in both summer and autumn seasons.

DISCUSSION

High summer temperatures did not affect the body status of ewes, since LW and BCS remained relatively constant during summer season. Studies conducted in sheep (Moura *et al*. 2014) have demonstrated that thermal stress causes a series of failures in the estrous behavior of females. These findings are not consistent with our results given that the percentages of Pelibuey ewes in estrous and ovulating were similar between summer (HS conditions) and autumn (reproductive season), observing 100% in both seasons.Pelibuey ewes also presented similar length of estrous cycle in summer and autumn, which suggest that the duration of this cycle was not affected by summer HS in this breed (Silanikove 2000). Published studies have not reported results close to this finding in hair ewes, Sejian *et al*. (2012).Many efforts have been directed to explain the HS effect on secretion of reproductive steroid hormones in ewes and cattle (Sejian *et al*. 2012).Findings of this study showed that since 100% of ewes presented estrous signs and ovulated, both under chronic HS of summer and during the natural reproductive season (autumn) in the zone of study. The capacity of the *corpus luteum* to secrete progesterone hormone was reduced by effect of summer thermal stress.

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THE INULIN CONCENTRATE FEEDING TO CALVES

A.Arne, A.Ilgaza

Department of Veterinary Medicine, Latvia University of Agriculture, Jelgava, Latvia

Summary

In order to achieve a faster animal growth and developmental in the European Union it prohibited to feed antibiotics to calves, it is important to find alternatives. The aim of the study was to find out how the feeding of *Helianthus tuberosus* flour, containing high concentration of prebiotics inulin (48.5 - 50.1%) affects the increase of live weight, overall health condition and colon histological status of calves. Our data show that compared with control group animals, the diarrheal cases of the probiotic group calves were relatively lighter and less frequent. Weight gain and carcass weight of prebiotic group is significantly ($p<0.05$) higher than in the control group calves.

Introduction

In the European Union the use of antibiotics in food for calves with an aim to promote a live weight increase is prohibited. Antibiotics affect both adverse and beneficial microflora, but does not improve the immune system of animals. Therefore alternative means of live weight gain, health condition improvement and mortality reduction are searched (Mathur et al., 2005; Verdonk, 2005, Heinrichs et al., 2009). Prebiotics could be used as one of such alternatives (Samanta, 2013; Masanetz et al., 2010).

Our **aim of the study** was to determine the produced in Latvia Helianthus tuberosus flour concentrate's (inulin 48.5-50.1%) feeding influence on calf health condition, live weight growth as well as on colon crypt status in the first four months of life.

Material and methods

The study included healthy 23 +/- 5 days-old calves, whose live weight was within 50 kg +/- 5 kg range. Two groups were created: a control group (CoG, n = 8) and a prebiotic group (PreG, n = 8), whom specially produced *H.tuberosus* flour (or flour 12g 6g inulin) was added to the consumed milk. The study lasted 56 days. Animals had free access to drinking water and hay, two weeks after the study start – to fodder as well.

Every day, the calves' health state was evaluated, with a particular focus on the defecation consistency. Animal faeces were assessed by Larson (1977) scale (from "0" - solid to "3" - watery faeces). Every 4 weeks (i.e. on the 1st, 28th, 56th day) test weightings were made. After 56 days, when the calves reached 12 weeks of age, a planned slaughter of calves was carried out. After slaughter, we obtained colon histological samples. They were dyed in hematoxilin / eosin and examined with a *Leica DM5000B* microscope at 20x magnification using a *Leica DFC490* camera and computerized digitization program *Im-Pro-Plus6.1*. In each sample 5 randomly selected fields were examined.

Results

The first table shows CoG and PreG group calf live weight (average and standard deviation) on the 1st, 28th, 56th research day, mean weight gain throughout the study period and the average cold carcass weight. It was stated that PreG weight gain in calves within 56 days was significantly higher ($p <0.05$) than of the CoG animals (Table 1). Also, average daily weight gain in PreG animals is 0.277 kg, thus significantly ($p <0.05$) higher than in the CoG. Cold carcass relative weight of the animals that were fed with prebiotics inulin was 5.72% higher than of the CoG animals (Table 1).

By analyzing faecal consistency changes, it was observed that at the study beginning, faecal mass assessment both of CoG and PreG calves met the 0.68 and 0.50 points (Fig.1). On the seventh week the CoG and PreG calves had more liquid in faeces (average 1.43 and 1.0 points). At this age, the animals started to receive a concentrated feed, which could be one of the causes of fecal liquefaction. After 3 weeks, both animal group faecal masses have become steadily more solid. At that time, the calves began to take hay more intensively, so their faecal consistency stabilized and, possibly, the digestive processes as well.

Investigating colon crypts, it was stated that in PreG animals they were deeper than in the CoG group (respectively 0.581mm and 0.448mm ± 0.34 ± 0.23). CoG animals on the investigated sample area (96143.54mm^2) had greater number of the crypts (375 crypts) than PreG animals (95649.48mm^2 and 301 crypts). Thus, the crypto density per 1 mm^2 of intestinal area in CoG is greater than in PreG animals. It should be noted that in 3 animals of the CoG and 1 from PreG leukocyte infiltration was found. It indicated a slight colon inflammation, which was not confirmed clinically.

Discussion

Evaluating our data, we can conclude that PreG calve live weight gain and carcass mass is significantly bigger ($p < 0.05$) than in the CoG. Also Stolić (2012) when feeding mannaoligosaccharide prebiotics to calves has shown that a significantly higher live weight gain can be reached.

Although in both groups of animals during the research period (on the 7th-9th week) after fodder intake, faecal mass consistence becomes liquid, our data indicate that it has been observed as the most stable one in PreG animals during the whole study period. Also Krol (2011) in his studies mentions that faecal mass consistency is better in animals that get inulin, than in the control group.

When making colon crypt measurements it was found that the prebiotic group of animals had fewer crypts, but they were deeper. It does not coincide with Masanetz (2010) research, where calves after inulin feeding had shallower crypts than in the control group. So prebiotics inulin feeding can affect the colon crypts, but it is important also to examine the histology of the small intestine, where, according to the data of other authors (Masanetz, 2010) the main prebiotic activity takes place and main differences are met.

Conclusions

The feeding of Jerusalem artichoke flour, containing high concentration of inulin, to calves from 4-12 weeks of age significantly increases the live weight gain and carcass weight outcome. It stabilizes the faecal mass texture and can serve as a safe feed additive, but studies in this area should be continued.

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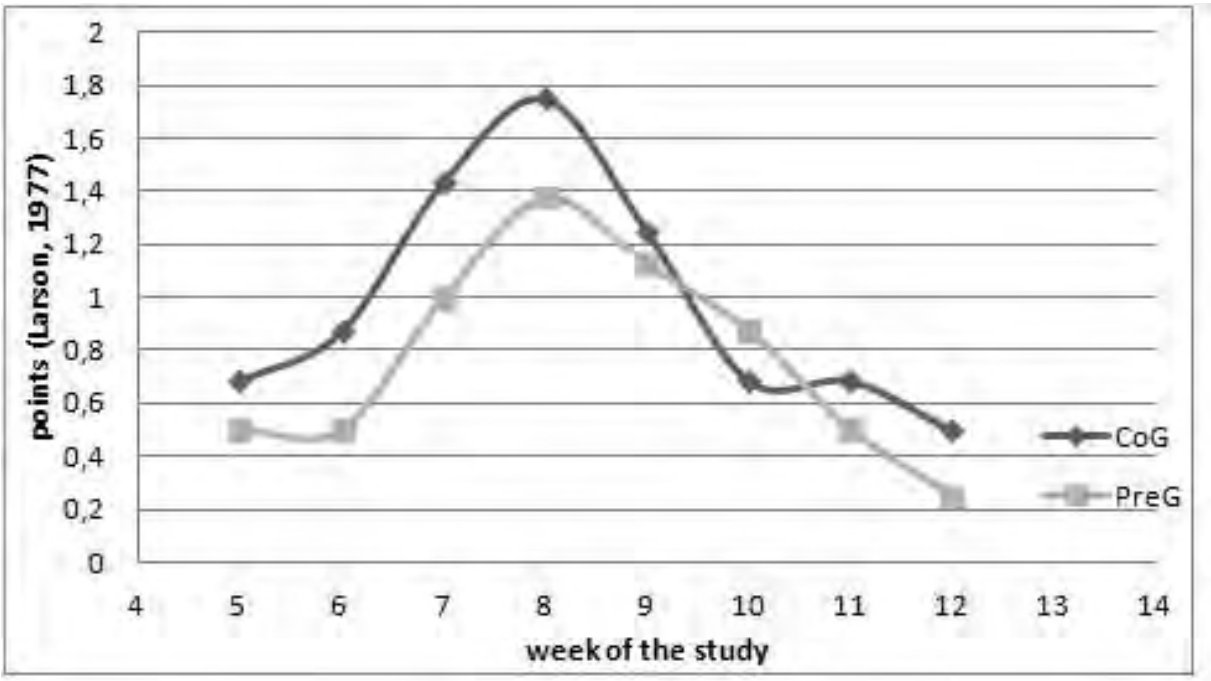

Calf growth performance

Groups	Average weight of the animal (kg), research day			Live weight gains (kg) 1-56 day	Average daily body weight gains (kg)	Average cold carcass weight, (kg)
	1	28	56			
Control	53.6±5.71	71.9±10.38	83.0±11.18	29.4±5.47	0.52 ± 0.183	43.57±8.16
Prebiotic	54.3±3.28	75.4±10.15	96.4±11.50	42.1±8.21	0.75* ± 0.201	49.14±8.07

GREENHOUSE GAS AND AMMONIA CONCENTRATIONS IN DAIRY COW BARNS ESTIMATED BY INFRARED SPECTROSCOPY (FTIR)

D. Witkowska, M. Steraniec, P. Orlich, K. Michalski, J. Sowińska

Department of Animal and Environmental Hygiene, University of Warmia and Mazury, Olsztyn, Poland

SUMMARY

The dairy sector is considered to be an important source of greenhouse gases, including CO_2, CH_4 and NO_X, which contribute to global warming. Atmospheric ammonia contaminate groundwater and soil. Monitoring and reducing gaseous emission by using best available techniques is a priority in animal production. The aim of the study was to estimate gas concentrations by FTIR method in two barns with a varied maintenance system of dairy cows.

The experiment was carried out from January to March in two naturally ventilated barns with straw litter (A. tied-stall, for 58 cows; B. free-stall, for 90 cows). Gas concentrations (CO_2, CH_4, NO_X, NH_3) were determined by Fourier Transform Infrared Spectroscopy (FTIR) using a portable multi-component Gasmet DX4030 analyzer for on-site measurements of chemical compounds at low concentrations in ambient air. Sample spectra were obtained 4 times (every two weeks) at 5 locations inside each building. After the measurements, sample spectra were analyzed with the use of Calcmet Professional software with a library of reference spectra for particular gases. The results were analyzed statistically using Statistica 10.0 software.

The average concentrations of CO_2, NH_3 and N_2O were statistically higher ($P \leq 0.01$) in the building A (1057 ppm, 2.85 ppm, 0.45 ppm, respectively) while in the barn B the mean concentrations of mentioned gases equalled 907 ppm, 1.78 ppm and 0.28 ppm. By contrast CH_4 concentration was statistically higher ($P \leq 0.01$) in B building (76 ppm) in comparison with A (65 ppm). The similar trend was noted with regard to NO_2 (B = 0.46; A = 0.13). The results indicate that the concentrations of gases in dairy sector depend on maintenance system and the FTIR method is useful for chemical analyze of barns air.

INTRODUCTION

The dairy sector is considered to be an important source of greenhouse gases, including CO_2, CH_4 and NO_X, which contribute to global warming (4, 5, 7). Atmospheric ammonia contaminate groundwater and soil, contributing to eutrophication and acidification (4). Monitoring and reducing gaseous emission by using best available techniques is a priority in animal production, so is required by international conventions such as the Kyoto protocol (7). The investigations demonstrate that the release rate of gases depends on many factors, including the regional climate, the type of building, floor type, the manure management system and the ventilation rate and system (2, 4, 6, 7). Some authors emphasize the need of more research concerning regional and global emission factors (4, 7). The aim of the study was to estimate gas concentrations by FTIR method in two naturally ventilated barns with a varied maintenance system of dairy cows.

MATERIAL AND METHODS

The experiment was carried out from January to March in two dairy barns. The building A, with tie-stall housing system, and natural ventilation (inflow by windows, outflow by roof ridge gap), was designed for 58 dairy cows. The animals were kept on the straw litter, which was changed twice a day. The building B was equipped with free-stalls and natural ventilation (inflow by curtains, outflow by roof ridge gap) and accommodating 90 dairy cows which were kept on straw bedded stalls. The litter on stalls was added every two days and changed once a week. The walking – manure concrete passages with full floor were in both objects. The faeces were removed by the scraper. The TMR feeding program was used in two barns.

Gas concentrations (CO_2, CH_4, NO_X, NH_3) were determined by Fourier Transform Infrared Spectroscopy (FTIR) using a portable multi-component Gasmet DX4030 analyzer for on-site measurements of chemical compounds at low concentrations in ambient air. Before each measurement series the device was zero calibrated using nitrogen gas. Sample spectra were obtained 4 times (every two weeks) at 5 locations inside each building, on the animal head level, and outside the buildings (at a distance 10m). After the measurements, sample spectra were analyzed with the use of Calcmet Professional software with a library of reference spectra for particular gases. During every sample spectra obtaining air temperature (ºC) relative humidity (%) and air speed (m/s) were measured using Kestrel 4000

weather meter and Hill's dry katathermometer. The ventilation rate was calculated from CO_2 mass balance according CIGR (1) formula. The results were analyzed statistically using Statistica 10.0 software.

RESULTS

The average concentrations of CO_2, NH_3 and N_2O were statistically higher ($P \leq 0.01$) in the building A (1057 ppm, 2.85 ppm, 0.45 ppm, respectively) while in the barn B the mean concentrations of mentioned gases equalled 907 ppm, 1.78 ppm and 0.28 ppm (Tab. 2). By contrast CH_4 concentration was statistically higher ($P \leq 0.01$) in B building (76 ppm) in comparison with A (65 ppm). The same trend was noted with regard to NO_2 (B = 0.46; A = 0.13). Outside the dairy cow barns CO_2, NH_3 and N_2O concentrations were also statistically higher in case of A farm, but the levels of CH_4 and N_2O, contrary to the interiors, were higher too. There were any statistic differences between NO concentrations.

DISCUSSION

In the building A, where the number of cows was about 36% lower, the concentration of most gases was higher. Probably it resulted from lower ventilation rate and higher temperature and humidity (Tab. 1). The carbon dioxide level confirm better air exchange in B building. Wu et al. (7) examinations show the significant relationship between ammonia emission rates and climatic factors. It should be mentioned that the concentrations of ammonia and carbon dioxide in both buildings did not exceeded the allowable for dairy cows levels (20 ppm for NH_3 and 3000 ppm for CO_2). Compared to other investigations conducted in winter seasons (3, 5, 6) the concentrations of ammonia in A and B barns was about 2-6 ppm lower. The N_2O concentration inside B building was about 0.1 ppm lower than outdoor values. Similarly to Ngwabie et al. (5) studies in both barns indoor N_2O concentrations were low and close to the outdoor concentrations, however in barn A the value was slightly higher. Wu et al. (7) report that the variation of CH_4 and CO_2 had a strong correlation. In contrast current results show significantly lower level of mean CH_4 in A barn (with higher level of CO_2), and the maximum value of methane concentration in B building was about 40% higher. It could be due to the lower number of animals, because the enteric and manure fermentation is the main source of methane. The mean values of methane concentrations (65-76 ppm) in A and B cow barns in were similar to Ngwabie et al. (4) results from winter research (67-77 ppm).

CONCLUSIONS

The results confirm that the concentrations of gases in dairy cow buildings depend on many factors and more measurements are needed for modelling and estimating greenhouse gas and ammonia immission and emission. Compatibility with other investigations results show that the FTIR method is useful for chemical analyze of barns air.

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Table 1. Temperature, relative humidity, air speed and ventilation rate in dairy cow barns

Parameters	Statistic measures	Inside		Outside	
		A	B	A	B
Temperature (°C)	mean range	12.7 7.7 – 15.3	9.4 0.5 – 17.2	1.6 -8.1 – 7.8	3.8 -7.3 – 13.4
Relative humidity (%)	mean range	70.6 68.4 – 75.2	57.9 41.0 – 74.0	83.5 69.1 – 89.8	55.2 37.8 – 64.2
Air speed (m/s)	mean range	0.2 0.1 – 0.3	0.2 0.1 – 0.3	1.1 0.4 – 1.9	1.4 0.6 – 2.3
Ventilation rate (m^3/h) according to CO_2 mass balance	mean	275.8	342.2	-	-

Table 2. Concentrations of greenhouse gases and ammonia in dairy cow barns

Gases	Statistic measures	Inside		Outside	
		A	B	A	B
NH_3	mean range SD	2.85^{A} 0.90 – 5.79 1.36	1.78^{B} 0.52 – 3.38 0.61	0.40^{**} 0.15 – 3.36 0.32	0.12^{**} 0.00 – 0.41 0.10
CO_2	mean range SD	1057.43^{A} 490 – 1766 279.96	906.95^{B} 411 – 2023 334.86	386.71^{**} 348 – 987 73.80	366.29^{**} 351 – 421 12.20
CH_4	mean range SD	65.26^{B} 12.20 – 117.00 23.21	76.12^{A} 5.19 – 191.00 43.58	4.45^{**} 2.16 – 54.80 3.21	2.29^{**} 1.77 – 9.57 1.17
NO	mean range SD	0.14 0.00 – 1.24 0.14	0.14 0.00 – 1.32 0.12	0.00 0.00 0.00	0.00 0.00 0.00
NO_2	mean range SD	0.13^{B} 0.00 – 1.79 0.10	0.46^{A} 0.00 – 4.87 0.37	0.10 0.00 – 1.43 0.08	0.08 0.00 – 0.88 0.07
N_2O	mean range SD	0.45^{A} 0.34 – 0.50 0.03	0.28^{B} 0.04 – 0.36 0.12	0.39^{*} 0.35 – 0.46 0.02	0.38^{*} 0.34 – 0.42 0.2

Values denoted by different letters or signs are different:

^{A,B} – at a level $P \leq 0.01$

** – at a level $P \leq 0.01$

* – at a level $P \leq 0.05$

Zoonoses, emerging diseases

LEISHMANIA SPP. IN DOMESTIC CATS WITH OR WITHOUT CLINICAL SIGNS FROM ILHA SOLTEIRA, SÃO PAULO, BRAZIL

S. B. Lucheis[1,2,3], M. F. Alves-Martín[3], M. S. Paixão[3], L. M. Guiraldi[3], W. J. Santos[3], G.P. Sánchez[1], V.B. Richini-Pereira[4]

[1]*Department of Veterinary Hygiene and Public Health, São Paulo State University, Botucatu, Brazil*
[2]*Paulista Agency of Agribusiness Technology – APTA, Bauru, Brazil*
[3]*Department of Tropical Diseases, São Paulo State University, Botucatu, Brazil*
[4]*Adolfo Lutz Institute, Regional Laboratory of Bauru, Brazil.*

SUMMARY

This paper presents data about the occurrence of feline leishmaniasis in 55 cats exhibiting or not exhibiting clinical signs in a Visceral Leishmaniasis (VL) area using parasitological (blood cultures), serological and molecular (PCR) techniques. Flagellate protozoa were found in blood cultures and PCR showed the detection of *Leishmania* spp. in blood samples. Positive serological exams were obtained by ELISA using extract soluble antigen (SE) and by recombinant k39 antigen (rK39), and by Indirect Immunofluorescent Antibody Test (IFAT) as well. Based on clinical evaluation, it was possible to detect the presence of clinical signs in most of the researched animals.

INTRODUCTION

Feline leishmaniasis (FL) cases have been reported in countries in South America [1], in Europe [2] and Asia [3]. These studies have reported the infection by several species of *Leishmania* in domestic cats, with the presence or absence of clinical signs The lack of an early diagnosis of feline leishmaniasis in endemic areas from Brazil can cause the animal to keep representing a potential risk of leishmanias transmission to the vectors [4].

MATERIAL AND METHODS

Fifty five cats with or without clinical signs was analysed by parasitological (haemoculture in Liver Infusion Tryptose Medium – LIT), serological (Indirect Fluorescent Antibody Test – IFAT, Indirect ELISA (ELISA-SE and ELISA-rK39) and molecular diagnostic technique by PCR using the 720-base pair (bp) region containing the sequence-specific minicircle kDNA of *Leishmania* spp. described by Ikonomopoulos et al. [5]. All animals were from Ilha Solteira, São Paulo, Brazil, which is an area endemic for canine visceral leishmaniasis.

RESULTS

Flagellate protozoa were found in nine blood cultures (16.4%). Seropositivity by the IFAT was observed in 32 serum samples (62.7%), with five animals showing a titre of 40 (15.6%), 18 animals showing a titre of 80 (56.2%), eight animals showing a titre of 160 (25%), and only one animal showing a titre of 320 (3.1%). ELISA-SE tests showed seropositivity in 37 animals (72.5%), while ELISA-rK39 tests revealed positive results in 11 animals (21.6%). *Leishmania* spp. was detected by PCR in blood samples from five animals (9.1%), with no positivity from blood cultures. Based on clinical evaluation, it was possible to detect the presence of clinical signs (alopecia, emaciation, pinna lesions, nose lesions, skin lesions) in 30 animals (54.5%). Symptomatic and asymptomatic animals, were positive for *Leishmania* spp., with a positivity in 90% and 76% of the animals, respectively in at least one diagnostic test. The distribution of clinical signals based on diagnosis of *Leishmania* spp. by blood culture techniques, indirect fluorescent antibody test (IFAT), ELISA-SE tests, indirect ELISA-rK39 and Polymerase Chain Reaction (PCR) from blood and blood cultures is demonstrated in **Table 1.**

DISCUSSION

The present study found, based on serological results, the presence of anti-*Leishmania* antibodies in most animals with greater index of positivity by ELISA-SE, with 72.5% (37/51) of reactivity, followed by IFAT, with 62.7% (32/51) reactivity. In contrast the lowest positivity was observed by ELISA-rK39, with 21.6% (11/51) of reactivity in the animals.

We observed that ELISA-rK39 was more effective in the detection of asymptomatic animals, which is uncommon in studies performed in dogs. Despite of the effectiveness in the detection of infection in symptomatic dogs, there was poor efficacy in the use of recombinant protein in asymptomatic animals [6]. Thus, we observed the effectiveness of the use of recombinant proteins for the early diagnosis of infection in cats, as the clinical status of these animals is poorly known regarding *Leishmania* infection.

The present study used a parasitological blood culture technique, which is a good technique for the isolation of the parasite but whose risk of contamination is high; additionally, its species identification is difficult, requiring molecular confirmation. Despite the difficulties, promastigotes were found in our cultures, obtaining positivity in 16.4% (9/55) of the animals. Our results agree with those of Braga et al. [1], who reported 4% (2/50) of cats as seropositive using the blood culture technique, with animals from a non-endemic region, suggesting good applicability of the technique in endemic regions.

Through clinical evaluation of the animals, cases of alopecia, weight loss, and pinna, nose and skin lesions were observed, which are clinical signs similar to those described in the findings of FL [2]. The association between skin lesions in cats from the endemic region and VL may exist because lesions can provide a gateway to sandflies, which can bite in areas with less hair.

CONCLUSIONS

The occurrence of *Leishmania* spp. infection in cats from an endemic region does not determine the presence of clinical signs, suggesting the association of parasitological, serological and molecular techniques to better elucidate the diagnostic and the need for further studies to clarify the epidemiological importance of domestic cats in the leishmaniasis cycle as well as the search for safer and more efficient diagnostic alternatives.

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Table 1: Distribution of clinical signals based on diagnosis of *Leishmania* spp. by blood culture techniques, indirect fluorescent antibody test (IFAT), ELISA-SE tests, indirect ELISA-rK39 and Polymerase Chain Reaction (PCR) from blood and blood cultures in 55 cats from the Animal Protection Association of Ilha Solteira (*Associação Protetora dos Animais de Ilha Solteira* – APAISA), São Paulo, Brazil.

Clinical signs	Animals n (%)	Blood Culture n (%)	IFAT n (%)	ELISA-SE n (%)	ELISA-rK39 n (%)	PCRb[a] n (%)	PCRc[b] n (%)
Alopecia	15 (27.3)	6 (40.0)	9 (60)	14 (93.3)	1 (66.7)	3 (20.0)	0 (0.00)
Emaciation	12 (21.8)	5 (41.7)	8 (66.7)	10 (83.3)	0 (0.00)	2 (16.7)	0 (0.00)
Nose lesions	2 (3.60)	1 (50.0)	1 (50.0)	1 (50.0)	0 (0.00)	0 (0.00)	0 (0.00)
Pinna lesions	10 (18.2)	2 (20.00)	7 (70)	7 (70.0)	2 (20.0)	0 (0.00)	0 (0.00)
Skin lesions	13 (23.6)	4 (30.8)	10 (76.9)	10 (76.9)	0 (0.00)	0 (0.00)	0 (0.00)
No clinical signs	25 (45.5)	1 (4.00)	12 (48.0)	17 (68.0)	8 (32.0)	2 (8.00)	0 (0.00)

[a] **PCR using whole blood samples**

[b] **PCR using blood culture**

AN ALTERNATIVE METHOD OF TREATING RINGWORM CALVES

V. Kvokacka[1], A. Ondrejkova[2], L. Mastalkova[3]

[1]*District Veterinary and Food Administration Presov, Presov, Slovak republic*
[2]*University of Veterinary Medicine and Pharmacy in Kosice, Kosice, Slovak republic*
[3]*Bio Agens Research and Development – BARD, Únětice, Czech Republic*

Summary: A preparation containing *Pythium oligandrum* was successfully used on a cattle farm as a possible alternative treatment of ringworm in the calves in case of failure of other treatment.

Introduction: Ringworm is a relatively common disease of calves caused by the *Trichophyton verrucosum* fungus. As well as through direct contact with a sick animal, the disease can also be transmitted as a result of environmental contamination. Dermatophytes are also responsible for severe skin diseases in humans (Rochette et al., 2003). In the case of immunosuppressed animals or in organic farms, alternative biological control methods can be used for managing the disease such as use of the micro-organism *P. oligandrum*. Preparations based on *P. oligandrum* have been used successfully in the treatment of dermatophytosis and yeast infection of rodents, dogs and cats. *P. oligandrum* uses its strict mycoparasitism to eliminate a broad spectrum of fungi, including the dermatophytes of the genera *Trichophyton and Microsporum. (*Mencl, 2002)

Material and Methods: Ringworm on the farm Š. remained in the calf stable from 23 October 2011. Almost all animals contracted the disease. Vaccination and disinfection on the holding was interrupted several times due to lack of money. Intensive treatment (vaccinotherapy using a live virulent vaccine with the help of supportive vitamin therapy) began in 2013 after a change of owner. Despite 3 vaccinations (according to the manufacturer's instructions) in the herd, improvements of health occurred in no more than 50% of the animals. The other half of the animals, however, did not show any improvement and all incoming animals became clinically ill. The situation was complicated by the milk powder machine, used for all incoming animals to the stable, and also by the reusable smart collars for the milk machine. A month after the last administration of the vaccine the most clinically affected animals were sprayed 3 times at weekly intervals. We used a water solution with a preparation containing *P. oligandrum*. After one month we applied the preparation several times at weekly intervals to all the animals in the infected barn. The success of the therapy was assessed clinically and recorded photographically.

Results: After three applications, only a few of the animals showed an improved clinical condition. It was only after the preparation had been applied to all the animals in the facility as well as to most exposed areas in the stable that the clinical signs of the disease disappeared and incoming animals were free of the disease. The outbreak of ringworm was declared over on 30 November 2013 after a 3-month observation period.

Discussion: Immunoprophylaxis of trichophytosis in cattle using specific vaccines was introduced into veterinary practice more than 30 years ago. The best results in the control of the disease were obtained using live vaccines. The immunity developed after administration of a live vaccine is similar to that developed after recovery from experimental infection (Faldyna et al., 2007). The high number of asymptomatic carriers is epidemiologically very important, as they can spread the spores in the environment and be a source of infection both to other calves and humans. The high positivity rate of the *T. verrucosum* infection found can be due to several factors, mainly the poor hygienic conditions of the stable, overcrowding and the non-use of a specific vaccine (Agnetti et al., 2014).There was no immunity against reinfection in animals having previous contact with sub-infectious doses through undamaged skin. The results obtained suggest that the level of post-infection immunity depends on the extent of the disease and the frequency of administration of the infectious agent (Rybnikar and Oborilova, 2008). The frequent occurrence of animals not responding to vaccination could not be explained. It should be assumed that the main factors responsible for this situation include poor handling of the vaccine strains and errors in application, especially the absence of continuous and systematic immune prophylaxis in the herds (Kielstein et al., 1999). The expected positive effect of vaccination and revaccination which we had recorded in other positive holdings in preceding years was not achieved in this case either. There had been reported shortcomings in animal nutrition, which were reflected in the condition of the animals and probably in their immunity. Preventive administration of preparations containing zinc and vitamins did not help despite the change in ownership and provision of full nutrition for the animals. Attempts to use a treatment composition containing Enilconazolum were not successful either. At the request of the holding owner we con-

sidered alternative therapy. Because of the number of animals, however, we excluded certain therapies / tea tree oil, vegetable extracts/ described in specialist literature. Due to overcrowding in the stable - the animals with clinical disease could not be transferred to other stables and chemical disinfection of the premises in the presence of animals was not considered. After three applications, only some of the animals showed an improved clinical condition. It was only after the preparation had been applied to all the animals in the facility as well as to most exposed areas in the stable that the clinical signs of the disease disappeared and incoming animals were free of the disease.

Conclusion: The biological treatment of ringworm of calves with a preparation containing *P. oligandrum* is an alternative option to currently used practices. When applied, the manufacturer's instructions for application and temperature concentration of preparation must be followed. One of its advantages is that it can be used by farmers for sanitation of the environment even in the presence of animals. As well as that, during the application by spraying, part of the product applied outside the animal also serves to disinfect the environment.

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SEROLOGICAL EVIDENCE OF BOVINE TUBERCULOSIS DISEASE IN ALBANIAN CATTLE BASED ON ELISA TEST RESULTS

A. Koni[1], A.Juma[2], I.Çera[2], St. Nardelli[3], R. Keçi[1], Xh. Koleci[1]

[1]*Faculty of Veterinary Medicine, Agricultural University of Tirana, Albania*
[2]*Food Safety and Veterinary Institute, Tirana Albania*
[3]*Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy*
Correspondent author: xhelil.koleci@gmail.com

Abstract

Bovine tuberculosis (bTB) is an important bacterial infectious disease in Albania, and an important concern for animal and human health, and the prevalence of bTB is poorly documented. The program for bovine tuberculosis control was not in place in particular last three years. Furthermore, in recent years in Albania, is recorded an increasing incidence of extra pulmonary tuberculosis and in randomly both *Mycobacterium bovis and M.caprae* are isolated from clinical human cases. In this longitudinal study were tested by ELISA 2661 sera blood samples, from 154 herds. Prevalence between herds range from zero to 40%, its average was 18.18%. The prevalence within herds ranged from zero to 4.6%, its average was 1.35%. Key words. Bovine tuberculosis, *Mycobacterium bovis* ELISA test, sero prevalence, Albania

Introduction

Bovine tuberculosis is an important bacterial diseases caused by *Mycobacterium bovis* (*M.bovis*). The disease is spread all over the world [3]. *M.bovis* affects a wide range of wild and domestic animals such as buffalo, sheep, goats, camels, pigs, wild boars, deer, antelopes, dogs, cats, foxes, minks, elephants, rhinoceroses, coyotes and several predatory animals, including tigers, lion and leopard [3]. Bovine tuberculosis is a zoonotic disease responsible per 5 - 10% of all human cases; man can become infected via the milk, aerosol, consumption of infected meat and accidentally laboratory exposure [3]. The main and most effective way of transmission of tuberculosis in cattle is aerosol. The density of animals, farm size and management practices (close contact during milking) facilitates the spread of the disease within herd. Calves can be infected by suckling of milk of infected animals. Despite that *M.bovis* is discovered since 1881 by Robert Koch, control programs began to apply only in the 1920s, initially in some developed countries. In Albania, the prevalence of bTB is poorly documented. However, at 1990 the prevalence of disease was low and was thought that it was successfully controlled. After 1990, the cattle management changed dramatically and from big collective size farms was moved in two small private farms. The structure of cattle farms in Albania is complicated. According statistics, there are 375217 cattle farms, 73% of them have 1-4 animals, and only 5% have more than 50 cattle. In epidemiological terms this affects negatively the opportunities for applying correctly disease control programs (many farms and spread out). In other hand the national cattle herd structure seems to help in cutting of disease transmission chain (small and separate flocks). During last three years it is not applied any control program for TB. Moreover, during those years, is obviously an increasing in the prevalence of extra-pulmonary tuberculosis in humans and from human clinical cases are isolated *M.bovis* and *M.caprae* (personal communication). Since May 2012 is available an IDEXX *M.bovis* ELISA test, it has relatively low cost, and it is recommended specifically for developing countries [1,4]. We carry out a longitudinal study in order to estimate the seroprevalence of bovine tuberculosis in Albania.

Materials and methods

Blood samples collected from jugular vein of 2661 healthy cattle collected during the bovine brucellosis survey, preserved at sera bank, located at Veterinary Medicine Faculty were stored at -20^{0}C until the ELISA procedure was performed. A longitudinal study was design and 2661 sera blood samples were tested by using IDEXX *M. bovis* Antibody (Ab) Test. The samples were randomely selected from 154 epidemiological units, 10 regions and 20 districts of Albania.

ELISA procedure. A tuberculosis antibody screening test was used in accordance with the manufacturer's instructions [a][1]. The results of the ELISA tests were expressed as the value of the sample (S) divided by value of the positive control serum (P), determined by measurement of the optical density (OD_{450}). The value of the sample divided by the value of the positive control gave the S/P value.

Results

The criteria used for determining the status of animals tested were as follows: an S/P value Mycobacterium bovis. The ELISA test results for tested samples are presented in Table 2. The number of animals tested in each district and the percentage of these animals which were serologically positive are shown. The presence of specific antibody against *M. bovis* was identified in all regions, 14 districts and 28 villages. In seven villages were identified cluster positive animals. Prevalence between herds range from zero to 40%, its average was 18.18% (Table 2). The prevalence within herds ranged from zero to 4.6%, its average was 1.35% (Table 2).

Discussion

Almost 1/3 of positive cases remain undetected. Many of the problems associated with Bovine tuberculosis control in cattle are due to the limitations of diagnostic tests. A comparison of comparative skin test, INF-g test and this test for diagnosis of BT in herd level, indicated that ELISA was the most sensitive and rapid method for identifying infected animals, combining of comparative skin test and ELISA increase the overall sensitivity [2]. ELISA IDEXX *M.bovis Ab*Test is useful and recomended by OIE for serological surveys of dairy herds [1, 2, 4]. **Conclusion**. Results of the serological survey demonstrated that approximately 1.35% of the cows sampled had high levels of antibody to *Mycobacterium bovis*. Although no other diagnostic test procedures were carried out in this serological survey, the results obtained are indicative of a potentially serious problem with tuberculosis in Albanian dairy cattle. Further work is required to determine the status of dairy cattle and to expand the range of tests available for the diagnosis of tuberculosis.

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Table 1. Status of animals tested based on ELISA results

Criteria used for determining status of animals	Status of animals	Number of animals
S/P value < 0.3	Negative	2625
S/P value > 0.3	Positive	36

Districts	Number of herds	Number of positive herds	% Positive Herds	Number of cattle tested	Positive/% Positive
Durrës	12	2	16.7	266	3/1.13
Krujë	2	0	0	10	0/0
Korcë	5	0	0	53	0/0
Shkodër	15	3	20	187	4/2.14
Fier	21	4	19	236	4/1.7
Lushnjë	6	0	0	123	0/0
Tepelenë	2	0	0	59	0/0
Tiranë	12	2	16., 7	63	2/3.2
Peshkopi	10	4	40	312	5/1.6
Sarandë	2	0	0	72	0/0
Vlorë	10	2	20	109	5/4.6
Elbasan	19	4	21.05	374	4/1.1
Lezhë	6	1	16, 7	93	1/1.1
Pukë	4	0	0	84	0/0
Librazhd	1	0	0	13	0/0
Tropojë	6	1	16, 7	38	1/2.6
Kukës	2	0	0	14	0/0
Bulqizë	6	2	33,3	148	2/1.4
Gjirokastër	5	1	20	126	1/0.8
Mat	8	2	25	281	4/1.4
Total	**154**	**28**	**18,8**	**2661**	**36/1.35**

Table 2. ELISA test results for five dairy herds

OCCURRENCE OF ENDOPARASITIC GERMS IN URBAN AND RURAL ENVIRONMENTS IN THE SLOVAK REPUBLIC

I. Papajová[1], J. Pipiková[1], P. Juriš[1], J. Venglovský[2], N. Sasáková[2]

[1]*Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic*
[2]*University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic*

SUMMARY

The objective of this study was to determine the possibility of soil contamination with propagative stages of intestinal endoparasites at different public places. Totally 578 dog's faecal samples from 8 towns (Košice, Trebišov, Veľké Kapušany, Prešov, Snina, Levoča, Zvolen and Trenčín) and 3 villages (Dlhé Stráže, Dravce, Valaliky) were examined for the presence of parasitic germs. 29.9 % of faecal samples were positive and eight different species of intestinal parasites were detected (*Toxocara canis*, *Trichuris vulpis*, Ancylostomatidae, *Taenia* spp., *Toxascaris leonina*, *Capillaria* spp., *Dipylidium caninum* and Coccidia oocysts). Additionally 285 sandpits were also examined. The parasitic eggs occurrence in the sandpits was as follows: *Toxocara* spp. (11.8 %), Ancylostomatidae (1.1 %), *Taenia* type (0.7 %) and *Trichuris*spp. (0.4 %). The occurrence of *Toxocara*spp. Varied between the urban and rural environments. The highest number of *Toxocar*a spp. Was found in villages (33.3 %) where ascities showed lesser prevalence. Based on the results we can conclude, that rural environment is more polluted with endoparasitic germs than urban. Increased risk of transmission of parasitoses in rural ecosystem is predominantly in the areas with low hygienic standards, low level of health awareness, and poor level of technical infrastructure.

INTRODUCTION

Dog's ownership in cities as well as in the countryside creates continuing problem and increasing risk. Contamination of environment with infectious stages of zoonoses in excrements originating from dogs and cats is related to this phenomenon (Sudhakar *et al.,* 2013). The problem of large towns is in a high density of dog's faeces and subsequent high environmental burden. Dog's excrements in environment present not only aesthetic problems, but it is also the problem of hygiene and epidemiology.

The objectives of this study were to determine the incidence of parasites in dogs faecal samples and monitor the level of soil contamination with parasitic germs at different public places in Slovakia.

MATERIAL AND METHODS

A total of 578 faecal samples of unknown dogs were collected randomly from the different public places in the Slovak Republic. 508 samples of dog's excrements were examined from 8 towns and 3 villages. Faecal samples were examined by flotation method for the presence of propagative stages of endoparasites.

Sand samples were collected from 285 children sandpits in order to identify the presence of parasite eggs in the environment. The sand samples were investigated according to Kazacos (1983).

RESULTS

A total of 578 dog's faecal samples were examined and 173 (29.9 %) were found to be positive for the presence of the propagative stages of endoparasites. In all examined samples 8 different species of intestinal parasites were detected. The occurrence of parasitic species in the excrements from the public places in selected towns is summarised in Table 1.

	Eggs/oocysts (n/p)							
	Toxocara canis	*Toxascaris leonina*	*Trichuris vulpis*	Family Ancylostomatidae	*Capillaria* spp.	*Dipilidium caninum*	Family Taeniidae	Coccidian oocysts
Košice	158/21	158/4	158/11	158/4	158/0	158/0	158/0	158/0
Trebišov	64/18	64/5	64/21	64/14	64/0	64/0	64/0	64/0
Veľké Kapušany	31/13	31/8	31/10	31/14	31/6	31/0	31/0	31/0
Prešov	100/10	100/3	100/2	100/5	100/0	100/0	100/0	100/0
Snina	23/4	23/0	23/0	23/5	23/0	23/0	23/0	23/0
Levoča	58/2	58/1	58/1	58/2	58/0	58/1	58/14	58/0
Zvolen	32/1	32/0	32/2	32/0	32/0	32/0	32/0	32/0
Trenčín	42/1	42/0	42/4	42/2	42/0	42/0	42/1	42/1
Valaliky	37/7	37/0	37/0	37/6	37/0	37/0	37/0	37/0
Dlhé Stráže	17/2	17/0	17/0	17/0	17/0	17/0	17/5	17/0
Dravce	16/0	16/0	16/0	16/0	16/0	16/0	16/3	16/0

*n - number of examined samples, p – number of positive samples

Table 1. Occurence of dog's endoparasites in **excrements from** the public places in selected towns and villages in the Slovak Republic

In rural areas only 70 dog's excrements were examined and 3 different species (eggs from the family Taeniidae, *T. canis* and eggs from the family Ancylostomatidae) were detected (Table 1). High prevalence of *T. canis* eggs and eggs of other intestinal helminths in dog populations pointed to the contamination of the environment, in which animals live. Therefore the contamination of sand samples from sandpits was also monitored. Out of 285 sandpits, parasite eggs were detected in 9.5 % of them. According to the conditions of sandpits maintenance sandpits were classified as fenced and unfenced. The unfenced sandpits were found to be more contaminated than fenced sandpits. In unfenced sandpits 12.5 % prevalence of the parasites were recorded, compared with fenced sandpits where only 1.3 % positivity was observed. The most abundant parasitic species was *Toxocara* (Table 2).

	Eggs														
	Toxocara spp.			*Toxascaris leonina*			*Trichuris vulpis*			Family Ancylostomatidae			Family Taeniidae		
	n/p	f/p	u/p	n/p	f/p	u/p	n/p	f/p	u/p	n/p	f/p	u/p	n/p	f/p	u/p
Košice	136/10	50/0	86/10	136/0	50/0	86/0	136/0	50/0	86/0	136/0	50/0	86/0	136/0	50/0	86/0
Veľké Kapušany	24/1	9/0	15/1	24/0	9/0	15/0	24/1	9/0	15/1	24/1	9/0	15/3	24/0	9/0	15/0
Prešov	30/3	0/0	30/3	30/0	0/0	30/0	30/3	0/0	30/0	30/2	0/0	30/2	30/0	0/0	30/0
Snina	15/0	5/0	10/0	15/0	5/0	10/0	15/0	5/0	10/0	15/0	5/0	10/0	15/0	5/0	10/0
Zvolen	38/2	3/0	35/2	38/0	3/0	35/0	38/0	3/0	35/0	38/0	3/0	35/0	38/2	3/0	35/2
Trenčín	36/2	6/1	30/1	36/0	6/0	30/0	36/0	6/0	30/0	36/0	6/0	30/0	36/0	6/0	30/0
Valaliky	6/2	4/0	2/2	6/0	4/0	2/0	6/0	4/0	2/0	6/0	4/0	2/0	6/0	4/0	2/0

n - number of examined sandpits, p – number of positive samples, u – number of unfenced sandpits, f – number of fenced sandpits

Table 2. Occurence of endoparasites in **sandpits from** the selected towns and villages

in the Slovak Republic

DISCUSSION

The present study was carried out to determine the presence of parasitic germs in dog's faeces collected randomly from the public places in urban and rural environments in the Slovak Republic. In the examined samples, 8 species of intestinal endoparasites were detected. *T.canis* eggs, *Trichuris* spp. eggs and eggs from the family Ancylostomatidae were the major source of contamination. Dogs from the large towns (Košice, Prešov, Zvolen and Trenčín) showed lower prevalence than dogs from smaller towns (Trebišov, Veľké Kapušany, Snina, Levoča) and villages (Valaliky, Dlhé Stráže, Dravce). Several factors could influence these differences. In fact, dogs from small towns and rural agglomerations are usually at high risk of infection. It is caused by frequent outdoors activities in gardens or large areas with majority of animals being without preventive care of veterinarians. This is probably also due to free dogs movement in the environment contaminated with faeces from wild animals and consumption of small mammals or wastes from dead and killed wild-boars (Antolová *et al.,* 2006). In localities such as Trebišov, Veľké Kapušany and Levoča, there are segregated Roma settlements with poor hygiene, bad socioeconomic status and lack of veterinary care for the household animals. The animals from these localities have free movement without restriction and they can contaminate public places with excrements.

The high prevalence of canine endoparasites presents a risk factor for dissemination of parasitic propagative stages into the environment. This was the reason why we monitored also contamination of sandpits in selected towns and villages in Slovakia.

Out of 285 soil samples examined from sandpits in Slovakia 27 were found positive for parasitic germs. The most frequent were the eggs of *Toxocara* spp. Animal faeces were not found in any of the sandpits. The higher prevalence of *Toxocara* spp. in sandpits may be also due stray cats.

CONCLUSIONS

It is evident, that the occurrence of intestinal parasites in dog's excrements poses a source of contamination of the environment in urban and rural ecosystems. In conclusion, intestinal parasites represent a silent hazard not only for other animals, but also for the general public health.

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ZOONOSES DENOUEMENT ON PUBLIC HEALTH AND ECONOMY OF INDIA: AN OVERVIEW

A.Meena

Department Of Animal Husbandry, Udaipur, Rajasthan, India

Zoonotic infections pose a significant public health challenge for low and middle income countries and have traditionally been a neglected area of research. India ranked topped in the list of countries worst affected by zoonotic diseases(those originating from animals).Livestock is an important sub sector of Indian agriculture contributing 6.71 billion dollar per annum accounting for 25% of the output of agricultural sector and about 22.45 million people work in livestock sector. In India livestock are important in supporting livelihood of poor farmers,consumers traders and labourers throughout the country.Close association between human population and animals, consumption of unpasteurized milk and dairy and inappropriate carcass disposal are some of the principle factors perpetuating infection in humans. The scope of this paper is restricted to the diseases present in India. The public health importance of these diseases are also emphasized. The clinical aspects, transmission, disease course, diagnosis and control of such diseases are discussed. Guidelines are presented for the inspection of the animal origin food for human consumption. The presence of Zoonotic diseases can be detected by observation of clinical signs ante mortem & by detection of localized or disseminated lesions in the carcass & viscera post mortem. Abnormal behaviour can indicate Rabies, Listeriosis, Tetanus or FMD. Skin lesions can result from TB, Paratuberculosis, Anthrax. Diarrhoea can suggest Salmonellosis, Colibacillosis etc. Based on epidemiological data of active surveillance programme it is estimated that due to Brucellosis there is loss of US $ 58.5 million per year and FMD causes $ 5 billion revenue loss in India. Therefore,necessary preventive measures are required to control these zoonotic diseases.

Keywords: anthrax, brucellosis, cysticercosis, India, leptospirosis rabies, salmonellosis, zoonotic diseases, zoonotic tuberculosis

Diseases and infections that are naturally transmitted between vertebrate animals and humans have been defined as Zoonoses.60% of all infectious disease pathogens and 75% of all emerging pathogens falls in the category of zoonotic diseases.(WHO,2010; Woolhouse and Gaunt,2007). Zoonotic diseases are of great public health importance in India (Chengappa et al,2007) which can be explained by the fact that livestock is an important sub sector of Indian agriculture contributing 6.71 billion dollar per annum accounting for 25% of the output of agricultural sector and 68% of the workforce relies on farming that is in close contact with domestic animals and poultry with frequent exposure to sick or infected animals.According to Sehgal and Bhatia (1990),intimate and prolong contact between man and animal facilitates the transmission of various communicable zoonotic diseases between man and animals. According to study conducted by International Livestock Research Institute (ILRI,2012) India is among the top geographical hotspots for zoonotic diseases followed by Ethiopia, Nigeria and Tanzania. With the world's second largest human population, two biodiversity hotspots (Myers et al 2000), and one of the world's greatest densities of tropical livestock (Thornton et al,2002), India possesses a favourable environment for the transmission of communicable diseases between man and animals (Jones et al,2008 ; Forman et al, 2008).Other factors responsible for dessiminating zoonotic diseases are: unhygienic living conditions, lack of education, poor personal hygiene, poverty and occupation. Wildlife also served as an important reservoir of zoonoses either infecting humans directly or indirectly through domestic animals. Furthermore, several relevant zoonotic infections may be vector borne, that is, transferred from animals to humans via, for example, arthropods or ticks. Many zoonotic infections actually are promoted by human behaviour such as bush-meat hunting (EBOLA fever), the farming and trade of live wild animals (SARS), close and repeated contacts with infected animals (avian influenza), deforestation, which brings humans closer to infected vectors and animal reservoirs (leishmaniasis),or building of dams, that favours the proliferation of mosquitoes (Rift Valley fever). All major zoonotic diseases prevent the efficient production of food of animal origin, particularly of much-needed proteins, and create obstacles to international trade in animals and animal products.They are thus an impediment to overall socioeconomic development in India.The presence of Zoonotic diseases can be detected by observation of clinical signs ante mortem & by detection of localized or disseminated lesions in the carcass & viscera post mortem. Abnormal behaviour can indicate Rabies,Listeriosis,Tetanus or FMD.Skin lesions can result from TB, Paratuberculosis, Anthrax. Diarrhoea can suggest Salmonellosis, Colibacillosis etc. Nine major zoonotic diseases, or classes of diseases, are accorded priority status in India by Roadmap to Combat Zoonotic Diseases in India (RCZI). Rabies is put up at the top among all, followed by anthrax, brucellosis, leptospirosis, zoonotic tuberculosis, Japanese encephalitis,cysticercosis,salmonellosis and rickettsial infections.Remaining zoonoses included food-borne illnesses,

emerging viruses, and plague while 13 zoonoses are identified as most important, by International Livestock Research Institute (ILRL) i.e zoonotic gastrointestinal disease;leptospirosis; cysticercosis; zoonotic tuberculosis; rabies; leishmaniasis; brucellosis; echinococcosis; toxoplasmosis;Q fever;zoonotic trypanosomosis,hepatitis E and anthrax.Heavy economic loss is caused to a varying degree due to different type of zoonotic diseases present in India.For example based on epidemiological data of active surveillance programme it is estimated that due to Brucellosis there is loss of US $ 58.5 million per year and FMD causes $ 5 billion revenue loss in India.Therefore present paper describes a brief review on public health importance,epidemiology and economic impact of these important zoonotic diseases (as mentioned by RCZI) in India in order to describe their importance and urgent need to take preventive measures effectively for their control. Rabies is one of the most important zoonotic diseases in India. Rabies is endemic in India (Sudarshan,2004).More than 99% of all human deaths from rabies occur in the developing world including India (WHO,1998). The endemic nature of rabies in India can be attributed to the prevalence of the disease in dogs as well as other species of domestic animals. Different scientists, organizations stated different datas on toll death of humans caused by rabies in India. According to the national survey by the Association of the Prevention and Control of Rabies in India (2003),it estimated that in India a total of 18,500 human deaths occur as a result of rabies each year. Whereas the World Health Organization (WHO,2002) estimated that rabies caused 30,000 human deaths per year in India, which accounted for approximately 60% of the estimated global total of rabies deaths.While Sudarshan(2004) stated that since 1985, India has reported an estimated 25 000–30 000 human deaths from rabies annually.On the contrary,the Central Bureau of Health Intelligence found an annual average of 249 deaths.At the same time the loss of livestock due to rabies is significant, there are few publications on estimates of the incidence of rabies in livestock (Knobel et al, 2005). Ghosh (2006) stated that the majority of people who die of rabies are people of poor or low-income socioeconomic status.Prevention of human rabies is possible through mass dog vaccination, promotion of responsible dog ownership and dog population control programmes with a partnership approach but in India this is a big challenge as it has a large population of dogs (around 25 million) and very low vaccination coverage. The government of India has still not made rabies a notifiable disease,so many deaths go unreported. Anthrax is a disease of herbivorous animals caused by Bacillus anthracis, and humans incidentally acquire the disease by handling infected dead animals and their products (Thappa and Karthikeyan, 2001, 2002; Hanna, 1998; Morton and Arnold,2003). In the states like Chattisgarh and Orissa Tribals or any given community, particularly if underprivileged, eat carcass of dead animals. Such people are vulnerable. According to a recent review of literature, there have been about 205 documented cases from India, the majority (109) of cutaneous anthrax (Lalitha, 2001).The characteristic clinical features of cutaneous anthrax are a painless ulcer with surrounding vesiculation along with massive edema and eschar formation (malignant pustule).Due to underreporting the actual incidence of anthrax in India is not known accurately mostly (Lalitha, 2001). Penicillin is the drug of choice for all forms of anthrax, beta-lactamase producing strains of B. anthracishave been reported (Bradaric and Punda-Polic,1992; Lalitha and Thomas,1997).In order to prevent anthrax there is need for proper legislation for meat handling as well as effective immunization of animals(Lalita,1996). Leptospirosis is an infectious disease caused by Leptospira interrogans complex which has over 20 sero groups and more than 200 serovars.Leptospirosis are excreted in the urine of the animals and they affect man when he comes into contact with urine of infected animals, directly or indirectly, when he is exposed to an environment contaminated by the urine of the infected animals such as soil and surface water following monsoon rains (Dutta and Christopher,2005). Therefore the illness commonly occurs during the monsoon.Leptospirosis has been under reported and under diagnosed from India due to a lack of awareness of the disease and a lack of appropriate laboratory diagnostic facility in most parts of the country.The outbreaks of leptospirosis have been reported from coastal Gujarat, Maharashtra, Kerala, Tamil nadu, Andhra Pradesh, Karnataka and Andaman periodically. In the last decade, there has been a rapid rise in the incidence of leptospirosis in north India(Sethi et a,l 2010). Since 1970, occupational exposure accounting for 30-50% of human cases and recreational activities are recognized as important causes of disease.Severe Leptospirosis can be diagnosed by the presence of fever, jaundice and renal failure.This is the pattern commonly seen in Kerala, Tamil Nadu and Gujarat. Atypical pneumonia has been reported from Andamans (Shivakumar,2013). The presence of brucellosis in India was first established early in the previous century and since then has been reported from almost all states(Renukaradhya et al,2002).In India Brucellosis is caused by mainly Brucella abortus and Brucella melitensis and is readily transmissible to man as an occupational hazard(Kollannur et al,2007). Public health significance of Brucellosis is well known (Koshi and Myers, 1969).The prevalence of brucellosis in cattle farms has long been recognised (Anon, 1918).), and several studies have confirmed widespread prevalence in different States in India.Due to Brucellosis in India there is loss of US $ 58.5 million per year. Losses are due to abortion in the affected animal population, loss of progeny, in-

fertility and reduced milk production. In humans, brucellosis is causing physical incapacity, loss of man days of labour. In India, effective control of brucellosis is a national problem. Tuberculosis (TB) is one of the most ancient diseases of mankind and has co-evolved with humans for many thousands of years or perhaps for several million years (Hirsh et al 2004).The zoonotic aspects of tuberculosis in human beings revolves around isolation of Mycobacterium bovis from their sputum specimen. Also it has been observed that not only M.bovis cause TB in animals but Mycobacterium tuberculosis too causes diseases in animals.The infection can get transmitted from animals to human beings and vice versa(Challu,2007).Prevalence of Bovine TB in India varies from 1.6 to 16% in cattle and 3 to 25% in Buffaloes(Mullick,1994).In India, increase of M. bovis infection in humans has manifested into a grave public health problem (Cosivi et al,1998; Cousins et al,1999, Kazwala et al,2001). Salmonellosis is a type of zoonoses, diseases which are transmittable from animals to human beings under natural circumstances (British Association for the Advancement of Science, 1977). Salmonellosis is one of the commonest and most widely disseminated diseases transmitted via food (WHO, 2005).In India many investigators have described the isolation of Salmonella from different sources (Ganguli,1958; Khera,1962; Sharma & Singh,1967).Places in India bear the highest risk of contracting salmonellosis during prolonged stays and can climb from 1: 30,000 in endemic regions to 1: 3,000 in high endemicity regions (Mayer et al, 2010).Case fatality rate due to salmonellosis has been varying between 1.1% to 2.5 % in last few years.Regarding prevention,no effective vaccination program has been developed. Therefore, control must be based on a strict hygiene program.Education of food handlers in aspects of preparation, refrigeration, and cooking of foods of animal origin as well as personal and environmental hygiene. Cysticercosis caused by larval stage of the tapeworm Taenia solium, is a major public health problem, especially in the developing world including India. Studies using neuroimaging techniques suggest that the disease burden in India surpasses many other developing countries.The annual societal cost (agriculture and health) of porcine cysticercosis/taenosis is estimated at about US$150 million in India alone(WHO,2013).There are certain unique features of the disease in India. The solitary form of the disease (solitary cysticercus granuloma, SCG) is the commonest presentation of the disease.Anywhere between 26 and 50% of all Indian patients presenting with partial seizures are diagnosed with a SCG on the CT scan (Wadia et al, 1987;Misra et al, 1994).The disease is prevalent in virtually all states of the country although it varies significantly between different states (Rajshekhar and Chandy, 2000).There are few reports of patients with cysticercosis from Jammu and Kashmir, a predominantly Muslim state, and Kerala where educational levels and hygienic standards are probably the highest in the country.Systematic population-based studies are lacking in most parts of the India; hence it is difficult to estimate the disease burden in India. Japanese Encephalitis(JE) was first recorded in Vellore and Puducherry in the mid 1950's and the first major outbreak occurred in 1973 in Bankura and Burdwan districts of West Bengal before spreading to other states.This disease has been reported from 26 states and UTs since 1978, only 15 states are reporting JE regularly. Most human cases reported from May–October, especially in northern India.The clinical manifestations of the disease are characterised with high-grade fever, convulsion, confusion, stiffness of neck and altered levels of consciousness from stupor to deep coma.The fatality rate varies between 10% - 40% and those who survive do so with various degrees of neurological complications like paralysis and cognitive deficiencies.In India the total population at risk is estimated 160 million. In India alone, the JE have claimed nearly 1,000 lives so far in 2012.The most disturbing feature of JE has been the regular occurrence of outbreak in different parts of the country.Govt. of India has constituted a Task Force at National Level which is in operation and reviews the JE situations and its control strategies from time to time. Rickettsial diseases are zoonoses caused by obligate intracellular bacteria grouped in the order Rickettsiales.According to RCZI important rickettsial diseases in India are Scrub Typhus; Epidemic Typhus; Endemic Typhus and Tick Typhus. For India, the reported numbers are an underestimate due to lack of community based data and non-availability of confirmatory laboratory tests (Chugh,2008). Therefore, necessary preventive measures are required to control these zoonotic diseases like awareness campaign, training for personal hygienic practises, development of more efficient diagnostic facilities, efficient reporting system and access to medical facilities particularly in rural areas which are still facing such challenges in developing country like India. To address these challenges the Government of India prepared a Roadmap to Combat Zoonoses Initiative in 2009 only which is the only standalone initiative on zoonoses in the country and modelled around "One World One Health" concept.

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RABIES – PREVALANCE ANALYSIS OF THE DISEASE IN POLAND

P. Cwynar, R. Kołacz

Department of Environmental Hygiene and Animal Welfare, Faculty of Biology and Animal Science, Wrocław University of Environmental and Life Sciences, J. Chełmońskiego 38C, 51-630 Wrocław, Poland

Rabies is a viral infectious disease of the central nervous system, causes a high risk to human life and health. Reservoir of the disease are both wild animals as well as accompanying animals. The virus is transmitted through the contact with the saliva of an infected animal, most commonly as a result of the bite or bitten by a sick animal. In Europe, the rabies virus is the most frequently recorded in wild animals, particularly in top predators, ie. foxes, badgers and raccoons.

The aim of this study is to analyze the incidence of rabies virus in Poland and the possibilities of its limitations, with particular emphasis on its presence in animals living in the wild. In 1990 the statistics of rabies in Poland indicated 2045 cases of the disease, including in 1668 occurred in wild animals and the 1374 cases were confirmed in foxes. Two years later, the population of animals infected by rabies significantly increased up to 3084 cases of rabies, including wild animals in 2549, of which 2079 confirmed cases were found in foxes. Since 1993, the government introduced the use of immunoprophylaxis against the occurrence of rabies through continuous and consistent administration of oral vaccines whose effectiveness established on the basis of the monitored the situation in the country. According to recent data from the year 2013, there were 160 confirmed cases in animals, including 126 wild animals, where only 103 foxes cases were found, mainly in poorly urbanized parts of the country.

Feed, water and waste hygiene and management

STORED GRAIN DAMAGE CAUSED BY SITOPHILUS GRANARIUS AND RESEARCH ON INSECTICIDAL PROPERTIES OF ESSENTIAL OILS

B. Bakutis, V. Ribikauskas, V. Baliukoniene, K. Venckunaite, J. Jovaisiene, J. Kucinskiene

Department of Food Safety and Quality, Veterinary academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

Summary

The aim of the work was to evaluate insecticidal activity of essential oils (EO) against *Sitophilus granarius* in stored grain. We used following EO: *Mentha piperita*, *Cupressus sempervireus*, *Origanum vulgare*, *Thymus vulgaris*, *Cymbopogon flex*, *Eugenia caryophyllata* and the mixture of *O. vulgare* and *C. sempervireus*. In wheat samples infected by *S. granarius* we found lesser quantities of fat, proteins, starch, moisture and ash compared with not infected samples. The highest insecticidal activity showed the mixture of *O. vulgare* and *C. sempervireus* and *C. sempervireus* EO. *C. sempervireus*, *E. caryophyllata* and *C. flex* EO's showed the best antibacterial activity, while *E. caryophyllata* and *C. sempervireus* showed the best antifungal activity.

Introduction

Insects are well-known grain pests causing damage to 10-30 % of all world grain production yearly. Granary weevil (*S. granarius*) is the most serious primary pest of stored grain, preferentially eat out grain embryos, thereby reducing the protein content of feed grain. This insect could be a vector for different kinds of pathological microorganisms. Feed infested with granary weevil could contain toxic metabolites and this could compromise health and welfare of farm animals. The best way to deal with such pest is to use chemical insecticides but they could be potentially toxic to humans and animals. Use of chemical pesticides is not allowed in organic farming and this could be serious problem to food and feed safety. It is found that some EO could be characterized with insecticidal, antibacterial and antifungal activities. Therefore, aim of this work was to evaluate insecticidal activity of EO against *S. granarius* in stored grain. Main tasks were: 1) estimate loses of some substances during storage of wheat grain infested with *S. granarius*; 2) to identify mycromycetes in stored grain; 3) to test insecticidal properties of some EO; 4) to test effect of EO on total amount of microbes and viable spores in infested stored wheat grain.

Materials and Methods

We used following 100 % EO: *M. piperita*, *C. sempervireus*, *O. vulgare*, *T. vulgaris*, *C. flex*, *E. caryophyllata* and the mixture of *O. vulgare* and *C. sempervireus*. There were three levels of dosage: 50µl, 100µl and 150µl. The properties of EO were investigated against *S. granarius* adults under laboratory conditions. Examples of wheat grain were taken from commercial grain storage according to procedures described in standard LST EN ISO 24333:2010. Chemical analysis of grain was performed with help of NIRS DS2500 equipment. The following components and properties have been determined: fat, proteins, starch, moisture and ash. Identification of mycromycetes have been performed by cultivating whole grains in Petri dishes with Czapek agar for 7-10 days at 26°C. Dishes were examined under microscope to observe the fungal structures and to identify genus. Insecticidal properties were estimated by placing 20 insects into sterile 500 ml glass containers and by adding separate small bucket with one chosen dosage of EO. Survival rate of insects was monitored every 4 h for 72 h. Infestation of grain was performed by adding 100 g of grain into 500 ml glass containers with insects. Estimation of total bacterial count was performed by cultivating grain extract (1:100) on agar medium as described in standard LST EN ISO 4833:2003. Estimation of total amount of fungi (number of viable spores) was performed by cultivating grain extract (1:100) on Czapek agar with chloramphenicol (50 mg/l) as described in standard ISO 21527-2:2008.

Results

After 2 months period in wheat samples infected by *S. granarius* we found lesser quantities of fat (1.87±0.11%-1.62±0.07%, $p>0.05$), proteins (10.98±0.40%-10.74±0.37%, $p<0.01$), starch (58.42±0.32%-57.85±0.41%, $p>0.05$), moisture (13.77±0.19%-13.15±0.15%, $p>0.05$) and ash (2.40±0.23%-2.27±0.13%, $p>0.05$) compared with not infected samples.

In wheat samples infected by *S. granarius* we found following kinds of fungi: *Mucor spp*. – 10.53%, *Fusarium spp*. – 15.79%, *Aspergillus spp*. – 73.68%. *Apergilus* was predominant and we distinguished separate species: *A. parasiticus* – 5.26%, *A. fumigatus* – 10.53%, *Aspergillus spp.* other. – 24.56%, *A. niger* – 33.33%.

High insecticidal activity showed mixture of *O. vulgare* and *C. sempervireus* EO: after 72 h application with 50µl EO mortality of insects was 53%. The highest insecticidal activity showed *C. sempervireus* EO - 93%. Lowest insecticidal activity showed 50µl *O. vulgare* and *M. piperita*: 10 and 8% respectively.

Application of 50µl *C. sempervireus* EO lowered total amount of bacteria by 91.9% (0.67±0.25 thous. CFU/g ($p>0.05$)), 100µl *E. caryophyllata* – by 85.1% (1.07±0.09 thous. CFU/g ($p>0.05$)), 150µl of *C. flex* and *C. sempervireus* EA – by 87.8% (0.90±0.20 thous. CFU/g ($p>0.05$) and 0.90±0.38 thous. CFU/g ($p<0.05$) respectively). *O. vulgare*, *M. piperita* and *T. vulgaris* EO's were found to have insignificant antibacterial properties.

EO's of *E. caryophyllata* (50µl) and *C. sempervireus* (100µl) showed the best antifungal activity – they lowered amount of fungi by 87.5% (0.17±0.03 thous. CFU/g ($p>0.05$)) and 89.4%, (0.63±0.23 thous. CFU/g ($p>0.05$)) respectively. *O. vulgare* and *T. vulgaris* showed insignificant antifungal properties.

Discussion

Different researches shows that we can expect insecticidal activity from EO's of clove (*E. caryophyllata*), eucalyptus, lemongrass (*C. flex*), lemon mint, pennyroyal, myrtle, thyme (*T. vulgaris*), calamus, cassia, turmeric (1, 2). Some of them are characterized with antibacterial and fungicidal properties as well. Other OE's with antimicrobial properties are sweet marjoram, rosemary, cypress (*C. sempervireus*), oregano (*O. vulgare*), peppermint (*M. piperita*), tea tree, orange, Siberian fir. In this research, we found that some of EO's showed considerable strong pesticidal properties. On other hand, very popular in Lithuania and well recognizable plant peppermint not showed expected qualities. In general, we consider that use of OE's has strong possibilities especially in organic farming. Besides that, OE's can help (at least in some degree) to prevent antibiotic resistance.

Conclusions

Internal infesters as *S. granarius* inflict their damage on stored grain mainly by direct feeding that causes chemical composition losses in stored grain. In addition, they transport various fungi in grains. Mixture of *O. vulgare* and *C. sempervireus* EO's, *C. flex*, *E. caryophyllata* and *C. sempervireus* EO's could be used as potential insecticides against granary weevil and these EO's showed some antimicrobial activity as well.

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EFFECTS OF PROTECTED FISH OIL ON FATTY ACID CONTENT IN THE GOAT MILK

R. Kupczyński[1], K. Śpitalniak[1], A. Zwyrzykowska[1], R. Bodkowsk[2], M. Szołtysik[3]

[1]Department of Environment Hygiene and Animal Welfare, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland
[2]Institute of Animal Breeding, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland
[3]Department of Animal Nutrition and Feed Quality, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland

In recent years there has been an increase in consumers' interest in so-called functional foods. In addition to its nutritional properties numerous studies have shown its capacity to benefit overall human health.

Goat milk is characterized by a high nutritional value. Comparing it to the cow's milk it has lower allergenicity and higher digestibility of proteins and fats. Studies on dairy cows show that by supplementing the fish oil and plant oils to feed, the fatty acid profile of the milk and the proportion of unsaturated to saturated fatty acids can be modified.

The aim of the study was to evaluate the impact of protected fish oil (FO), used as a feed additive for Polish refined goats, on the concentration of fatty acids in milk fat. The study involved two groups of 10 heat each.

The animals were assigned to groups by the analogue method. Goats of experimental group from first month of lactation received fish oil supplement in a dose of 50 g /d for 4 weeks of the experiment. Fatty acid composition was determined in representative samples following gas chromatography method with the use of gas chromatograph Agilent Technologies 5973.

It was found that the supplementation used there resulted in adecrease in fat content of milk in goats receiving FO. There were also differences in the fatty acid profile. The amount of unsaturated fatty acids and long chain fatty acids increased. There was also a decrease in saturated fatty acids levels, including palmitic and stearic acids, as well as short-chain fatty acids. Fish oil supplementation increased the concentration of isomers: cis-9 and trans-11 CLA in milk (1.08 g / 100 g fatty acids). The amount of vaccenic acid increased (up to 5.38 g / 100 g fatty acids), as well as n-3 acids including EPA (up to 0.51 g / 100 g fatty acid) and DHA (up to 0.57 g / 100 g fatty acids). The use of fish oil as a feed additive had a positive effect on the modification of the content of fatty acids characterized by biological properties in the milk of goats.

DISPOSAL OF ANIMAL WASTES TO SOIL AND THE RELATED RISK TO ANIMAL AND HUMAN HEALTH

J. Kachnič[1], N. Sasáková[1], J. Venglovský[1], I. Papajová[2], G. Gregová[1], R. Hromada[1], M. Ondrašovič[1]

[1]*University of Veterinary Medicine and Pharmacy in Kosice, Department of Environment, Veterinary Legislation and Economics, Komenského 73, 041 81 Košice, The Slovak Republic*
[2]*Parasitological Institute of the Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice; The Slovak Republic*

Summary

The study investigated microbiological and parasitical risks related to disposal of animal manure to soil after storage at temperatures 4°, 20° and 42°C for 115 days. Plate counts of *Salmonella typhimurium* and number of devitalised non-embryonated model *Ascaris suum* eggs were determined on days 0, 7, 12, 22, 32, 40, 55, 90 and 115 of storage. At the same intervals level of selected physico-chemical parameters were determined. Microbiological examination showed that *S. typhimurium* survived in the slurry for less than 115 days at 4°C and for less than 90 days at 20°C and 42°C. Devitalization of *A. suum* eggs increased with temperature and time of storage but complete devitalization was not achieved even after 115 days at 42°C. Physico-chemical parameters showed changes related to decomposition processes but did not allowed us to draw definite conclusion regarding their influence on devitalization of pathogens. The results indicate potential risk to human food chain that can be prevented by strict observation of legislative provisions and appropriate treatment of animal manure.

Introduction

The optimum way of use of animal excrements is their application to soil that allows one to improve its structure and supplement nutrients important for growing crops Safe processing of animal excrements is one of the key factors, because there are significant microbiological risks related to animal wastes spread onto land subsequently used for crop production or livestock grazing. Unsuitable manipulation may frequently result in environmental pollution with respect to noxious gasses and odours, contamination of surface and ground water and hygiene risks related to micro-organisms and various parasitic stages (Venglovský et al., 2009). With regard to animal wastes we are concerned particularly with representatives of the family *Enterobacteriacea*, the majority of which have zoonotic character.

Animal excrements of farm animals are also a source of endoparasites An important factor in spreading of endoparasitoses is high tenacity of some propagative stages of parasites (Papajová and Juriš, 2009).

The study was conducted to investigate the microbiological and parasitical risk related to application of pig slurry to soil, particularly to survival of *S. typhimurium* and non-ebryonated eggs of *A. suum* in the pig slurry stored under laboratory conditions at temperatures 4°, 20° and 42°C.

Material and methods

Raw pig slurry was inoculated with *S.typhimurium at a dose of* 3.6 x 10^5 CFU.ml^{-1}. Non-embryonated *A. suum* eggs were inserted to raw slurry on polyurethane carriers. Plate counts of *Salmonella typhimurium* were determined on days 0, 7, 12, 22, 32, 40, 55, 90 and 115 of storage. At the same intervals we investigated also devitalisation of introduced non-embryonated *Ascaris suum* eggs. Physico-chemical examination included determination of pH, dry matter (DM), chemical oxygen demand (COD) and ammonium ions (NH_4^+).

Results

Results of microbiological examination show survival of *S. typhimurium* in the slurry during 115 days of storage. The initial concentration of the tested *S. typhimurium* strain (3.6 x 10^5 CFU.ml^{-1}) in pig slurry stored at 4° C decreased by day 90 by three orders of magnitude (3.1 x 10^2 CFU.ml^{-1}) and on day 115 of storage the test strain was no more recovered. The tested strain survived in slurry for less than 40 days at 20°C. The most marked decrease in plate counts of test bacteria was recorded in pig slurry stored at 42°C At this temperature the test bacteria survived for less than 32 days. This indicated that viability of bacteria in stored pig slurry was affected first of all by the temperature during the storage. Increased temperature is an important factor contributing to devitalization of indicator micro-organisms.

Devitalization of *Ascaris suum* eggs increased with temperature and time of storage but complete devitalization was not achieved even after 115 days at 42°C. Parasite survival in animal manures may also be related to temperature, but the trends are not as pronounced as those reported for bacterial pathogens. This is likely due to their ability to form cysts and oocysts for protection from environmental pressures.

Discussion

Besides temperature and time of storage the survival of pathogens in the slurry may well depend on factors other than temperature and duration of heat treatment, e.g. moisture content, free ammonia concentration, pH, the presence of other micro-organisms and other physico-chemical properties (Turner, 2002, Venglovský *et al.,* 2006). In our study we observed a pH increase in stored pig slurry at all three temperatures. This increase was not in correlation with the level of ammonium which varied considerably. This could be due to its release as ammonia and the related decrease in total nitrogen by the end of the experiment except for temperature 4°C. Dry matter content decreased according to expectations, so did other parameters, which may be related to production and release of some volatile compounds during the storage. The processes that take place in slurry are, however, very complex and the extent of our examinations did not allow us to draw any definite conclusions in this respect.

Conclusions

Legislation in advanced countries requires acceptable procedures for the disposal, processing and application of animal manures. However, there are still aspects that may raise some risk for safety of human food chain and require further investigations. Our results showed that the risk to public health arising from application of insufficiently treated animal manure to soil may be higher than detected by the common methods.

The best way is to put stress on preventive actions and measures that may eliminate any known or suspected danger resulting from pathogens present in animal manures applied to the soil that is used for animal grazing or growing of crops for human consumption.

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PERSISTENCE OF SALMONELLA DERBY AND LISTERIA MONOCYTOGENES IN DIGESTATES DERIVED FROM PIG AND DAIRY FARMS

A-M. Pourcher[1, 2], C. Ziebal[1,2], G. Maynaud[3], C. Druilhe [1,2], E. Latrille[3], D. Patureau[3], J. Jimenez[3], N. Wery[3]

[1] *Irstea, UR GERE, 17 avenue de Cucillé, CS 64427, F-35044 Rennes, France*
[2] *Université européenne de Bretagne, F-35000 Rennes France*
[3] *INRA, LBE UR050, Avenue des Etangs, F-11100 Narbonne, France*

Summary

Digestates produced from the anaerobic digestion (AD) of manure can be reused as fertilizer. However the potential presence of pathogens in these organic waste products (OWP) and their ability to regrow during long-term storage may constitute a health hazard. This study aims to investigate the factors which may influence the survival of pathogenic bacteria during storage of by-product of AD. Eight OWP were sampled from agricultural AD plants. They were inoculated with *Salmonella* Derby and *L. monocytogenes* and incubated at 24°C. The results show a decline in the number of the two strains during storage (4.5-6 Log reduction in 7-20 days). Pathogen survival has been analyzed with regards to the degree of stabilization of organic matter and the residual microbial activity. The results showed that both biotic and abiotic factors have an effect on the survival of the pathogens and that the survival of *Salmonella* Derby is not influenced by the same factors as *L. monocytogenes*.

Introduction

Livestock manures are co-digested with various organic feedstocks to enhance the biogas production. The resulting digestates are interesting feedstocks for nutrient recovery. However, questions remain on technical and environmental impacts of biogas plants. Indeed, digestates used as organic fertilizers may contain pathogens which may be spread together with the digestate onto agricultural soils. Thermophilic AD is more effective for pathogen inactivation than mesophilic AD (Smith *et al*., 2005). Nevertheless, even after thermophilic AD, there is a risk of recontamination and regrowth of pathogens during the storage of digestates (Bagge *et al*., 2005). Although the most important factors affecting the survival of pathogens during AD have been reported, there is little information on the behaviour of pathogens during storage of OWP. This study aims to investigate abiotic and biotic factors that may influence the survival of pathogenic bacteria during storage of organic waste products (OWP) obtained from on-farm biogas plants.

Material and method

Eight types of OWP were sampled from mesophilic on-farm AD processes: three raw digestates (R3, R4, R9), two liquid fractions of digestates (L2, L7) and three composted solid fraction of digestates (C1, C5, C7). OWP (125 mL) were inoculated with rifampicin-resistant strains of *L. monocytogenes* and *Salmonella* Derby at an initial level of 10^6-10^7 CFU/g. They were stored at 24°C for 41 days. *L. monocytogenes* and *Salmonella* were enumerated using Palcam and XLD agar supplemented with 100 mg/L of rifampicin and 50 mg/L of cycloheximide. Media were incubated at 37°C for 72h and 24h, respectively.

The degree of stabilization of organic matter (OM) was determined by a chemical sequential extraction based on a modified Van Soest method. Two compartments were extracted: (i) the easily accessible fraction of OM assimilated to particulate extracellular OM and (ii) the slowly accessible fraction assimilated to humic substance-like, cellulose and hemicellulose. The no extractible fraction was assimilated to lignin-like compounds. Microbial activities were estimated by (i) the residual microbial activity measured by methane production during 40 days under anaerobic conditions, and (ii) the non-specific enzymatic activity performed by fluorescein diacetate (FDA) hydrolysis.

Results and discussion

Digestate characteristics of the investigated plants are presented in Table 1.

Table 1. Characteristics of the OWP

sample	pH	Moisture content %	Fraction easily accessible %	Fraction slowly accessible %	Fraction No extractible %	CH_4 prod. (L/Kg VM)	FDA activity (µg/g RM)
R3	8.1	96	44.3	12.3	43.4	35.9	73.5
R9	7.7	90	9.5	30.5	60.0	53.7	168
R4	8	91	33.1	13.8	53.2	64.4	300
L2	8.3	94	45.0	13.2	41.8	53.1	83
L7	7	89	62.9	22.2	14.9	40.9	117
C1	7.3	25	4.0	53.4	42.6	19.1	182
C5	7.8	56	6.9	77.8	15.3	17.5	270
C7	8	59	5.4	39.7	54.9	0	233

The liquid fraction and the raw digestates contain a high level of easily accessible fraction of organic matter which was not degraded during the anaerobic digestion, independently of the hydraulic retention time (ranged between 20 and 90 days). Their residual methane productions is in the same order of magnitude than those reported by Rico *et al.* (2011) for digestates of liquid fraction of dairy manure. As expected, composted digestates contain the lowest easily accessible fraction and the lowest residual methane production. The lowest enzymatic activity, measured by FDA hydrolysis, is observed in the liquid fractions.

The log reduction of both bacteria ranged between 4.5 and 6 (Fig.1) within 20 days.

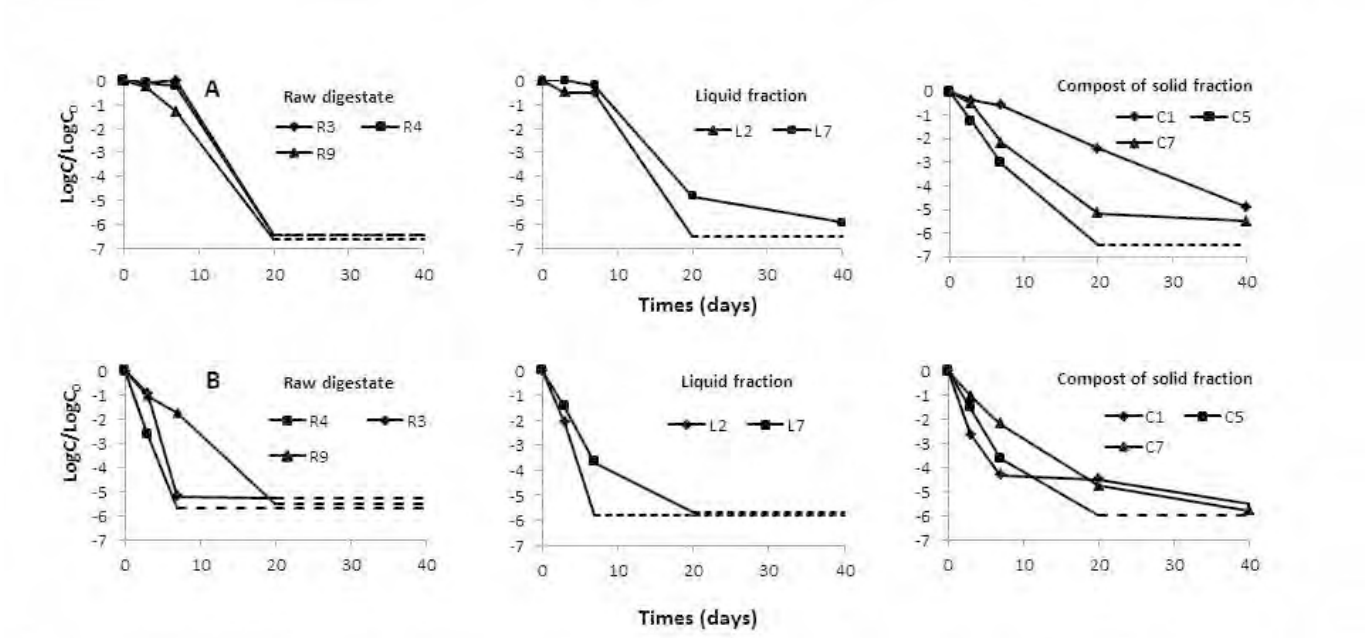

Figure 1. Kinetics of survival of *L. monocytogenes* (A) and *Salmonella* Derby (B) in 8 OWP. Dotted lines indicate the limit of detection.

Overall, *L. monocytogenes* survived longer than *Salmonella.* This is in accordance with the shorter persistence of *Salmonella*, compared to that of *L. monocytogenes*, observed in soil by Brennan *et al.* (2014). Interestingly, the level of *L. monocytogenes* remained stable during the first week of incubation in raw digestates and in liquid fractions whereas it decreased in composted digestates which had less easily accessible fraction (Table 1). However, *L. monocytogenes* was still detected in two of the composted digestates after 41 days. No lag phase was observed for *Salmonella* which survived longer in composted digestates.

A principal component analysis was performed on variables describing the behaviour of the strains and the characteristics of the OWP (Fig. 2).

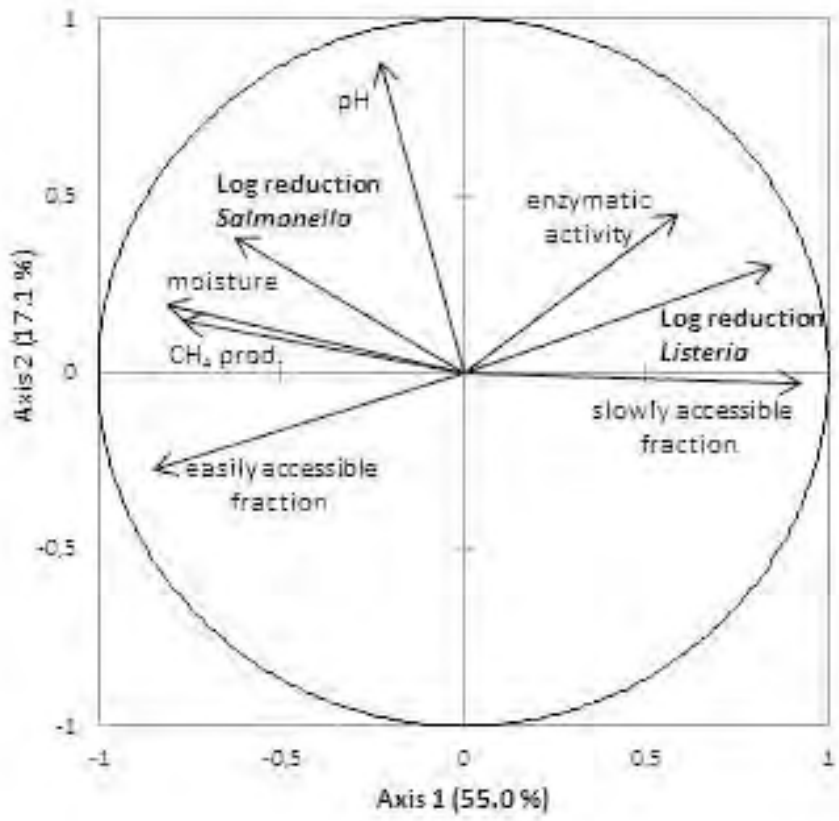

Figure 2. PCA of data for chemical, biochemical and microbial characteristics of the 8 OWP.

The persistence of the strains was estimated by their log reduction after 7 days. The decay of *Salmonella* is positively correlated to the moisture content and to the anaerobic activity (residual CH_4 production) whereas the decay of *L. monocytogenes* was correlated to the slowly accessible fraction and negatively correlated to the easily accessible fraction of the OM (humic substance-like, cellulose and hemicellulose).

Conclusion

The decay of *L. monocytogenes* and *Salmonella* is influenced by a combination of biotic and abiotic factors including substrate accessibility to microbes and microbial activity. Nevertheless, other factors such as microbial diversity may also interact and will be further analysed.

Acknowledgements

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THE COMPARATIVE STUDY ON METHODS FOR MICROBIOLOGICAL ANALYSIS OF WATER FOR ANIMAL DRINKING

Silvia-Cornelia Antoniu, Constantin Lupescu

Institute of Diagnosis and Animal Health, Bucharest, Romania

Summary

Introduction

The main indicators for microbiological analysis of drinking water are: coliform bacteria, *Escherichia coli* and enterococci. *Escherichia coli* is the best indicator for fecal pollution. Its presence indicates a recent fecal pollution due to inadequate treatment of water or existence of breaks in the distribution network. The coliform bacteria and enterococci are relative specific indicators for fecal pollution, because they can originate from another habitat (soil), in absence of fecal pollution.

Animal, material and methods

The scope of this paper consisted in comparison of two methods for microbiological analysis of animal drinking water: method by inoculation in liquid medium and membrane filtration method.

Results

In the lab of hygiene from Institute of Diagnosis and Animal Health Bucharest, in the period 2012-2014, there were analyzed 62 samples of water from local (35,49%) and central (64,51%) sources of water, providing from animal farms (32,26%), population households (29,03%) and IDAH laboratory of experimental veterinary medicine (38,71%).

The interpretation of results was performed according to national legislation: Law 311/2004 for modifying and completion of Law 458/2002 concerning quality of drinking water because, in Romania, there isn't any rule concerning water for animal drinking; it is considered that the water for animal drinking must have the same quality as the water for human consumption.

Conclusions

Since 29.03% of the samples analyzed by membrane filtration method have been inadequate in terms of all the parameters of microbiological analysis (coliform bacteria, *Escherichia coli*, enterococci), it is necessary to monitor the water sources for animal drinking in order to maintain the animal health, to prevent the occurrence of transmissible diseases from animal to human, to protect of environment and, not at least, to protect public health.

Introduction

The main indicators for microbiological analysis of drinking water are: coliform bacteria, *Escherichia coli* and enterococci. *Escherichia coli* is the best indicator for fecal pollution. Its presence indicates a recent fecal pollution due to inadequate treatment of water or existence of breaks in the distribution network. The coliform bacteria and enterococci are relative specific indicators for fecal pollution, because they can originate from another habitat (soil), in absence of fecal pollution.

The scope of this paper consisted in comparison of two methods for microbiological analysis of animal drinking water: method by inoculation in liquid medium and membrane filtration method.

Animal, material and methods

The detection and enumeration of coliform bacteria and *Escherichia coli* in water by *membrane filtration method* consisted in filtration of sample, incubation of membrane on a selective lactose agar medium at 36±2 °C, for 21±3 h, confirmation of presumtive colonies by biochemical tests: oxidase, indol and glucuronidase test and couting of coliform bacteria (lactose-positive colonies, giving a negative oxidase reaction) and *Escherichia coli* (lactose-positive colonies, giving a negative oxidase, a positive indole and a positive glucuronidase reaction) in 2-3 days.

The detection and enumeration of enterococci in water by *membrane filtration method* consisted in filtration of sample, incubation of membrane on a solid selective medium containing sodium azide (to suppress the growth of GRAM-

negative bacteria) and de 2,3,5-triphenyltetrazolium chloride (a colourless dye) that is reduced to red formazan by intestinal enterococci. Typical colonies are raised, with a red, maroon or pink colour, either in the centre of the colony or throughout. By transfer of membrane, with all the colonies, onto bile-aesculine-azide agar, preheated at 44°C, intestinal enterococci hydrolyse aesculin on this medium in 2 h. The end-product 6,7-dihydroxycoumarin, combines with iron(III) ions to give a tan-coloured to black compound which diffuses into the medium.

The counting of coliform bacteria in water by *multiple tubes method* consisted in inoculation of test portions and decimal dilutions of them in tubes with the liquid selective medium (simple or double concentrated laurylsulphate broth), incubation at 37±0.5°C, for 48 h and biochemical confirmation by sub-culture from tubes with turbidity and gas production on solid selective culture medium (lactose-eosin- methylene blue agar).

Counting of fecal streptococci in water by *multiple tubes methods* was performed inoculation of test portions and decimal dilutions of them in tubes with the liquid selective medium (simple or double concentrated sodium azide broth), incubation at 37±0.5°C, for 48 h and biochemical confirmation by sub-culture from tubes with turbidity in liquid selective medium (sodium azide-bromocresol purple broth) or on solid selective culture medium (glucose-sodium azide-triphenyltetrazolium chloride agar).

In case of *multiple tubes method,* the appreciation of target microorganisms is performed by statistical analysis of counts of positive and negative portions-test, observed after incubation.

Results

- The results of 2 tests are mentioned in the table 1.
- In the lab of hygiene from Institute of Diagnosis and Animal Health Bucharest, in the period 2012-2014, there were analyzed 62 samples of water from local (35,49%) and central (64,51%) sources of water, providing from animal farms (32,26%), population households (29,03%) and IDAH laboratory of experimental veterinary medicine (38,71%).
- The interpretation of results was performed according to national legislation: Law 311/2004 for modifying and completion of Law 458/2002 concerning quality of drinking water, where the maximum accepted limits for microbiological analyzed parameters are mentioned: coliform bacteria /100 ml = 0, *Escherichia coli*/100 ml = 0 and intestinal enterococci/100 ml = 0.
- 18 out of all tested samples, by membrane filtration method, were inadequate for all microbiological parameters (coliform bacteria, *Escherichia coli* and intestinal enterococci) (29,03%).
- For coliform bacteria, the results obtained by performing of both tests coincided in 96,77% of cases, but for enterococci/ fecal streptococci - in 91,93% of cases. The last percentage was lower because of difference between enterococci and fecal streptococci, intestinal enterococci being a category of fecal streptococci.

Discussions

- The advantages of *membrane filtration method* are: easy for implementation in the lab, easy to perform, low time and high precision. The disadvantages are: special equipment as membrane filtration system, narrow field of application (for waters with low bacterial background flora).
- *Multiple tubes method* become sensible for a value more than 2 CFU/100 ml and it is impossible to detect target microorganisms in waters with 1 UFC/100 ml).

Conclusions

- *Escherichia coli* - the best indicator for fecal pollution. Its presence indicates a recent fecal pollution due to inadequate treatment of water or existence of breaks in the distribution network. The coliform bacteria and enterococci are relative specific indicators for fecal pollution, because they can originate from another habitat (soil), in absence of fecal pollution.
- Because, in Romania, there isn't any rule concerning water for animal drinking, it is considered that the water for animal drinking must have the same quality as the water for human consumption. Therefore, interpretation of results was performed according to national legislation: Law 311/2004 for modifying and completion of Law 458/2002 concerning quality of drinking water.

- Since 29.03% of the samples analyzed by membrane filtration method have been inadequate in terms of all the parameters of microbiological analysis (coliform bacteria, *Escherichia coli*, enterococci), it is necessary to monitor the water sources for animal drinking in order to maintain the animal health, to prevent the occurrence of transmissible diseases from animal to human, to protect of environment and, not at least, to protect public health.

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EN ISO 9308-1:2000 /AC:2008 Water quality. Detection and enumeration of *Escherichia coli* and coliform bacteria. Partea 1: Membrane filtration method (cancelled)

EN ISO 9308-1:2000 Water quality. Detection and enumeration of *Escherichia coli* and coliform bacteria. Partea 1: Membrane filtration method (cancelled)

DISINFECTION OF DRINKING WATER USED FOR WATERING OF FARM ANIMALS

N. Sasáková[1], T. Hrušková[2], Z. Bujdošová[2], N. Bozakova[3], J. Venglovský[1], G. Gregová[1], T. Čornejová[1], M. Ondrašovič[1]

[1]University of Veterinary Medicine and Pharmacy in Kosice, Department of Environment, Veterinary Legislation and Economics, Komenského 73, 041 81 Košice, The Slovak Republic
[2]University of Veterinary Medicine and Pharmacy in Košice, Department of Chemistry, Biochemistry and Biophysics, Institute of Medical Chemistry, Komenského 73, 041 81 Košice, The Slovak Republic
[3]Faculty of Veterinary Medicine, Trakia University - Stara Zagora, Bulgaria

Summary

Quality of water sources was investigated on 3 farms in eastern Slovakia focusing on chemical indicators of faecal contamination and on determination of counts of coliform bacteria, *E.coli,* enterococci and bacteria cultivated at 22 and 37 °C. Due to potential contamination, particularly in rainy period, we determined experimentally suitable doses of chlorine disinfectants and tested their effectiveness. On two farms it was necessary to increase the doses. On the basis of the results we recommended to disinfect the sources more frequently using smaller doses of disinfectant.

Introduction

Many infectious diseases of animals and humans are transmitted by contaminated water. To eliminate the risk of disease transfer, water intended for mass consumption is treated and disinfected before use. Treatment of water depends on the source and the requirements on treatment and quality are set by relevant legislation. Regular monitoring of water is necessary, particularly water from individual sources - wells. Monitoring includes many parameters focused on indicators of pollution, chemical and bacterial contamination and disinfection byproducts (Zhao et al., 2012).

Disinfection with active chlorine preparations is considered the most suitable way of disinfection of on farm drinking water sources. The dose of chlorine depends on the quality of treated water and the preparation used. Both these factors affect the level of residual active chlorine by the consumer. If disinfection is not done properly, disinfectants can affect the health of consumers of disinfected water or induce various responses (Michalus, 2000).

Materials and methods

We investigated quality of water used on 3 farms in eastern Slovakia. Two farms were keeping only cattle and one both cattle and sheep. Water was sampled several times in the period of January-May, 2014. Chlorination with calculated doses of Chloramine T started in March and continued through May.

Chemical examination focused on parameters indicating faecal pollution: ammonium ions, nitrites, nitrates, phosphates and chlorides. Bacteriological examination was carried out according to Regulation of the government of the SR 496/2010 Coll. determination of counts of coliform bacteria, *E.coli* and enterococci and bacteria cultivated at 22 and 37 °C. In addition, COD_{Mn} and free chlorine was determined and experimental chlorination was performed to determine the optimum single dose of Chloramine T for disinfection of the investigated water sources (HORÁKOVÁ et al., 2003). COD_{Mn} (oxidizability) is an important parameter indicating risk of formation of byproducts. The calculated dose of Chloramine T was used to chlorinate water in the wells and one week later the water was tested bacteriologically and for presence of residual (free) chlorine.

Results

Good quality of water intended for human consumption and watering of animals is essential for their health and for prevention of contamination of the food chain. Disinfection of water, that serves as the final measure against spreading of diseases, should be carried out when necessary, keeping in mind all associated risks and trying to prevent them.

Doses of Chloramine T based on experimental chlorination appeared relatively efficient on Farm 1 and 3, while on Farm 2 the estimated dose had to be increased, but still was much lower than that recommended by the manufacturer of this preparation. Adequate disinfection ensures hygiene safety of water and does not exceed the limit for residual active chlorine (0.3 mg·L^{-1}). Because our evaluations concerned water that should comply with the limits for drinking water, we compared our results with those set by the relevant legislation.

Chemical examination of water sources on Farms 1 and 3 did not indicate faecal pollution of water and the results corresponded to the requirements on drinking water. Water source on Farm 2 exceeded the limit for COD_{Mn} in January (3.7 compared to 3.0 mg/l) and for nitrates at all samplings (72-88 mg/l compared to the limit of 50 mg/l).

Results of initial bacteriological examination of water from all water sources on investigated farms exceeded the maximum acceptable limit at all samplings.

Experimental chlorination of source on Farm 1 set the dose of Chloramine T to 20 g per well. This dose was subsequently doubled to 40 g in the period of heavy rains. The dose calculated for wells on Farms 2 and 3 (180 g) appeared sufficient for water on Farm 3 but had to be doubled on farm 2.

As all three wells were situated in an agricultural area there existed some risk of contamination with animal manure or fertilizers especially in spring and in the periods of heavy rains. In order to decrease the risk of production of disinfection byproducts more frequent disinfection with lower doses of chlorine preparations could be recommended especially during wet periods when wells could be contaminated with some run-off.

Conclusions

Chemical and microbiological examinations performed on the three investigated farms showed that the water source on Farm 3 provided water of better quality than sources on Farms 1 and 2. Our results indicated that weather (precipitation) affected quality of water on all three farms and was associated with some risk to animals consuming this water.

Doses of Chloramine T based on experimental chlorination and used for disinfection on investigated farms appeared relatively efficient on Farm 1 and Farm 3, while on Farm 2 the estimated Chloramine T dose had to be increased considerably, but still was much lower than that recommended by the manufacturer of this preparation. It appeared desirable to adjust the intervals between individual treatments to weather conditions (heavy rain) instead of increasing the active chlorine doses.

However, protection of individual water sources against contamination (protection zones, elimination of seepage of rain or run-off from fields) is always the best approach in prevention of human and animal diseases.

Acknowledgement

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HYGIENE ASPECTS OF DRINKING WATER SOURCES USED IN PRIMARY MILK PRODUCTION

N. Sasáková, J. Venglovský, G. Gregová, I. Papajová[1], B. Nowakowicz-Debek[2], K. Veszelits Laktičová, R. Hromada, D. Chvojka, J. Koščo

[1]University of Veterinary Medicine and Pharmacy in Kosice, Department of Environment, Veterinary Legislation and Economics, Komenského 73, 041 81 Košice [1]Parasitological Institute of the Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice; The Slovak Republic
[2]Department of Animal Hygiene and Environment, University of Life Sciences in Lublin ul. Akademicka 13, 20-950 Lublin, Poland

Summary

We examined the quality of drinking water intended for primary milk production from 5 sources on agricultural farms in industrially contaminated area. Chemical investigation showed that limits for nitrates and chlorides set by legislation were exceeded in sources for individual supply. Coliform bacteria as an indicator of faecal contamination were present only in sources for individual supply and the limits were exceeded particularly in spring, summer and fall. Limits for bacteria cultivated at 37°C were exceeded only in source 4 in autumn. The samples from individual supply showed the highest bacteriological contamination

Keywords: drinking water, sources, quality, coliform bacteria, *E. coli*

Introduction

Protection of water is currently a priority and a basic factor of environmental management. Today we commonly find territories where drinking water sources are contaminated by anthropogenic activities (Sasáková, 2009). The Regulation No. 199/2008 Coll. establishes Programme of agricultural activities in declared vulnerable areas where the level of nitrates in surface and ground water or in lakes exceeds 50 $mg.l^{-1}$. Hygiene rules apply to wells as sources of drinking water for individual and mass supply. The surroundings of wells must be checked regularly for any sources of potential contamination, such as septic tanks, sewage pipelines, liquid fuel tanks, animal houses, manure heaps and similar (Klinda, 2010).

Drinking water used in milk production this process must be safe and regularly monitored.The aim of the study was to investigate chemically and bacteriologically the quality of water sources on selected farms and evaluate their suitability.

Materials and methods

Sources of drinking water intended for individual supply (IS) and mass supply (MS) used on agricultural farms for primary milk production (5 farms) were evaluated according to Governmental Order of SR No. 496/2010 Coll.

Chemical examination focused on indicators of faecal contamination of water sources and presence of residual chlorine.

Bacteriological examination included parameters indicating general contamination (BC22 and BC37), potential contamination with faeces or sewage (coliform bacteria), and presence of micro-organisms that are part of the digestive tract of man and animals (*E. coli*). We counted bacterial colonies (CFU) after inoculation on relevant nutrient agars and cultivation for prescribed time at optimum temperatures.

Results

Ammonium ions (NH_4^+) were detected only in samples from source 2 and reached the highest concentration in spring which could be related to melting of snow or extensive rainfall. Nitrates (NO_3^-) were found in all sources at every sampling in spring and only in traces in the remaining seasons. The limit value for nitrates was exceeded in source 4 (78.3 mg/l) and for chlorides in source 3 (475.03 mg/l) in autumn.

In sources for individual supply (MLV = 0/10 ml) the MLV for EC was exceeded in sources 3 and 4 throughout the sampling, in the source 3 in winter (1 CFU/10 ml), summer (6 CFU/10 ml) and autumn (13 CFU/10 ml) and in source 4 in winter (8 CFU/10 ml), summer (66 CFU/10 ml) and autumn (130 CFU/10 ml).

Coliform bacteria (CB) were present only in individual sources (sample 1, 3 and 4). In all sources for IS coliform bacteria were present in 10 ml of water in all seasons (source 2: 8 CFU in winter, 220 in spring, 60 in summer and 48 in autumn; source 3: 26 CFU in winter, 240 in spring, 138 in summer and 35 in autumn; source 4: 80 CFU in winter, 260 in spring, 288 in summer and 240 in autumn).

The limit for plate counts of BC37 was exceeded in source 4 in summer (290 CFU/1 ml) and autumn (150 CFU/1 ml).

In sources for individual supply free chlorine was present only in source 2 (0.1 mg/l in summer and autumn).

Despite the fact that sources for individual supply 3 and 4 were fenced, their surroundings were not kept up and protected adequately. These sources could be contaminated by grazing of farm animals or application of their excrements on soil. This may result in contamination of ground water in the entire location.

Discussion

Evaluation of risks resulting from exposure to chemical substances and other contaminants is a complex task. Moreover, the quality of data on toxicity is very variable. Studies of toxicity in animals are the biggest source of data for risk evaluation (Howd & Fan 2008).

Polluted water can affect adversely the animals that have to consume it (exitus of calves, ketosis or acetonaemia of cattle, chronic diarrhoea, liver damage, spreading of infections) (Bitton 1999).

Because all activities in primary milk production affect essentially the safety of milk products, one must eliminate or decrease potential microbial contamination from all sources already in this phase of food chain. This includes farm environment, water, milking personnel, animals and complying with the rules of good hygiene practice. Low level of hygiene during milking and treatment of milk presents a risk already in the phase of primary production (Code 2004).

Conclusions

The water sources for individual supply were fenced, but their surroundings were not kept up and protected adequately. Their potential contamination could result from grazing of farm animals or application of their excrements on soil.

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The Regulation No. 199/2008 Coll. establishes Programme of agricultural activities.

DISINFECTION OF ANIMAL EXCRETA AS ONE OF THE MEASURES ELIMINATING INFECTIOUS DISEASES

Dušan Chvojka, Silvia Ondrašovičová, Rudolf Hromada, Naďa Sasáková, Katarína Veszelits Laktičová, Ján Koščo, Miloslav Ondrašovič

University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice, The Slovak Republic

Summary

Disposal of excrements is an important problem in the livestock management. Manure is widely used as the fertilizer in crop production, but its incorrect treatment leads to loss of nutrients what can then be an important contributor to environmental pollution. In this paper, the results of disinfection of manure are presented thus a risk of the outbreaks of same infectious diseases in livestock management can be predicted.

Introduction

One of the issues how to prevent the spreading of infectious diseases in the livestock management is the disinfection of the manure. When choosing a suitable disinfectant is necessary to focus on the fact whether the disinfectant is capable to devitalize the infectious disease agent, but do not contribute to the creation of residues, which could have same adverse affects on the environment, or if its application can enriched the fertilizing properties of the manure. Such disinfectants are as follows: NH_4, H_3PO_4 and $Ca(OH)_2$. Currently, the incidence of paratuberculosis is an important problem in herds of cattle, because the agent has a high resistance to environmental influences as well as to the antimicrobial activity of disinfectants. The abovementioned disinfectants do not devitalize this pathogen. However, the required devitalization effect on this microorganism was achieved after the application of the peroxyacetic acid, which is rapidly degraded in the environment without leaving undesirable residues.

Material and method

The physico-chemical analysis of slurry was carried according to STN 83 0540 (1982) and Horáková et al. (2003) and STN 46 5735 Industrial compost, (1991).

Plate counts of *E.coli* and total coliforms were determined using Endo agar (BioMark Laboratories, India) with incubation at 37°C for 24 h. Plate counts of faecal streptococci were determined using selective agar Slanetz-Bartley (BioMark Laboratories, India) and incubation at 37°C for 24-48 h.

These chemical parameters (pH, dry matter, BOD, COD, N-NH_4 and total phosphorus) were monitored and evaluated in slurry as well.

Results

The application of chemical disinfectants in liquid manure triggered the complex of chemical-physical changes, which has effect on the amount of hydrogen ions. Two days after the administration of 3% $Ca(OH)_2$, the pH was 12.0, and decreased to 2.5 after the addition of phosphoric acid. After the addition of 1% formaldehyde and 3% $Ca(OH)_2$, the dry matter content increased by more than 2%.

The most significant increase in the value of BOD compared to the control (9930 $mg.l^{-1}$) occurred after the disinfection of slurry with the Persteril, 48 hours after its addition was the BOD value around 18 480 $mg.l^{-1}$, while after the disinfection with formalin was the BOD value lower (13 430 $mg.l^{-1}$). The storage within six weeks caused a progressive decline up to 7580 $mg.l^{-1}$ in the Persteril and to 11 140 $mg.l^{-1}$ in formaldehyde treated slurry. We presume, that the changes that have seen using the Persteril are related to its strong oxidant properties. Similar results were also recorded in determining the values of COD.

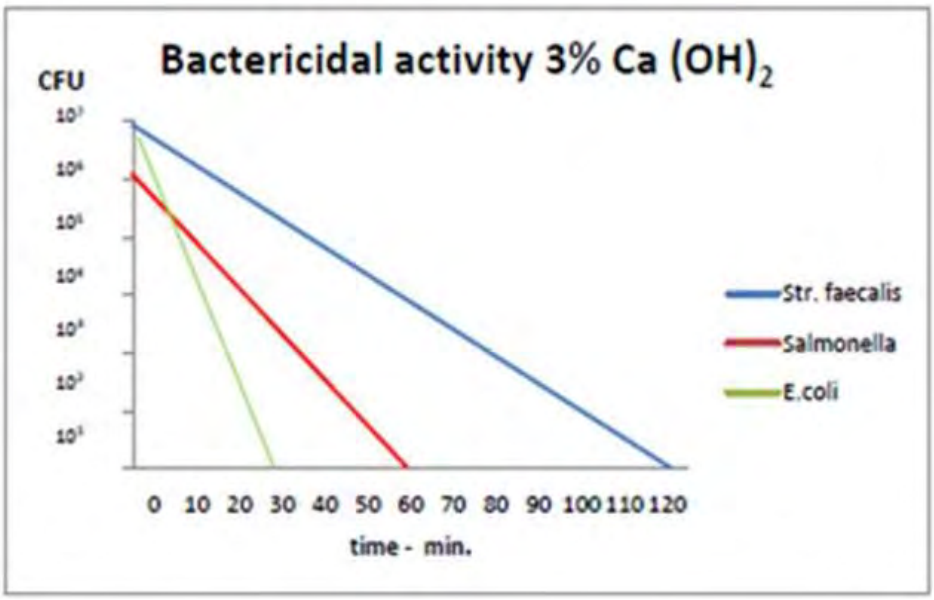

Figure 1. Bactericidal activity 3% $Ca(OH)_2$

Bactericidal activity of 3% $Ca(OH)_2$ was most pronounced for *E coli*, which has been devitalized to 30 minutes, meanwhile, the salmonella was devitalized in 60 minutes. *Str. feacalis* resisted the calcium hydroxide treatment for 120 minutes (Fig.1.).

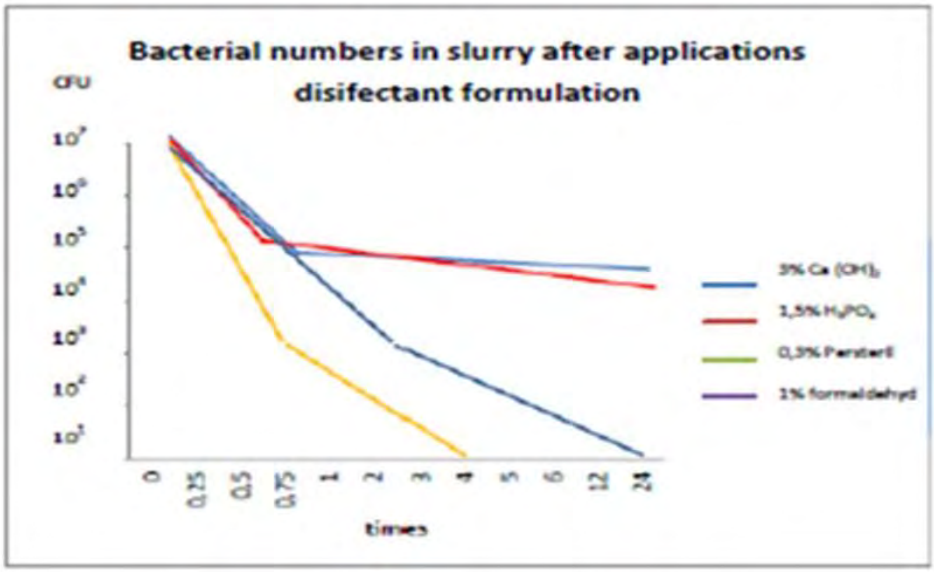

Figure 2. Bacterial numbers in slurry

The results of the devitalization of micro-organisms are given in Figure 2. The most effective impact was observed after the administration of peroxyacetic acid (Persteril), when the bacterial numbers was negative within 4 hours. The formalin treatment exhibit a negative finding in 24 hours. The 24 h exposure to phosphoric acid and calcium hydroxide, decreased the total numbers of microorganisms from 10^7 to 10^5.

Discusion

As reported by Turner and Burton (1997), in the intensive livestock production using a grid housing is produced manure which can cause further environmental problems, especially water pollution, due to its incorrect treatment. Generally, liquid manure from healthy animals is not routinely disinfected but its incorrect storage or its wrongly chosen time and place of an application may cause the aforementioned environmental problem. In the case of the slurry obtained from infected animals, this represents the epidemiological problem and it is necessary to disinfect such a material prior its application to the soil. When selecting the efficient disinfection procedure and preparation, the focus should be on such a way that ensures the devitalization of pathogens but do not contribute to the presence of undesirable residues in the manure (Singh akol.2006).

Kneflová et al. (1992) describe the devitalizing effect of the disinfectant (Lautericid) when its concentration of 5% devitalized the mycobacterium within 60 minutes. The active substance in this product is coconut acid amine acetate. After the treatment of pig and cattle slurry, the devitalization effect of hydrogen peroxide with a catalytic effect of iron and silver ions was observed by PROFANT et al. (2006). The mycobactericidal properties of preparations (such as: formalin, glutaraldehyde, phenol and peroxides) are well described in The good practice for biosecurity in the pig sector published in 2010 by The food and agriculture organization of the united nations and the world organization for animal health.

Conclusions

The application of disinfectants into the slurry leads to different chemical changes which caused mainly the fall of pH but also the decrease of dry matter, BOD and COD value. In the evaluation of bactericidal activity of 3% $Ca(OH)_2$, the devitalization of microorganisms occurs within 2 hours after its administration. The devitalization of microorganisms originated from the slurry was most effective using the 0.3% concentration of the peroxyacetic acid up to 4 hours after the treatment and after the application of 1% formaldehyde within 24 hours.

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HYGIENICALLY SAFE RECYCLING OF SLURRY, SEWAGE SLUDGE AND FERMENTATION PRODUCTS IN AGRICULTURE

W. Philipp, R. Eling, N. Hartmann, T. Schilling, L. E. Hoelzle

Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

Summary
Sewage sludge, manure and fermentation products should only be used as organic fertilizers if they do not contain pathogens. This goal can be achieved by heating > 53 °C, anaerobic thermophilic digestion and long-term storage.

An alternative to treatment of organic fertilizers could be application restrictions based on determined critical values of Salmonella or Escherichia coli (100 per gram for salmonella and 1000 per gram for E. coli, respectively).

In future, quality labels common for compost should be prevailed for all organic fertilizers including sewage sludge and fermentation products. The basic investigations ruled out to achieve this label include the screening of microbiological parameters in addition to the measurement of nutrients and pollutants.

1 Introduction

Slurry, sewage sludge and fermentation products can contain pathogenic microorganisms including zoonotic agents and therefore, represent a health hazard to humans and animals (Appel et al., 2011).

In 2011, an epidemic-like EHEC-outbreak (EAHEC O104:H4) happened in Germany resulting in serious illnesses. This strain combined features both of classical enteroaggregative E. coli as well as classical enterohemorrhagic E. coli.

However, tenacity of EAHEC O104:H4 in the environment is unknown (Appel et al., 2011). According to Appel and co-workers (2011) and Beutin et al. (2011) it seems that the pathogen has its main reservoir in man, multiplies in man, and gets into the environment via feces and sewage sludge. It is assumed that the minimum infective dose of EAHEC O104:H4 is approx. less than 100 bacteria. The risk of contamination of the environment, plants and soil with organic fertilizers containing this pathogen during application of farmyard manure and sewage sludge or by irrigations must be considered (Beutin et al., 2011).

In this study, laboratory experiments were performed to investigate the inactivation of E. coli (EAHEC) O104: H4 and Salmonella Typhimurium in organic substrates by heating at 53 °C and by long time storage. The temperature of 53 °C was chosen due to the fact that the majority of thermophilic biogas plants are operated at temperatures between 52-55 °C.

2 Material and methods

Test samples of the respective substrate were inoculated with 1 ml of a freshly prepared suspension of the pathogens. The quantitative detection of EHEC O104:H4 was performed in accordance to BEUTIN et al. (2011). Salmonella was detected quantitatively using the MPN technique.

3 Results

Saline, sewage sludge, slurry were spiked with EAHEC suspensions resulting in end concentrations ranging from $1.7x10^8$ to $3.0x10^8$ cfu/ml.

After heating for four hours the EAHEC concentrations in the substrates were reduced to $9.3x10^5$ cfu/ml in saline, $9.9x10^6$ cfu/ml in sewage sludge, and $1.5x10^7$ cfu/ml in slurry. Heating for 18 hours led to concentrations which were below the detection limit of the test assay. The concentrations of the control samples at 6 °C were in all cases between $2.3x10^8$ and $4.3x10^8$ cfu/ml. Mean reduction values of EAHEC after four hours of heating at 53 °C were $1.4x\log_{10}$ in sewage sludge, $2.2x\log_{10}$ in slurry, and $4.6x\log_{10}$ in saline solution. After eighteen hours the reduction rates were $8.4x\log_{10}$, $8.1x\log_{10}$, and $8.5x\log_{10}$ for sewage sludge, saline solution and slurry. In control samples the EAHEC concentrations remained more or less unchanged.

In the inoculated starting substrates, Salmonella concentrations ranged from $9.3x10^7$ to$1.7x10^8$ cfu/ml. After heating for four hours Salmonella concentrations between $2.0x10^3$ and $4.3x10^3$ cfu/ml could be detected. After eighteen hours of heating, the concentrations were below the detection limit of 3.0 cfu/ml in all three substrates. Mean reduc-

tion values ranged between 4.6 and 4.8 x log_{10} after heating for four hours. After eighteen hours, the reduction rates were in all three substrates between 7.9 and 8.2 x log_{10}.

In addition, EAHEC and Salmonella were stored in the different substrates (sewage sludge, slurry, and fermentation products) for 36 weeks at 10 °C to detect the inactivation potency of this long-term storage on these two pathogens.

In principle, long-term storage allowed a reduction of EAHEC of about 5 log_{10}-steps in all tested substrates. This reduction rate was achieved in sewage sludge after at least 36 weeks, in slurry after 8 weeks, and in fermentation products after 36 weeks of storage.In Salmonella Typhimurium DT120 reduction rates of at least 5 log_{10}-steps could only be achieved after 36 weeks of storage time in all substrates. In contrast to the EAHEC, salmonella were not detectable in the fermentation products after 36 weeks.

4 Discussion

In this study a reduction by five log_{10}-steps (defined in Regulation (EU) No 142/2011) of E. coli (EAHEC) O104:H4 and Salmonella Typhimurium DT120 could be achieved in all substrates at the temperature/time combination of 53 °C and 18 hours.

A long-term storage for approx. 4 month of organic fertilizers contaminated with EAHEC O104:H4 and Salmonella Typhimurium DT120 was not sufficient to reduce these bacteria for at least five log_{10} steps. Exceptions from this were EAHEC in slurry and Salmonella Typhimurium in fermentates. Avery et al. (2005), Himathongkham et al. (1999, 2000) and Mannion et al. (2007) assumed that a reduction of the concentrations of pathogens as EAHEC and Salmonella in fertilizers is given by a long-term storage. Therefore, six months are required and should be postulated.

5 Conclusions

Organic fertilizers (e.g. slurry, sewage sludge, fermentation products) should only be applied after a sufficient reduction of pathogens. In principle, this can be achieved by heating, thermophilic digestion or long-term storage. Alternatively, analyses of the organic fertilizers with regard to the content of selected infectious agents (i.e. salmonellae and E. coli) could be performed (potential critical values: 10^2 cfu/g for salmonellae; 10^3 cfu/g for E. coli). These investigations can lead to a quality label for the fertilizers which can be connected to certain ways of application regulations.

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Prevention of disease, biosecurity – vaccination

RESULTS OF THE IMPLEMENTATION OF A RB51 VACCINATION PROGRAMME IN COMBINATION WITH SANITARY MEASURES FOR THE CONTROL OF BOVINE BRUCELLOSIS IN A SMALLHOLDING AREA IN PORTUGAL

A.P. Figueira[1], R. Ribeiro[2], A.P. Fonseca[3], S. Madeira[2], F. Boinas[2].

[1]Direção Geral de Alimentação e Veterinária, Direção de Serviços de Alimentação e Veterinária da Região Norte, Divisão de Alimentação e Veterinária de Vila Real, Praça do Campo da Fonte, 5400-160 Chaves, Portugal
[2]Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica de Lisboa, 1300-477 Lisboa, Portugal
[3]Direção Geral de Alimentação e Veterinária, Direção de Serviços de Alimentação e Veterinária da Região Centro, Bairro da Senhora dos Remédios, 6300-535 Guarda, Portugal

Summary
Bovine brucellosis (BB) due to *Brucella abortus* infection is an important contagious disease that can cause abortions and infertility in cattle, is a zoonosis and, it eradication is difficult to achieve.

In Northern Portugal, in the county of Montalegre, difficulties to disease eradication due to high prevalence of infection, high number of smallholdings, practice of mountain communal grazing, the existence of a certified type of bovine production with high genetic value and the constrains of implementing B19 vaccination, led to the local implementation of a special five year eradication programme (SEP) for BB, which combined the application of sanitary measures with vaccination of bovines with RB51.

Results achieved in BB prevalence and incidence with implementation of the five year SEP (2005 to 2010) in Montalegre region, were evaluated. Secondary effects of the application of RB51 in pregnant females were also taken into account.

There was a very favorable evolution of the disease. Herd prevalence decreased from 10% to 0.3% and animal prevalence dropped from 3.3% to 0.05%, in this last case with significant differences between Montalegre and the rest of the DIV (official Direction of Veterinary Intervention). Herd incidence decrease from 2.6% in 2004, to 0%, achieved in 2009 and 2010. The rate of reported abortion after vaccination of pregnant females was 0.16%, 12.5 times less than the rate referenced by the manufacturer.

The success of this programme in Montalegre provided a valuable case study to the official veterinary services by illustrating the value of RB51 in combination with sanitary measures as an important measure for sustainable disease control, in high risk areas for BB.

Introduction
BB has been eradicated from many member states of the European Union, and national programmes, governed by country and European legislation, are employed where it persists. Control methods include test-and-slaughter or stamping-out when the prevalence is low or vaccination, in combination with sanitary measures, when the prevalence is high or control resources are constrained [1]. Vaccination increases resistance to infection and reduces both the risk of abortions and excretion of the organism. Two vaccines against BB are commercially available: B19 and RB51. Despite effectiveness of B19, it induces abortion if administered to pregnant animals and creates persistent titres that confound identification of infected cattle by most diagnostic tests (especially adult cattle), contrary to RB51 [1].

Portugal has an eradication programme for BB based on sanitary measures as serological testing of all susceptible cattle, slaughter of seropositive animals, depopulation under certain circumstances, laboratory confirmation of infection, premovement testing and post-abortion reporting [2]. However, in some regions of the country where the control of BB represents a serious challenge, combined vaccination programmes have been implemented with good results [1; 3].

In the county of Montalegre, the high number of smallholdings that are the producer's source of subsistence, the practice of mountain communal grazing, the existence of a certified type of bovine production, the lack of B19 and the high prevalence of infection, led to the local implementation of a SEP for BB, which combines the application of sanitary measures with bovine vaccination with RB51.

Material and methods

One year after interrupting B19 programme (Fig. 1) it was implemented a massive vaccination programme of bovine with RB51 (with vaccination of all female cattle aged over 4 months and revaccination of the females after 6 to 12 months whenever it was epidemiologically justified) in conjunction with a set of sanitary measures (serological surveys of the bovine herds, sanitary slaughter of the positive animals, bacteriology of the samples collected at slaughter, isolation of herds and control of animal movement). In the case of occurrence of abortions after vaccination there were performed bacteriological examinations for detection of *Brucella* vaccine strain in materials from abortions and vaginal swabs.

Results

A programme with B19 vaccination was implemented in 2002 and 2003. In 2004 herd prevalence of BB infection achieved 10% and animal prevalence 3.3% (Fig. 1). In the five years of SEP, herd prevalence decrease to 0.34% and animal prevalence to 0.05% (Fig. 1 and 2). Herd incidence decrease from 2.6% in 2004, to 0%, achieved in 2009 and 2010. Comparing herd and animal prevalence in Montalegre with the rest of DIV, DIV has always recorded, previous to SEP, a lower prevalence. However, two years after the implementation of SEP, there was a shift in this trend with Montalegre showing lower prevalence values. There were statistical significant differences in animal prevalence between Montalegre and the rest of DIV (*p-value*=0.04, Fig.2).

The rate of reported abortion after vaccination of adult females with RB51 was 0.16% (7389 adult females that were vaccinated and 12 abortions with isolation of *Brucella* vaccine strain). Abortions occurred frequently at the 7 month of pregnancy (with a range of 6 and 8 months) and the average time after vaccination and the occurrence of abortion was 4 months (3 to 5 months).

Discussion

A drop in herd and animal prevalence occurred in 2003, possibly associated with B19 vaccination (Fig. 1). However, due to lack of vaccine production, the programme was interrupted in 2004.

In 2005 it was decided to implement a five year SEP with RB51 vaccination for BB eradication. After implementation of SEP there was verified a favorable trend in the evolution of the disease in Montalegre, comparatively in the rest of the DIV. The sharp decrease in animal prevalence leads to a lower rate of excretion of the organism and consequently, less transmission in a region where communal grazing represents a risk factor to BB infection.

The rate of reported abortion of pregnant females associated with RB51 was 12.5 less in comparison with the reference rate indicated by the producer laboratory [4].

Conclusions

In situations where risk factors are present, as communal grazing, vaccination is an important measure that helps to reduce the excretion rate of *Brucella abortus* and subsequent environmental contamination. This case study in continental Portugal provided valuable support to the veterinary services as an example to other vaccination programmes.

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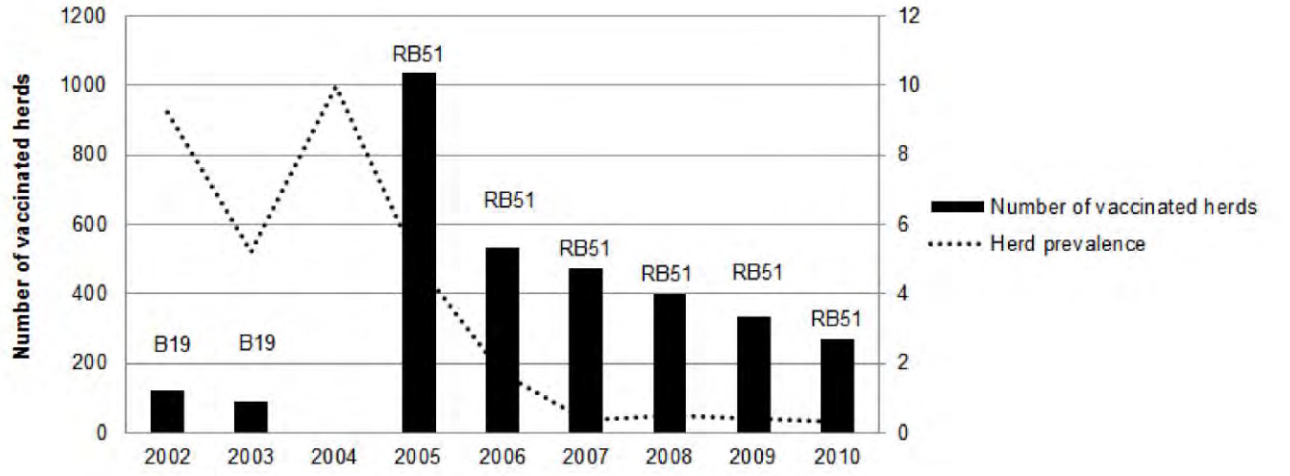

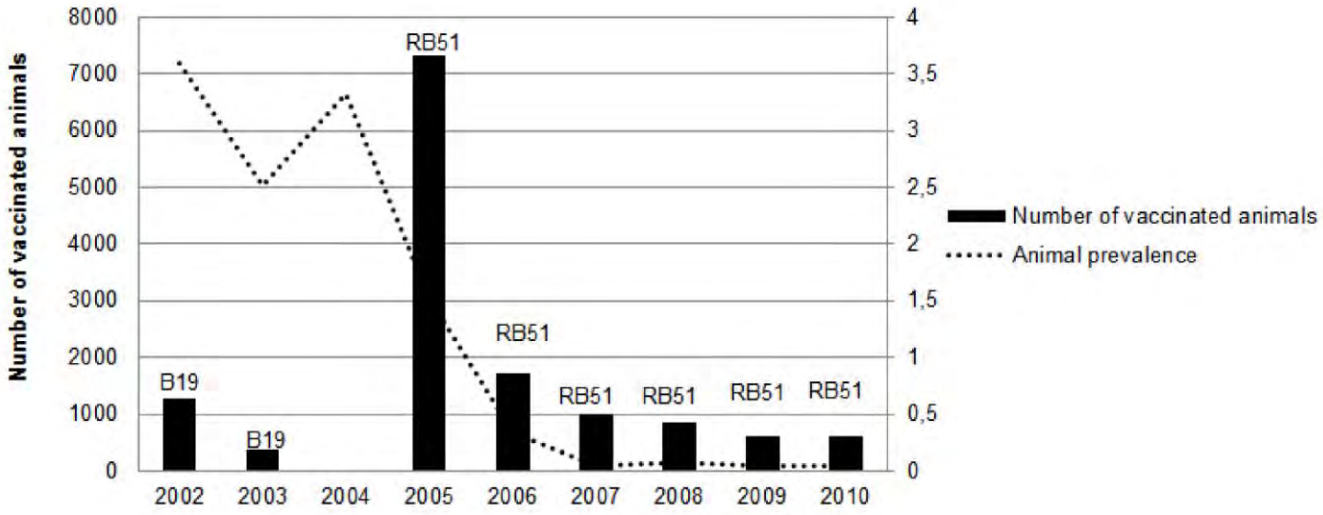

	Region	2005	2006	2007	2008	2009	2010	Median value	Difference between regions (*Wilcoxon rank sum test*)
Herd prevalence (%)	Montalegre	4.82	1.71	0.38	0.50	0.42	0.34	0.46	*p-value* = 0.24
	Rest of DIV of Vila Real	0.58	1.21	0.86	1.12	4.54	2.16	1.16	
Animal prevalence (%)	Montalegre	1.53	0.35	0.05	0.08	0.05	0.05	0.07	*p-value* = 0.04
	Rest of DIV of Vila Real	0.79	1.02	0.71	0.87	3.02	1.33	0.94	

EFFECTIVE CLEANING OF ANIMAL HOUSES EFFECTIVE CLEANING STABLES - A PREREQUISITE FOR EFFECTIVE DISINFECTION

Karel Tittl[1], Pavel Novak[1], Gabriela Mala[2]

[1] *Tekro spol. s r.o., Prague, Czech Republic*
[2] *Institute of Animal Science, Prague Uhříněves, Czech Republic*

Summary

Washing is a key part of each animal hygiene programme. High pressure washing above 120 bar results in the transfer of contaminated water droplers over long distances. The least effective is high-pressure washing using cold water without pre-soaking. Washing with hot water is more effective but more expensive than other methods. Correctly pre-soaked pens can be washed much easily and safely.

Introduction

Cleaning and washing stables after the removal of animals represents a major step towards effective reduction of infection pressure of rearing environment between two runs. Pre-wetting pens in the stables before washing is recommended as an integral part of the farm hygiene programme.

Animals, Materials and Methods

The presented paper is focused on comparing different methods of washing and determines the optimal ratio between effective cleaning and consumption of working time in the stables for pre-fattening pigs. The secondary objective was to determine the potential impingement distance of aerosol depending on the washing pressure.

Monitoring was realized at the animal house of pre-fattening pigs with all in – all out breeding system. The field trial was carried out in five separated pens in time after removing of pigs. Materials of section: half slatted floor – plastic statts and concrete floors, plastic barriers with zinc tubes, tille wall facing in living zone combined with rendering iron-plate ceilings.

Basic parameters of each section: length = 8.0 m; width = 10.0 m; height = 2.3 m

Inside area surface = 322.8 m^2. The surface of the technological facilities was 145.3 m^2. Total surface of each section was 468.1 m^2.

There were compared the time required for the following cleaning methods:

1) Without pre-soaking

2) Water pre-wetting; 1 day

3) Pre-soaking low alkaline detergent (Shift, 1:200); 20 – 60 min

4) Pre-soaking medium alkaline adhesive gel detergent (Target Powergel, 1:50); 20 – 60 min

5) Combination of water (2 hours) + after gel (20-60 min)

In all experiments always used washing with cold/hot water.

Cold washing was performed with high-pressure washer Comet K901 after calibration:

1) Without pre-wetting with the performance of 19 l/min at 160 bar; rotary nozzle.

2) After pre-soaking of 15 l/min at 100 bar; linear nozzle.

Washing with hot water (90°C) was performed with high-pressure washer Comet KF Premium 9.21 after calibration:

1) Without pre-wetting of 20 l/min at 170 bar; rotary nozzle; 90°C

2) After pre-soaking of 16 l/min at 100 bar; linear nozzle; 90°C.

Results

The study results are shown in tables 1 and 2.

When washing with cold water only, without any pre-soaking, the average time for one section was 6h52m with 7,828 l of water. Hot water reduced time by 18.9% and water by 14.7% but was spend 33.4 l of fuel for hot water. Water pre-wetting brought the time/water savings by 9.0/9.0% for cold and 26.9/23.1% for hot water (30.1 l oil). Using Shift with cold water has been shortened time of 52 minutes and water saved of 2,428 l; hot water 4h 56m and 4,736 l of water was used (23.7 l of oil). The same technique using Target Powergel the savings grew (cold/hot) to 37.6/48.8% of time and 50.8/56.9% of water, 16.9 l of fuel used. Combined pre-soaking water + gel reduced time for cold/hot washing by 49.5/52.7%, water consumption decreased of 60.1 % for both (cold/hot) and 15.6 l of diesel used. Surfaces pre-soaking using alkaline detergent foam applications in order to dilute the dried organic soiling prior to pressure washing significantly influenced the time, when the floors before it were pre-soaked with water. When working with cold water, washing process was shortened by almost half. Using of proper alkaline surfactant significantly accelerates the process of washing and even allows us to get almost identical times for both hot and cold water even when it is optimally combined the pre-wetting system with correctly formulated pre-soaking agents.

All monitored savings can be achieved using combination of water pre-wetting with an stronger alkaline non-ionic surfactant of more 50 %. Interesting results were also recalculated in total costs for high-pressure washer operators, water, fuel, depreciation of washers and cleaners. The total costs are showed in table 2. Taking the fact, restocking in pre-fattening pigs is performed six times a year, into consideration farmer can save at only one section 28.25 m^3 of water, more than 20 hours of work and 200 liters of fuel, what can be also represented by amount of 158.80 EUR per one section and year.

Discussion

Using proper alkaline detergent for pre-soaking with sticky foam was found as the most effective because it adheres better to any surfaces can break the biofilm up and removes fats and protein deposits. The use of non-ionic detergents for absorbing surface (wood, concrete, plastic) is also achieved by hitting the microscopic pores in the material below the surface. It helps to reduce the adhesion of organic residues in further batch. Moreover, it permits the use of the subsequent washing surfaces using cold water at a safe pressure of water up to 100 bars.

Conclusions

Pre-soaking pens before their high pressure washing is recommended by professionals worldwide. Such a method of washing offers the farmers many advantages. The most notable benefits that bring prewashing using the alkaline surfactant is reduction of working time and amount of water used for cleaning and washing. Correct implementation of wet cleaning have simultaneously a decisive influence on the effectiveness of disinfectants. On the other hand, however, this phase of the disinfection program entails the risk of potential contamination of other stables in the farm area and even outside of the farm.

Acknowledgements

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APPLICATION OF HERBAL EXTRACTS AND PROPOLIS FOR FODDER IN PROPHYLAXIS OF DIARRHEAL DISEASE IN RABBITS

R. Kupczyński[1], K. Śpitalniak[1], A. Zwyrzykowska[1], T. Piasecki[2], M. Bednarski[2]

[1]Department of Environment Hygiene and Animal Welfare, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland
[2]Department of Epizootiology and Clinic of Bird and Exotic Animals, Faculty Wrocław University of Environmental and Life Sciences, Wrocław, Poland

Earlier stage of the study involved the use of propolis and herbs extract to the water for rabbits. Due to the positive effects of the research it was decided to use these preparations as an additive formulations of complete feed.

The aim of this study was to evaluate the influence of biopharmaceutics based on the extract of propolis (EEP) and extract of herbs in rabbit meat on the antioxidant status, blood biochemical parameters and animal health. The experiment was performed on 48 rabbits assigned to four groups (n = 12). Experimental desing: group I- propolis extract, group II - herbal extract, propolis group III - propolis extract and herbal extract. 2 ml of propolis 20ml of herbal extract was used for 1 kg of feed. The control group received standard pelleted feed without additives. The preparations were applied to the feed using spraying method. After two weeks of additives applications, the rabbits were infected with *E. coli* isolated at an earlier stage of the research. Oral infectious dose was 5 x 10^5 CFU/cm^3.

In our study we determined the hematological and biochemical blood parameters, also the parameters of acid-base balance and antioxidant status. During the experiment the body weight, feed and water intake were monitored. Dead rabbits were subject to dissection and bacteriological examinations were performed on the samples from the small intestine and the cecum. These tests also were performed in the rest of animals used in the research, out the end of the experiment, after euthanasia. The use of ethanol extract of propolis (EEP) and herbs in rabbits infected with *E. coli* resulted in reduced number of falls and a positive effect on weight gains. The highest weight gain was observed after an application of propolis only to feed. The application of feed additives resulted in an increase in the concentration of total protein and hemoglobin in the blood. There was beneficial effect of applicated propolis extract and EEP including herbs on blood antioxidant status.

INVESTIGATION ON EFFICACY OF INACTIVATED VACCINES AGAINST KHVD UNDER LABORATORY CONDITIONS

L. Gaede[1], T. Schmid[1], G. Bräuer[2], K. Böttcher[2], S. Bergmann[3], D. Fichtner[3], R. Beckmann[4], S. Speck[1], U. Truyen[1]

[1] Institute for Animal Hygiene and Veterinary Public Health, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany
[2] Sächsische Tierseuchenkasse, Fischgesundheitsdienst, Königswartha, Germany
[3] Institute of Infectology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald – Insel Riems, Germany
[4] Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, Division Veterinary Medicine, Viral Vaccines II, Langen, Germany

Introduction: Koi-Herpes Virus (Cyprinid herpesvirus 3) disease (KHVD) is an economically important disease in carp (*Cyprinus carpio)* with mortality rates up to 100%. In German federal state Saxony, KHVD caused substantial losses in commercial carp farming with significant impact on the total carp production.

Virus dissemination via water, carrier fish, predators and other factors hinder eradication programs. Therefore, inactivated vaccines were applied as a promising strategy to control KHVD from 2011 - 2014 in field studies, approved by the Saxonian Ministry of Social Affairs and Consumer Protection. Efficacy of antigen preparations (AGP) used, was assessed by challenge infections under controlled conditions in the laboratory.

Animals, Materials and Methods: Three KHV-AGP, differing in virus isolates used (Taiwan or Israel), antigen concentration and amount of adjuvant, were examined for efficacy in two-year-old carp after intra-abdominal injection. Fish were challenged by immersion with 2-3x10^2 $TCID_{50}$/ml KHV Israel 60 or 120 days post vaccination, respectively. Animals were euthanized at seven day intervals (7, 14, 21 dpi). DNA was extracted from gill and kidney tissue to compare contents of viral DNA in vaccinated and non-vaccinated fish by quantitative real-time PCR. In addition, neutralizing antibodies were determined using a serum neutralisation test (SNT).

Results: Clinical symptoms of KHVD were observed in all experimental groups, without significant differences in frequency or severity between vaccinated and non-vaccinated animals. KHV-DNA was detected in similar concentrations in the different groups. By SNT, neutralizing antibodies were detected in carp sera, obtained from animals vaccinated with two out of three KHV-AGP examined.

Conclusions: Although the different AGP were able to elicit antibody titers in vaccinated carp, protection against KHVD after virus challenge was not observed under the experimental conditions applied. Further studies on the development of more potent vaccines appear necessary.

SANITATION IN ANIMAL BREEDING AS AN IMPORTANT PART OF PREVENTION AGAINST ZOONOTIC DISEASES

Ján Koščo, Dušan Chvojka, Rudolf Hromada, Ľuboš Korytár, Katarína Veszelits Laktičová, Naďa Sasáková, Vladimír Petrovič, Miloslav Ondrašovič

The University of Veterinary Medicine and Pharmacy in Košice Department of Environment, Veterinary Legislation and Economics, Slovak Republic

Summary

A good animal hygiene and a suitable environmental conditions are considered as a basic requirement in all farms. The essential technical operations in animal husbandry include sanitation measures. The complex of sanitation consists of disinfection (destruction of pathogenic and potentially pathogenic microorganisms), disinsection (elimination of insects and vermin) and deratisation (rodent control). The sanitation includes also a waste removal, the removal of carcasses, a compliance with hygiene standards and a creation of optimal environmental conditions for the animals.

Introduction

Farmers and holders of animals have to accept, that a prevention of infectious diseases plays an important role in the protection of animal health and in the protection of human public health too. The aim of a preventive sanitation is the destruction of potential zoonotic agents. It is aimed at the suppression of an aggressive microflora in animal stables. A focal sanitation is targeted at destroying of disease outbreaks. The rodent control is an essential step in the prevention and in the elimination of infectious diseases. It is known that rodents play an important role as reservoir of the infection. Using of chemical rodenticides is the most frequent method of the rodent control. A population density of rodents may be higher in poultry and swine farms. It is due to presence of a feed on qualitative good level. Therefore, the highest requirements for rodent control must be used in poultry farms and in piggeries.

Disinfection is an essential step towards the removal of pathogenic and harmful microorganisms in the external environment. Disinfection is a complex process whose ultimate effect depends on many changing factors. The effectiveness of disinfection affects the resistance of microorganisms, selection and the use of disinfectant and the external environment in which the disinfection process takes place.

Disinsection is a set of measures aimed at combating harmful insects and other arthropods transmitting infective agents to humans, animals and causing economic damage and damage of the food and feed as well as other products.

Rodent control in the process of deratisaton is an essential step in the prevention as well as elimination of infectious diseases that rodents can be passive or active propagator.

Material and methods

The shortcomings of disinfection as not appropriate mechanical cleaning of areas that should be disinfected and the amount of disinfectant solution are the most important before starting the process of disinfection. In the focus disinfection we must take into account the fact that the pathogen as a cause of an infectious disease stays there even after removal of the animals. On this basis, after removal of the animals the whole house is disinfected before cleaning. After disinfection the excrements are removed but we handle them as with infectious material. The pathogen is then devitalised by composting or chemical treatment. Only after these steps of mechanical cleaning we can proceed to the disinfection of the object.

Another drawback is the amount of applied disinfectant. For spray disinfection the dose from 0.5 to 1.0 liter.m^{-2} is required and for aerosol disinfection recommended dose is 10 ml.m^{-3}. The cause is often inadequate and inefficient application technique. If these measures are not professionally guided, breeder often due to financial reasons and also timing reasons, choose an inefficient process for disinfection. On Fig. 1, an inappropriate treatment of disinfection is documented.

Epidemiological significance of disinsection is that the insect can be a vector, respectively, reservoir of infectious zoonotic disease. Transmission can be active or passive. Tab. 1 shows the most common diseases transmitted by insects and ticks. Chemical method of disposing of insects in the protection of livestock has the widest application

and is based on the use of chemicals - insecticides. The most used insecticides are made of substances which chemically belong to the group of organophosphates, carbamates or pyrethroids.

The last step of sanitation process is deratisation. Harmful rodents are an important reservoir of pathogens from the genus *Leptospira*. A rat is a ubiquitous carrier of serotype *L. icterohaemorragie,* but also other leptospira serotypes. Actively can carry pathogens as causal agents of salmonellosis, tularemy, listeriosis, pasteurellosis, etc. and passive virtually all infectious diseases. Tab. 2 shows the most common diseases caused by harmful rodents. The most used method is chemical deratisation. According to final effect, rodenticides are divided to acute acting rodenticides, that cause symptoms of poisoning or death of rodents in several hours and chronic rodenticides. The most common occurrence of rodents is in poultry and pig farms, where rodents have access to food. Preventive deratisation of these farms is made usually twice per year but despite of this rodents can reproduce. Therefore, in such objects is necessary to apply a permanent care facility against harmful rodents. The overgrowth of field hamster occurs usually in three to five-year intervals, especially after mild winters.

Results

Mosquitoes	*Eastern Equine Encephalitis*
	Western Equine Encephalitis
	West Nile Encephalitis
	Malary
	Denque Fever
Ticks	*Tularemy*
	Tick Borne Recurring Fever
	Babesiosis
	Lyme Boreliosis
	Ehrlichiosis
Fleas	*Cryptosporidium parvum*
	Salmonella spp.
	Shigella spp.
	Bacillus anthracis
	polioviruses
	Entamoeba spp.
	Giardia spp.

Tab. 1: List of pathogens and diseases usually transmitted by various insects

Helminths	*Capillaria* spp.
	Nippostronglyus brasiliensis
	Toxocariasis
	Heterakis spp.
	Hymenolepsis nana
	Taenia taeniaeformis
Protozoa	*Cryptosporidium parvum*
	Toxoplasma gondii
	Trypanosoma lewisii
	Eimeria spp.
	Sarcocystis spp.
Bacteria	*Leptospira* spp.
	Salmonella spp.
	Listeria spp.
	Yersinia spp.
	Pasturella spp.
	Pseudomonas spp.
Rickettsia	*Coxiella burnetti*
Viruses	Hepatitis E
	Hantavirus

Tab. 2: List of pathogens usually transmitted by rodents

Conclusions

The efficacy of disinfection, disinsection and deratisation depends on the active ingredient but also on accuracy of those procedures and environmental cleanup. It is known that the use of insecticides creating resistance, especially in flies and therefore substituting of used insecticides is recommended.

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PRINCIPLES OF BIOSECURITY IN POULTRY BREEDING FARMS

P. Novak[1], G. Mala[2], K. Tittl[1]

[1] Tekro Ltd., Prague, Czech Republic; [2] Institute of Animal Science, Prague Uhrineves, Czech Republic

Summary

A prevention of diseases of poultry flocks is one of the prerequisites for ensuring good health and high yield of housed animals as well as achievement of economic profit. Many diseases of poultry can be avoided by using good animal breeding practices as well as introduction of the management of the health status of the entire flock. The main focus of our work was to create a complex of preventive possibilities to minimise the potential disease-causing microorganisms in all steps of poultry breeding. That is based on the principle of timing. It starts in a hatchery through the rearing chickens to the broilers or egg laying flocks with emphasis on an animal health and a quality of production.

Introduction

Infectious disease agents of poultry are a threat to poultry health and, sometimes to human health. Those have significant social and economic implications [1,2]. Most air pathogens are spread by more than one route. Viral respiratory infections, such as Newcastle and infectious bronchitis can be transmitted over distances up to 5 kilometres by wind dispersal of virus laden dust particles [3].

In poultry production, especially under intensive conditions, prevention is the most viable and economically feasible approach to the control of infectious disease agents [1,2]. A prevention of diseases of flocks of poultry is one of the prerequisites for ensuring good health and high yield of housed animals as well as achievement of economic profit. A treatment of disease is not as efficient and economical in relation to prevention. There is a principle that prevention is cheaper than a cure [4]. Many diseases of poultry breeds can be avoided by using good animal breeding practices as well as introduction of the management of the health status of the entire flock.

Material and Methods

The main focus of our work was to create a complex of preventive possibilities to minimise the potential disease-causing micro-organisms in all steps of poultry breeding. It starts in a hatchery (the quality of grandparental and parental flocks) through the rearing chickens to the broilers or egg laying hen flocks with emphasis on an animal health and a quality of production. There was collected and analysed a complex of preventive measures designed to prevent the penetration of infectious agents into animals by persons, animals, technological systems and equipment, transport, and the farm sanitation.

Results

The poultry breeding from the biosecurity point of view can be understood as well-oiled machine, which consists of individual gearwheels (i.e. the different stages of breeding), and they are interconnected and interdependent. Any change in one of them subsequently induces changes in the other wheels. The result is a disruption of clockwork, which will lead to a complete stop of the whole machine (clockwork). It works very similar in the poultry farming. When something gets neglected in grandparent or parent flock or in a hatchery, the consequences can occur at rearing chickens, broiler fattening stations or egg laying hens (Scheme).

The hatchery is a potential source of direct or indirect spread of infection. In the operation of hatchery must focus on checking compliance with critical control points in individual stages of the technological process with emphasis on monitoring eggs, surfaces and equipment that they may contaminate eggs, contamination of air, including the ventilation system, technological systems and equipment, and personnel.

Hatchery sanitation is significant factor in reducing the risk of disease day-old chickens. Low levels of sanitation may also cause increasing mortality of the rearing.

There is paid attention in the poultry farms for laying hens and broilers. It is focused on implementation of the preventive measures against the introduction of infection and its spread in farm by animals, people, dead chickens, technological systems, sanitation, wild animals, feed and water at last bedding.

Discussion

Flock housing and management systems can affect opportunities for the introduction, transmission, and persistence of foodborne pathogens in poultry [5].

Hygiene and sanitation play a major role in any effective disease control programme for poultry production premises [6]. One of the important requirements to facilitate hygiene and sanitation is adoption of the 'all-in/all-out' method, together with the restriction of each enterprise to a single type or species of bird [6].

Strict compliance with hygienic measures, the level of veterinary and nursing work, with an emphasis on friendly way of handling and optimal storage conditions of hatching eggs are a crucial factor in the production of quality one-day old chicks, which are a prerequisite for achieving economic efficiency in hatchery.

The major emphasis for preventing infections is to avoid the introduction of pathogens into the farms, hatcheries and feed mill. This requires the establishment and implementation of biosecurity practices aimed at suppressing the most common sources of pathogens [3]. Biosecurity is the cheapest, most effective means of disease control available [7]. Biosecurity planning and implementation reduces the risk of infectious disease transfer within and among poultry flocks [8].

Conclusions

An adequate level of hygiene is a prerequisite for maintaining good health of poultry breeds and achieving a high yield of production and reproductive performance of flocks. Underestimating the individual biosecurity measures brings down first of all yield of profit, it will increase the frequency of the disease occurrence and thus consequently will increase management costs, while increasing the death risk of poultry, which reduces the economic profits of farmers. A breeder should develop and implement individual plan biosecurity farm as part of an overall management strategy of health, production and reproduction.

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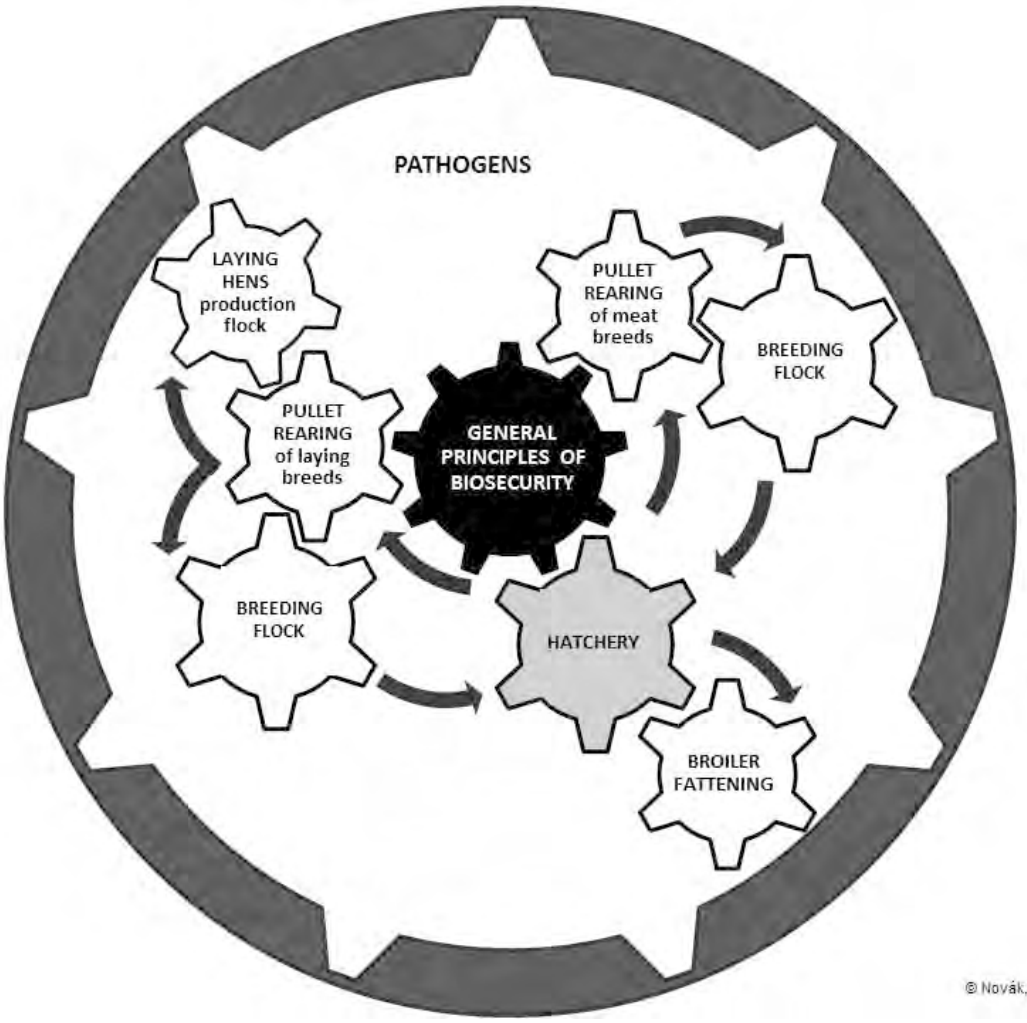
PATHOGENS
LAYING HENS production flock
PULLET REARING of meat breeds
BREEDING FLOCK
PULLET REARING of laying breeds
GENERAL PRINCIPLES OF BIOSECURITY
BREEDING FLOCK
HATCHERY
BROILER FATTENING
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ARE THE PRINCIPLES OF BIOSECURITY IMPORTANT FOR BEEKEEPERS?

P. Novak[1], K. Tittl[1], V. Pazout[2], G. Mala[3]

[1] Tekro Ltd., Prague, Czech Republic
[2]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[3]Institute of Animal Science, Prague Uhrineves, Czech Republic

Summary

Biosecurity in beekeeping as an integral part of bee colony health management is an important requirement for the production of healthy and biologically wholesome raw materials and foodstuffs of animal origin. Biosecurity management practices are designed to prevent the spread of diseases by minimizing the movement of microorganisms and their vectors (viruses, bacteria, moulds, etc.) into the honey bee farm. Biosecurity principles are intended to prevent the introduction of infectious agents into honey bee farms and its dissemination by person, bees, feed, technological systems of bee breeding in order to prevent threat animal health or honey quality.

Introduction

In recent years, bee colonies around the world have been affected by the invasion of new disease, primarily due to the influence of anthropogenic activities. The most bee mortality is due to common diseases, caused by Acarapis woodi, Paenibacillus larvae, Melissococcus plutonius, Aethina tumida, Tropilaelaps spp. and Varroa spp. [1]

Not only the spread of pathogens and unwanted chemicals as well as present methods of treating bees, affects not only the reduction of disease incidents but also the bee immunity. The incidence of the bee disease causes significant direct and indirect losses, in connection with the liquidation of foci and eradication of diseases [2].

The incidence of disease of colony of bees causes significant loss, which is not only direct, i.e. a loss of good condition and performance of entire colony of bees. Also indirect, i.e. comes in connection with the liquidation of disease outbreaks [3,4]. Recently there have been large losses of bees in the world. The cause of the loss is so-called "Colony-Collapse Disorder" is so far unknown [5].

Material and methods

This study presents a brief overview of the principles of external and internal biosecurity as a basis for beekeeping prevention, with emphasis on the importance of sanitation in beekeeping. Processing study is based on the establishment of a database of information resources and their subsequent analysis.

Results

Biosecurity (biosecurity) represents a management of beekeeping strategy, aiming to minimize the possibility of penetration of pathogenic microorganisms (external biosecurity) and disseminated to the bee-colonies (internal biosecurity) in order to prevent health hazards.

The external part of biosecurity is primarily focused on the following areas:

1. Preventing the introduction of infectious agents into beekeeping - all the queen bee, bees (swarm etc.), but also all the equipment and supplies entering the breeding - should come from trusted sources;
2. Limited access of visitors to breed and create effective barriers;
3. Elaboration of rules for materials handling - transmission of infection may occur when moving sick bee colonies;
4. Veterinary protection zones (distance between apiaries of different breeders, etc.). It is necessary to ensure a sufficient distance from the hives from all potential sources of pollution (roads, urban areas, industrial plants, etc.), which could adversely affect not only the health of the bees, but also the quality of bee products.

The internal part of biosecurity is primarily focused on the following areas:

1. Optimize production technology systems are based on the regular renewal of beeswax. It works every three years. The principles of the right procedure beekeeping must be based on strong breeding colonies with developing cleaning instinct and regular monitoring of their condition and health status.

2. Regular sanitation of breeding establishment, including beekeeping supplies and equipment. Attention should be given to frequent cleaning, washing and disinfection of all parts of the breeding establishment, including utilities. Also, beekeepers must not forget on insects and rodent control.
3. The periodic monitoring of the health status must be focused on the detection of clinical signs of disease) with respecting of the EU and Czech Republic legislation.
4. Checking the quality of feedstock as a prerequisite for production of healthy and biologically wholesome final products (honey, bee wax, royal jelly, propolis, bee venoms).

Discussion

The diagnosis and control of honey bee diseases at the colony level is quite difficult. More than with other animals, the possibilities and the methods applied for clinical observation and diagnosis depend on seasonal conditions [8].

Effective bee health checking program must include an adequate level of hygiene in breeding facilities including the proper nutrition [2,4]. It also ensures preventive precautions against of disease and harm (overheating, hypothermia, chemicals). Many diseases can be avoided by using good bee keeping practices. Biosecurity is an important integral part of beekeeping health management [3]. Another important part of prevention and compliance with animal hygiene conditions in the environment and breeding areas is a regular sanitation of all warehouses [4].

Conclusions

Bee keeping biosecurity can be summarized into ten points:

1. Breeding colonies with developed strong cleaning instinct, periodic inspection bee condition;
2. Use trusted sources of the queen bee and bees;
3. Respect the principles of adequate procedure of beekeeping treatment during the whole year;
4. Breeding colonies on suitable sites (concentration, nutrition capacity of the landscape);
5. Burning of old unused and non-functional hives;
6. Timely, proper and correct supplemental feeding of bee colony in late summer;
7. Creation of divisions - reducing infection pressure – “recovery" environment conditions;
8. Periodic monitoring of health status and health situation;
9. Therapeutic treatment of hives with approved products;
10. Respect of EU and national laws, regulations and guidelines.

Acknowledgements

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Sustainability in livestock production

MILK YIELD, COMPOSITION AND BLOOD SERUM CONSTITUENTS OF OSSIMI EWES AS AFFECTED BY YEAST SUPPLEMENTATION

A. A. Baiomy[1], and A. A. Mottelib[2]

[1]Anim. Prod. Dept., Fac. Of Agric., South Vally Univ., Qena, Egypt
[2]Dept. of Anim. Med. Assiut Univ., Assiut, Egypt

SUMMARY

Feeding trail was conducted to evaluate influence of Saccharomyces cerevisiae on milk production, composition and some blood metabolites of ossimi ewes during milking period. Control group was fed a concentrate mixure and huy and grazed twice daily, while second group and third were fed same diet supplemented with 3 and 6 gm of live yeast culture respectively. Treated groups had significantly higher values for fat corrected milk 740,605 g/day for G3 and G2 respectively, while values of milk, fat and lactose yield were higher only in G3 as compared with G1. Milk urea values were significantly lowered in G3. Yeast administration influenced B-Hydroxy-butyrate which were significantly higher in the treated groups, and non-esterified fatty acids values which were significantly higher only in G3 compared with G1.

Keywords: Live yeast culture, ossimi ewes, milk and blood.

INTRODUCTION

Sheep milk has about twice the fat of cow milk and 40% more protein than cow milk. In the last twenty years some probiotics such as Aspergillus or A.Niger, (saccharomyces cerevisiae) (3), and some microbial growth promoters e.e. thiamine, niacin were used as feed additives in order to improve rumen conditions and cellulose digestion in dairy cows.

Inactive dry yeast was only used to improve the yield and composition of milk in sheep and also as a source of protein and Vitamins of B-Complex when added to rations.

Data indicate that supplementation of yeast in ruminant diet may improve feed intake (6), milk production digestion.

A feeding trail was conducted to evaluate influence of Saccharomyces cerevisiae on milk production, composition and some blood metabolites of dairy ossimi ewes.

MATERIALS AND METHODS

Sixty ossimi ewes (aged 3-3.5 years, average body weight 48±3.13kg) were used. All ewes were in second lactation and the trail was done from the 42th to the 182th day of lactation. Animals were divided into three groups (20 animals, each) on the 42th day (Peak of Lactation) to carry out the experiment. Control group (G1) received only 100% of NRC nutrient allowances of dairy sheep without live yeast culture for 6 weeks after parthrition.G2 and G3 received the same NRC nutrient in addition to 3 and 6 gm/day/sheep of live yeast culture respectively for 6 weeks after parturition. Total mixed ration was comprised of 65% forage and 35% concentrate mixture to formulate diets to meet NRC.Animals were fed at 7.00 a.m and 5.00 p.m the ration of concentrated mixture according to their body weight (1kg/ ewe/ day).

Beside the concentrate mixture, animals were fed mixed grass pasture. Water was available all day and minerals were supplied in salt licking blocks. Animals were adopted the double daily milking at 6.00 a.m and 5.00 p.m.

Individual milk samples consisted of proportional volumes of morning and evening milk were collected (5ml/kg of produced milk). A composed milk sample of each ewe was analyzed weekly. Fat (%), protein (%), lactose (%), total solids (%), somatic cell count, urea and PH were determined. Solid non fat was calculated the difference (T.S % - Fat %). Milk yield was corrected to 7% fat (4). Blood sample were collected on the 42th, 112th and 182th day of lactation prior to morning feeding. The blood was allowed to clot and the serum was obtained by centrifugation. The obtained

serum was stored at - 20°C until analysis. Many organic biochemical constituents of blood serum were determined using commercial test kits of reagents.

The obtained data were statically analyzed according to (1).

RESULTS

The obtained data for milk yield and composition as well as Blood serum constituents were recoroled in tables 1 and 2.

DISCUSSION

Ration supplemented with live yeast culture that given to ossimi ewes has a positive effect on their milk yield during lactation. This is in agreement with (5) in dairy cows. Live yeast cultures were most efficient when small ruminants fed on high concentrates (7). Our animals were fed on relatively high concentrate ration (1 kg/ animal/ day) which could lead to improve buffering capacity in the rumen. Our results were also dose-dependent, because 3gm of live yeast culture was not enough to improve milk yield in G2.

The non-significant elevatyion in fat and total solids percentages in milk of G3 may be due to the used supplementation. Milk protein %, lactose %, SCC (No./m1) and PH did not changed. Milk protein yield and lactose yield were obviously increased in G3. This is in agreement with (8). Milk urea values were significantly lowered in G3. It has been reported that yeast supplementation reduce ruminal ammonia by 10% and this lowering affects on ruminal microbial activity that causes drop of urea in milk and blood. The significant elevation in B-HB and NEFA agreed with (2) who explained rise in NEFA to fat mobilization and that of B-HB to increase ruminat butyrate. The remaining blood parameters were remained nearly without obvious changes.

CONCLUSION

It could be concluded that rumen micro-flora plays a key role in the mode action of yeast in rumen digestion for better performance and best daily weight gain.

Live yeast culture (yea sacc 1026, alltech, Kentucky. USA) supplementation has a significant beneficial effect on milk yield in ossimi ewes fed pastures and concentrate mixture during milking period.

Since the influence was dose-dependent 6 gm/ animal/ day, thus additional studies must be conducted to clarify more and more the important of yeast as feed additive under different feeding conditions.

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Composition of milk	G1	G2	G3
	0g of yeast culture/day	3g of yeast culture/day	6g of yeast culture/day
No. of sample	20	20	20
Milk yield (g/day)	527±55b	564±53ab	670±76a
Fat (%)	7.8±0.3	7.7±0.3	8.0±0.1
Protein (%)	5.8±0.1	5.7±0.35	5.7±0.5
Lactose (%)	4.4±0.47	4.4±0.2	4.3±0.2
Total solids (%)	19.0±0.8	18. 8±0.5	19.5±0.8
Non fat solids (%)	11.2±0.4	11.0±0.3	11.1±0.6
FCM7 (g/day)	571.6±63b	605.5±73a	740.4±81a
Fat yield (g/day)	73±8b	76±8b	85±15a
Protein yield (g/day)	54±5	56±6	60±8
Lactose yield (g/day)	38±5b	43±3ab	47±8a
Scc (x 103/ml)	405±169	380±75	490±152
Urea N mg/100ml	27.4±2.46a	27.2±1.95a	25.0±0.4a
PH	6.76±0.44a	6.72±0.36a	6.74±0.43a

Table (1) Mean values ± SE for milk yield and composition of ossimi ewes as affected by yeast supplementation in the ration

Blood serum parameters	G1	G2	G3
	0g of live yeast culture/day	3g of live yeast culture/day	6g of live yeast culture/day
No. of sample	20	20	20
NEFA (mm01/L)	0.30±0.10b	0.37±0.2ab	0.38±0.12a
BHB (mm01/L)	0.51±0.08b	0.61±0.14a	0.60±0.2a
Urea (mm01/L)	7.40±1.39	7.31±0.86	6.94±1.17
Triglycerides (mm01/L)	0.25±0.06	0.24±0.06	0.28±0.07
Cholesterol (mm01/L)	1.92±0.23	1.91±0.25	1.96±0.16
VLDL (%)	6.3±4.1	6.1±4.0	7.7±4.0
HDL (%)	44.5±12.1	46.6±8.7	48.5±8.8
LDL (%)	49.1±12.6	47.2±7.9	43.7±10.0
AST (µ Kat/L)	2.55±1.01	2.78±1.30	2.45±0.95
ALT (µ Kat/L)	0.31±0.15	0.23±0.12	0.26±0.13
GGT (µ Kat/L)	0.82±0.07	1.07±0.20	0.93±0.33
ALP (µ Kat/L)	3.82±0.98	3.75±1.43	3.28±1.30

Table (2) Mean values ± SE for blood serum constituents of ossimi ewes as affected by yeast supplementation in ration

EFFECT OF PROPYLENE GLYCOL ON THE METABOLIC STATUS AND MILK PRODUCTION OF DAIRY BUFFALOES

Hussein Awad Hussein[a,*], Sh. M. Abdel-Raheem[b], M. Abd-Allah[c], W. Senosy[d]

[a] *Internal Veterinary Medicine, Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt*
[b] *Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt*
[c] *Animal Production Department, Faculty of Agriculture, Al-Azhar University Assiut, Egypt*
[d] *Department of Theriogenology, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt*
**Correspondence: hhussein@aun.edu.eg*

Summary: This study was designed to investigate the effects of drenching with propylene glycol (PG) on the body condition, serum metabolites and milk production during transition period of dairy buffaloes. Animals were randomly allocated to: a control group of 5 buffaloes and PG group of 10 buffaloes that were drenched with 500 ml of propylene glycol once daily from 10 (9±3) days prepartum until 2 weeks postpartum. Ultrasound measurements of backfat thickness (BFT) were measured weekly, while blood samples were taken at - 4, - 2, 2, 4, 6, and 8 weeks from parturition for estimation of hematological and biochemical metabolites. At – 4, -3, and -2 weeks from calving, BFT did not differ between the two groups, but decreased after calving and was higher for control than PG group at week – 1 and 1. Hematological analysis revealed insignificant changes between the two groups. Serum non-estrified fatty acid (NEFA) concentrations, β-hydroxy butyric acid (BHBA), and glucose did not differ between the two groups before parturition. At 2, and 4 weeks from parturition, NEFA was higher for control than PG group, while BHBA were higher at 2, 4, 6, and 8 weeks for control than for treated buffaloes. In contrast, glucose level was significantly increased in PG group than control one at week 2 postpartum ($P < 0.05$). Serum concentrations of total cholesterol, triglycerides, total proteins, albumin, and globulins did not differ significantly between the two groups ($P > 0.05$). Serum enzyme activities of asparate aminotransferase and γ-glutamyl transferase were significantly increased in control than PG group. Average 60-day milk yields were 8.4±0.22 and 10.7±0.40 kg/day for control and PG groups, respectively, and higher in treated buffaloes ($P < 0.05$). In addition, milk compositions did not differ between the two groups. In conclusion, drenching of dairy buffaloes with propylene glycol may reduce the risk of ketosis, improve the metabolic status, and increase the milk yield.

Introduction: During early lactation, the amount of energy required for maintenance of body tissues and milk production exceeds the amount of energy the cow can obtain from dietary sources. To the best of authors' knowledge, no studies have reported the effect of PG on metabolic status and milk production in buffaloes. Therefore, this study was designed to investigate the effects of PG drenching during transition period on the metabolic status and milk production traits in dairy buffaloes.

Materials and methods: This experiment was carried out on multiparous pregnant dairy buffaloes (*Bubalus bubalis*) (average body weight 550 kg; 9 months pregnancy), which were randomly allocated to a control group of 5 buffalo cows and a treated group of 10 buffalo cows. The age and lactation numbers of animals ranged from 5 to 9 years and 3 to 7, respectively. The herd average milk production of the previous lactation season was 1792.41±43.52/265 days. The diet was formulated according to recommendations (1). Buffalo cows were milked twice daily at 08.00 and 13.00 h and milk production was recorded. Buffalo cows in the treated group were drenched with 500 ml of propylene glycol once daily from 10 (9±3) days before expected calving date until 2 weeks postpartum. Buffalo cows of the control group were drenched with 500 ml of water. The drenching time was set at 1.5 h after the morning feeding. Ultrasound measurements of backfat thickness (BFT) were carried out using a portable Beta-mode ultrasound generator with a 6-MHz linear transducer (2). Two blood samples, one with heparin and the other without an anticoagulant, were collected by jugular venipuncture every two weeks. Hematological analysis was performed by automated cell counter. Serum metabolites were measured using commercial test kits. Milk acidity, pH and percentages of fat, ash, total solids and protein were determined (3). Milk energy was calculated as described elsewhere (4). Data were analysed statistically using SPSS software.

Results: Figure 1 shows the ultrasound illustrations of BFT in treated and control group. Figure 2 exhibits the effect of PG drenching on the serum concentrations of NEFA, BHBA, glucose and cholesterol. Milk yield of PG group was significantly greater than that of control group ($P<0.05$).

Discussion: Buffaloes drenched with PG had lower serum NEFA concentrations at either 2 or 4 weeks after parturition, consistent with a previous study (5). Since PG rapidly supplies energy, this improved the degree of negative energy balance, resulting in decreased body fat catabolism and serum NEFA levels (6). In the present study, the increase of serum BHBA concentrations in control group corresponded well with the results of NEFA levels. It was observed that increased BHBA concentrations in the blood predispose a cow to clinical ketosis (6). At 2 weeks postpartum, the serum glucose concentrations were higher in PG group than control one. Such increased could be attributed to the fact that PG is rapidly absorbed and metabolized as a source of energy. High concentrations of NEFA and BHBA and low concentrations of glucose in control dairy buffaloes at postpartum period, indicating they suffered from subclinical ketosis. Increased serum activities of AST and GGT may due to impaired liver function. In the present study, the increased milk yield without subsequent increase in NEFA and BHBA might indicate improvement of metabolic status as a result of PG supplementation.

Conclusion: Results of this study suggest that supplementation with PG during transition period of buffaloes can reduce the risk of ketosis, improve the metabolic health status and increase the milk yield

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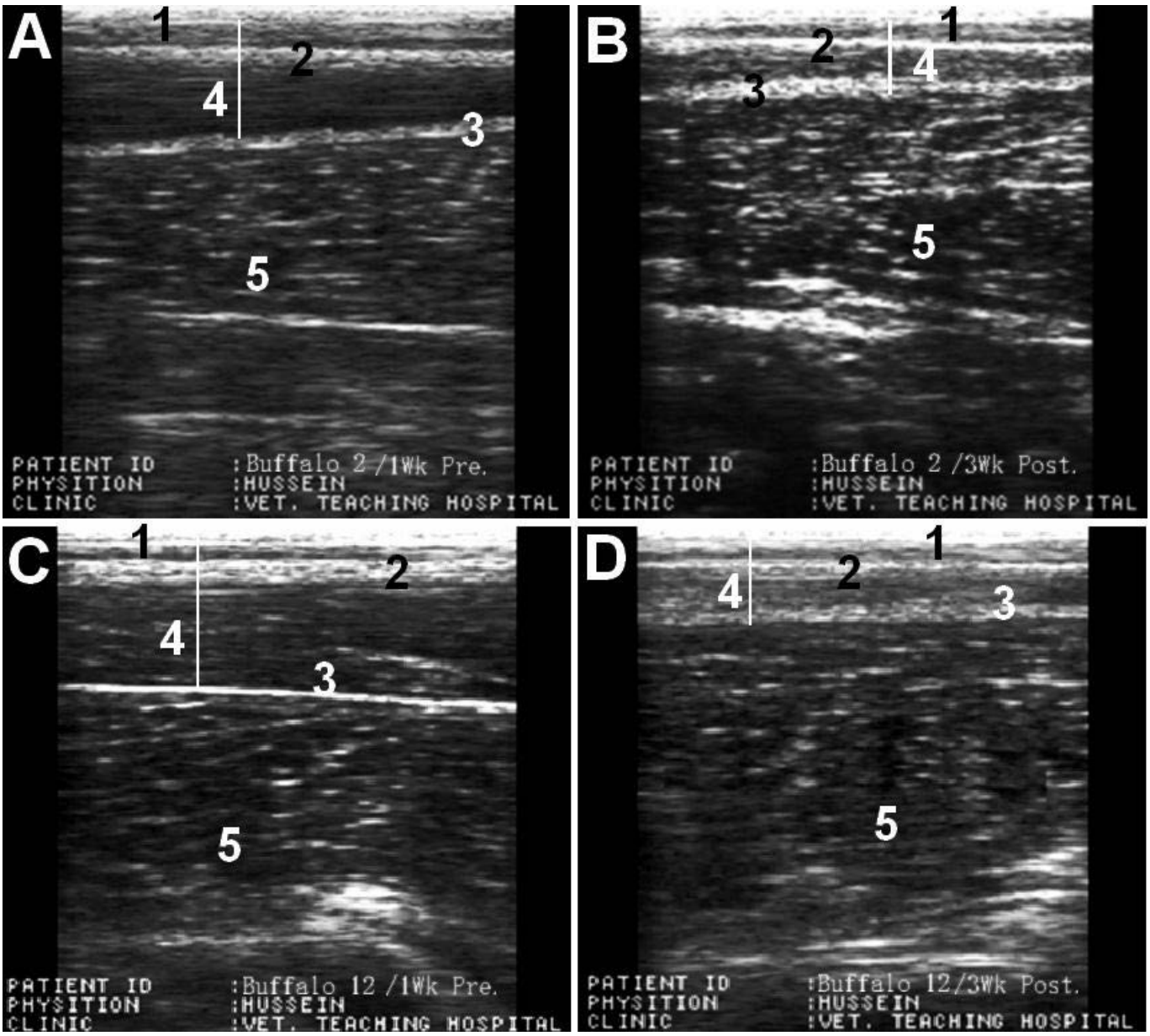
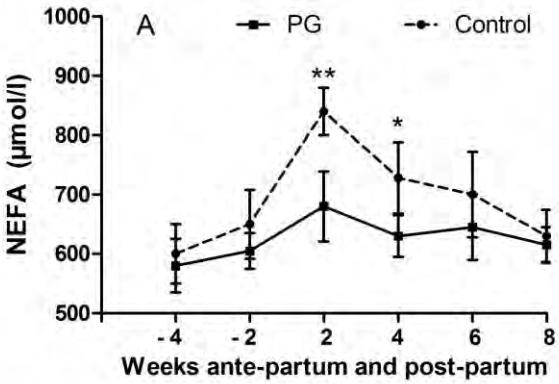
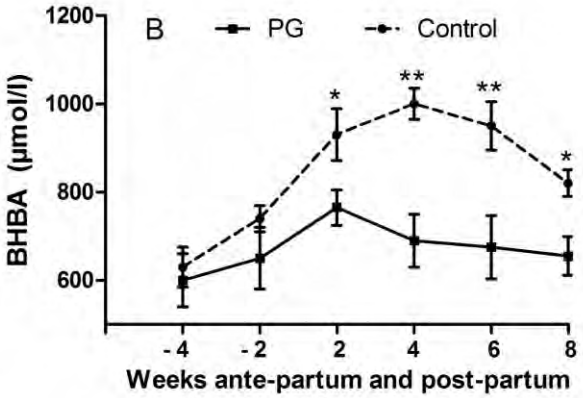
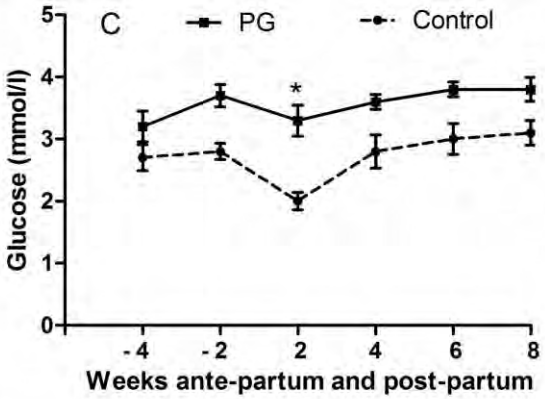
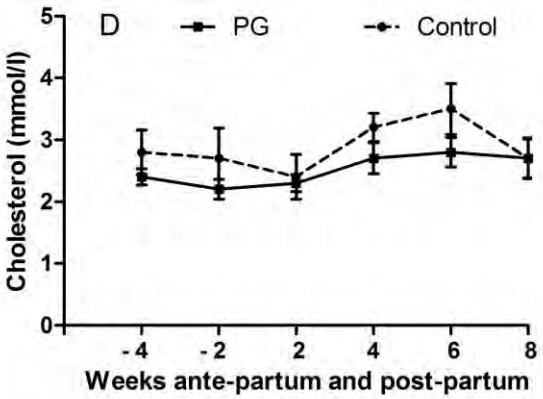
Figure legends: Fig. 1: Ultrasound images of backfat thickness of 22 and 18 mm in a buffalo administered PG at week 1 prepartum (A) and week 3 postpartum (B), respectively. Ultrasound images of backfat thickness of 33 and 15 mm in a control buffalo at week 1 prepartum (C) and week 3 postpartum (D), respectively. 1, skin; 2, superficial fascia; 3, deep fascia; 4, subcutaneous fat; 5, gluteal muscle. **Fig. 2:** Concentrations of NEFA, BHBA, glucose and cholesterol in control and PPG groups


A
1
2
4
3
5
PATIENT ID :Buffalo 2 /1Wk Pre.
PHYSITION :HUSSEIN
CLINIC :VET. TEACHING HOSPITAL
B
1
2
4
3
5
PATIENT ID :Buffalo 2 /3Wk Post.
PHYSITION :HUSSEIN
CLINIC :VET. TEACHING HOSPITAL
C
1
2
4
3
5
PATIENT ID :Buffalo 12 /1Wk Pre.
PHYSITION :HUSSEIN
CLINIC :VET. TEACHING HOSPITAL
D
1
2
4
3
5
PATIENT ID :Buffalo 12/3Wk Post.
PHYSITION :HUSSEIN
CLINIC :VET. TEACHING HOSPITAL


A
PG
Control
NEFA (µmol/l)
1000
900
800
700
600
500
**
*
-4
-2
2
4
6
8
Weeks ante-partum and post-partum


B
PG
Control
BHBA (µmol/l)
1200
1000
800
600
*
**
**
*
-4
-2
2
4
6
8
Weeks ante-partum and post-partum


C
PG
Control
Glucose (mmol/l)
5
4
3
2
1
0
*
-4
-2
2
4
6
8
Weeks ante-partum and post-partum


D
PG
Control
Cholesterol (mmol/l)
5
4
3
2
1
0
-4
-2
2
4
6
8
Weeks ante-partum and post-partum

THE EFFECT OF MONENSIN SUPPLEMENTATION ON RUMINAL FERMENTATION PARAMETERS OF SHEEP

A. Taghizadeh[1], M. Besharati[2*], V. Hesni[1]

[1]*Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran*
[2]*Department of Animal Science, Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Ahar, Iran*

Summary

This study was carried out to determine of the effects of monensin on ruminal fermentation parameters in Ghizel sheep. Sixteen male Ghizel sheep were used allocated in completely randomized design. The diet containing of 2.9 ME (Mcal/kg DM) and 150 g/kg DM CP was formulated based on NRC requirement consisting of 400 g kg^{-1} DM alfalfa, 488 g kg^{-1} DM barley grain, 200 g kg^{-1} DM soybean meal, 589 g kg^{-1} DM, corn grain and 20 g kg^{-1} DM limestone containing predicted metabolizable energy 2.9 Mcal kg^{-1} DM and containing crude protein 150 g kg^{-1} DM. The treatments containing different level of ionophores (20ppm, 25ppm, 30ppm and 35ppm). The experiment period was 21 days. The rumen fluid was collected using stomach tube at 2 and 4 h after morning feeding. The floatation and sedimentation time, methylene blue reduction time, pH, total ammonia nitrogen and total VFA in ruminal samples were determined. The ruminal pH and total VFA were not significant differences. The effect of monensin on ruminal ammonia concentration was significant difference ($P<0.05$). The results showed that the monensin had not effect on ruminal parameters except of ruminal ammonia-N resulted low proteolytic bacteria activity.

Introduction

Ionophores are used widely in fattening animal production. Ionophores biological reactions contain: increasing of energy metabolism efficiency in bacteria and host animal, improvement of nitrogen metabolism of ruminal bacteria and animals and removing or decreasing of digestive disorders in ruminants. Ionophores decrease some bacteria that produce acetate, while the proportion of propionate producer bacteria is increased. Monensin and lasalocid are as inophores that used extensive in animal nutrition. Ionophores increase propionate production resulting high blood glucose that can be improved energy supply in transit period and early lactation period (Meyer et al., 2009).

The objective of this study was to determine of effect of monensin on rumen fermentation parameters (pH, total ammonia nitrogen and total VFA) in Ghizel sheep.

Materials and methods

16 Ghizel sheep (45±6.59 kg) are randomly received one of four diets in a completely randomized design. The composition of diet based on NRC (1985) containing predicted metabolizable energy 2.9 Mcal kg^{-1} DM and containing crude protein 150 g kg^{-1} DM. The treatments containing different level of ionophores (20ppm, 25ppm, 30ppm and 35 ppm). The period of present study was 21 days for each ionophore. The rumen fluid of each treatment was obtained by stomach tube at 2 and 4 hours after feeding. The effect of treatment was determined on rumen pH, ammonia-N, total volatile fatty acid (VFA) (Stuchbury & Sake, 2001.

Statistical analysis

Data obtained from *in vivo* study was subjected to ANOVA as a completely randomized design with 4 replicates by the GLM procedure of SAS (2002), and treatment means were compared by the Duncan test.

Results and Discussion:

The obtained results are shown on Tables 1, 2, and 3. The ruminal pH at 2 and 4 h after feeding were not significant differences due to supplemented monensin. Ives et al. (2002) showed when ionophores added in the diet ruminal pH was decreased, that is in contrast to our results. These reported that with altering of sampling time from 2 h to 4 h after feeding, ruminal pH was increased that confirm our obtained data. Also, these finding have been reported by Erickson et al. (2003). Domescik and Martin (1999) indicated increased ruminal pH in *in vitro* experiment due to added ionophores. The similar results were confirmed by Baran (1988). These finding were in contrast with, compared to obtained data in present experiment. The effect of monensin on ruminal VFA concentration was not significant difference (Table 2). The ruminal ammonia-N was decreased significantly when the monensin was fed (Table 3).

Ives et al. (2002) showed the reduced ammonia-nitrogen when ionophores was fed. The obtained data in present study is in agreement with that were reported by Han et al. (2002) and Domescik and Martin (1999), who indicated low ammonia-N concentration for animal fed ionophores. Baran (1988) stated that reduced ammonia-N concentration and deamination of amino acids can be resulted from decreased ruminal proteolytic bacteria population.

Conclusion

As a whole the monensin can be manipulate rumen ecosystem and decrease ruminal ammonia-N, resulting high ruminal undegradable protein and increasing the ruminal escaped protein.

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Table 1. The effect of monensin on ruminal pH.

Sampling hour	Monensin (ppm)	SEM			
20	25	30	35		
2	5.77	5.72	5.71	5.69	0.05
4	6	5.92	5.85	5.79	0.13

The means within a column without common letter differ (p<0.05).

Table 2. The effect of monensin on ruminal VFA(mMol/L)

Sampling hour	Monensin (ppm)	SEM			
20	25				
2	124.75	125.25	126.75	127.5	3.31
4	111.75	113.75	117.5	119.5	4.58

The means within a column without common letter differ (p<0.05).

Table 3. The effect of monensin on ruminal ammonia-N (mg/L)

Time (after feeding, h)	Monensin (ppm)	SEM			
20	25	30	35		
2	101[a]	97.75[ab]	91.75[bc]	86.37[c]	2.85
4	105.5[a]	100.37[ab]	96.25[bc]	92.37[c]	2.03

The means within a column without common letter differ (p<0.05).

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