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ASSOCIATIONS OF COWS' NON-SPECIFIC INFLAMMATORY RESPONSE WITH INFECTIOUS DISEASE STATUS OF THE HERD

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SUMMARY

The study investigated the effect of herd's bovine rhinotracheitis virus 1 (BHV1), bovine viral diarrhoea virus (BVDV), bovine respiratory syncytial virus (BRSV) and *Mycoplasma bovis* antibody status and various farm-level management factors on cows' serum amyloid A (SAA) concentrations. Data and serum samples were collected from 100 Estonian dairy herds and analysed for presence of abovementioned pathogens' antibodies and SAA concentrations. For statistical analysis, a linear random-intercept model was used where logarithmically transformed SAA was the outcome variable and farm a random factor, with other variables as fixed factors. Herds with a prevalence of BHV1 antibodies >50% had significantly higher SAA concentrations than BHV1 negative herds. Herds with presence of BRSV antibodies and herds with *M. bovis* antibodies in >30 % cows demonstrated higher SAA concentrations. SAA concentrations in a herd were found to be lower if a veterinarian was permanently employed by the farm.

INTRODUCTION

Bovine respiratory disease (BRD) is a major global health concern affecting cattle [1]. It has a considerable economic impact [2] and is regarded as the most common cause of mortality in dairy cattle [3].

Various predisposing factors together with several pathogens like BRSV, BHV1, BVDV, mycoplasmas and others have been shown to be involved in the BRD complex [1, 4, 5].

Acute phase proteins (APPs), including SAA, are produced in the liver as a part of acute phase response to infection, stress or other tissue damage [6]. Several studies have demonstrated the sensitivity of APPs as markers of respiratory infection [7, 8] and suggested their usefulness as a tool for monitoring herd health and detecting clinical and subclinical disease [9, 10]. However, most of these studies have involved calves and there is paucity of information regarding adult dairy cows.

The aim of this study was to investigate the impact of the herd's antibody status for various BRD-related pathogens and other farm-level factors on the non-specific inflammatory status of cows, measured by SAA concentrations.

MATERIAL AND METHODS

Serum samples from a representative number of randomly selected cows and heifers (age 6 months to calving) were collected between September 2006 and April 2008 from 100 Estonian dairy farms to estimate herd health status.

All cow samples (5174 in total) were analysed for BHV1 antibodies, using a commercial BHV-1 gB ELISA test kit, HerdChek* (IDEXX, Switzerland). 20 random cow samples from each farm were investigated for *M. bovis* antibodies, for which the BIO K 260 ELISA test (Bio-X Diagnostics, Belgium) was used. Ten serum samples from randomly selected heifers per herd were tested for BVDV, using the PrioCheck BVDV Ab test kit (Prionics AG, Switzerland) and 20 random heifer samples from each herd were analysed with the Svanovir ELISA test (Svanova Biotech AB, Sweden) for BRSV antibodies to establish herd infection status.

Herd BHV1 status was divided into 3 categories: negative herds (n = 38), prevalence <50% (n = 28) and prevalence >50% (n = 34). Herd infection status for other three pathogens was categorised into two groups for each, as follows: BVDV – antibodies detected (n = 76) or not (n = 16); BRSV – antibodies detected (n = 51) or not (n = 43), and for *M. bovis*, presence of antibodies in <31% samples (n = 69) and in >30% samples (n = 31).

A questionnaire for recording other herd data, covering herd size, housing type and management etc., occurrence of clinical diseases, animal purchase and reproduction history as well as employment of a veterinarian and/or inseminator was filled for every farm.

SAA was measured from 10 randomly selected samples from each farm using ELISA test (Phase SAA Assay, Tridelta Development Ltd., Ireland) according to manufacturer instructions.

For statistical analysis, a linear random-intercept model was used where logarithmically transformed SAA was the outcome variable and farm a random factor. Crossly haemolysed samples were excluded from analysis (n = 17). Categorised herd infectious disease statuses and other variables were included as fixed factors. Categorised farm size (<50, 50 - 99, 100 - 199, 200 - 399, >400) was controlled for in the model (multiple Wald test p = 0.034). Stepwise backward method was used to fit the final model.

RESULTS

As shown below in Table 1, herds where the prevalence of BHV1 antibodies in cows was >50% demonstrated significantly higher SAA concentrations than BHV1 negative herds. Herds with heifers that were BRSV-antibody positive and herds with *M. bovis* antibodies in >30 % cow samples had higher SAA concentrations. SAA concentrations were found to be lower in a herd if a veterinarian was in permanent employ at the farm. No statistically significant association was found between BVDV antibody status and SAA concentrations.

DISCUSSION

In the present study, cows in herds with BHV1 antibody prevalence >50% had significantly higher SAA concentrations than BHV1 negative herds. This is consistent with the pathogenesis of BHV1, where the virus stays latent after infection and may re-activate upon immunosuppressive stimuli, e.g. calving [11], leading to the situation where the higher prevalence of BHV1 among cows also leads to higher incidence of clinical or disease.

Higher SAA concentrations were also observed in herds where heifers were BRSV-antibody positive. As BRSV is often considered to be the pathogen most often associated with severe respiratory disease especially in younger animals and immunologically naïve herds [12, 13], this may reflect a previously occurred outbreak of clinical disease, as SAA is shown to respond rapidly to infection [7] but Larsen *et al*. [14] also suggest that a previously subclinical BRSV infection may trigger an outbreak of clinical disease due to secondary bacterial infections.

Another factor influencing increase of SAA concentrations was detection of *M. bovis* antibodies in >30% of samples in a herd. While mycoplasmas are also found in healthy animals, they may induce disease together with bacteria and viruses [15] and *M. bovis* is associated with mastitis and arthritis, as well as respiratory and reproductive diseases [16].

Table 1. Herd-level factors influencing serum amyloid A (SAA) concentrations of cows.

Variable (*n* = number of herds)	Regression coefficient*	P value	95% confidence interval for regression coefficient
Own veterinarian employed by farm:			
no (*n* = 77)	0		
yes (*n* = 23)	-0.667	0.001	-1.123, -0.271
Heifers with bovine respiratory syncytial virus (BRSV) antibodies in herds:			
no positive animals in herd (*n* = 48)	0		
positive animals in herd (*n* = 51)	0.427	0.009	0.107, 0.733
Prevalence of cows with *M. bovis* antibodies in herds:			
0-30 % of positive animals (*n* = 69)	0		
>30 % of positive animals (*n* = 31)	0.434	0.009	0.106, 0.761
Prevalence of cows with bovine herpes virus 1 (BHV1) antibodies in herds:			
no positive animals in herd (*n* = 38)	0		
≤50 % positive animals (*n* = 28)	0.088	0.642	-0.285, 0.462
>50 % positive animals (*n* = 34)	0.735	0.000	0.323, 1.146

* Regression coefficient expressed on the logarithmic scale

Regarding the impact of management factors, SAA concentrations were found to be lower in a herd if a veterinarian was permanently employed by that farm, instead of using the services of an outside practitioner. This may reflect the better ability of a veterinary professional to detect symptoms of early disease and/or management problems potentially compromising herd health compared to an employee without veterinary background. Other explanations to consider involve the possible time difference from an observation of a health problem to the veterinary assistance being sought and the possible difference in motivation and/or general workload in a permanently employed *vs* contracted professional.

CONCLUSIONS

Results of the study demonstrate an impact of herd's infectious disease status on the general health of cows, as expressed by inflammatory status. In addition, results suggest that the level of availability of veterinary services has an impact on herd health.

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MACROSCOPIC AND MICROSCOPIC ASPECTS OF AIRSACCULITIS IN SLAUGHTERED BROILERS IN BRAZIL

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SUMMARY

Brazil is one of the most important poultry producers and the largest exporter of chicken meat in the world. The occurrence of respiratory diseases represents serious economic losses in poultry production. Airsacculitis is the main gross lesion of broilers respiratory system and results in substantial damages to the broiler industry due to condemnations at slaughter. This study was conducted to establish macroscopic and microscopic aspects of airsacculitis in broilers slaughtered under federal inspection in Bahia state, Brazil. Photos were taken and collected samples of air sacs lesions of birds condemned during the inspection line. The photographs were analyzed to determine the degree of severity and the samples were sent to laboratory for analysis and identification of bacteria isolated. In the present work, *Pseudomonas* spp. was found in 53.3% of airsacculitis studied and in the other 46.7% was found, isolated or associated between them, bacteria from the Enterobacteriaceae family. It was possible to identify the following bacterial genera: *Escherichia coli (50.0*%), *Enterobacter* spp. (28.4%), *Citrobacter* spp. (21.4%), *Serratia* spp. (14.3%), *Yersinia* spp. (14.3%), *Leclercia* spp. (14.3%), *Klebsiella* spp. (14.3%) and *Morganella* spp. (7.1%). This study indicates the *Pseudomonas* spp. as the main bacteria in broilers airsacculitis in Brazil, and confirms the findings of the literature regarding the involvement of *E. coli* as an important enteric bacteria involved in broilers airsacculitis, while revealing the involvement of other bacteria in this respiratory disease of birds.

Keywords: 1. Airsacculitis; 2. Broilers; 3. Bacteria.

INTRODUCTION

As a result of technological advances, the complex Brazilian poultry experienced significant development process in the past three decades. In terms of production and operational organization, the production of chicken meat has reached high levels, making Brazil a major producer of poultry and the largest exporter of poultry meat in the world. In this sense, has changed inspection system also seeking progress of the investigation of the killing and condemnations of carcasses to determine their causes in order to reduce or eliminate the losses occurred and its effects on the economy of the poultry sector. Despite all these precautions, the number of convictions in slaughterhouses is still large, which makes it necessary to carry out ongoing research aimed at identifying the main problems associated with and, thus, contribute to decline. In a previous study in poultry slaughterhouse under federal inspection in Bahia state, found that the appearance, cachexia and airsacculitis were main causes of total condemnation of carcasses of slaughtered chickens (7).

Thus, considering that the airsacculitis have been one of the major causes of condemnation of broilers slaughtered in specialized industry, this work was carried out focusing on the macroscopic and microscopic aspects of airsacculitis broilers slaughtered in a slaughter plant under federal inspection in Bahia state, Brazil.

MATERIAL AND METHODS

This study was conducted in a poultry slaughter plant located in Bahia state, Brazil, where were collected 30 samples of air sacs along the inspection line during the slaughter. Then, in an appropriate place, there was gross examination of each of the condemned carcasses, writing down the main lesions and obtaining photographic images in order to classify the lesions found during the process of killing broilers (1, 2). Using gloves, the air sacs were removed from their anatomical parts and transferred to sterile plastic containers, marked, sealed with tape, placed in sealed plastic bags and also stored in cool boxes with ice and transported to the Bahia's Avian Health Laboratory for microbiological analysis.

In the laboratory, in conditions of safety and sterility, were performed the isolation of the material collected on agar plates containing eosin methylene blue and the plates incubated at 35^{o}C for 24 to 48 hours. After this period, the colonies were analyzed according to their morphological aspects and Gram staining, following the oxidase test and biochemical identification in order to identify bacteria from the Enterobacteriaceae family (4).

RESULTS

As the macroscopic aspects, the photographs taken during the study were assessed for the lesions, where they established the severity of airsacculitis found considering the following changes: opacity, thickening, swelling of the capillaries and the presence of purulent content/cheesy in air sacs. The air sacs are thin-walled structures, transparent, empty inside, as can be seen in Figure 1. According to the changes found the airsacculitis were classified as slight, moderate and severe (Figures 2, 3 and 4, respectively).

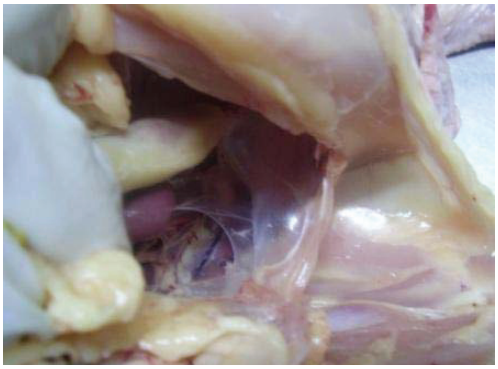

Figure 1: Normal air sacs of broilers slaughtered in Bahia state, Brazil.

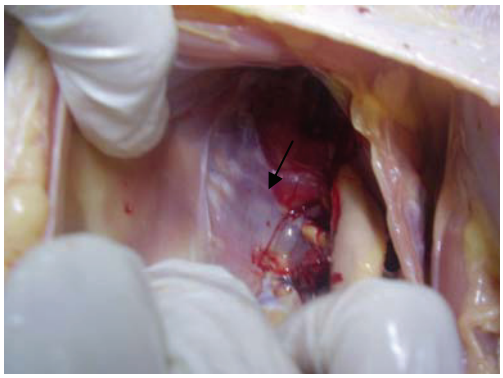

Figure 2: Air sacs with a slight opacity and thickening (arrow) obtained from broilers slaughtered in Bahia state, Brazil.

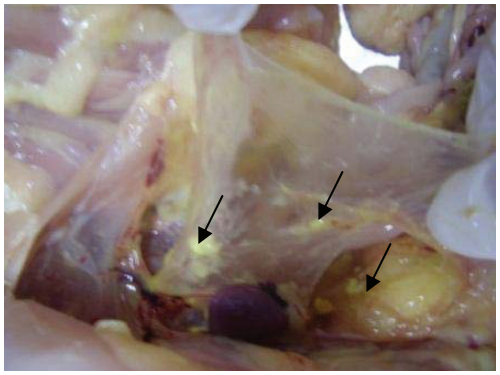

Figure 3: Air sacs showing opacity and thickening with moderate yellowish purulent foci located (arrows) obtained from broilers slaughtered in Bahia state, Brazil.

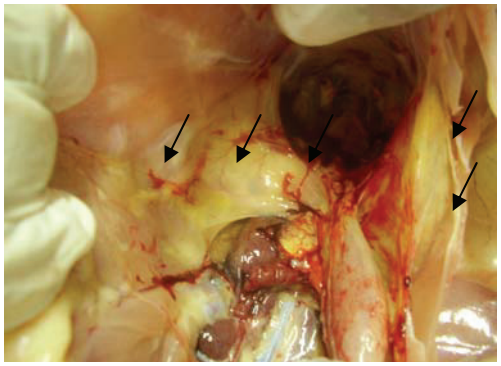

Figure 4: Air sacs with intense opacity and thickening (arrows), which characterizes the most severe grade, obtained from broilers slaughtered in Bahia state, Brazil.

With regard to microbiological aspects of airsacculitis were isolated and stored 105 morphologically distinct bacterial colonies. Of these, it was found that 67 colonies (64%) belonged to the genus *Pseudomonas* spp., while the remaining 38 colonies (36%) belonged to different genera of the family Enterobacteriaceae (Table 1).

Table 1: Number and percentage of Enterobacteriaceae isolated from airsacculitis in broilers slaughtered in Bahia state, Brazil.

Microorganisms	Number of colonies	Percentage
Escherichia coli	08	21%
Enterobacter spp.	04	10.5%
Citrobacter spp.	04	10.5%
Serratia spp.	04	10.5%
Yersinia spp.	03	7.9%
Leclercia spp.	02	5.3%
Klebsiella spp.	02	5.3%
Morganella spp.	01	2.6%
Enteric Group 69	01	2.6%
Not conclusive*	09	23.7%

*Not identified by biochemical tests performed

Besides airsacculitis caused by simple infections, was also found associations among Enterobacteriaceae bacteria isolated from the air sacs, as shown in Table 2.

Table 2: Presence of Enterobacteriaceae in single or mixed airsacculitis infections of broilers slaughtered in Bahia state, Brazil.

Microorganisms	Absolute Frequency	Relative Frequency
E.coli (single infection)	04	28.6%
Serratia spp. (single infection)	01	7.1%
Not conclusive (single infection)*	02	14.2%
E.coli + Enteric Group 69	01	7.1%
E.coli + *Serratia* spp. + *Enterobacter* spp.	01	7.1%
E.coli + *Leclercia* spp. + *Citrobacter* spp.	01	7.1%
Enterobacter spp. + *Leclercia* spp.	01	7.1%
Enterobacter spp. + *Klebsiella* spp.	01	7.1%
Enterobacter spp. + *Citrobacter* spp. + *Yersínia* spp.	01	7.1%
Citrobacter spp. + *Yersínia* spp. + *Morganella* spp. + *Klebsiella* spp.	01	7.1%

*Not identified by biochemical tests performed

DISCUSSION

The results of this study are consistent with a previous work performed in Brazil, where the *E. coli* was the most common lesions of air sacs, which was isolated in 25 (80.64%) of the 31 samples studied, in simple or mixed infections (6). Another study about etiology of 170 bacteria isolated from airsacculitis of broilers in Jordan, of which 150 (88.2%) were identified as *E. coli* (3). In Argentina, similar results were found about the correlation between *E. coli* and *Mycoplasma* spp. in 12 (66.6%) of 18 cases of broilers with chronic respiratory diseases (5). In another study about air sac lesions, in five cases (2.9%) were found association of *E. coli* and *Ornithobacterium rhinotracheale* and in two (1.2%) was found association between *E. coli* and *Bordetella avium* (3).

CONCLUSIONS

This study confirms the findings of the literature regarding the involvement of *Escherichia coli* as a major bacteria involved in airsacculitis broilers in Brazil, while revealing the involvement of other bacteria in this important respiratory disease of chickens.

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MONITORING ON HYGIENE MANAGEMENT IN ANIMAL SHELTERS

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SUMMARY

A study was conducted to investigate hygiene management in animal shelters. Therefore, samples were obtained by swabbing different surfaces. The total plate counts were determined and a screening for different viruses was conducted. Subsequent to the common hygiene procedures, coronavirus was detected in each of the four participating animal shelters, parvorvirus was found in two of them. In general, total bacterial counts were not reduced more than 10^2 cfu (colony forming units) / cm^2.

The study shows that further investigations on hygiene management in animal shelters and a better training of the shelter staff are required.

INTRODUCTION

Hygiene management is of great importance concerning the quality management in animal shelters [6]. The prevention of infectious diseases requires an efficient hygiene management [6]. Aim of the study was to monitor critical points in four animal shelters in the area of Leipzig (Germany) in order to improve hygiene management strategies. Samples of selected spots within the facilities were taken for bacteriological and virological examinations. Additionally, questionnaires were used to evaluate the hygiene situation in the participating shelters.

MATERIAL AND METHODS

We visited four animal shelters for monitoring the situation of the hygiene management. The shelters referred to as A, B and D are private shelters, shelter C w is municipal. Dog capacity ranges from <50 (B), 50-100 (A, C); to > 100 (D). Cat capacity ranges from <50 (A), 50-100 (B) to >100 (C, D). Every visited shelter has separate quarantine facilities for cats, and three of the shelters provide quarantine facilities also for dogs (A, C, D). Facilities for diseased animals are provided in just one shelter (C). Only shelter D has a plan for cleaning and hygiene procedures. In shelter A and D sanitizers are used for daily hygiene procedures, whereas detergents are used in shelter B and C.

Samples were obtained by swabbing surfaces with both, wet and dry swabs [1, 2]. Therefore, areas of several locations within dog and cat facilities with a size of about 10 cm^2 were swabbed before and after the common hygiene procedures. Additionally, the disinfection mat located in front of the cat facilities was sampled twice at each shelter.

Serial 10-fold dilutions were prepared and the total bacterial count was determined using plate count agar [2]. We also identified gram positive bacteria on Columbia agar + 5% sheep-blood and gram negative bacteria on Endoagar. Selected bacteria were biotyped applying the Matrix-Assisted-Laser-Desorption/Ionization Time-Of-Flight Mass-Spectrometry (MALDI-TOF). The monitoring for viral pathogens was focused on corona-, parvo- and calicivirus using PCR-technique [3, 4, 5].

Additionally, Crandell Reese Feline Kidney cells were infected with sterile filtered swab media to isolate infectious parvo- and calicivirus.

RESULTS

Subsequent to the hygiene procedures coronavirus-RNA was detected in shelter A at two sampled spots, in shelter B at five spots (one of these was also sampled after disinfection) and in the shelters C and D at one spot. In shelter B and D coronavirus was also detected on the disinfection mat.

Parvovirus was found at one single spot after cleaning in shelters B and C. No other spot sampled were tested positive for corona- or parvovirus.

The microbiological testing of cleaned areas showed a total bacterial count of $>10^2$ cfu (colony forming units) / cm^2 in shelter A with 33,33 % of the sampled spots (4), in shelter B with 85,71% of the spots (12, 3 of them even after using a disinfectant), in shelter C with 36,36% of the spots (4) and in shelter D with 16,67% of the sampled spots (2).

The disinfection mat in the shelters A und B contained more than 10^2 cfu / cm^2. Bacteria involved were mainly opportunistic pathogens like coagulase-negative and -

positive *Staphylococci, Enterobacteriaceae, Acinetobacter spp., Pseudomonas spp. and Enterococcus spp.*

DISCUSSION

Although hygiene management is very important for disease prevention in animal shelters, many shelters do not focus strictly on it. The poor financial situation and low budgets may contribute to the situation.

Coyne et al. [9] compared animal shelters with human health care institutions. Both have high turnover rates of patients and therefore a need of implementing strict hygiene measures. Quarantine, vaccination and isolation wards are demanded as well as hygiene are needed to minimize pathogen transmission. There is sparsely literature about hygiene measurement in animal shelters. No benchmark for the bacterial load on surfaces is available for animal shelters. For the hospital environment, bacterial loads from <2,5 to 5 cfu / cm^2 after hygiene procedures are mentioned [7, 8]. In pigfarms total plate counts of 10^3 cfu / cm^2 after disinfection have been reported. These benchmarks may be indicatory for surfaces in animal shelters as well. However, the implementation of hygiene procedures should reduce bacterial loads to a considerable degree and prevent the transmission of pathogens. Although the detected viruses and the high bacterial counts can be accounted for a poor hygiene management (especially in shelter B), further investigations are necessary.

CONCLUSIONS

The preliminary results show that the hygiene management in three of the examined animal shelters, especially in shelter B, is insufficient. There is a potential risk of pathogens spreading all over the animal shelters, mainly presumed by the results of coronavirus-detection. Further investigations will be done to get more information about hygiene management. The obtained results will be discussed with the participating shelters and necessary changes in hygiene management as well as the training of the staff in hygiene procedures have to be revised.

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SERUM CALCIUM, PHOSPHORUS AND MAGNESIUM CONCENTRATIONS OF DAIRY CATTLE IN CITY OF GARMSAR

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SUMMARY

Calcium, phosphorus and magnesium are main mineral elements of body in dairy cattle; especially high producing ones. In order to measure concentrations of serum calcium, phosphorus and magnesium of dairy cattle in city of Garmsar, coccygeal blood samples were collected from four hundreds dairy cattle of hybrid Holstein cattle from 40 farms in Garmsar during four different seasons. Biochemical analysis were carried out on the serum samples for measurement of minerals using commercial kits. Commercial reagent kits, based on spectophotometeric methods, were used to assay calcium , phosphorus and magnesium. The results were analysed with Student's *t*-test, followed by Post-hoc test, and a value of $P< 0.05$ was considered to be significant. Biochemical analysis of serum samples revealed that the calcium , phosphorus and magnesium concentration of dairy cattle were 8.76 ± 0.068 , 6.25 ± 0.059 and 2.074± 0.018 mg/dl respectively. It has been found that the mean calcium concentration of cows in Garmsar region was lower than that reported for dairy cattle but phosphorus and magnesium concentrations were in normal range of dairy cattle. Furthermore this study defined that the lowest calcium and phosphorus concentrations of dairy cattle were in winter and the lowest serum magnesium concentration was in spring. As *well* , the highest calcium and phosphorus concentrations were in spring and the highest magnesium was encountered in autumn. Although serum calcium concentration of dairy cattle in Garmsar was lower than normal range reported for cattle, but clinical hypocalcemia is not a common finding in this region, but a subclinical hypocalcemia can cause a lot of economic losses due to loss of productivity.

INTRODUCTION

Calcium, phosphorus and magnesium are the main mineral elements of body which have many function in dairy cattle especially high producing ones. A dietary deficiency or disturbance in metabolism of calcium, phosphorus or vitamin D including imbalance of calcium-phosphorus ratio is the principle cause of osteodystrophies and periparturient hypocalcemia [2]. Magnesium deficiency cause lactation tetany in adult dairy cows and hypomagnesemic tetany of calves. Dairy cows during lactation absorb1.71 g calcium in turn of each gram phosphorous absorption. Body calcium store during gestation and especially in last two months of pregnancy decreases to a very low level. Each kilogram of milk with 4% fat approximately has 1.22 g calcium [1]. Then pregnancy and lactation were the most important causes of hypocalcemia in dairy cattle. Amount of phosphorous in dairy cow's feed must be very high because of following reasons: 1- losing high amount of endogenous phosphorous in feces, 2- absorption of phosphorous from alimentary tract is approximately low, 3- high concentration of phosphorous in milk. In contrast to calcium there was not any mechanism for transfer of bone phosphorous to blood stream [3]. According to NRC, daily requirement of a 450 kg dairy cow with 18 kg milk production is 4 grams [4].

MATERIAL AND METHODS

During the present study 400 hybrid Holstein dairy cattle from 40 dairy farms in city of Garmsar (Semnan province, Iran) were studied. Blood samples from coccygeal vein of dairy cattle were collected using vacutainers (Pars Khavar Co. Qazvin, Iran). Age, number of calving, date of last calving, pregnancy status, and recently received medicines of each sampled cows were recorded. One hundred dairy cows were sampled in each season and 400 dairy cattle were totally sampled in this study. Blood samples were transferred to laboratory after two hours of collection and sera were isolated by centrifugation and kept at – 20 °C until analysis. Biochemical analysis were carried out on the serum samples for measurement of calcium, phosphorous and magnesium minerals using commercial kits. Commercial reagent kits (Zist Shimi Co. Tehran, Iran), based on spectophotometeric methods, were used to assay calcium , phosphorus and magnesium. The results of biochemical examination of serum samples were analysed with Student's *t*-test, followed by Post-hoc test, and a value of $P< 0.05$ was considered to be significant.

RESULTS

Mean serum calcium , phosphorus and magnesium concentration of dairy cattle in Garmsar were 8.76 ± 0.068 , 6.25 ± 0.059 and 2.074± 0.018 mg/dl respectively.

Table 1. Mean serum concentration of calcium, phosphorous and magnesium of dairy cows in different seasons (Mean± SE).

Element / Season	Calcium	Phosphorou	Magnesium
Spring	9.57± 0.13	7.24± 0.08	1.84± 0.03
Summer	8.93± 0.14	6.73± 0.15	2.08± 0.04
Autumn	8.55± 0.11	6.05± 0.07	2.21±0.02
Winter	7.90±0.08	5.34±0.07	2.16±0.03

Table 1 shows the mean serum calcium, phosphorous and magnesium concentrations of dairy cattle in the present study during four different seasons. Statistic analysis showed that serum calcium and phosphorous concentration of dairy cattle in spring was significantly higher than those in summer, autumn and winter seasons ($P< 0.05$). Serum magnesium concentration of dairy cows durin spring was significantly lower than in summer, autum and winter ($P< 0.05$). Mean serum phosphorous concentration of sampled cows in summer was significantly higher than that in autumn season ($P< 0.05$). Mean serum magnesium concentration in summer was lower than that in autumn season but the difference was not significant ($P> 0.05$). Serum calcium and phosphorous concentrations of dairy cows during autumn were significantly higer than those in winter season ($P< 0.05$).

Table 2 shows the mean serum calcium, phosphorous and magnesium concentration of pregnant and nonpregnant dairy cattle of Garmsar in different seasons. Statistic analysis revealed that mean serum calcium and phosphorous concentration of pregnant dairy cattle in autumn were significantly higher than those in nonpregnant dairy cattle ($P< 0.05$).

Table 2. Mean serum concentration of calcium, phosphorous and magnesium of pregnant and nonpregnant dairy cattle during different seasons (Mean± SE).

Element / Season		Calcium	Phosphorou	Magnesium
Pregnant	Spring	9.54± 0.16	7.25± 0.08	1.83± 0.03
	Summer	8.86± 0.19	6.70± 0.18	2.07± 0.05
	Autumn	8.59± 0.14	6.12± 0.08	2.201±0.03
	Winter	8.00±0.11	5.41±0.07	2.18±0.04
Nonpregnant	Spring	9.47± 0.26	7.26± 0.18	1.86± 0.06
	Summer	9.06± 0.20	6.79± 0.27	2.10± 0.07
	Autumn	8.46± 0.20	5.88± 0.15	2.23±0.05
	Winter	7.76±0.13	5.25±0.13	2.12±0.04

DISCUSSION

From the results of biochemical analysis of serum samples in the present study it has been found that the mean serum calcium concentration of cows in Garmsar region was lower than that reported for dairy cattle (9- 12 mg/dl) [2,3] but mean serum phosphorus and magnesium concentrations were in defined normal range for dairy cattle (4- 7 mg/dl and 1.9- 3.2 mg/dl respectively) [2,4]. Furthermore this study defined that the lowest calcium and phosphorus concentrations of dairy cattles was in winter and the lowest serum magnesium concentration was in spring. As well , the highest calcium and phosphorus concentrations were in spring and the highest magnesium was encountered in autumn.

CONCLUSION

Although serum calcium concentration of dairy cattle in Garmsar was lower than normal range reported for cattle, but clinical hypocalcemia is not a common finding in this region. Although a subclinical hypocalcemia can cause a lot of economic losses due to loss of productivity.

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POST RACE TRACHEAL ENDOSCOPY IN KURDISH HORSES

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SUMMARY

Exercise-induced pulmonary hemorrhage (EIPH) is a common occurance in race horses. The Kurdish horse is one of the oldest and fast breeds in Iran. Sixty pure Kurdish healthy horses aged 4 to 13 years and different sexes were examined endoscopically for detection of exercise-induced pulmonary hemorrhage within 2 hours after racing. 17(28.3%) of these animals had various degrees of hemorrhage in the tracheal lumen and no epistaxis detected in them. Statistically, a significant relationship observed between the occurance of EIPH and the age of examined horses, but there was no significant relationship between EIPH occurance and sex.

INTRODUCTION

Exercise-induced pulmonary hemorrhage (EIPH) occurs in horses throughout the world and does not appear to have any geographic distribution and it is a disorder of horses that run at high speed, such as thoroughbred or standardbred racehorses. Epistaxis due to EIPH occurs during or shortly after exercise and is usually first noticed at the end of a race, particulary when the horse is returned to the paddock or winner's circle and is allowed to lower its head (5,10,15). The cause of this disorder is tremendous increase in blood pressure and rupture of alveolar capillary membranes with subsequent extravasation of blood into interstitial and alveolar spaces (6,8,12). Although rupture of alveolar capillaries occurs secondary to an exercise-induced increase in transmural pressure (pressure difference between the incide of the capillary and the alveolar lumen). The prevalence of EIPH varies with the method used to detect it and the frequency with which horses are examined (15). There are a variety of techniques available for determining the presence and severity of EIPH including direct examination of the airways through a flexible endoscope or examination of bronchial lavage or tracheal aspirates for evidence of hemorrhage. Endoscopic examination of the upper respiratory tract and detection of frank blood within the trachea is the usual method of diagnosis (14). Age in considered a risk factor for EIPH, with the prevalence of the disorder being higher in older horses. There is no consistent association of sex with prevalence of EIPH. The objective of this study was to evaluate the prevalence of EIPH in one of the Iranian sport horse breeds for the first time.

MATERIALS AND METHODS

Sixty healthy pure Kurdish horses, thirty males and thirty females, wheithing between 350- 500 kg and aged 4-13 were examined endoscopically to detect of EIPH after exercise. All of the animals were in stables with optimal ventilation and fed on a diet based on grain and alfalfa hay, as well as mineral and vitamin supplements. After taking history of any recently disorder or diasese or exist of poor racing performance and trusting of absecence any problem in them, the complete examination of body organs were performed and recorded. After confirming of health in them, horses got a few minutes in warming-up phase of walking and trotting in the racecourse prior to testing. Horses galloped 1600 meters long at 12m/s. Respiratory endoscopic evaluation was carried out in all horses 90-120 minutes after exercise using a flexible fibreoptic endoscope introduced through one of the nostrils and passed down to the carina. The level of blood presence was determined. Animals which would not accept the endoscope, were sedated with single dose of xylazine hydrochloride (1mg/kg IV). The presence of blood for assessment of severity of EIPH using a 0-4 grading as follows: Grade 0 – no blood detected in the pharynx, larynx, trachea or main-stem bronchi, Grade 1 – Presence of one or more flecks of blood, Grade 2 – one long steram of blood, Grade 3 – Multiple distinct streams of blood covering more than one-third of the tracheal circumference; no blood pooling at the thoracic inlet, Grade 4 – Multuple, coalescing streams of blood covering >90% of the tracheal surface with pooling of blood at the thoracic inlet (10).

RESULTS

Seventeen (28.3%) horses of the examined animals showed some degree of bleeding on endoscopic examination. Our results from respiratory endoscopy after exercise showed the increasing number of bleeders and severity of hemorrhage in aged horses. So on the basis of the data obtained there was a significan relationship between the increased values of EIPH prevalence and the age of animals ($r=0.343$, $p\leq0.01$). But there was no significant corrolation between horse`s sex and prevalence of EIPH (Table 1).

Table 1: Age distribution of bleeders and the severity of EIPH in them

Age	4	5	6	7	8	9	10	11	12	13
number	5	5	6	7	6	6	8	5	5	7
Degree 1 EIPH	0	0	1	2	2	2	3	1	0	0
Degree 2 EIPH	0	0	0	0	0	0	0	1	2	2
degree 3 EIPH	0	0	0	0	0	0	0	0	0	1
Total	0%	0%	16.6%	28.5%	33.3%	33.3%	37.5%	40%	40%	42.8%

DISCUSSION

The breed of horses is an important risk factor for prevalence of EIPH (15). Based on a single endoscopic examination within 2 hours of racing, coducted by Pascoe et al. in 1981 43.8% of thoroughbred horses had various degrees of hemorrhage in the tracheal lumen and 0.8% of them had blood flow from the nostrils (11). In the same manner, Raphel and Soma in 1982 reported that 75.4% of thoroughbred racehorses had some degrees of EIPH and 9.0% of them had blood at the nostrils (13). Mason et al. in 1984 showed that from 1093 post-race endoscopic examination of thoroughbred racehorses in Hong Kong, 46.8% of them had EIPH (9). In 2006 Costa and Thomassian showed that 62% of thoroughbred racehorses in Brazil had some degrees of EIPH (3). Therefore, the prevalence of EIPH in thoroughbred racehorses in single endoscopic examination within 2 hours of race is 43-75%, While the prevalense of EIPH in standardberd racehorses, in same method is 26-43% (10,15). Lapointe et al. in 1994 reported that 87% of Quebec standardbred racehorses had some degrees of hemorrhage in endoscopic examination 1 hour after racing on at least 3 occations. They concluded that no significant relation detected between the frequency of EIPH and age, sex on gait (7). Birks et al. in 2002 compared standardbreds and thoroughbred from this view point and showed that there was no apparent effect of breed (or possibly racing gait) on EIPH (2). Considering the findings of the mentioned studies we suggest that this disorder is a common disorder in standardbred racehorses as it is in thoroughbred racehorses. Hillidge et al. in 1985 at retrospective survey of EIPH based on endoscopic examination within 30-90 minutes after racing in 94 appaloosa horses reported that approximately 52% of racing appaloosa horses had some degrees of EIPH and there was a significantly increased prevalence of EIPH with increasing age in that population (4). The prevalence of this disorder is approximately 11% in polo ponies and 62% in Quarter horses used for barrel racing (15). Araya et al. in 2005 showed that the prevalence of EIPH in Chilean Criollo horses in endoscopic determination within 90-120 minutes after exercise is 60.8% (1). To the best of our knowledg there is no published article about prevalence of EIPH in Iranian horses of any breeds. The kurdish horse is one of the oldest and fast breeds of horses from Iran that used in flat racing and game of polo. Our results showed that 17 horses from 60 had some degree of hemorrhage in trachea and bronchi in endoscopic determination, so the prevalence of EIPH in Kurdish horse is 28.3%. In addition, our findings showed a significant relationship between increasing prevalence of EIPH and increasing age but there was no significant relationship between prevalence of EIPH and sex that is in agreement with previous studies (4,11,13).

CONCLUSION

From this study it can be concluded that the Kurdish horse suffers from EIPH same as throughbred or other sporting breeds.

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EFFECT OF INTRAVENOUS (IV) INJECTION OF OXYTETRACYCLINE ON SERUM CALCIUM, PHOSPHOROUS AND MAGNESIUM IN CATTLE

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SUMMARY

This study was carried out to investigate the effect of oxytetracycline on blood serum Ca, P and Mg in cattle. The dose of 10mg/kgBW oxytetracycline was injected IV in five cattle for four days. Oxytetracycline and Ca (ionized and total), P and Mg were measured in blood serum which were taken before and 1, 3, 6, 12 and 24 hours after injection. Oxytetracycline measured by HPLC and Ca, P and Mg were measured by biochemical method. The level of oxytetracycline increased immediately one hour after injection and then reduced. The level of Ca (ionized and total), P, and Mg were significantly decreased.

INTRODUCTION

Oxytetracycline (OTC) is one of the important members of the tetracycline group of antibiotics which is routinely used in animal husbandry. It is used for the prophylaxis and treatment of a great number of diseases since this antibiotic possesses a broad spectrum activity against many pathogenic organisms. OTC can be used in veterinary formulations for the prevention and control of disease and added to feed for such a purpose. It is licensed for use in a wide variety of food-producing animals such as cattle, pigs, sheep, poultry and it is a principal antibiotic used in fish farming too.(2)

Calcium (Ca) is the major cation required in the mammalian diet, and is the most abundant mineral element in the body. The skeleton, an articulated framework that facilitates locomotion and provides some support for the vital internal organs, contains about 98% of the body Ca as calcium phosphate. The remaining Ca, about 2%, is distributed in the extracellular and cellular fluids, and has essential roles in metabolism, blood clotting, enzyme activation and neuromuscular function. The metabolism of Ca and phosphorus (P) is closely related, and a deficiency or an excess of either one will interfere with the utilization and metabolism of the other. Phosphorus is second only to Ca in abundance in the body, with about 80% of the body P located in the skeleton, the remaining 20% having essential metabolic functions in cell contents and cell walls. Phosphorus functions as a component of the nucleic acids which are the basis of genetics, and in nucleotides, such as adenosine triphosphate (ATP), which function in energy metabolism. Phosphorus is a component of both cell walls and cell contents as phospholipids and phosphoproteins. In addition, P functions in acid–base buffer systems of blood and body fl uids, in cell differentiation and in maintaining the structural integrity of cells(4).

Calcium chelation is the side effect of oxytetracycline. There for this study was carried out in cattle to investigate the effect of intravenous (iv) injection of oxytetracycline on serum level of calcium, phosphorous and magnesium.

MATERIAL AND METHODS

Five cross breed, non lactating and non pregnant cattle were used for this study. The dose of 10mg/kgBW oxytetracycline (1) was injected IV in these cattle for four days. Blood samples were taken before and 1, 3, 6, 12 and 24 hours after injection. Sera were stored in -20°c until examination. The level of Oxytetracycline was measured by HPLC. The level of Ca (ionized and total), P and Mg were measured by biochemical method.

Data were analyzed by a one-way analysis of variance (ANOVA) and LSD test using soft ware SPSS version 16.

RESULTS

The level of oxytetracycline increased immediately one hour after injection and then reduced as showed in figure 1.

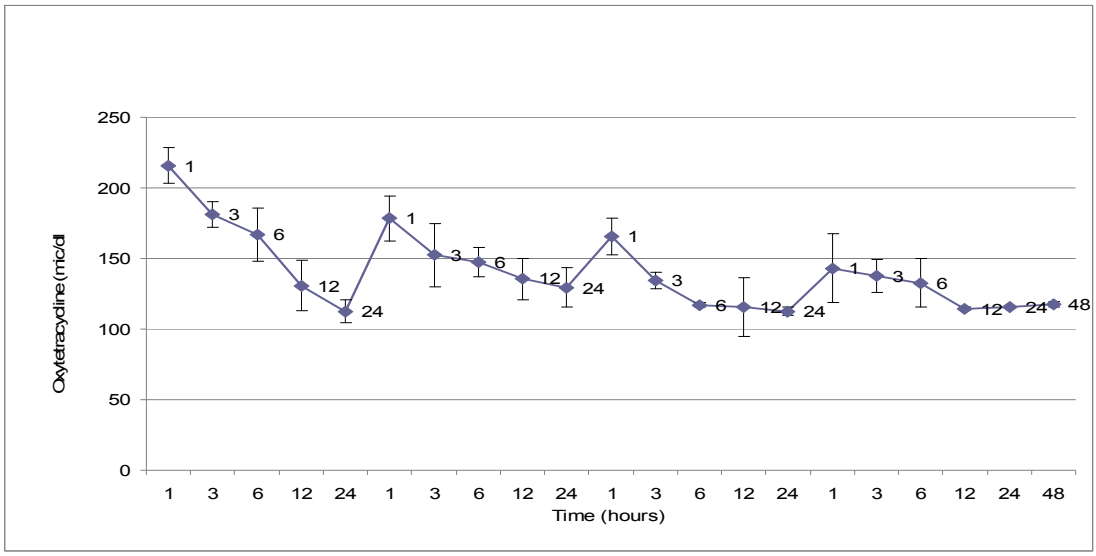

Figure 1: The level of Oxytetracycline (mg/dl) in serum after the IV injection of Oxytetracycline

The level of total calcium reduced after the 1, 3 and 6 hours after the injection of OTC and then increased to the level of before injection of OTC at the time of 24 (figure 2).

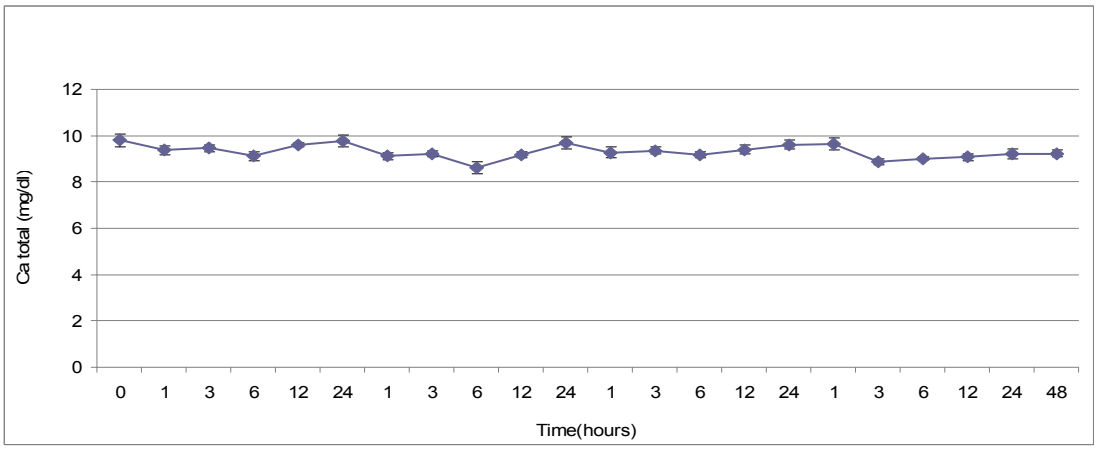

Figure 2: The level of total Ca (mg/dl) in serum after the IV injection of Oxytetracycline

Ionized Ca was reduced after the Iv injection of OTC (figure 3), but this reduction was not significant.

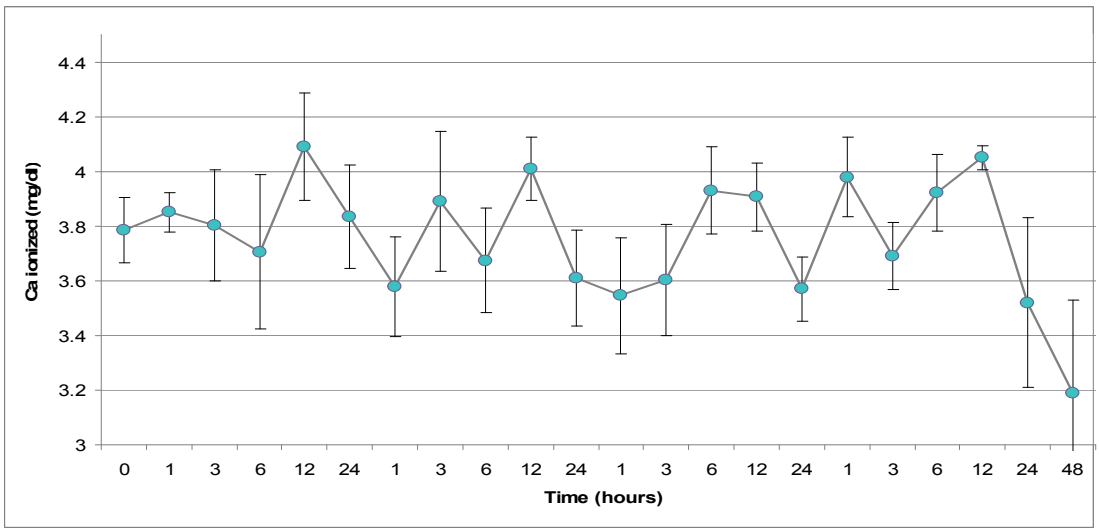

Figure 3: The level of ionized Ca (mg/dl) in serum after the IV injection of Oxytetracycline

The level of P was increased at the time of 1, 3, 6, 12 and 24 after the first(day 1) of IV injection of OTC , but in the another days (days 2, 3, 4), was significantly decreased (figure4).

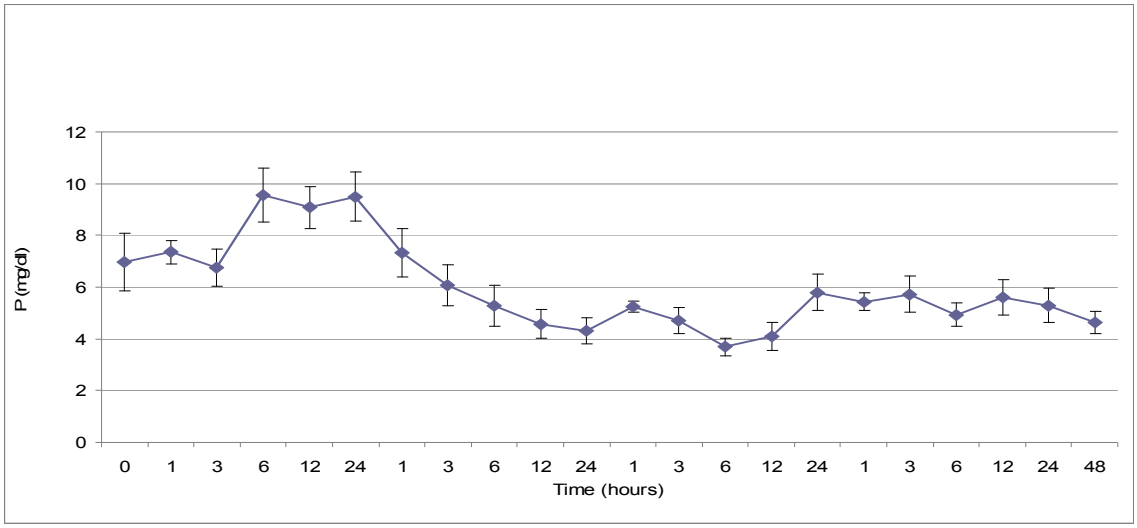

Figure 4: The level of P (mg/dl) in serum after the IV injection of Oxytetracycline

The level of Mg as same as of P, was increased at the time of 1, 3, 6, 12 and 24 after the first (day 1) of IV injection of OTC , but in the another days (days 2, 3, 4), was significantly decreased (figure5).

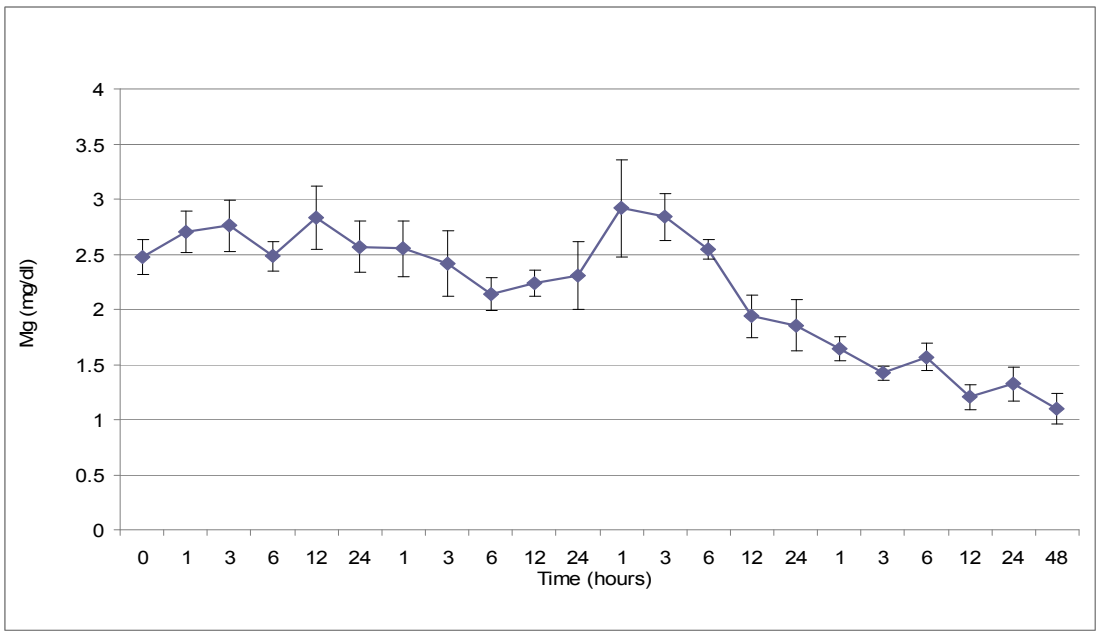

Figure 5: The level of Mg(mg/dl) in serum after the IV injection of Oxytetracycline

DISCUSSION

Button and Mulders Showed intravenous injection of OTC in propylene glycol (PG), OTC in saline solution, and PG alone in sheep had no significant effects on total plasma calcium concentrations over a 60-minute period. In contrast, ionized calcium concentrations in whole blood were significantly (P less than 0.01) depressed for approximately 3 minutes after OTC in PG and OTC in saline solution, IV. A slight depression of ionized calcium concentrations was noticed after injection of PG alone. Seemingly, calcium chelation by OTC may be a major factor in the collapse syndrome of ungulates given preparations containing OTC by rapid IV injection (3). The result of this study showed IV injection of oxytetracycline had significantly effects on Ca, P and Mg. It is resulted from divalent metal ion chelation by oxytetracycline. It is concluded that in treatment with oxytetracycline, the level of blood serum Ca, P and Mg of patient must be noted.

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EFFECT OF PANTOPRAZOLE ON RATE OF IMMUNOGLOBULINES ABSORPTION IN THE NEWBORN CALVES (Abstract)

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OBJECTIVE

Newborn calves, due to not receiving immunoglobulin from the mother in uterus, are agammaglobulinemic and gain immunity immediately after birth through colostrum intake. Whereas 24 hours after birth, abomasum produces more acid, the probability of colostrum globulin destruction increases. Blocking acid secretion through proton pump inhibitors might prevent colostrum immunoglobulin destruction.

ANIMALS, MATERIALS AND METHODS

To study this issue 15 newly-born male Holstein calves were studied in five three-member groups, 3 being control groups and 2 were experimental groups. The calves under study were fed colostrum and milk at zero, 12, 24, 36, 48, 60, 72 and 84 hours after birth using esophageal tube as follows: Control groups: A- calves were fed with milk for 24 hours after birth then with colostrum for 72 hours after birth; B- calves were fed with milk for 48 hours after birth and then with colostrum for 72 hours after birth; C- calves were fed with colostrum for 72 hours after birth. Experimental groups: A- pantoprazole was injected intravenously each 24 hours (2 mg/kg) and the calves were fed with milk for 24 hours after birth and then colostrum for 72 hours after birth. B- pantoprazole was injected intravenously each 24 hours (2 mg/kg) and the calves were fed with milk for 48 hours after birth and then with colostrum for 72 hours after birth. Immunoglobulins G, M and A of serum were measured using ELISA.

RESULTS & CONCLUSION

The results didn't show any significant difference in concentration of blood immunoglobulin of control and experimental groups. Thus, it is assumed that high pH of abomasum has no significant effect on immunoglobulin intake.

EVALUATION OF BLOOD GLUCOSE LEVEL FOR DETECTION SUBCLINICAL KETOSIS IN DAIRY HERDS

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SUMMARY

In early lactation, there is a heavy demand for glucose and the energy intake is likely to be lower than necessary to meet requirements. Glucose level decline in ketotic cows . The aim of this study was to evaluate serum glucose level as a screening test for detection of subclinical ketosis in postpartum cows in dairy herds. One – hundred ninety lactating cows in 2-4 wks of lactation From fifteen herds in Nishaboor were included in this study . Blood samples were collected & transported on ice to laboratory and serum separation was done in 60 min. Serum samples were kept at -20 till the laboratory analysis . BHBA was determined by enzymatic method & by commercial kit (Ranbut , Randox® , UK) . Glucose was determined by commercial kit (Pars Azmoon®, Iran) . Photometer Riele 5010 was used for photometric evaluation. Sensitivity & specificity , Positive predictive value , negative predictive value & true prevalence of blood glucose level as a screening test were determined with the cut-off point of 30 mg/dl, 32 mg/dl & 35 mg/dl . Apparent prevalence in the threshold (cut-off point) of 1400 & 1200 µmol/L (with gold standard) were 15.26 & 18.42 respectively . The mean level of BHBA & glucose in ketotic and non ketotic cows were 2.7 ± 0.26 µmol/L ,0.637 ± 0.637±0.2 µmol/L & 30.72 ± 1.47 , 43.9±0.8 respectively . Specificity & sensitivity of blood glucose level in various cut-off point (30mg/dl,32mg/dl,35mg/dl) determined . If 1400 µmol/L were assumed as threshold , sensitivity & specificity in cut-off point of 30 mg/dl , 32 mg/dl & 35 mg/dl were 95.6 %, 91 % , 83.5 % (sensitivity) & 72.55 , 78.4%, 80.5% (specificity) respectively . If 1200 µmol/L were assumed as threshold , sensitivity & specificity in cut-off point 30mg/dl,32mg/dl,35mg/dl were 95.4% , 80.6%,84.8% (sensitivity) & 68.6%, 74.4%, 72.5%(specificity) respectively . There were a negative correlation between glucose level & BHBA (r = -0.457) . If 1400 µmol/L were assumed as threshold , true prevalence in cut-off point 30 mg/dl, 32mg/dl and 35 mg/dl were 22.19% 22.40% & 23.2 % respectively . If 1200 µmol/L were assumed is as threshold , true prevalence in cut-off point 30 mg/dl, 32mg/dl and 35 mg/dl were 28.8% , 33 % 34.4% respectively . The result of this study showed that blood glucose determination is a sensitive test with low specificity .

INTRODUCTION

Glucose plays a key role in the metabolism of the animal body. It is an essential source of energy for the maintenance of many tissues, e.g. the nervous system, red blood cells, the placenta and the mammary gland. It also serves as a precursor for the biosynthesis of essential cell components. Impaired gluconeogenesis, a shortage of carbohydrate precursors, or both, are believed to contribute to various metabolic disorders frequently seen in ruminants, such as acetonemia in dairy cows and pregnancy toxemia in sheep [Veterinry Medicine]. Ketosis causes economic losses to the dairy industry because of impaired milk production, decreased reproductive efficiency, increased involuntary culling, and increased treatment costs (Detilleux 1994 ; Dohoo1984; Dohoo1984a; Miettinen 1990; 1991 ;1994; Plym Forshell 1991) .Subclinical disease incidence is far more common than clinical disease, frequently goes unnoticed and may be associated with significant clinical disease risks, impaired production and reduced reproductive performance (Duffield 2004) .Subclinical ketosis is defined as elevated concentrations of circulating ketone bodies in the absence of clinical signs (Andersson, 1988). The aim of this study was to evaluate blood glucose level as a new screening test to detect subclinical ketosis.

MATERIAL AND METHODS

A total of 190 cows with high producing records(> 9000 Liter/ year , 305 day milk production) from Sixteen Industrial dairy herds in Nishaboor with a total of 8354 cows were included in this study. All herds were equipped to a computerized Health & fertility records system so Peripartum disease information was recorded in on-farm data sheets, veterinary records, and on-farm computer record systems & Reproductive data including DIM at first insemination, conception date, number of inseminations, herd removal date, and pregnancy status at removal, were recorded using the same methods.

Similar calving management program was performed in all herds. The study was conducted from June – August 2010. The blood samples were taken during 2-4 wks postpartum. To avoid the postprandial effects , all samples were taken 4 hours after feeding. The blood was drawn from coccygeal vein , transferred on ice to laboratory . Samples were centrifuged at 2000g for 15 min & harvested serum were kept at -20 until analysis . BHBA was determined by BHB dehydrogenase (Ranbut, Randox , Uk) , Glucose was determined by glucose oxidase method (Glucose , Pars Azmun , Iran) . A semi automated photometer (RIELE 5010, Germany) was used for photometric methods. All statistical analyses were performed using Analyse it® software. Both serum BHB & glucose concentrations were normally distributed; therefore, data from each parameter were expressed as mean±SD . In order to evaluate the correlation between BHBA & glucose level , Pearson correlation (r) was determined . To compare blood glucose between ketotic and non ketotic cows , independent sample t test was performed. In order to evaluate the validity of test, sensitivity, specificity, positive and negative predictive values were determined in various cut off points.

RESULTS

The distribution of BHB concentration & lactation number in 190 cows are shown in Figure 1 . The mean±SD of serum BHB and glucose concentrations in cows with and without SCK is shown in Table 1. Blood Glucose concentration in subclinical ketotic cows were significantly lower ($p \leq 0.05$) than in non ketotic cows . The coefficient correlation between BHB & glucose is shown in Fig-2. The concentrations of BHB and glucose in serum were significantly ($p \leq 0.01$) and inversely correlated ($r = -0.457$; $P \leq 0.05$) in the tested cows. Specificity & sensitivity of blood glucose level in various cut-off point (30mg/dl,32mg/dl,35mg/dl) were determined . If 1400 µmol/L were assumed as threshold , sensitivity & specificity in cut-off point of 30 mg/dl , 32 mg/dl & 35 mg/dl were 95.6 %, 91 % , 83.5 % (sensitivity) & 72.55 , 78.4%, 80.5% (specificity) respectively . If 1200 µmol/L were assumed as threshold , sensitivity & specificity in cut-off point 30mg/dl,32mg/dl,35mg/dl were 95.4% , 80.6%,84.8% (sensitivity) & 68.6%, 74.4%, 72.5%(specificity) respectively .

Table 1: The mean±SD of serum BHB and glucose concentrations in cows with and without SCK

	Subclinically Ketotic 1400 µmol/L (n= 29)	Non ketotic < 1400 µmol/L (n= 161)	All cows (n= 190)
	Mean±SD	Mean±SD	Mean±SD
BHB (µmol/L)	2.4±0.42	0.637±0.08	0.982±0.07
Glucose (mmol/L)	1.70± 0.08	2.42±0.04	2.30±0.09

Table -2: Apparent & true prevalence of SCK in various cut off points of blood glucose levels

Cut off point	Apparent prevalence (%)	True Prevalence(%)
30 mg/dl (1200 µmol/L)	18.42	28.28
30 mg/dl (1400 µmol/L)	15.26	22.19
32 mg/dl (1200 µmol/L)	18.42	33
32 mg/dl (1400 µmol/L)	15.26	21.79
35 mg/dl (1200 µmol/L)	18.42	31.8
35 mg/dl (1400 µmol/L)	15.26	23.2

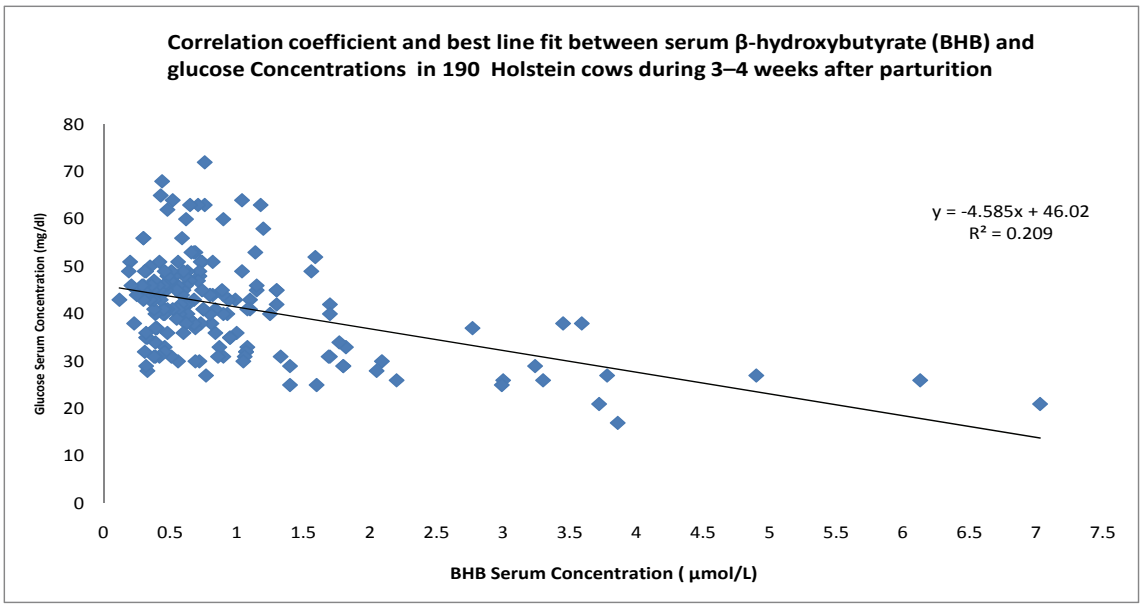

Figure 2: Correlation coefficient and best line fit between serum β-hydroxybutyrate (BHB) and glucose Concentrations in 190 Holstein cows during 3–4 weeks after parturition

DISCUSSION

The gold standard test for subclinical ketosis is blood β-Hydroxy Butyrate (BHB) which is more stable ketone body than acetone or acetoacetate. This method is expensive , time consuming & required equipments so a reliable , precise , fast and economic method should be used. There are various in farm tests for screening subclinical ketosis in dairy herds. Semi-quantitative milk tests such as keto test are approved and used commonly for monitoring energy balance during transition period . These methods are simple but there are not available in many developing countries. To our knowledge there are not any published document that evaluate blood glucose level as a screening test for detecting subclinical ketosis. The major cause of variation in blood glucose may be the major fluctuations in daily feed intake. Investigations of feed intake of dairy cows on commercial farms have shown that concentrate dispensers are commonly incorrectly adjusted and errors of more than 50% in feed intake are sometimes found. In situations of marginal energy imbalance, blood glucose concentration levels may be unreliable as an index of the adequacy of energy intake. here is some conflicting evidence about the relationship between mean values of blood glucose of a lactational group and insufficient energy intake and reproductive inefficiency. In some work, there is an expected relationship between low blood glucose and an increased incidence of ketosis. In others, the relationship is not dear, however there was a more consistent relationship between the actual energy intake as a percentage of requirement and the plasma non-esterified fatty acids, but this finding was not sufficiently reliable to be useful[veterinary Medicine] .The results of this study showed that there is a weak correlation between BHB and glucose level in subclinical ketotic cows and blood glucose levels is not a precise and reliable index for evaluating SCK In dairy herds.

CONCLUSION

The result of this study showed that blood glucose determination is a sensitive test with low specificity .

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A STUDY CONCERNING THE DYNAMIC OF AMYLASE AND LIPASE ACTIVITY FROM PANCREATIC TISSUE IN DIFFERENT SPECIES OF BIRDS (Abstract)

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ABSTRACT

Dosing and interpreting some enzymes in the blood serum (plasma) has always been a current methodology with a great value and clinical signification. The enzymes actively secreted in the blood by the exocrine glands, considered as *markers* of a good pancreatic functioning, the amylase and the lipase, have the property to metabolise the nutrients, facilitating their digestion and absorbtion. Their presence in big concentrations in the plasma or blood serum represents the consequence of the permeability changes of the cell membranes, changes that occur because of some affections that involve a cell damage. 12 samples of frozen pancreatic tissue and 15 samples of raw pancreatic tissue from 4 species of birds (pullets, ducks, turkeys, geese) were analysed. A more intense release of the enzymes in the plasma corelates with processes of cytolysis, as a reflection of some pathological events accompanied by the growth in the membrane permeability. Generally, during the *cytolysis*, the enzymes of an organ are released in the blood, their detection in the blood serum reflecting an „*enzymatic spectrum*" of the affected organ, as well as the intensity of the cell permeability changes in direct relationship with the speed of enzymes that pass from the damaged cell into the blood. The mal-digestion and mal-absorbtion of the nutritive principles represent the consequence of the pancreatic affections, being noticed a direct relationship between the cell lesion and the growth in activity of the enzymes in the plasma or serum. One of the objectives of this study is getting the most accurate information for diagnosis and prognosis by determining the enzymatic activity which has an important clinical significance for establishing a differential diagnosis or the evolution of a disease.

COMPARISON OF THE ANALGESIC EFFECT OF ELECTEOACUPUNCTURE AND TRAMADOLE ON VISCERAL PAIN IN RAT (Abstract)

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INTRODUCTION:

Since the use of electroacupuncture (EA) may be suitable for animals in veterinary medicine, effect of this technique on control of visceral pain was compared to tramadol in present study. Acetic acid was intraperitoneally injected in 3 groups of rats (10 rat each group) and writhing was countered every minutes until 40 minutes after acetic acid injection. First group was kept as control.

ANIMALS, MATERIALS AND METHODS

Tramadol was administered at dose 10mg/kg intramuscularly in group 2 and rats of group 3 received EA and ST-36 (Zu San Li) and SP-6 (San Yin Jiao) points were stimulated at 1ms, 0.3mA and 0.3Hz frequency, intensity and duration respectively. The amount of serum cortisol was measured by ELISA kit at end of study.

RESULTS

The results shown the mean of writhing was significantly decreased in group 2 and 3 with comparison to group 1. The mean (± SE) writhing was 22.3± 3.67, 3.4 ± 1.13 and 3.6 ± 0.8 per 10 minutes respectively. The mean of serum cortisol was greater in negative control group than other groups (1.36 ±0.04 ng/ml). This mean was significantly was lesser in group 2 and 3 in comparision to control group 1. In other hands, the mean (± SE) cortisol was 0.4 ± 0.02, 0.7 ± 0.04 and 1.16 ± 0.07 ng/ml in groups 1, 2 and 3 respectively.

CONCLUSIONS

Since corticosteron is major corticosteroid in rats and the precursor of corticosteron and cortisol is same, thus the amount of cortisol is decreased by elevation of corticosteron synthesis during stress and pain. Present study shown tramadol and EA has similar analgesic effect on writhing test in rats**.**

OCCURRENCE OF PULMONARY EMPHYSEMA (PE) IN SHEEP OF ANIMAL RESEARCH INSTITUTE

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SUMMARY

Pulmonary emphysema (PE) is an incurable condition in which alveoli and the bronchioles are irreversibly destroyed. For diagnosis, the affected lungs were sent to pathology laboratory and the disease was diagnosed as PE. Every year around 10% of sheep of this institute according to different causes such as infectious and metabolic diseases, physical injuries, surplus lambs and ewes is slaughtered (culled).Nine cases were diagnosed as PE which was 6.8 % of all cases of pneumonia and 1.3 % of all inspected carcasses.

INTRODUCTION

This disease causes stress on heart and makes it work harder to pump blood into the lungs (Wise and Tashkin, 2007). The disease causes permanent holes in the lungs and the airways become obstructed. It causes great difficulty for breathing and generally impaired functioning in animal's entire body. It is an irreversible process and once lung cells are affected, there is no way to rehabilitate them. It causes air to get trapped in the lungs and blow up like a balloon and getting space for healthy lung tissue to work (Judd *et al*., 1993).

MATERIAL AND METHODS

A sheep flock in Animal Science Research Institute was evaluated. For diagnosis of emphysema in the flock, the adult sheep were tested periodically. Those which suffer from pulmonary distress such as difficult breathing, tachypenia, abdominal breathings and emaciation were examined thoroughly.

RESULTS

Among total number of 650 sheep slaughtered for different reasons during 5 years from 2002 to 2007, 132 sheep were affected with different types of pneumonia (20.3%). Nine cases were diagnosed to have emphysema, which was 6.8 % of all cases of pneumonia and 1.3 % of all inspected carcasses.

DISCUSSION

The cause of disease can be the high level of L-tryptophan in crops that most likely can be high in lush, rapidly growing pastures, particularly (but not exclusively) in the fall (Breez, 1975).

CONCLUSIONS

One approach is to control dietary management including avoiding growing lush pastures in fall, feeding hay before turn out on pasture and limiting exposure time on suspect pastures, limiting grazing time and gradually increasing exposure to the pasture daily and using pastures before they become lush (Ohfuji, 1993).

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DOES TEAT POSITION INFLUENCE TRAUMATISATION OF MAMMARY GLAND IN MACHINE MILKED EWES?

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SUMMARY

The aim of this work was to find the influence of horizontal and vertical teat position of ewes on traumatisation of mammary gland in the process of milking. The measurements took place in two breeds of sheep (East Friesian sheep - EF, crossbreed Sumavska sheep and Lacaune - SxLC). The surface temperature of the udder of 20 ewes with teats positioned horizontally and 20 ewes with teats positioned vertically was monitored by thermographic method before and after milking in the middle lactation period. Temperature records were evaluated by computer program TermaCAM Reporter 2000 and the data were statistically analysed by the Statistica software. The surface temperature of udders with horizontally situated teats decreased by -1.53 K in sheep SxLC, and by -0.55 K in EF sheep after milking, the surface temperature of udders with vertically situated teats decreased by -1.56 K in SxLC and by -0.16 K in EF sheep. Significant difference ($p<0.01$) in the surface temperature measured after milking was proved between udders with vertically situated teats in comparison to udders with horizontally situated teats only in the EF sheep. Statistically significant traumatisation of the mammary gland was found in East Friesian ewes with vertically situated teats of mammary glands in comparison to the mammary glands with horizontally situated teats.

INTRODUCTION

At the same time increasing requirements for quality livestock products also increase the demands on breeding environment, animal welfare, as well as on milking process itself. Poor welfare in dairy sheep has a number of deleterious effects on the quality of ovine milk and cheese [1]. Milking characteristics and udder morphology are one of the factors determining milkability in dairy sheep [2] and the work routine during milking [3]. The teats are the most stressed part of the udder because they come into direct contact with the milking machine [4] therefore differences in milking techniques influence the reaction of teats on milking and the rate of recovery after milking [5]. The inadequate milking procedures (poorly designed or managed milking equipment) lead to teat injuries, pain and udder diseases in dairy animal [6] and result in reduced level of welfare. Teat position (angle) of ewes is very important parameter in term of machine milking, which influences the process of milking and welfare of ewes during milking. The ideal teat position of udder of ewes is vertical which enables the milk removal without any functional inhibitions. Horizontal teats are also more susceptible to distortion during machine milking. The milk flow is thus arrested. This inhibits the ejection reflex and thus increases alveolar milk retention [3].

The aim of this work was to find the influence of horizontal and vertical teat position of ewes on traumatisation of mammary gland in the process of milking.

MATERIAL AND METHODS

Udder morphology traits were measured in two flocks of purebred East Friesian - EF (farm I., n=103 ewes), Sumavska sheep and their crosses with Lacaune - SxLC (farm II., n=239 ewes) in the middle lactation period (102nd-134th day after lambing). Udder measurements of four traits were performed by one technician and they included udder length, udder width, teat length and teat angle from the vertical. Based on measurements of the teat angles there were selected 20 ewes of EF sheep and 20 of crossbreed SxLC sheep for the experiment. First group (H) had 10 ewes with teats positioned horizontally (from 55° to 70°), the other group (V) had 10 ewes with teats positioned vertically (from 20° to 40°). All ewes were clinically healthy during the same milking period and they were not pregnant during the experimental period. There were milked in the milking parlours with dosing of the concentrate mixture from the tank at the beginning of milking. Parameters of milking machines on both farms were following: the vacuum level – 40.0 kPa, level of pulsation rate 150 pulses per minute, pulsation ratio (intake and press) 50:50. The measurements of surface temperature took place once during a period of lactation (102nd-134th day after lambing), always on two consecutive days and included 2 morning and 2 evening milkings from each farm. The surface temperature of the sheep udder was monitored by thermographic method (thermo camera Flir P 45) before and after milking. The length of milking and the climatic conditions in the place of milking were measured simultaneously. Temperature records were evaluated by computer program TermaCAM Reporter 2000 and the data were statistically analysed by Statistica software (analysis of covariance).

RESULTS

Crossbreed SxLC with horizontal teats had significantly ($p<0.05$) higher angle of the teat than EF sheep (Table 1). The significantly ($p<0.01$) shorter teats (by 10.1 mm – horizontal teats, by 7.6 mm – vertical teats) were found in crossbreed SxLC in contrast to EF sheep. The differences in udder morphological traits between EF sheep with horizontal and vertical positions of the teats were not statistically significant while the udder length in crossbreed SxLC with vertical teats was found significantly lower ($p<0.05$) by 21.1 mm in comparison to crossbreed with horizontal teats.

EF sheep with horizontal teat position had lower milk yield per milking (374 ml) than EF sheep with vertical teat position (528 ml) while crossbreed SxLC with horizontal teats gave higher milk yield per milking (441 ml) compared to crossbreed with vertical teats (374 ml). These differences were below the level of statistical significance.

Table 1: Udder morphology traits in ewes with extreme positions of the teats

Parameter		Breed			
		EF		SxLC	
Teat position		Horizontal	Vertical	Horizontal	Vertical
Teat angle	[°]	56±3 [a]	34±7	62±4 [a]	39±2
Udder length	[mm]	156.7±22.8	154.0±17.3	157.5±24.0 [A]	136.4±13.6 [A]
Udder width	[mm]	133.3±20.2	139.0±15.2	133.9±10.4	134.1±13.4
Teat length	[mm]	32.2±4.4 [b]	34.0±3.9 [c]	22.1±3.2 [b]	26.4±6.0 [c]

[A] Means followed by different letters are significantly different at ($p<0.05$); [a,b,c] ($p<0.01$)

The surface temperature of the teats before milking was significantly ($p<0.01$) higher in EF sheep with horizontal teats than in EF sheep with vertical teats (Table 1). The significantly higher ($p<0.01$) surface temperature of udder was detected in EF sheep with horizontal teat position before milking compared to temperature measured after milking. Likewise, the surface temperature of udder in SxLC with horizontal and vertical teats was found to be statistically significantly ($p<0.01$) higher before milking compared to the temperature state after milking. In EF sheep with horizontal teats the surface temperature of udder was significantly ($p<0.01$) lower than in sheep with vertical teats after milking.

Table 2: The surface temperature of udder of ewes depending on the teat position

The measuring point	Breed	Teat position	Surface temperature [°C]		Temperature differences before and after milking [K]
			before milking	after milking	
Teat	EF	H	32.29±2.458 [A]	31.70±0.953	-0.59 [a]
		V	31.22±2.444 [A]	31.82±0.932	+0.60 [a]
	SxLC	H	28.89±3.234	28.39±1.680	-0.50
		V	28.48±4.208	29.08±1.855	+0.60
Udder	EF	H	33.72±0.779 [b]	33.17±0.542 [b,c]	-0.55 [f]
		V	33.73±0.558	33.57±0.713 [c]	-0.16 [f]
	SxLC	H	31.67±2.001 [d]	30.14±1.599 [d]	-1.53
		V	32.35±1.780 [e]	30.79±1.823 [e]	-1.56

[A] Means followed by different letters are significantly different at ($p<0.05$); [a,b,c,d,e,f] ($p<0.01$)

The length of milking in EF sheep with horizontally situated teats was shorter (73 ± 24 seconds) than in EF sheep with vertical teats (82 ± 27 seconds). In contrast crossbreed SxLC with horizontal teat position were milked longer time (89 ± 47 seconds) in comparison to crossbreed with vertical teats (52 ± 2 seconds).

The surface temperature of horizontally situated teats decreased (by -0.50 K in SxLC, by -0.59 K in EF sheep) after milking whereas the surface temperature of vertical teats increased (by +0.60 K in SxLC, by +0.60 K in EF sheep). The surface temperature of udders with horizontally situated teats decreased by -1.53 in sheep SxLC, and by -0.55 K in EF sheep after milking, the surface temperature of udders with vertically situated teats decreased by -1.56 K in SxLC and by -0.16 K in EF sheep. Significant difference ($p<0.01$) in the surface temperature measured after milking was proved between udders with vertically situated teats in comparison to udders with horizontally situated teats only in the EF sheep (Table 2).

DISCUSSION

Skin temperature is used as a parameter that relates to the intensity of the circulation of blood [7]. An increase of udder temperature is an important indicator of mastitis [8]. Studies [9, 10] carried out in dairy cows, showed that conventional milking machines may cause an increase of teat–end temperature by 2 °C. In our work there was found significantly higher temperature of horizontally situated teats of EF sheep even before milking when compared to sheep with vertically situated teats. This can be explained by higher level of mechanical stress as a

result of higher rubbing of these teats against hind legs when walking. The width of udder including teats was 197.7 mm in EF sheep with horizontal teats and 139.0 mm in EF sheep with vertical teats (178.1 mm in SxLC with horizontal teats and 134.1 mm in SxLC with vertical teats). In accordance with the results of Stellett et al. [11] this work showed lower temperatures of udder in EF ewes and as well in crossbreeds SxLC after milking. The decrease of the udder temperature immediately after milking can be explained by both shorter duration of milking and related shorter period of udder load in comparison to dairy cows and the evaporation of water from the udder after the wet toilet of udder. During ewe milking the weight of the claw can provoke a painful twist at the base of the most inclined teats [3] and this can cause decrease of milk yield. Significantly lower temperatures of udders in EF with horizontally positioned teats was caused by bad adjustment of the teat cups to the teats and subsequent air intake and more frequent falling down of the milking set. On the contrary in vertically positioned teats there were found similar results like in the work of Vegricht et al. who stated that the average temperature of the teat tip after milking increased [10]. Teats, which are placed too horizontally or too vertically can cause problems both when being looked for by lambs immediately after parturition and at suckling itself [12].

CONCLUSIONS

The angle is one of the morphological traits of udder which is suitable to be included among selection markers. The change of the teat surface temperature during ewe milking can be used as a parameter of evaluation of level of welfare in sheep breeds. Statistically significant traumatisation of the mammary gland was found in East Friesian ewes with vertically situated teats of mammary glands in comparison to the mammary glands with horizontally situated teats.

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BETA-CAROTENE AND VITAMIN A CONTENT OF SERUM OF DROMEDARY CAMEL IN YAZD PROVINCE (IRAN)

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SUMMARY

In the present study, the values of beta- carotene and vitamin A in serum of dromedary camel (*Camelus dromedarius*) was investigated in Yazd (Iran). The blood samples from one hundred sixty-eight camels were randomly collected in Yazd slaughterhouse and this province camle breeding farms, from February 2009 to July 2010. Spectrophotometery was used for measuring of beta-carotene and vitamin A levels. The results were analyzed statistically with multifactorial repeated measures (ANOVA). Results showed that the mean values of serum beta- carotene and vitamin A were 8.9 µg/dl and 63. 9 µg/dl, respectively. There wasn't significant difference in serum beta-carotene and vitamin A between two sexes. Although, there was significant difference in serum beta-carotene between hot (summer) and cold (winter) seasons, the levels of serum vitamin A didn't show significant difference between these seasons.The results of this study showed that vitamin A content of serum of dromedary camel in Yazd province is similar to normal ranges of vitamin A in cattle but the value of beta-carotene is very lower than its normal range in cattle.

INTRODUCTION

Vitamin A (retinol) is one of the most important fat-soluble vitamins and is found in green plants and can be synthesizes by the small intestinal mucosal cells and the liver from plant carotenoid precursors.[4] Because of vitamin A particular roles in different tissues and organs, in deficiency conditions various clinical signs are seen. In addition, sometimes the marginal deficiency is present and although, clinical signs are not visible but performance defects such as infertility is seen. Vitamin A deficiency may be primary disease,due to an absolute deficiency of vitamin A or its precursor carotene in the diet, or a secondary disease in which the dietary supply of the vitamin or its precursor is adequate, but their digestion,absorption, or metabolism is interfered with to produce a deficiency at the tissue levels. Primary vitamin deficiency is of major economic importance in groups of young animals on pasture or fed diets deficient in the vitamin or its precursor[3]. Some usual dietary or management conditions that favor the vitamin deficiency are gazing on dry pastures or cereal grains other than corn, exclusive feeding of grains that have been stored at high temperature and humidity.[4].

In large animals, most studies about beta-carotene and vitamin A is done in cattle and information about other animals such as camel is rare. In some regions of Iran, dromedary camel(*Camelus dromedarius*) is breeding for production of meat. In the present study the values of beta- carotene and vitamin A in serum of dromedary camel was investigated in Yazd.

MATERIAL AND METHODS

The blood samples were collected from one hundred sixty-eight camels (male: 129 and female 39) that were randomly selected at Yazd slaughterhouse and some camel breeding farms in this province from February 2009 to July 2010. The samples were obtained from jagular veins and were collected in tubes and placed on ice in the dark. A spectrophotometery method was used for measuring of beta-carotene and vitamin A levels [5]. The results were analyzed statistically with multifactorial repeated measures (ANOVA).

RESULTS

Results showed that the values of mean ± SE of serum beta- carotene and vitamin A were 8.9 ±1.1 µg/dl and 63. 9 ± 4.7 µg/dl, respectively.The mean ± SE values of beta-carotene and vitamin A in male camels were 9.3 ± 1.4 µg/dl and 63.6 ± 5.8 µg/dl,respectively. In female camels, the mean ± SE values of beta-carotene and vitamin A were 7.3 ± 1.7 µg/dl and 64.9 ± 6.9 µg/dl, respectively. There wasn't significant difference in serum beta-carotene and vitamin A between two sexes. The mean ± SE values of beta-carotene and vitamin A in

summer were 12.9 ± 2.1 µg/dl and 86.6 ± 5.6 µg/dl, respectively. In winter, the mean ± SE values of beta-carotene and vitamin A were 5.1 ± 0.8 µg/dl and 42.8 ± 6.8 µg/dl, respectively. . Although, There was significant difference in serum beta-carotene between hot (summer) and cold (winter) seasons, the levels of serum vitamin A didn't show significant difference between two seasons.

DISCUSSION

The determination of normal values of serum beta-carotene and vitamin A in camel, is essential for diagnosis of deficiencies in this animal. Unfortunately, studies are rare about this subject. In two available published investigations, the mean of serum beta-carotene were 21.5 µg/dl [1] and, and the mean of serum vitamin A were 173 µg/dl [1] and 479.5 ± 69.4 ng/L [3].

Dietary and management conditions are the most important factors that influence the levels of beta-carotene and vitamin A in body. Serum beta-carotene levels vary largely with diet, but the serum vitamin A levels do not commence to fall until the hepatic stores are depleted [3].It seems, this is the reason that why there was significant difference between beta-carotene values of serum in summer and winter and there was not significant changes between serum vitamin A values of serum in these seasons. In this study, there was not significant difference between beta-carotene and vitamin A values of the serum in two sexes. This showed that in studied camels, the dietary management same in two sexes. It is said that females are slightly more resistant to the vitamin deficiency than males, presumably because of the interconversions of estrogenic hormones into vitamin A.[4]

CONCLUSIONS

The results of this study showed that vitamin A content of serum of dromedary camel in Yazd province is similar to normal ranges of vitamin A in cattle (25-85 µg/dl) but the value of beta-carotene is very lower than its normal range in cattle (150-397 µg/dl) .

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FIELD STUDY FOR EVALUATION OF TREATED WASTE WATER IN MILKING GOAT FARM (Abstract)

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INTRODUCTION

The process of re-used waste water recycling is connected with risk of spreading pathogens to animals and human if has been involved. The zoonotic infection with Bacterial, viral, fungal and parasitic agents can be occur. The present study aims to investigate upon the safety of treated waste water (Tww) reuse in milking goat farm regarding breeding and water supply for drinking and irrigation of green fodder.

ANIMALS, MATERIALS AND METHODS

2.1. Treated waste water final effluent: A total of 486 water samples were obtained from Helwan Treatment Plant, south Cairo. The Tww samples were subjected to chemical, bacteriological and parasitological examinations.

2.2. The field experiment: It was carried out on goat farm and extended for six months.Twenty-seven, 8-12 years old milking goats were selected from the herd and supplied with Tww for drinking and irrigation. Fecal examination and blood analysis were done to animals, beside bacteriological examination to the harvested milk during the period of experiment.

RESULTS

The analysis of Tww revealed the presence of *Fe,Cu,Zn,Mn,Co* and *Pb* with the acceptable limits. The bacteriological examination of Tww revealed decreases in heterophic plate count, total count, fecal count and fecal strept count. The parasitological examination showed *Ascaris,Trichostrongylus* eggs,*Coccidian* and *Cryptosporidium* oocysts.The Tww had no significant adverse effect on fertility, pregnancy and birth. The bacteriological examination of milk was free from *T. B* and *Brucella* microorganisms.The average daily milk yield was slightly decreased with no changes in its normal constituents and the udder was healthy and normal in size[no mastitis].The blood profiles of goat were within the normal levels except, there was leuckocytosis[9.11x10 with neutrophilia,$4.5x10^3$ and eosinophilia,$1.1x10^3$].

CONCLUSIONS

Our results showed succeeding in use of Tww in milking goat farm breeding without changes in behaviour,health status, physiology and reproductive patterns. It was concluded that the use of Tww in goat farms must be done under health precautions and the sewage treatment plant must be with high technology of performance.

EFFICACY ASSESSMENT OF TEAT DISINFECTION IN LACTATING SOWS

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SUMMARY

This paper describes investigation of introduction of teat disinfection in lactating sows in order to reduce piglet mortality due to alimentary infections. The investigation was carried out at pig-breeding farm and included 50 sows devided in 2 equal groups. Group 1 served as control and in group 2 teats of the sows were daily treated with commercial antiseptic. The diagnosis of piglet death due to alimentary infections was based on post-mortem analysis. Piglet death percentage due to alimentary infections was lower in experimental group compared to the control. The results demonstrated that teat disinfection in lactating sows may serve as efficient biosecurity measure with the aim of reduction and prevention of piglet death due to alimentary infections.

INTRODUCTION

Suckling pig mortality presents important economic and welfare issue. Along with starvation and crushing by the sow, alimentary infections are major contributor to piglet mortality [1, 2]. Moreover, more than 40% of total piglet losses may be ascribed to alimentary infections [3]. In intensive pig production farrowing crate is still the prevalent form of housing used for the sows and their litters during lactation period [4], whereat teats of the sows are exposed to numerous microbes accumulated on the floor of the farrowing crate. This makes predisposition for the piglet alimentary infections, because various microbes through contaminated teats may enter into piglet alimentary system. Taking into account that usual hygiene of the farrowing crate, by washing with disinfection solution under pressure, is not performed during animals' stay in the farrowing crate due to sensitive piglets, it is necessary to look for other effective procedures to reduce microbes on the sow teats, what should consequently reduce the piglet mortality due to alimentary infections. The aim of the investigation was to determine whether and to what extent the teat disinfection in lactating sows may reduce suckling pig mortality caused by the alimentary infections.

MATERIAL AND METHODS

The investigation was carried out at pig-breeding farm with high incidence of suckling pig mortality due to alimentary infections. It included 50 sows of the same breeding lot, each with 10 piglets on an average, during 28 days averagely of their stay in the farrowing unit. The sows were devided in 2 groups. Group 1 served as control and was left untreated. In group 2 sow teats were daily treated with commercial antiseptic with potent germicid action. The teat disinfection was done using disposable napkin soaked in antiseptic. The diagnosis of piglet death due to alimentary infections was based on post-mortem analysis, and colibacilosis was found in most cases**.** Statistical analysis was performed using the Statistica 9.0 (Statsoft Inc., 2011) statistical software and methods of Mann-Whitney U-test.

RESULTS

Piglet death percentage due to alimentary infections was lower in the experimental group (median value = 0) compared to the control (median value = 1) although this difference was not statistically significant ($P>0.05$). It is also noticeable that the percentage of the farrowing crates with dead piglets was lower in the experimental group (48%) compared to the control (72%) (Figure 1).

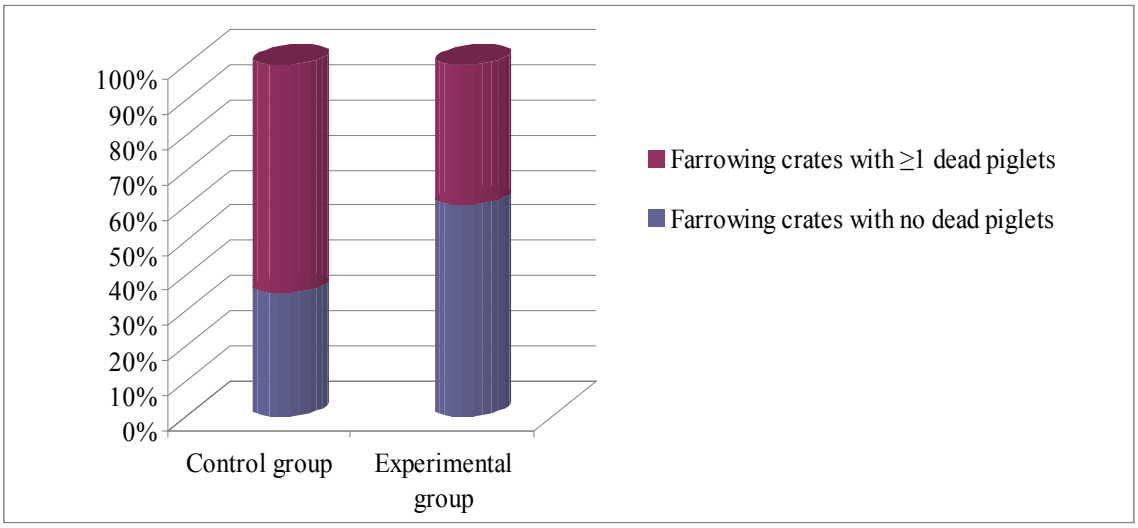

Figure 1: Percentage of farrowing crates with dead piglets due to alimentary infections in control and experimental group

DISCUSSION

Udder hygiene in dairy farming is a rutine procedure before and after milking in order to reduce post-secretion contamination of fresh milk and to prevent mastitis. It is performed by dipping the teats into antiseptic on the basis of the active foam, by wiping the udder with disposable napkin soaked into the antiseptic or with incorporated antiseptic, or by washing the udder in disinfection solution, what depends on the level of the udder contamination [5]. The described procedure of the teat sanitation could also be the method of choice in the pig production. Previous investigations showed the teat disinfection in lactating sows to reduce the bacterial counts on the teats [2, 6], however it was necessary to assess to what extent it may affect the piglet mortality due to alimentary infections. According to this investigation it appears that teat sanitation in lactating sows may reduce the piglet losses. This confirms the result that in the experimental group, where teats of the sows were treated with antiseptic**,** lower percentage of dead piglets, in other words the farrowing crates with dead piglets, was found in relation to the control group (Figure 1).

CONCLUSIONS

The investigation demonstrated the introduction of teat disinfection in lactating sows as effective biosecurity measure to reduce and prevent piglet death caused by alimentary infections.

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THE USE OF ESSENTIAL OILS TO IMPROVE OF ENVIRONMENT QUALITY IN POULTRY HOUSES

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SUMMARY

The purpose of this study was to evaluate the effect of essential oils to improve of environment quality in henhouse. Two essential oils (*PINUS SILVESTRIS L.* (pine) and *EUCALYPTUS POLYBRETEA L.* (eucalyptus)) and their combinations were used to improve the air quality in the experimental henhouse. The henhouse is 2.97 x 5.02 in size, 2.50 m in height. Hens were kept in battery cages. Essential oils (1:500) were evaporated and were sprayed to air of henhouse. In the henhouse temperature was 17-20°C, relative humidity ranged between 40-50%, the airflow – between 0.08-0.09 m/s during experiment. Positive effect for total bacterial (3.5-50.0%) and fungi (49.6-61.3%) counts in air of henhouse was essential oil *PINUS SILVESTRIS L.* (pine). Essential oil of pine had no effect on ammonia in air. Primary results of our study show that: the best characterized effect for air quality of henhouse had of combination of essential oils *PINUS SILVESTRIS L.* (pine) and *EUCALYPTUS POLYBRETEA L.* (eucalyptus).

INTRODUCTION

European consumers show growing concerns regarding the welfare of domestic animals. Many health problems are likely related to high levels of ammonia, dust and airborne microorganisms in poultry houses [8]. A main clinical symptom found in poultry exposed to ammonia is keratoconjunctivitis [4].

High dust concentration is another major problem in poultry houses. The dust particles originate from poultry feed and litter material, poultries (feathers, skin scales and faeces), soil, microorganisms (bacteria and fungi), insects, and mites [1; 5]. Dust in poultry houses is mainly of organic origin, and components of the dust can be biologically active and cause hypersensitivity reactions. Airborne microorganisms are adsorbed on dust particle smaller than 5 μm in diameter, inhaled by respiration. Many particles are antigenic and can activate the innate and the adaptive immune systems, causing inflammation. Bacterial endotoxin, a potent non-specific immunostimulant, is an important pathogenic agent in animal houses. Thus, to alleviate the potential for farmers to be exposed to dust and bioaerosols, it is essentially important to control and manage the air quality in the poultry houses [4; 6].

For this reason, there exist several methods for air disinfection such as air filtration, air washing or regular water spraying to reduce the concentration of airborne particle. But since the application of disinfectant agents is restricted when living animals may be involved especially in case they are for food production it seems to be necessary to investigate new, eco-friendly, unobjectionable substances with a potential for air-disinfection.

The purpose of this study was to evaluate the effect of essential oils to improve of environment quality in henhouse.

MATERIAL AND METHODS

Two essential oils (*PINUS SILVESTRIS L.* (pine) and *EUCALYPTUS POLYBRETEA L.* (eucalyptus)) and their combinations were used to improve the air quality in the experimental henhouse of Veterinary Academy. The henhouse is 2.97 x 5.02 in size, 2.50 m in height. Hens were kept in battery cages. The concentration of essential oils and their combinations were 1:500. Essential oils were evaporated and were sprayed to air of henhouse. The evaporation of essential oil was started at 8 o'clock in the morning and steaming for eight hours. The essential oil was spraying intervals every three hours.

Bacterial counts and viable spores of fungi in air samples were determined sedimentation method on a commercial nutrient agar (Biolife, Milan, Italy). The medium was incubated for 24 hours in an incubator at 37°C. The grown colonies (CFU/m^3) were counted. Viable spores of fungi in air samples were determined sedimentation method on a standard agar Czapek-Dox (Oxoid) supplied with chloramphenicol (50 mg/l) (Sigma). Petri dishes with medium were incubated for 7-8 days at 26±2ºC, viable spores of fungi in air samples were counted CFU/m^3.

Air temperature (°C), relative humidity (%) and airflow rate (m/s) were determined with a TST device (Testo Inc., Germany). The concentration of ammonia was determined with a Dräger-Multiwarn II device (Dräger, Darmstadt, Germany). All data were analyzed with "SPSS for Windows", version 12.0.

RESULTS AND DISCUSSION

Indoor air quality in animal housings can be influenced by many factors, such as air temperature, humidity, ventilation rate and type, type and amount of feed provided, type of feed delivery system, type of floors and litter used and type and activity of the animals [9]. Dust concentration depends also on air distribution, relative location to the dust sources, and occupants' activity levels in the building. Hartung (2007) has estimated that about 80-90% of the dust in animal houses comes from feed, 2-12% from animals, 2-8% from manure, and a certain portion from bedding materials.

Gram-negative bacteria such as *Klebsiella, Pseudomonas*, *Escherichia coli, Salmonella or Shigella* species and other leading pathogens are ubiquitous, i.e. omnipresent in our immediate environment [2]. As important decomposers, filamentous fungi and their air-borne spores are ubiquitous in nature. Most of the prevalent fungal species found outdoors and indoors belong to *Alternaria*, *Aspergillus, Cladosporium* and *Penicillium* species [3].

During experiment temperature was 17-20°C, relative humidity ranged between 40-50%, the airflow – between 0.08-0.09 m/s in the henhouse.

Effect of with different essential oils *PINUS SILVESTRIS L.* (pine) and *EUCALYPTUS POLYBRETEA L.* (eucalyptus) are summarized in Table 1.

Table1. Essential oils *PINUS SILVESTRIS L.* (pine) and *EUCALYPTUS POLYBRETEA L.* (eucalyptus) effect for ammonia, total bacterial count, fungi count in air of henhouse

Effect %	Air samples collection time after evaporation				
	hour	3 hours	5 hours	7 hours	24 hours
Essential oil *PINUS SILVESTRIS* L.					
NH_3 concentration	12.9	103.1*	107.2*	117.0*	117.0*
Bacterial count	39.9	50.0	46.4	3.5	20.3
Fungi count	49.7	61.3	58.9	49.6	58.9
Essential oil *EUCALYPTUS POLYBRETEA* L.					
NH_3 concentration	111.7*	104.4*	101.9*	115.8*	115.0*
Bacterial count	120.0*	26.8	0.2	8.9	9.8
Fungi count	185.3*	102.7*	162.9*	111.2*	138.8*

* did no effect

Positive effect for total bacterial and fungi counts in air of henhouse was essential oil *PINUS SILVESTRIS L.* (pine). Essential oil had no effect on ammonia in air.

In table 2 are summarized results effect of combination essential oils *PINUS SILVESTRIS L.* (pine) and *EUCALYPTUS POLYBRETEA L.* (eucalyptus).

Table 2. Combination of essential oils *PINUS SILVESTRIS L.* (pine) and *EUCALYPTUS POLYBRETEA L.* (eucalyptus) effect for ammonia, total bacterial count, fungi count in air of henhouse

Effect %	Air samples collection time after evaporation				
	hour	3 hours	5 hours	7 hours	24 hours
NH_3 concentration	9.8	15.9	0.0	108.9*	6.4
Bacterial count	105.0*	103.0*	10.7	0.8	10.0
Fungi count	163.0*	137.0*	168.0*	120.0*	16.9
	Air samples collection time after spraying				
	hour	3 hours	5 hours	7 hours	24 hours
NH_3 concentration	13.0	13.0	103.5*	11.6	15.2
Bacterial count	39.7	43.0	49.4	32.3	20.8
Fungi count	28.1	48,9	39.0	17.4	40.9

* did no effect

Positive effect was the spraying of combination of essential oils *PINUS SILVESTRIS L.* (pine) and *EUCALYPTUS POLYBRETEA L.* (eucalyptus) for ammonia (11.6-15.2%) total bacterial count (20.8-49.4%), fungi count (17.4-28.1%) in air of henhouse.

Ventilation plays a main role in sustaining the welfare and performance of confined livestock, by affecting thermal exchanges between the animal's body surface and the environment and by removing air pollutants [7]. Poor ventilation can lead to increased airborne particulate and gaseous pollutant concentrations [1]. Poor ventilation is also responsible for increased airborne concentrations of viable microbes NH_3 and CO_2, reduced feed efficiency, and enhanced aggressive interactions in cattle, in pigs, and in broiler chickens.

Ventilation control includes higher ventilation rate and effective room-air distribution systems. Air cleaning strategies include use of air filters, ionizers, or wet scrubbers.

Essential oils are a potent source of environmentally and ecologically safe biocides and could be exploited for commercialization.

Active constituent of pinene is used in agricultural chemical products. The chemical compound pinene is a bicyclic terpene known as a monoterpene.

Eucalyptol is a natural organic compound. Eucalyptol has a fresh camphor-like smell and a spicy, cooling taste.

Eucalyptol is the chemical compound from *Eucalyptus globulus* oil has antibacterial effects on pathogenic bacteria in the respiratory tract. The Eucalyptus oil can also be used as an insect repellent and biopesticide.

In conclusion, the primary results of our study show that: the best characterized effect for air quality of henhouse had of combination of essential oils *PINUS SILVESTRIS L.* (pine) and *EUCALYPTUS POLYBRETEA L.* (eucalyptus).

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AUTOMATIC MILKING SYSTEM: EFFECT OF USED VACUUM LEVEL ON BOVINE TEATS

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SUMMARY

The objective of this study was to determine the effect of vacuum applied to bovine teats in two types of AMS under development using infrared thermography, and compare it with a conventional tandem milking parlor. Two AMS prototypes were evaluated: AMS-O (older, made in 2003) and AMS-N (newer, made in 2005), along with a conventional tandem milking parlor 2x10 (TMP). The vacuum level in all systems was 42 kPa. Temperature profiles of teats (thermograms) were recorded by a Flir 45 P thermographic camera immediately before and after milking with no preparation. The differences in teat temperature before and after milking in the AMS-O group averaged 2.36 ± 1.63 K, in the AMS-N group 1.62 ± 2.17 K and in the TMP 1.80 ± 1.40 K. A statistically significant difference was observed between the AMS-O and the AMS-N groups ($P<0.05$). The other differences in teat temperature changes were statistically insignificant. The newer AMS prototype placed a significantly smaller stress on the teats, and it may be said that it also showed a sub-significantly better result in comparison with the tandem milking parlor.

INTRODUCTION

Since their introduction, automatic milking systems (AMS) have been the subject of research. The scientific and technical evaluation focused mainly on the frequency of milking, the ejection effect, milk quality (especially in terms of somatic cell count and fat content), the hygiene of teat and udder prior to robotic milking, milking management of AMS, and the welfare of dairy cows kept in AMS-equipped stalls [1].

However, little is known about the influence of robotic milking on the mammary gland. The udder parts that experience the greatest stress are the teats, and their condition changes in the course of milking. The evaluation of teat condition before and after milking is done mostly by classification system [2], by cutimeter [3], by ultrasonographic scanning [4], and lately also by means of infrared thermography (IRT) [5]. So far, the IRT has not been deployed to study the relationship between robotic milking and udders/teats even though many authors recommend it as a method suitable to evaluate the effect of milking machines on mammary glands because it captures the visually elusive teat tissue trauma in a non-invasive, contact-less and objective manner. Milking does affect the teat temperature and these changes, or more precisely their magnitude, can be used to evaluate the effect of milking on mammary glands and assess the comfort level of cows being milked in a certain way. The objective of this study was to determine the effect of vacuum applied to bovine teats in two types of AMS under development using infrared thermography, and compare it with a conventional tandem milking parlor.

MATERIAL AND METHODS

Two AMS prototypes from the same manufacturer were evaluated: AMS-O (older, made in 2003) and AMS-N (newer, made in 2005), along with a conventional tandem milking parlor 2x10 (TMP). The vacuum level in all systems was 42 kPa.

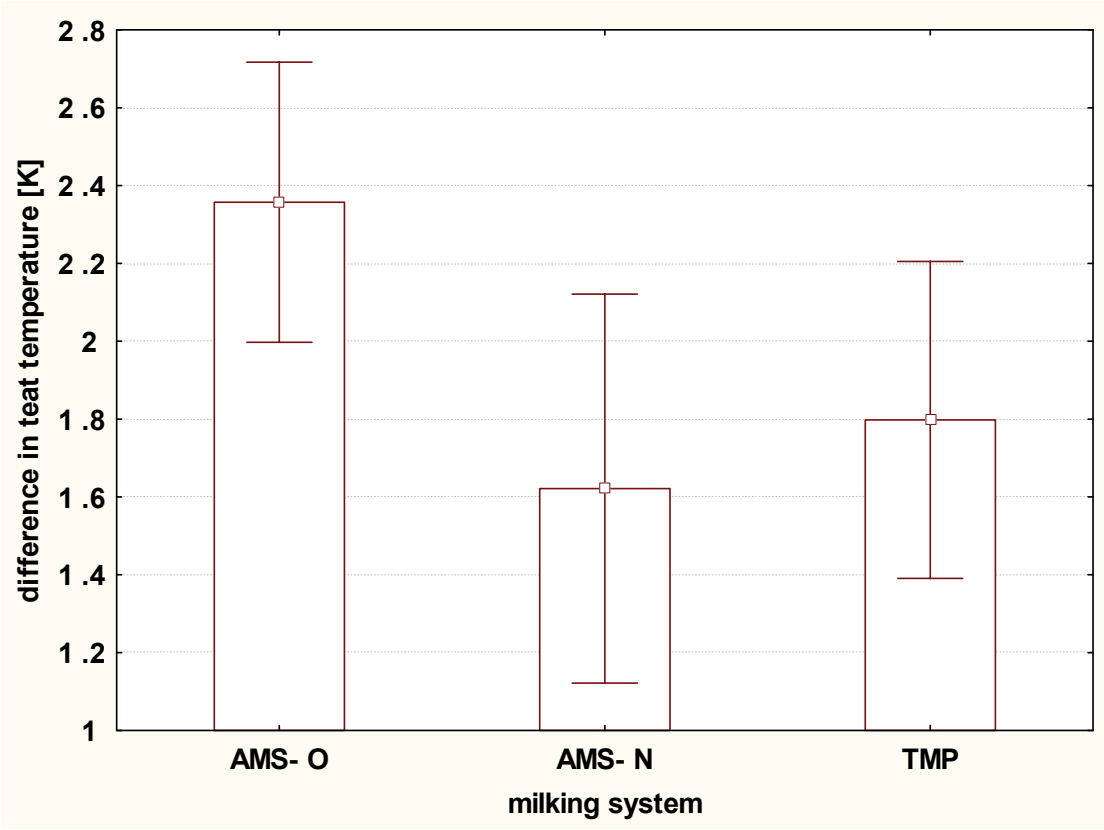
All equipment was tested under dairy farm conditions. The experiment involved cows of Holstein breed (n = 20, first lactation stage). Teat temperatures in both AMS groups were measured in the course of one day, those in the tandem parlor at the morning and afternoon milking.

Temperature profiles of teats (thermograms) were recorded by a Flir 45 P thermographic camera immediately before and after milking with no preparation. The camera lens was 50 cm distant from the teat, the emissivity was 0.95. Measured concurrently were air temperature, reflected temperature, and relative humidity at the place of measurement.

The thermograms so obtained were evaluated by a special computer program called ThermaCAM Reporter 2000, and the resultant data was processed by Statistica.cz (StatSoft, USA) - procedure ANOVA, POST-HOC tests.

RESULTS

The difference in teat temperature before and after milking in the AMS-O group averaged 2.36 ± 1.63 K. In the AMS-N group, the teat temperature rose by an average of 1.62 ± 2.17 K and the average difference in the TMP group was found to be 1.80 ± 1.40 K. A statistically significant difference was observed between the AMS-O and the AMS-N groups (Tukey, at $P<0.05$). The other differences in teat temperature changes were statistically insignificant. The results are shown in Graph 1.



Graph 1: The changes of teat temperature depending on milking system

DISCUSSION

In general, machine milking elevates the teat temperature of dairy cows [6 - 10] (Hamann, 1985, Eichel, 1992, Paulurd et.al, 1992, Kunc et al., 1999, Kunc et al., 2000). That was ascertained in conventional milking parlors. This study yielded the same results, for both the tandem milking parlor and the AMS. It is obvious from the results though that the older AMS prototype stresses the teats significantly more than the newer type or the tandem milking parlor. The newly developed AMS thus demonstrated better results than the older one, and was also sub-significantly better than the tandem milking parlor.

CONCLUSIONS

The newer AMS prototype placed a significantly smaller stress on the teats, and it may be said that it also showed a sub-significantly better result in comparison with the tandem milking parlor.

These results are attributable to the innovations and technical improvements in the development of AMS for dairies. However, more research has to be done in order to further advance the technology and the process.

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ALTERNATIVE LAYING HENS SYSTEMS AT FAMILY HUSBANDRIES IN CROATIA – HOW TO BECOME PROFITABLE

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SUMMARY

This paper discuss the introduction of new laying hens housing systems due to legislation which the Republic of Croatia adopted. According to the laws by 2012, all layers must be switched from conventional cage system to enriched cages or alternative systems. Regart to expensive investments in new equipment for the small producers on family farms in Croatia, authors consider how much it is profitable to select some of the alternative systems. Small producers may see their chance in the mutual integration of the cooperative and joint participation in domestic and especially foreign markets. Assumption is that eggs from alternative systems, if more, have an organic mark, could become an export product of RH.

INTRODUCTION

European consumers have induced a new approach in laying hens housing, especially with regard to welfare, in the context of food safety [10]. Now, Croatian legislation, harmonized with the European, issue ways of housing which will provide acceptable hens welfare and appropriate production results. The ability of different alternative (non-cage) housing systems provides a flexible choice for producers which now can balance their production goals with market demands as well as with regulations. In short time all the current cage systems will have to be replaced by prescribed systems. Family husbandries will be able to, regardless of the number of layers, choose a system that will provide their income.

As European Directive uses the term «alternative system» for any kind of non-cage holding comprising indoor and outdoors rearing on natural or concrete floors with up to four levels above the stable floor [5].

Many European countries such as Holland, Germany and Austria are considering a ban of any cage and propose only the alternative housing systems, others still argue the use of both.

Considering the significantly more expensive production in alternative systems, as well as greater technological and hygienic demands, paper discuss the feasibility of introducing such systems.

CURRENT KNOWLEDGE

Transition to an alternative system of keeping can be a challenge, and achievement of balance between the welfare of laying hens, environmental protection and the successful production, exceptional reward. Recorded results of alternative systems give the possibility for a conclusion on the appropriate productivity, low mortality and a safe working environment, however, the choice of alternative systems requires knowledge of various management skills. Perchery barn, Semi Intensive, Deep litter or free range systems, will significantly improve the welfare of laying hens and, at the same time, ensure quality eggs. In addition, alternative systems complement the idea of good agricultural and good environmental practice.

These new system include construction of tiers and perches above the littered area, so hens could move in all directions, also insure space for foraging and dust bathing [2]. These facts represent significant advantage of aviaries compared to furnished or enriched cages.

Aviaries could be in combination with sheltered outdoor areas (wintergarten, verandas), or free range. So it can be sad that aviaries provide the larges amount of freedom for laying hens of all alternative systems, because of possibility to express all spectrum of physiologically behavior [9,11]. Conducted research refereed to ANI (animals need index) proved that alternative system achieve best scores in comparison with cage systems, which was predictable.

Considering laying performance of hens in different housing systems there were no significant differences.

Worse health status and higher mortality are noticed in alternative systems [3].

DISCUSSION

Since the discussion about welfare threats of laying hens housed in cages started, also started reflection about new systems that will ensure natural conditions of housing, breeding and production, and reduce any stressful situation, pain, suffering and fear. At the same time the general public become increasingly sensibilisied on the life quality of hens housed in cages [8).

Today it is still very low production of eggs from alternative systems, in the world 0.9% and 4.8% in Europe. As reasons for the higher price of eggs from such farming are cited, increased work, nutrition, hygiene, etc. [12]. Furthermore, it is proved that the higher mortality is in alternative systems than in conventional, because the hens are exposed to different causes of diseases, increased possibility of pecking and cannibalism, parasites emerge, the more difficult maintenance of air quality hygienic conditions and other microclimate factors. In alternative systems is higher percentage of dirty and cracked eggs, very few of them layed in the nest, and more on the floor. Furthermore, according to published studies, eggs that originate from hens housed conventionally often are identified by A label from eggs originating from alternative systems. The reason is because of a large percentage of dirty and cracked eggs in alternative farming. This is particularly true in a free range. There is evidence that the indices of shell hardness, brittleness, egg weight, shell color are better in the cage housed hens eggs than those held alternatively [13]. Guesdon and Faure (2004) in one study noted a higher mortality of laying hens in conventional cages and more expressed welfare in enriched cages but did not noticed the impact of cages on the productivity. Similar studies have been conducted by experts of the EU and noted that the number of bacteria on the eggs and the percentage of damaged eggs was significantly higher, although not generally high, with eggs coming from enriched cages.

Enriched cages, aviaries and other alternative systems are better only in terms of welfare. The fact that public opinion, especially European, despite the scientifically proven facts, mentioned above, does not want to buy eggs from any cage system. That opens the possibility of countries that exceed the new system by 2012 year, to consider worthwile and sustainability of the transition to alternative systems.

Enriched systems, aviaries and other alternative forms of laying hens housing are much better in terms of welfare. However, there is a problem of immediate environment pollution, in particular, expressed as air pollution by bacteria, fungi, dust and ammonia, which is significantly higher in alternative than in conventional systems [10].

The possibility of achieving higher standards of hygiene, better control of laying hens behavior, a smaller proportion of damaged and dirty eggs are just some of the features of the cage to justify their use [1, 14].

Cages are, also, still the most economical way of producing eggs [6], and the best housing system in terms of disease prevention [7].

According to available data from a Ministry of agriculture, fishing and rural development, Croatia currently has 160 registered producers of eggs for food. Of these, ten are large manufacturers (over 10,000 hens), and the rest are small producers. Current housing systems were in 90% conventional cages. According to new legislation, all they have to move on new systems (enriched cages or alternative). The question is how many small producers on the family husbandries worthwhile the investment in new cage systems due to the cost of the equipment and need for increased space, to maintain the same number of hens. Alternative systems require less investment, but on the other hand, require a change in the approach to managing and technology.

The introduction of alternative laying hens housing systems opens up the possibility of registration and transition to organic production with pleased the other criteria (maximum of 230 hens per hectare which is equivalent to 170 kg N / ha / year, a total of 3000 hens by farming and 6 beaks per m^2 in the house and 4 beak per m^2 outside the building). In this case, the eggs get eco bonus, which ensures safety and traceability of food, nutritional value, recognition and noncontamination. By imminent accession of Croatia to the EU thus produced eggs could become a distinctive and competitive export product.

CONCLUSIONS

Eggs produced in alternative systems will carry the tag: 0 - organic farming, 1 - free-range, 2 - floor housing. For customers, which in Croatia, are increasingly adopting a modern approach regarding animal welfare, with emphasis on food safety, the supply of eggs from alternative farming provides greater choice. Eggs that carry such labels achieve a higher price, and opens up the possibility of placements in foreign markets.

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AN OVERVIEW ON DAIRY COWS SHELTERING IN TRANSYLVANIA (ROMANIA)

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SUMMARY

In the present study we aimed at performing an overview on dairy cows sheltering in Transylvania. Out of the 44 shelters studied, 20 were provided with tie-stalls system and 24 had free-standing stalls system. 60% of the tie-stalls shelters had short stops and 35% medium stops, while only one shelter had long stops. 90% of these shelters used straw beds (straw, wood dust), but it was insufficient and not properly stored. 60% of the shelters had organized natural ventilation, while 40% had an unorganized ventilation system. Only one shelter was equipped also with mechanic ventilation. In 70% of the tie-stalls shelters waste products were evacuated manually, and not frequently enough, which caused fowl air and improper hygienic conditions. 10 out of the 24 free-standing stalls shelters could house over 100 animals. In the case of 3 shelters the minimum required resting surface was not complied with, which had negative consequences over the health state and production. The beds were made up of straw and wood dust, while in 2 shelters there were rubber mattresses. In 20 shelters the ventilation was natural but not organized, only 4 of them had organized ventilation. Waste products were mechanically disposed of in 24 free-standing stalls shelters, with a frequency ranging form 2-6 times/day. We noticed an increase in the number of free-standing stalls shelters and a decrease of the tie-stalls shelters, which may improve the sheltering conditions of animals.

INTRODUCTION

The appropriate care of domestic animals in shelters corresponding to the species, breed, productive type and age category, results in positive effects and economic advantages: a decrease of the impact the unfavourable conditions in the natural environment have over animal productions, better working conditions for the caretakers due to mechanisation, saving fodder due to controlled feeding, etc. [2]. At the same time, keeping animals indoors for a long period of time, even in appropriate shelters, presents some drawbacks: the animals are deprived of the stimulatory effect of moderate temperature variations, of direct solar radiation, of breathing fresh air and of continuous movement, which may lead to a decrease of their physical resistance and to an increase of germs in the crowded shelters, etc.. These drawbacks are amplified considerably in poorly designed shelters and in those with poor ventilation and waste disposal systems.

Our study aimed at performing an overview on dairy cows sheltering in Transylvania.

MATERIAL AND METHODS

The study was carried out in the period between June 2009-September 2010 in 44 shelters, in the following counties: Alba, Bihor, Bistriţa-Năsăud, Braşov, Cluj, Covasna, Mureş, Satu-Mare and Sibiu. Out of these shelters, 20 were provided with tie-stalls system, and the number of cows within them ranged between 22 and 200, and 24 shelters had free-standing stalls system housing between 60 and 170 cows.

Data were obtained by interviewing farmers and by observations on the spot.

RESULTS

The data obtained in shelters with tie-stalls shelters are presented in table 1.

Table 1. Tie-stalls shelters

Shelter	No. of cows	Resting surface (m^2/head)	Beds type	Ventilation type	Manure evacuation
1	42	2.16	Straw + wood dust	Unorganized natural ventilation	Manual
2	50	2.79	Straw + wood dust	Organized natural system	Manual
3	32	1.49	-	Organized natural system	Manual
4	22	2.62	-	Organized natural system	Manual
5	113	1.53	Straw	Organized natural system	Mechanic
6	200	1.8	Straw	Organized natural system	Mechanic
7	94	2.8	Straw	Organized natural system	Mechanic
8	98	2.8	Straw	Organized natural + mechanic system	Mechanic
9	99	2.8	Straw	Organized natural system	Mechanic
10	64	1.58	Wood dust	Organized natural system	Manual
11	41	3.23	Straw + wood dust	Unorganized natural ventilation	Manual
12	44	4.5	Wood dust	Unorganized natural ventilation	Manual
13	44	1.98	Wood dust	Unorganized natural ventilation	Manual
14	40	1.8	Straw	Unorganized natural ventilation	Manual
15	56	1.76	Wood dust	Organized natural system	Manual
16	47	2.4	Wood dust	Unorganized natural ventilation	Manual
17	104	2.28	Wood dust	Organized natural system	Manual
18	104	2.28	Wood dust	Organized natural system	Manual
19	30	1.87	Straw	Unorganized natural ventilation	Manual
20	27	1.6	Wood dust	Unorganized natural ventilation	Manual

The data obtained in shelters with free-standing stalls system are presented in table 2.

Table 2. Free-standing stalls shelters

Shelter	No. of cows	Resting surface (m^2/head)	Beds type	Ventilation type	Manure evacuation
1′-8′	70	2.36	Straw	Unorganized natural ventilation	Mechanic
9′	120	2.68	Straw	Organized natural system	Mechanic
10′-11′	92	2.68	Straw	Unorganized natural ventilation	Mechanic
12′-13′	170	2.64	Straw	Unorganized natural ventilation	Mechanic
14′-15′	150	2.2	Straw	Organized natural system	Mechanic
16′	150	2.68	Straw	Organized natural system	Mechanic
17′	152	2.68	Rubber mattresses	Unorganized natural ventilation	Mechanic
18′	154	2.53	Rubber mattresses	Unorganized natural ventilation	Mechanic
19′	96	2.5	Straw	Unorganized natural ventilation	Mechanic
20′	60	2.36	Wood dust	Unorganized natural ventilation	Mechanic
21′	90	2.16	Wood dust	Unorganized natural ventilation	Mechanic
22′	104	1.99	Straw	Unorganized natural ventilation	Mechanic
23′	90	2.2	Wood dust	Unorganized natural ventilation	Mechanic
24′	119	1.99	Straw	Unorganized natural ventilation	Mechanic

DISCUSSION

Out of the 20 tie-stalls shelters studied, 60% had short stops and 35% medium stops, while only one shelter had long stops. From the point of view of animal welfare, short stops are not recommended as the may lead to an increase of mastitis occurrence, lameness, metrites, vaginal and uterine prolapse [1]. When comparing the resting surface in these shelters with the minimum required area, which is of 1.76 m^2/animal [3], we can notice that in shelters no. 3, 5, 10 and 20 this value is not reached. 90% of free-standing stalls shelters used straw beds (straw, wood dust), but it was insufficient and not properly stored. 60% of the shelters had organized natural ventilation, while 40% had an unorganized ventilation system. Only one shelter was equipped also with mechanic ventilation. In 70% of the tie-stalls shelters waste products were evacuated manually, and not frequently enough, which caused fowl air and improper hygienic conditions.

In the case of 3 free-standing stalls shelters (no. 21′, 22′ and 24′) the minimum required resting surface (2.25 m^2/head) [3] was not complied with, which had negative consequences over the health state and production. In other 3 shelters (no. 14′, 15′ and 23′) the resting surface was close to this value. Figure 1 presents the percentage distribution of all shelters under study depending on whether or not they comply with the minimum required resting surface.

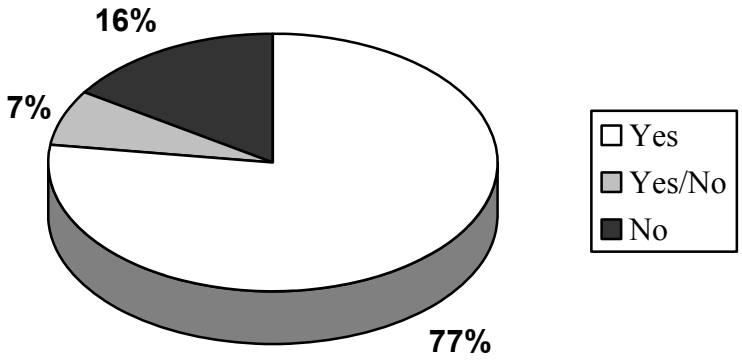

Fig. 1. Percentage distribution of studied shelters acording with minimum required resting surface

The beds were made up of straw and wood dust, while in 2 shelters there were rubber mattresses. In 20 shelters the ventilation was natural but not organized, only 4 of them had organized ventilation. Waste products were mechanically disposed of in 24 free-standing stalls shelters, with a frequency ranging form 2-6 times/day.

CONCLUSIONS

Failure to comply with the recommeded sheltering area.
Widespread use of unorganized natural ventilation.
Manual evacuation of waste in many of the tie-stalls shelters.
Increased number of shelters with free-standing stalls at the expense of those with tie-stalls, which is likely to improve housing conditions of dairy cows.

ACKNOWLEDGMENTS

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DRINKING WATER INTAKE MANAGEMENT WITHIN DAIRY COWS SHELTERS IN TRANSYLVANIA (ROMANIA)

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SUMMARY

This study aims at tackling drinking water intake management in 44 dairy cows shelters in Transylvania. Out of these shelters, 20 were provided with tie-stalls system, and 24 shelters had free-standing stalls system. In 8 out of the 20 tie-stalls shelters, water intake was only carried out twice a day, while in the others whenever needed. According to the welfare regulations, water intake should be unlimited and should be performed from a water intake device for each individual cow. None of the tie-stalls shelters complies with this provision. Out of the free-standing stalls shelters, 20 were connected to the public water system, while 4 had their own water sources. The number of animals allocated for each individual watering hole ranged between 9 and 24. In the case of the collective cradles, there were between 2.66 and 5.71 cm available for each cow. If we consider that the minimum required is one individual watering device for 15 cows, that is to say 4 cm of collective watering device for each cow, then we can conclude that 7 out of the 24 free-standing stalls shelters do not comply with these provisions. We noticed that 27 out of the 44 shelters studied were poorly managed from the point of view of the water intake.

INTRODUCTION

Water is one of the main environmental factors that deeply influences all living creatures. Water is the solvent and vehicle for most biogenic substances and the medium for the main biochemical reactions. Animal water requirements vary and depend on production levels. In cows there is a relation between body weight and water demand, and between the quantity of milk produced and the water consumed [1]. The amount of water consumed by animals also depends on how water is supplied. For example, in the case of automatic watering, cows consume approximately 5 more liters of water per day, as compared to the case of manual watering [1]. Failure to meet the biological consumption needs (drinking water and animal food preparation) leads to a decrease in animal production and to organic disorders. Failure to meet the technological needs (sanitation water, animal products processing, etc.) negatively reflects on the shelter environment, and respectively on animal hygiene and machinery [2].

The present study aims at tackling drinking water intake management in dairy cows shelters in Transylvania.

MATERIAL AND METHODS

The study was carried out in the period between June 2009-September 2010 in 44 shelters, in the following counties: Alba, Bihor, Bistriţa-Năsăud, Braşov, Cluj, Covasna, Mureş, Satu-Mare and Sibiu. Out of these shelters, 20 were provided with tie-stalls system, and the number of cows within them ranged between 22 and 200, and 24 shelters had free-standing stalls system housing between 60 and 170 cows.

Data were obtained by interviewing farmers and by observations on the spot.

RESULTS

The data obtained in shelters with tie-stalls shelters are presented in table 1.

Table 1: Tie-stalls shelters

Shelter	No. of dairy cows	Water source	Type of watering devices	Frequency of watering
1	42	Own source	Cradles	Twice/day
2	50	Own source	Cradles	Twice a day
3	32	Own source	Cradles	Twice a day
4	22	Own source	Cradles	Twice a day
5	113	Own source	Water bowls	Freely
6	200	Own source	Water bowls	Freely
7	94	Own source	Water bowls	Freely
8	98	Own source	Water bowls	Freely
9	99	Own source	Water bowls	Freely
10	64	Own source	Water bowls	Freely
11	41	Own source	Water bowls	Freely
12	44	Own source	Water bowls	Freely
13	44	Own source	Water bowls	Freely
14	40	Own source	Water bowls	Freely
15	56	Own source	Cradles	Twice a day
16	47	Own source	Cradles	Twice a day
17	104	Public system	Cradles	Twice a day
18	104	Public system	Water bowls	Freely
19	30	Own source	Cradles	Twice a day
20	27	Own source	Water bowls	Freely

The data obtained in shelters with free-standing stalls system are presented in table 2.

Table 2: Free-standing stalls shelters

Shelter	No. of dairy cows	Water source	Type of watering devices	No. of cows/ watering device	Cradles length/ cow (cm)
1′-8′	70	Public system	Cradles	-	5.71
9′	120	Own source	Frost-proof	15	-
10′-11′	92	Public system	Water bowls	23	-
12′-13′	170	Public system	Frost-proof	21.25	-
14′-15′	150	Public system	Frost-proof	9.37	-
16′	150	Public system	Cradles	-	2.66
17′	152	Public system	Cradles	-	5.52
18′	154	Public system	Cradles	-	5.45
19′	96	Own source	With push paddle	24	-
20′	60	Own source	With push paddle	7.5	-
21′	90	Own source	Frost-proof	11.25	-
22′	104	Public system	Water bowls	17.33	-
23′	90	Public system	Cradles	-	4.44
24′	119	Public system	Water bowls	14.87	-

DISCUSSION

Out of the 20 tie-stalls shelters, only two were connected to the public water supply, with frequent monitoring of the water quality. The other 18 had their own water supply (well drilling or well). In all shelters there is one constant level watering device (water bowl, cradle) for two animals. In as far as watering is concerned, it was performed freely in 12 of the investigated shelters, while in the remaining 8 it was performed twice a day (figure 1).

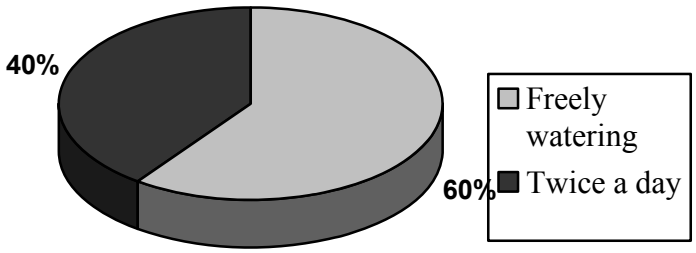

Fig. 1. Percentage distribution of tie-stalls shelters acording watering frequency

According to the welfare regulations [3], water intake should be unlimited and should be performed from a water intake device for each individual cow. None of the tie-stalls shelters complies with the last provision.

In the case of shelters with free-standing stalls, 20 were connected to public water supply networks, while 4 used their own water supply. Figure 2 shows the percentage distribution of all the 44 studied shelters, from the point of view of the water supply used.

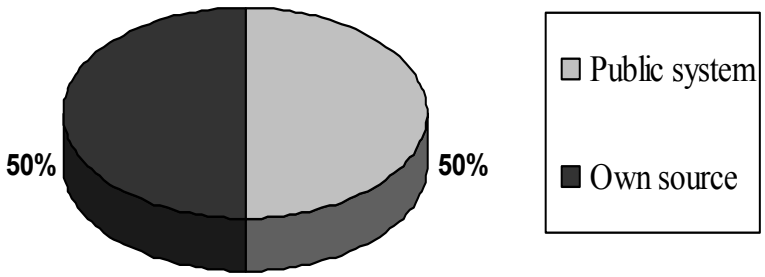

Fig. 2. Percentage distribution of all shelters acording water suply used

Watering was performed freely in all 24 shelters. Considering that the minimum required is one watering device for every 15 animals, and 4 cm of cradle for each animal (in the cradle-type watering) [3], we could notice that in 7 of the shelters with free standing stalls (no. 10′, 11′, 12′, 13′, 16′, 19′ and 22′) these requirements were not met (figure 3.). This could lead to overcrowding and competition between animals when drinking, which would affect them in a negative way.

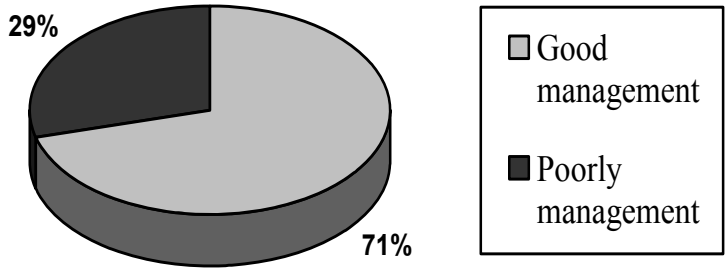

Fig. 3. Percentage distribution of free-standing stalls shelters acording watering management

CONCLUSIONS

27 out of the 44 shelters studied were poorly managed from the point of view of the water intake. Recommendations were put forward to remedy this situation.

ACKNOWLEDGMENTS

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STUDIES ON THE IMPROVEMENT OF FARM ANIMALS IN TURKEY

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INTRODUCTION

The number of inhabitants and the rates of population growth and development are 72.561.312, %1.45, %8,9 respectively, in Turkey. Numerous investigations on animal improvement have been conducted by the Agricultural Ministry and Universities to compensate the requirements of emerging markets in the country. A number of studies related with animal improvement in Turkey were summarized in this paper.

ANIMALS, MATERIALS AND METHODS

The cattle breeds such as Holstein, Brown Swiss, Jersey, Charolais, Simmental were imported and used for both pure breeding and crossing. Anatolian Brown was the most successful crossbred.
The crossing experiments were performed to improve the economic traits of native sheep breeds by using German Mutton Merino, Rambouillet, Lincoln, Hampshire Down and Ost Friz. New types such as Karacabey Merino, Konya Merino, Ramlıç, Tahirova, Sönmez, Acıpayam and Türkgeldi were developed at the end of these works.
At the beginning of the 20th century, purebred horses such as Arabian, Nonius, Percheron and Ardene were used in crossing investigations for fulfilling the demands of Turkish Military. Arabian horses are maintained at state farms and some private farms are also breeding Thoroughbreds for only racing purposes.

RESULTS

New cattle and sheep types for Turkish breeders were distributed via the state farms. In addition, Erbro broilers and Atak, Atak-s and Atabey laying commercial hybrids were produced for the poultry industry in Turkey.

CONCLUSIONS

More investigations for animal production will be useful to cover the demand of increasing population of Turkey.

Key words: *Turkey, improvement, cattle, sheep, horse, poultry*

INTRODUCTION

The number of inhabitants and the rates of population growth and development are 72.561.312, %1.45, %8,9 respectively, in Turkey [5,6]. According to 2009 statistics, Turkey's cattle, sheep, goat, horse and poultry population are projected to be about 10.703.958, 21.749.508, 5.128.285, 166.753 heads and 234.082.206 peace respectively[33]. Increasing demand for animal products cannot be fulfilled due to inadequate yields of native breeds in Turkey [9,20]. Deficiencies in meat and milk productions are pronounced in some periods [8,21]. Also the lack of pastures contributes to this insufficiency in animal production. These problems of the animal industry can be resolved by improvement of genetic structure, better management and increasing the number of livestock [12].
A number of studies related with animal improvement by government and universities in Turkey were summarized in this paper.

CATTLE BREEDING

Montafon, Holstein, Jersey, Aberdeen Angus, Hereford and Bonihad cattle was imported to Turkey for improving the yields of native breeds and resolve the insufficiency of animal products after establishing new Turkish state in 1923 [11]. In 1935, primary studies at the Karacabey State Farm in South Marmara region were intiated. A new type called as Karacabey Montafon Cattle by people were obtained from Montafon X Native Grey crossings. They were formerly named as Anatolian Brown. The lactation milk yield, daily weight gain and adult body weight of Native Greys are 600 - 1000 kg, 800 g and 300 kg respectively as the dam line of Anatolian Brown. The figures of these traits in Anatolian Brown are 3000 kg, 1000 g and 500 kg in 17-18 months aged males respectively [4]. The purpose of this crossing was to combine early growth ability, higher milk and meat yields of Brown Swiss breed with the adaptation to climate, resistance to disease and parasite, roughage evaluation, high survival rate and reproductive performance abilities of Native Grey breed [19]. Milk yields of Different

originated Swiss Brown cattle maintained at the farms of General Directorate of Agricultural Enterprises (TIGEM) are between 4853 and 6674 kg [31]. Pure breeds, Brown X Native Grey, Brown X East Anatolian Red, Holstein X South Anatolian Red and Jersey X Native Black crosses were spread out to Turkey [2]. Today, %41.09 of the cattle population are consisted of crosses in the country [32]. The various studies were conducted on the fattening performance and crossing [10,23,24,26,27,34]. Arpacık et al. [7] reported slaughter weight, hot dressing percentages and the meat ratio 226.3 kg, % 53.4 and %70.3 respectively in East Anatolian Red used for the beef production. The same researchers found the figures of same traits for Montafon crosses 288.8 kg, %54.8 and %72.2 respectively. In comparative studies Kocak and Ozbeyaz [20] reported that the adjusted milk yields were 1490.52 kg, 2437.24 kg, 2557.89 kg and 2159.27 kg respectively for Kilis known as the highest milk yielding native breeds, Simmental X Kilis F_1, B_1 and F_1 X B_1 genotypes respectively. Similarly, Kilis cattle was used for increasing the resistance of Holstein cows to the subtropical conditions. The lactation length and milk yield were reached up to 319 days and 5638 kg in B_1 cows [9]. General Directorate of Agricultural Research (TAGEM)of Agricultural Ministry started the Conservation of Domestic Farm Animal Genetic Resources project for native cattle breeds in 1995. By the way of this project different native cattle breeds were put under protection in Cukurova Agricultural Research Institute (South Anatolian Red cattle), Eastern Anatolia Agricultural Research Institute (East Anatolian Red cattle), Lalahan Livestock Research Institute (Native Black cattle) and in the Marmara Livestock Research Institute (Native Gray cattle) [15].

SHEEP and GOAT BREEDING

The back crossing between German Mutton Merino sheep and Native Kivircik sheep was started In 1934 for the aim of sheep improvement in Turkey. Karacabey Merino sheep was developed at the end of these studies. The aim of this crossing was to produce quality wool. Then in the 1950s the Central Anatolia Region were added to the program and Anatolian Merino breed was developed by the crossing German Meat Merino sheep and Native White Karaman. New sheep types such as Konya Merino, Acıpayam, Ramlic, Tahirova and Türkgeldi were developed at same years. Crossing researches were continued after importation of Dorset Down, Hampshire Down, Lincoln, German Blackhead Mutton Merino and Ile de France rams and ewes in 1986 [11].

Different yield purposed sheep types were obtained after this crossing researches. Mature weight of Native Kivircik sheep is between 40-50 kg. Whereas the same trait was found among 50-55 kg in Karacabey Merino sheep which was improved by crossing German Mutton Merino rams and Kivircik ewes. Acipayam sheep of 60-70 kg were obtained by crossing Asaf rams carrying 50% East Friesian and 50% Awassi genotypes and Awassi X Daglic F_1 crosses. Acıpayam sheep are approximately 58% heavier then Native Daglics. Ramlic breed have 68.75% Rambouillet and 31.25% Daglic genotype. Live weight and lactation yield are 50 and 70 kg respectively in this breed. Tahirova sheep were developed by crossing East Friesian and Kivircik breeds. Tahirovas have possession of 75% East Friesian and 25% Kivircik genotypes. Mature weight and lactation yield was found to be 55-60 kg and 250-300 kg respectively [3,17]. Additionally some dual-purpose types such as Menemen (75% Ile de France, %18.75 East Friesian, %6.25 Kivircik), Hasmer (%37,5 merino, %31,25 Hampshire Down, %31,25 German Blackhead Mutton Merino), Hasak (%37,5 Akkaraman, %31,25 Hampshire Down, %31,25 German Blackhead Mutton Merino), Hasiv (%37,5 Awassi , %31,25 Hampshire Down, %31,25 German Blackhead Mutton Merino), Linmer (%50 Lincoln, %50 Merino) for meat and wool production were developed [18,30]. Different crossing studies with Saanen goats were conducted to increase the milk production of native Black goats in last decades. Sengonca and Kosum [28] reported that The Saanen goats can be successfully used in crossing with the native goats of Aegean region. Due to protection of native breeds is obligatory for developing resistance to harsh conditions and new breeds, General Directorate of Agricultural Research of Turkish Agricultural Ministry started a project named Conservation of Domestic Farm Animal Genetic Resources [18]. Different native sheep and goat breeds were put under protection in Marmara Animal Research Institute (Merino, İmroz, Kivircik and Sakiz), Konya Animal Research Institute (south Karaman), Lalahan Research Institute (Angora goats) [15]. In addition General Directorate of Agricultural Research prepared another project to improve Akkaraman, Morkaraman, Anatolian Merino, Hemsin, Awassi, Kangal Akkaraman, Karacabey Merino, Karayaka, Karya, Pırlak sheep breeds and Angora goats in individual breeders via Breeder Association [29].

HORSE BREEDING

At the beginning of the 20th century, Purebred Arabian, Nonius, Percheron and Arden breeds were brought to Turkey for riding and draft puposes in private farms and army. They were also used in crossing. In early periods of republic, because of the insufficiency agricultural technology and motorization was not to be passed Austrian originated Haflinger horses was imported to Karacabey State Farm in 1961. The purpose was to compensate the demand of farmers. Haflinger was used both pure breeding and crossing with the Arabian horses in Karacabey State Farm. The performance tests indicated that the half blood Haflingers were akin to pure bloods. Haflinger and crosses was requested by the public because they have nerveless temperament, easier management, resistant against diseases. Because of these features haflinger breed reached today. However, the other breeds except for Haflinger and Arabian breeds were not useful for breeders [13,15]. Since 1928 Arabian horses was included in official competitions and started to being bred as a race horse. Arabian horse breeding is still

continued in Karacabey, Sultansuyu, Cifteler agricultural enterprises and some private farms [22]. Some private thoroughbred farms are also available [14].

POULTRY BREEDING

A broiler commercial hybrid named Erbro were developed in Erbeyli Fig Research Institute. Adalı and Yelmen [1] compared R1 and R2 parent lines of erbro with Hybro and Ross parent lines in an investigation. They reported R2 parent line was 132 g and 119 g lighter than Hybro and Ross. R1 parent line was 201 g and 188 g lighter than Hybro and Ross. They reported that R1 and R2 can compete with foreign lines. Fathel and Elibol [16] compared Atak and Atak-s brown layers improved in Ankara Poultry Research Institute and commercial hybrids Nick Brown and Lohmann Brown. In this study, egg yields were (hen-day) 278.4, 290.5, 305.4 and 315.5 pieces respectively. Age of maturity of these lines were 154.3, 152.1, 149.2 and 146.8 days respectively. Daily feed consumption were 119.2, 125.8, 120.1 and 124.5 g respectively. White layer hybrit was improved in Ankara Poultry Research Institute and compared with imported hybrids Hy-line and Lohmann LSL. In this study, egg yield (hen-day), age of maturity, daily feed consumption for Atabey and two foreign layers were found 297.9, 293.4 and 320 pieces; 151.5, 150.6 and 149.1 days; 117.2, 114.6 and 118.3 g respectively [32].

Native Denizli and Gerze breeds were put under protection in Lalahan Livestock Research Institute as part of the Conservation of Domestic Farm Animal Genetic Resources project [15].

RESULT

As a result, the meat, dairy and egg production has reached important levels with the improvement attempts in Turkey since the establishment of the republic. But animal originated protein consumption for per capita in Turkey is in the short of European Union average. The livestock production and improvement activities are needed to be supported by the government for this reason.

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STUDY ON THE EFFICACY OF TRICHOBEN® VACCINE IN CALVES

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SUMMARY

The efficacy of Trichoben®, a live anti- ringworm vaccine, were examine in calves. In the present study 30 calves between 1 to 4 months age were studied into two different groups. In the first group vaccination was carried out by two intramascular injections of Trichoben® 14 days apart. The second group was considered as control group and did not received any injection. Jugular blood samples with and without anticoagulant were taken from calves of two groups before injection of vaccine and once again 14 days after last vaccination. Stimulation of cell mediated immunity in vaccinated and unvaccinated calves were measured by lymphocyte transformation test (LTT) followed by calculating the lymphocyte stimulation index (SI). Serum specific immunoglobulin G (IgG) concentration in vaccinated and unvaccinated groups were measured to evaluate the humoral immunity stimulation induced by vaccine. The results of the present study showed that lymphocyte stimulation index (SI) in vaccinated calves was significantly increased during 14 days post vaccination as compared with the calves in the control group ($P< 0.05$). Serum specific immunoglobulin G concentration against *Tr. verucosum* and *Tr. mentagrophytes* in vaccinated group was also significantly higher than in control group ($P< 0.05$). From the results of the present study it can be concluded that Trichoben® stimulates cell mediated immunity as well as humoral immunity in vaccinated calves against *Tr. verucosum* and *Tr. mentagrophytes* two important fungi in cattle dermatophytosis.

INTRODUCTION

Dermatomycosis occurs in all animal species in all countries but more commonly when animals are accommodated in dense groups, especially indoors. *Trichophyton verucosum* and *Tr. mentagrophytes* are more common fungi which grow on hair, skin or both in cattle. Spread between species occurs readily and in rural areas 80% of human ringworm may derive from animals [4]. Injury to infected animals is of minor importance but suuficient damage to hides occur to warrant some attempt at control of the disease. Widespread transmission, zoonotic importance and damage to hide and leather industeries needs the control program for the ringworm. Vaccination of cattle against ringworm has been widely carried out in common veterinary practice since 1971 [6]. Live vaccines yielded the best results under both experimental and practical conditions [3]. It was found that a single dose of these vaccines is not sufficient for the development of satisfactory immunity against trichophytosis; double administration of these biopreparations has to be done to obtain convenient vaccine efficacy [5]. The interval between vaccination and re-vaccination is mostly from 10 to 14 days in registered antimycotic vaccines. Trichoben® (Bioveta, a.s.,Czech Republic) is a commercially avirulent vaccine against bovine trichphytosis. This vaccine contains *Trichophyton verucosum* (N. 765 and N. 8166) and *Tr. mentagrophytes* (N. 202) antigens and can not sporoulate in the environment. The present study has been conducted to evaluate the efficacy of Trichoben® vaccine against bovine ringworm in calves.

MATERIAL AND METHODS

During the present study 30 calves between 1 to 4 months old were studied into two uniform groups. In the first group vaccination was carried out by two intramascular injections of 2.5 ml of Trichoben® 14 days apart. The second group were considered as control group and did not received any injection. Blood samples were taken by jugular venepuncture using vacutainers (Pars Khavar Co., Qazvin, Iran) from vaccinated and unvaccinated animals before vaccination and once again 14 days after second vaccination. Serum samples for immunoglobulin analyses were removed by centrifugation shortly after bleeding. Cell mediated immunity in animals of two groups were measured in heparinized blood samples shortly after bleeding. Stimulation of cell mediated immunity in vaccination and unvaccinated calves were measured by lymphocyte transformation test (LTT) and calculating the lymphocyte stimulation index (SI). Humoral immunity response were assessed by measuremen of serum specific immunoglobulin G (IgG) concentration in vaccinated and unvaccinated groups. Indirect enzyme linked immunosorbent assay (ELISA) technique was used to measure the specific IgG concentration in sera of vaccinated and unvaccinated animals in the presenty study. The results of SI and immunoglobulin examination of samples were analysed with Student's *t*-test, and a value of $P< 0.05$ was considered to be significant.

RESULTS

The results of present study showed in tables 1 and 2. As can be seen in table 1 the mean serum specific IgG concentration against dermatomycosis in animals of two groups before vaccination was very low and statistic analyses between two groups didn't show any significant difference. The corresponding average IgG values were 1.73± 0.38 pg/dl for vaccinated animals, and 0.43± 0.36 for unvaccinated animals. Student's *t*-test for mean values showed significant increases in specific IgG concentration in sera of vaccinated group as compared with the unvaccinated group ($P< 0.05$).

Table 1. Mean values of serum IgG concentration (pg/dl) of vaccinated and unvaccinated calves before and after vaccination (Mean± SE).

Group	No	Before vaccination	After vaccination
Vaccinated	15	0.07± 0.04	1.73± 0.38*
Unvaccinated	15	0.18± 0.16	0.43± 0.36

* Significantly different ($P< 0.05$).

Table 2 shows the lymphocyte stimulation index (SI) of vaccinated and unvaccinated calves in the present study. As it appear in table 2 the mean value of SI in vaccinated calves before vaccination was 0.24± 0.08 which is similar to mean value of SI in unvaccinated group (0.21± 0.06). Mean value of SI in vaccinated animals 2 weeks after second injection of Trichoben (2.62± 0.38) was significantly different as compared with the unvaccinated group (0.57± 0.22) ($P< 0.05$). The results of the present study showed that lymphocyte stimulation index (SI) in vaccinated calves was significantly increased during 14 days post vaccination as compared with the calves in control group (P< 0.05).

Table 2. Mean values of lymphocyte stimulation index (SI) of vaccinated and unvaccinated calves before and after vaccination (Mean± SE).

Group	No	Before vaccination	After vaccination
Vaccinated	15	0.24± 0.08	2.62± 0.38*
Unvaccinated	15	0.21± 0.06	0.57± 0.22

* Significantly different ($P< 0.05$).

DISCUSSION

Vaccination against dermatomycosis has achieved a great deal of success in preventing infection in cattle in most countries of Europe anf Scandinavia. Vaccination include those containing highly immunongenic, non- virulent strains of fungi, or those killed vaccine containing specific fractions of mycelia[2]. Trichoben® (Bioveta, a.s.,Czech Republic) is a commercially avirulent vaccine against bovine trichphytosis. This vaccine contains *Trichophyton verucosum* (N. 765 and N. 8166) and *Tr. mentagrophytes* (N. 202) antigens and can not sporoulate in the environment. Problems concerning the immunity rise after a single administration of the vaccine against trichophytosis in cattle were dealt with in a previous study[5]. The Russian vaccine LTF-130 against trichophytosis in cattle injected once at a double dose did not raise sufficient immunity response, either [1]. Sarkisov (1979) regards a single vaccination against trichophytosis unacceptable and thus explains the cases of insufficient efficacy of LTF-130 in practice. For other mycotic vaccines for cattle the following period between vaccination and re-vaccination is recommended: for the Polish vaccines Trichovac 10 - 12 days [9] and Bovitrichovac 10 - 14 days [8]; for the Russian Vermet vaccine 10 - 16 days (Sarkisov et al. 2000); and for the German Insol Trichophyton vaccine 14 days. Twice injection of Trichoben in young calves with 2 weeks interwal in the present study increased serum specific immunoglobulin G together with, circulating lymphocyte stimulation index (SI) in the vaccinated group as compared with non- injected animals ($P< 0.05$).

CONCLUSION

On the basis of the results of the present study it can be concluded that the Trichoben® (Bioveta, a.s.,Czech Republic) is an efficient anti- dermatomycosis vaccine in cattle, which can stimulate humoral and cell mediated immunity in calves when injected twice with 2 weeks interval.

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ANTIVIRAL POTENTIAL OF DIFFERENT BACTERIA SPECIES AND BACTERIAL METABOLITES

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SUMMARY

The antiviral potential of different bacteria species and of their metabolites was investigated in a research project. In first *in-vitro* experiments the antiviral effects of different bacteriocins against enveloped and non-enveloped viruses (influenzavirus H5N6 and H1N1, Feline herpesvirus, Newcastle disease virus and Murine norovirus) were tested. Only for influenzavirus H5N6 a possible antiviral effect of sakacin A and nisin can be supposed. Nevertheless, the virus inactivation by different pH values should be clarified in further experiments. In a second part of this study a variety of culture supernatants of different bacteria species, mainly lactic acid bacteria and staphylococci, was tested for their antiviral activity against enveloped and non-enveloped viruses. Until now only one supernatant of a *Lactobacillus curvatus* strain showed an antiviral effect against the Murine norovirus, widely used as a surrogate for human noroviruses. A selection of relevant bacterial starter and protective cultures will be tested in following experiments for their potential of virus inactivation in raw food products.

INTRODUCTION

An antiviral effect of bacteriocins as well as bacterial supernatants, e.g. originated from lactic acid bacteria or staphylococci, was described in different studies for example with herpes simplex virus [1], influenzavirus A [2] and Newcastle disease virus [3]. Application of these bacteria as starter and protective cultures and their metabolites during food processing or as potential probiotics added to food or animal feed can therefore be a promising measure to reduce virus infections in humans and animals. The aim of this research project is to investigate the antiviral potential of different bacteria commonly used as starter and protective cultures during food processing as well as of related bacteriocins.

MATERIAL AND METHODS

The antiviral effect of bacteriocins, potentially produced by lactic acid bacteria, was investigated *in-vitro* towards enveloped viruses (influenzavirus H5N6 and H1N1; Newcastle disease virus, NDV; Feline herpesvirus, FHV) and towards the non-enveloped Murine norovirus (MNV). The different viruses were incubated 1:10 with the bacteriocins sakacin A, sakacin P (GenScript, USA) and nisin (Nisitrol, Schechen) solved in PBS for 3 days at 24 °C at a pH range from 5.8 - 6.3. The reduction of the virus titer was subsequently determined by titration of incubated samples in corresponding cell culture with MDCK+, CRFK or macrophage cells. Furthermore, the antiviral potential of 35 cell-free supernatants originated from different species of lactic acid bacteria (*Lb. plantarum*, *Lb. paracasei*, *Lb. curvatus*, *Lb. sakei*, *Ped. acidilactici* and *Ped. pentosaceus)* as well as from *Staphylococcus xylosus/carnosus* and *Kocura varians* was tested against MNV, H1N1 and H5N6. The incubation and titration of the virus-supernatant-mixture was done like described above. The analysis for cytopathogenic effect and the calculation of the virus titer followed 3 to 5 days after titration of the samples.

RESULTS

All tested bacteriocines showed no antiviral effect against non-enveloped MNV. For the enveloped viruses a reduction of the virus titer in PBS supplemented with nisin or sakacin A could only been shown for H5N6 with a virus titer reduction between 1.5 and 2 log units. However, because of the increasing instabilty of H5N6 at pH < 6.2 results are difficult to interpret. Furthermore, recent experiments did not indicate a strong antiviral effect of nearly all tested cell-free supernatants derived from *Lb. plantarum*, *Lb. paracasei*, *Lb. curvatus*, *Lb. sakei*, *Ped. acidilactici* and *Ped. pentosaceus* as well as from *Staphylococcus xylosus/carnosus* and *Kocura varians*. The supernatent derived from a *Lb. curvatus* strain revealed a significant antiviral effect against MNV with a reduction of virus titer between 0.8 and 1.4 log units.

DISCUSSION

First results of the study did not confirm the expected antiviral potential of bacteriocins in case of MNV, NDV, FHV and H1N1. More research work is needed to examine the potential effect of the bacteriocins sakacin A and nisin on influenza viruses. Therefore experiments with bacteriocins and H5N6 should be repeated at higher pH values (7.0 until 6.5), where the virus remains more stable. The screening for suitable bacteria which may be used for virus inactivation during food processing revealed one supernatant from *Lb. curvatus* with an antiviral activity against MNV. Which substance of the supernatant contributes to inactivation of MNV will be investigated in further experiments.

CONCLUSIONS

According to the shown *in-vitro* experiments no clearly antiviral effect of bacteriocins and no high antiviral potential of nearly all tested starter and protective cultures against enveloped and non-enveloped viruses can be assumed. The antiviral potential of *Lb. curvatus* against MNV will be further characterized. Additional experiments will include investigations concerning the influence of starter and protective cultures on virus inactivation in the complex food matrix of raw sausage products. Therefore, selected starter cultures with different acidification properties will be tested in various viral contaminated raw sausage products. If bacterial cultures and their metabolites (for example lactic acid) reveal an additional antiviral effect, their use can be a promising measure in regard to consumer protection and product safety.

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ANTI-VIRUS ACTIVITY INDUCED BY BCG-PSN IN CHICK EMBRYO FIBROBLAST CELLS *IN VITRO*

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SUMMARY

The effect of polysaccharide nucleic acid of Bacillus Calmette Guerin (BCG-PSN) on chick embryo fibroblast (CEF) cell viability, anti-Vesicular stomatits virus (VSV) activity and on IFN-α and IFN-β levels were determined in vitro. BCG-PSN inhibited VSV growth in CEF cells in the range of 18.0-712.5 μg/mL and over 12-48 h of treatment. The anti-VSV activity was maximal for 144.0 μg/mL of BCG-PSN treatment at 24 h, after which it declined. The amount of IFN-α and IFN-β in cell supernatant and the transcription levels of IFN-α mRNA and IFN-β mRNA increased after BCG-PSN treatment, indicating that the anti-viral activity was relatived to the production of type I IFN. However BCG-PSN also showed a toxic effect by inhibiting CEF cell proliferation in a dose-dependent manner. The results indicate that the dosage level of BCG-PSN must be carefully considered when used in a clinical setting.

INTRODUCTION

Bacillus Calmette Guerin (BCG) immunotherapy has been demonstrated to stimulate the immune system to fight infection and disease [1-2]. However, severe side effects hamper its field application. The polysaccharide nucleic acid of Bacillus Calmette Guerin (BCG-PSN) is extracted from BCG and without severe side effects, and has been patented by the Ministry of Public Health of PRC (People's Republic of China). It stimulates the production of type I interferon (IFN-α and IFN-β) and enhances immunocyte activity and has been demonstrated to be potent activators of antiviral immunity. It has been used in the treatment of atopic disorders, conjunctivitis, mastitis, chronic bronchitis and other conditions in humans [3-6]. In this study, we chose vesicular stomatitis virus (VSV) as experimental subject to investigate the anti-virus activity of BCG-PSN and assessed the contribution of IFN to the effect.

MATERIAL AND METHODS

Influence of BCG-PSN on CEF cells viability using the MTT assay

To observe the influence of BCG-PSN on CEF cell viability, a colorimetric assay was used that measures the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) into an insoluble formazan product by the mitochondria of viable cells. The level of viability determined correlates with proliferative and survival ability and the metabolic activity of the cells. cell viability = (mean absorbency in test wells)/(mean absorbency in control wells)×100

Virus protection assays

To measure the anti-viral ability of BCG-PSN, briefly, CEF cells were first seeded into 96-well flat-bottomed plates and cultured to confluence. The cells were then incubated with 100 μL of different concentration of BCG-PSN then challenged with 100 × TCID50 of VSV. Cells were incubated with MTT (0.5 mg/mL) for 4 h, DMSO was added to dissolve the formazan in cells, the absorbance at 490 nm was then measured with a Multi-well Microtiter Plate Reader, and the morphology of cells was observed in microscope.

Analysis of IFN-α and IFN-β induced by BCG-PSN

CEF cells were treated with different concentration BCG-PSN for 24 h and the supernatants harvested. An enzyme-linked immunosorbent assay (ELISA) kit (R&B System, USA) was used to detect IFN-α and IFN-β in the supernatants which applys sandwich technique.

Quantitative real-time RT-PCR fluorescence detection of IFN mRNA (FQ-PCR)

The primers for IFN-α (GenBank accession number: X92476), IFN-β (GenBank accession number: AY974089) and the housekeeping gene GAPDH (glyceraldehydes-3-phosphate dehydrogease) (GenBank accession number: K01458) were designed according to previous reported in GenBank. Primer sequences for these genes were: IFN-α Sense: 5'GTCTTGCTCCTTCAACGACA3', Anti-sense: 5'GCGCTGTAATCGTTGTCTTG3', IFN-β Sense:

5'TCCAGGTCCTTCAGAATACG3',Anti-sense: 5'TGCGGTCAATCCAGTGTT3'

GAPDH Sense: 5′TGAAAGTCGGAGTCAACGGAT3′, Anti-sense: 5′ACGCTCCTGGAAGATAGTGAT3′.

RESULTS

Effect of BCG-PSN on cell viability

The effect of BCG-PSN on cell viability assessed using the MTT assay is shown in Fig.1. BCG-PSN inhibited proliferation of the CEF cells in a dose-dependent manner. Increasing the BCG-PSN concentration of treatment led to a decrease in cell viability.

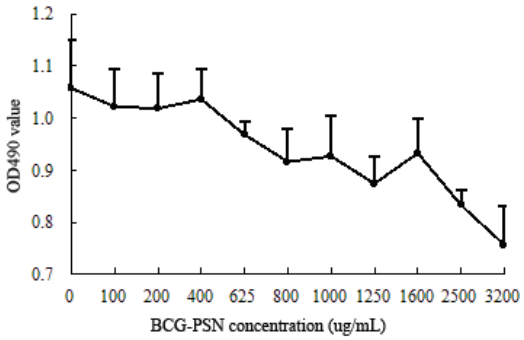

Fig.1 The effect of different concentrations of BCG-PSN on CEF cells viability.

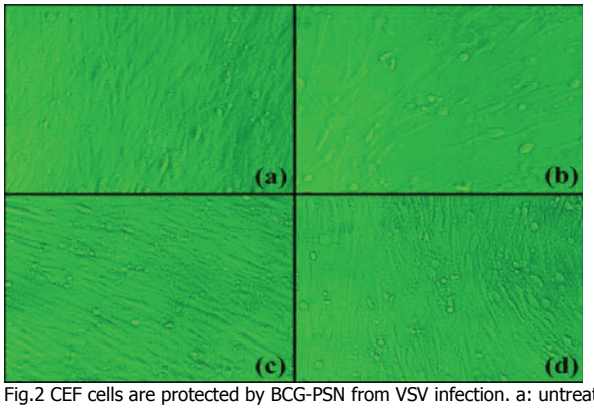

Fig.2 CEF cells are protected by BCG-PSN from VSV infection. a: untreated cells; b: cells exposed to VSV; c: CEF cells exposed to VSV and treated with 100 μg/mL BCG-PSN; d: CEF cells exposed to VSV and treated with 200 μg/mL BCG-PSN.

Anti-viral ability induced by BCG-PSN

After CEF cells were incubated with 100 μg/mL or 200 μg/mL BCG-PSN for 24 h, it was found that it could protect the cells from a challenge of 100 × TCID50 of VSV, as shows in Fig. 2. To determine the time-dependent effect of BCG-PSN on anti-VSV activity, CEF cells were incubated with 144 μg/mL BCG-PSN for different times (12 h to 24 h) and then challenged with 100 × TCID50 VSV. The presence of anti-viral activity is reflected by OD570 values (Fig. 3a). After treatment for 12 h, an anti-viral activity in CEF cells was apparent higher than that of the control without BCG-PSN ($P < 0.05$). With an increase of treatment time up to 48 h the anti-viral activity of CEF cells gradually increased. The anti-VSV activity reached a peak at 24 h and then decreased out to 48 h, although the level was still higher than that of the control without the BCG-PSN ($P < 0.05$). When the cells were treated with different concentrations of BCG-PSN for 24 h, significant anti-VSV activity was detected when for 18 μg/mL BCG-PSN (Fig 3b), the effect increasing with BCG-PSN levels and reaching a peak level at 144.0 μg/mL, although there was no significant difference between the activity of 287.0 μg/mL, 575.0 μg/mL and 712.5 μg/mL ($P > 0.05$). The anti-VSV activity decreased when dosage levels were higher than 144.0 μg/mL. Based on the incubate time and dose effect, the anti-viral activity was strongest after CEF cells incubated with 144.0 μg/mL of BCG-PSN for 24 h.

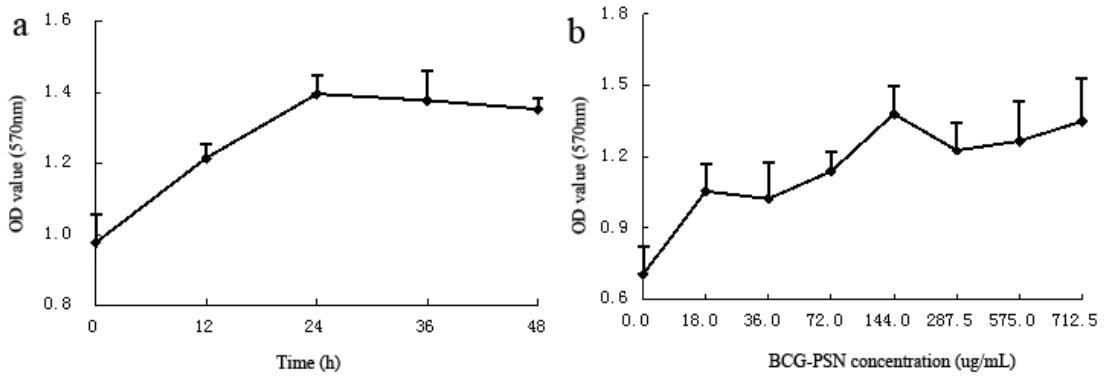

Fig.3 Effect of incubation time and dosage of BCG-PSN on anti-VSV ability.

BCG-PSN induce CEF cells to produce IFN-αand IFN-β

The amount of IFN-αand IFN-β in the supernatants after CEF cells cultured with 36.0 μg/mL BCG-PSN for 24 h were 9.42 ng/mL and 8.27ng respectively, which was significant higher than the control without BCG-PSN (P < 0.05) (Fig.4a). The amount of IFN-αand IFN-β increased with the increase of BCG-PSN concentration. The amount of IFN-α reached peak at 144.0μg/mL, while The amount of IFN-βreached peak at 287.0μg/mL.

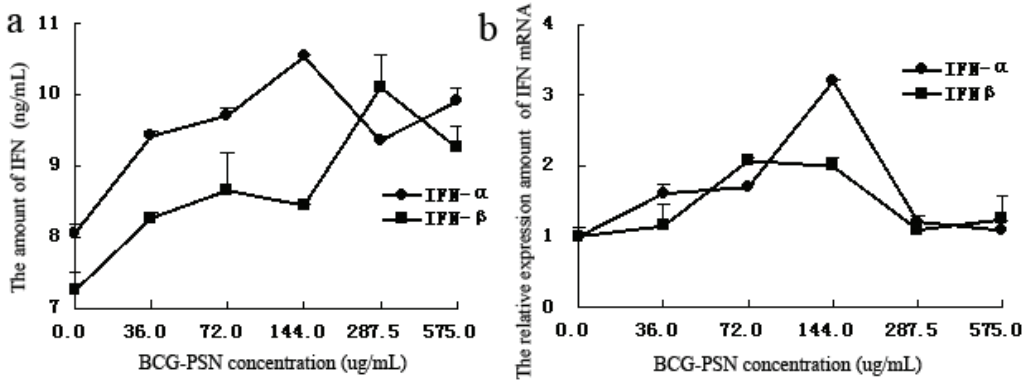

Fig.4 The amount of IFN and IFN mRNA after CEF cells treated with different BCG-PSN concentration.

BCG-PSN induces CEF cells to enhance transcription levels of type I IFN mRNA

After CEF cells were treated with different concentrations of BCG-PSN for 24 h, it was found that both the transcription levels of IFN-α mRNA and IFN-β mRNA increased in the similar tendency with induction of dosage of BCG-PSN (Fig.4b), the levels of IFN-α mRNA and IFN-β mRNA reached a peak at 144μg/mL, but there were no statistic difference between the groups with 287.0 μg/mL to 575.0 μg/mL BCG-PSN treated (P > 0.05).

DISCUSSION

In this study it was found that the anti-VSV activity increased as a result of BCG-PSN treatment. The effects were dependent on the concentration and time of exposure to the BCG-PSN. BCG-PSN inhibited VSV growing on CEF cells with concentration and incubated time at the range of CEF cells incubated with 18.0 μg/mL to 712.5 μg/mL for 12 h to 48 h. After exceeding the range, the anti-VSV activity decreased. It reveals that proper BCG-PSN concentration and incubate time were important to anti-VSV activity. The anti-VSV activity reached a peak level when VSV-infected CEF cells incubated with 144.0 μg/mL of BCG-PSN for 24 h. Previously it had been reported that the anti-viral effect was connected with IFN production. Type I IFN promotes an antiviral state in neighbouring cells by inducing the expression of several antiviral proteins such as protein kinase R (PKR), 2′-5′-oligoadenylate synthetase (OAS), RNnase L and Mx proteins [7]. Here both increase of the production amount of IFN-αand IFN-β in supernatants and the transcription levels of IFN-α mRNA and IFN-β mRNA showed that the anti-viral activity of BCG-PSN was related to production of type I IFN. Our results, however, also showed that the anti-VSV activity did not increase continuously with increasing time of exposure, concentration of IFN-α and transcription levels of IFN-α mRNA and IFN-β mRNA. This may be partially explained on the basis of the toxic effect of BCG-PSN on CEF cells, as shown by a decreased viability of the CEF cells with high concentration of BCG-PSN, or long exposure times. In our study, anti-VSV activity was maximal when cells were exposed to 144.0 μg/mL of BCG-PSN for 24 h, a level at which the higher level of IFN induced was sufficient to resist VSV growth with minimal cell damage. The dosage of BCG-PSN is a critical factor and one that must be considered when used in a clinical setting.

CONCLUSIONS

BCG-PSN supplys a new choice for us to resist some virus infection in livestock, such as herpes simplex virus, hepatitis virus, respiratory virus, or enhances the anti-viral activity when combining with antiviral drugs. BCG-PSN also has some toxic effect, the dosage and the frequency should be considered when used.

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PRINCIPLES OF BIOSECURITY IN SHEEP FARMS

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SUMMARY

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 or compromise the quality of the products produced in the farm. The aim of our work was to assess the main way of complex preventive measures to minimise the potential disease-causing microorganisms in 41 sheep farms with emphasis on animal health and product quality. 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 point of farm sanitation was also taken into consideration. The infection pressure in farms and in stables proportionally increases with animal concentration, duration of their stay in the stable. This may cause growth of depression and health problems in sheep. Therefore the sanitary control measures should be incorporated in all houses, so that a good hygienic standard can be easily maintained.

INTRODUCTION

Optimal environmental conditions are essential if animals are to reach their genetic potential. 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 or compromise the quality of the products produced in the farm. Disease transmission from just one newly introduced animal to another animal in the flock can affect the health of the entire flock [1].

Implementing and maintaining a biosecurity plan is important as the introduction of new diseases into a herd can be expensive in terms of decreased production due to the illness an possibly death of animals including additional costs associated with treatment [2]. In principle it is a complex of preventive measures designed to prevent the penetration of infectious agents in animals by persons (hygienic filter), animals (wildlife, birds, pets, rodents, insects), technological systems and equipment, transport, airborne infections, contaminated feed and water, dead animals [3,4]. Good management can do much to reduce the effect of adverse environmental factors. Biosecurity is an integral part of health herd management.

ANIMALS, MATERIAL AND METHODS

The aim of our work was to assess the main way of complex preventive measures to minimise the potential disease-causing microorganisms in 41 sheep farms with emphasis on animal health and product quality. Sheep farms were grouped by the size of the herd into small (up to 10 ewes), medium (11-50 ewes) and large (above 50 ewes) flocks. Based on the sheep checklists [5,6,7] we created the Investigator's schedule, which was filled in by farmers in cooperation with the vets and sheep specialists. There was collected and analysed a complex of preventive measures designed to prevent the penetration of infectious agents into animals by persons (sheep farmers, seasonal workers, vets, visitors, etc.), animals (quarantine, purchase of animals, exhibitions, sharing), health condition (health control, deworming, vaccinations, preventive health herd management), transport (care of vehicles), breeding technological systems and equipment (technology of winter housing, pasture management, feeding, drinking, removing of excrements, ventilations) and the point of farm sanitation (cleaning and disinfection, handling with cadavers) was also taken into consideration. The obtained information was entered into a database, which was statistically analysed by Statistica software (non parametric tests).

RESULTS

The results are summarised in the following table according the Criteria of biosecurity and size of the flocks including a statement of statistical significance.

Table: Evaluation of the level of biosecurity in sheep farms

Criteria of biosecurity	Size of the flock					
	less than 10 ewes		11 – 50 ewes		more than 50 ewes	
	mean	s.d.	mean	s.d.	mean	s.d.
Personnel	2.2 ^{A,B}	1.8	3.2 ^{A}	1.0	3.2 ^{B}	1.3
Animal	10.4	4.4	10.2	4.3	12.2	4.7
Health state	8.0	1.9	8.8	2.4	9.9	2.8
Transport	3.3	0.8	3.2	1.1	3.4	1.4
Breeding, technology	8.6	1.5	8.4	2.3	8.5	2.4
Sanitation	6.3	2.4	6.9	4.2	8.9	3.8
Total evaluation	39.3 ^{C}	8.6	40.6 ^{D}	9.7	46.1 $^{C, D}$	9.6

^{A,B,C,D} Means followed by different letters are significantly different at ($p<0.05$)

Small flocks were found to have significantly lower level of security of herds against contact with other persons (vets, farm visitors, season workers etc.) who come into direct contact with the animals. The possibility of importation of an infection to farms by animal purchases, animals from animal exhibitions, including quarantine measures, is a problem mainly in small and medium sheep herds, as well as questions of possibility of maintaining good herd health status by preventive and therapeutical measures. Flock managers should only buy animals from farms with good biosecurity and general health management. Transport conditions stated in legislation were fulfilled in all breeds adequately. Technological systems of breeding used for winter stabling of sheep as well as during grazing period were not proved to be statistically different in all monitored breeds. Level of sanitary measures used in monitored farms increases with increasing size of basic herd. Based on analysis of collected data it can be stated that breeds with higher number of sheep have better conditions for application of principles of breed security against importation of infectious agents to the farm as well as their spread within the farm. Breeders of small and medium flocks are not able to fulfil more than 50% of required markers. On the other hand they can pay attention to the sheep individually.

DISCUSSION

The Good biosecurity practices should be part of the preventive health management plan of all operations. The infection pressure in farms and in stables proportionally increases with animal concentration, duration of their stay in the animal house. This may cause growth of depression and health problems in sheep. Therefore the sanitary control measures should be incorporated in all houses, so that a good hygienic standard can be easily maintained. Therapeutic arrangement is usually too expensive. The use of good breeding practices and correct Biosecurity rules usually prevents the herd from health disorders [1]. Good management can do much to reduce the effect of adverse environmental factors. Biosecurity is an integral part of health herd management. The degree of veterinary hygienic protection depends on the concentration of the parent herd of sheep, the disease situation in the locality, as well as the level of immunity of the organisms of animals kept in the site. According to Gray [8] and Stear et al. [9] endemic diseases as well as epidemic diseases that are insect borne require keeping level of immunity in the flock at the required level by vaccination. However general immunity needs to be managed equally well by ensuring adequate nutrition, reducing parasite levels that could have negative impact on immunity and even selecting animals with natural immunity and resistance to disease. Adequate hygienic level of breed is the presumption of achieving the high level of production and reproduction parameters of the sheep and the economical rentability for the stockman [10]. Only standard practice keeps on good results for a long time.

CONCLUSIONS

Although Basic principles of sheep farm biosecurity providing the appropriate level of health in the sheep are summarized in the following areas:

1. Maintaining a closed herd turnover;
2. Prevention of the contamination of sheep by farm visitors and other people;
3. Not using the workers, who were in contact with sheep from other flocks;
4. Using the four week quarantine for purchased sheep including rams and the animals returning from exhibitions;
5. Using regular health herd management control including the parasitic control programme;
6. Transporting sheep only in clean trucks;
7. Minimising contact with wildlife, pets and stray animals, which may transmit sheep disease;
8. Breeding and pasture management with emphasis on the quality of feed and water sources;
9. Aiming vaccination programme to minimise the risk of introduction of disease and parasites to the flock;
10. Implementing strict sanitation program for whole farm (disinfection, disinsection and deratisation).

Veterinarians should focus on the implementation of a flock biosecurity plan as a part of the overall strategy to control hazards to production and reproduction.

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THE INFLUENCE OF FASTING ON SOME BIOCHEMICAL FACTORS OF SERUM IN CATTLE

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SUMMARY

This study was carried to investigate the influence of fasting (as a model of anorexia) on some biochemical factors of serum in cattle. Five cross-breeds, non-lactating and non-pregnant cattle with BCS 2-2.5 were fasted for 8 days. Blood samples wer taken from the jugular vein during fasting. Glucose, NEFA, ApoA1, ApoB, TG, cholestrol, BHBA, total lipid of blood serum were taken by biochemical methods and laboratory kites. The results of this study showed the concentrations of the NEFA and ß-hydroxybutyrate (BHBA) increased significantly ($p<0.001$) at the end of fasting. There were no significant difference in the concentrations of serum TG, cholesterol, glucose, total lipid, ApoA1 and ApoB at the prefasting and end of fasting. The results of this study showed, fasting like anorexia (as a result of natural disease) induces ketonemia and fat mobilization from adipose tissue in response to the negative energy balance.

INTRODUCTION

Negative energy balance has been implicated in the development of fatty liver, insulin resistance, and impaired health and milk production in dairy cows. It is caused by several complicated factors, including excessive feeding before parturition, stress, feed deprivation, hormonal imbalance, decrease in feed intake, low-energy feed intake.

The concentration of glucose, NEFA, ApoA1, ApoB, TG, cholestrol, BHBA, total lipid in serum are the indicators of energy balance. Fasting as a model of anorexia is used for induced negative energy balance. Therefore, this study was carried out to investigate the influence of fasting on some biochemical factors of serum in cattle.

MATERIAL AND METHODS

Five cross-breeds, non-lactating and non-pregnant cattle weighing on average of 304/6 kg (293kg to 310kg) were used for this study. cows were evaluated for healt problems by physical examination and they fasted for 8 days, but water were freely available.

Blood samples wer taken from the jugular vein the day before and 8 days during fasting. Glucose, NEFA, ApoA1, ApoB, TG, cholestrol, BHBA, total lipid of blood serum were taken by biochemical methods and laboratory kites.

Data were analyzed using a one –way analysis of variance (ANOVA) and the post. hoc Tukey test. Values are expressed as mean ± standard deviation (S. D.) in the text and in the figures. All statistical analyses were performed using sigma stat 2 software (copy right 1992-1995 Jandel corporation). Significance was accepted at the level of $p< 0.05$.

RESULTS

The results of this study showed the concentrations of the NEFA and ß-hydroxybutyrate (BHBA) increased significantly ($p<0.001$) at the end of fasting.

The level of NEFA was increased from 0.77±0.31mmol/l at the day before fasting to 1.27±0.31 mmol/l at the day 8 after fasting (Figure 1).

The level of BHBA was increased from 0.23±0.02 mmol/l at the day before fasting to 0.73±0.09 mmol/l at the day 8 after fasting (Figure 1).

The results of this study showed the concentration of total lipid, glucose, TG, cholestrol, ApoA1 and ApoB at the day before fasting were 273.5±45.32, 55.17±11.98, 25.63±5.8, 65.37±13.11, 3.58±0.01 and 7.44±3.75, and the 8 days after fasting were 269.77±65.62, 42.63±5.02, 15.21±2.71, 73.17±3.5, 3.58±0.01 and 7.64±1.12, respectively. There were no significant difference in the concentrations of serum TG, cholesterol, glucose, total lipid, ApoA1 and ApoB at the prefasting and end of fasting.

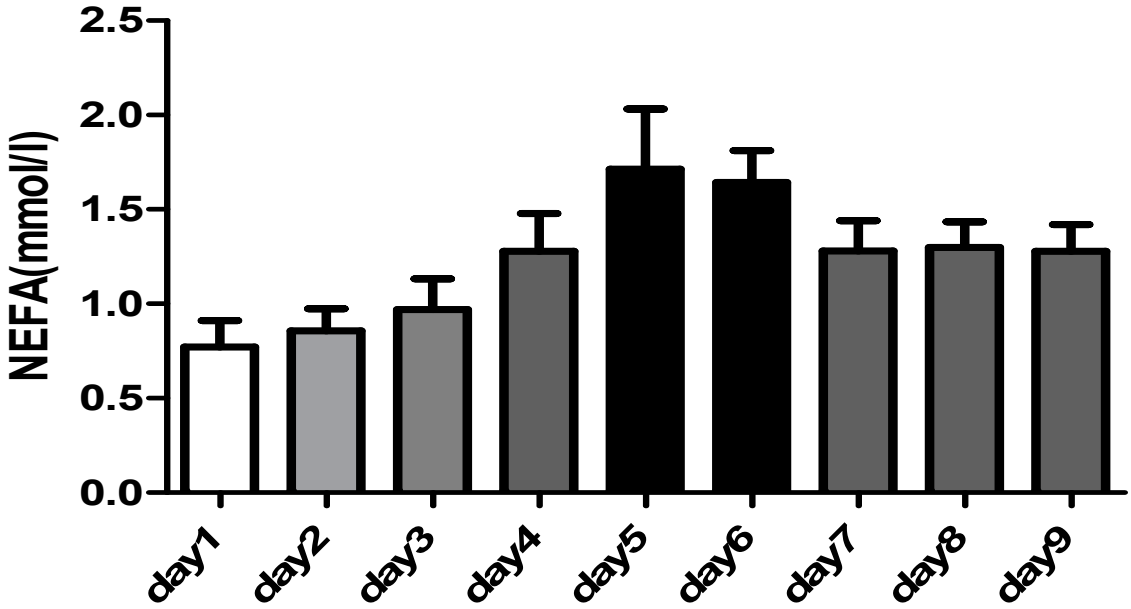

Figure 1: The concentration of NEFA at the day before (1) and duration of fasting

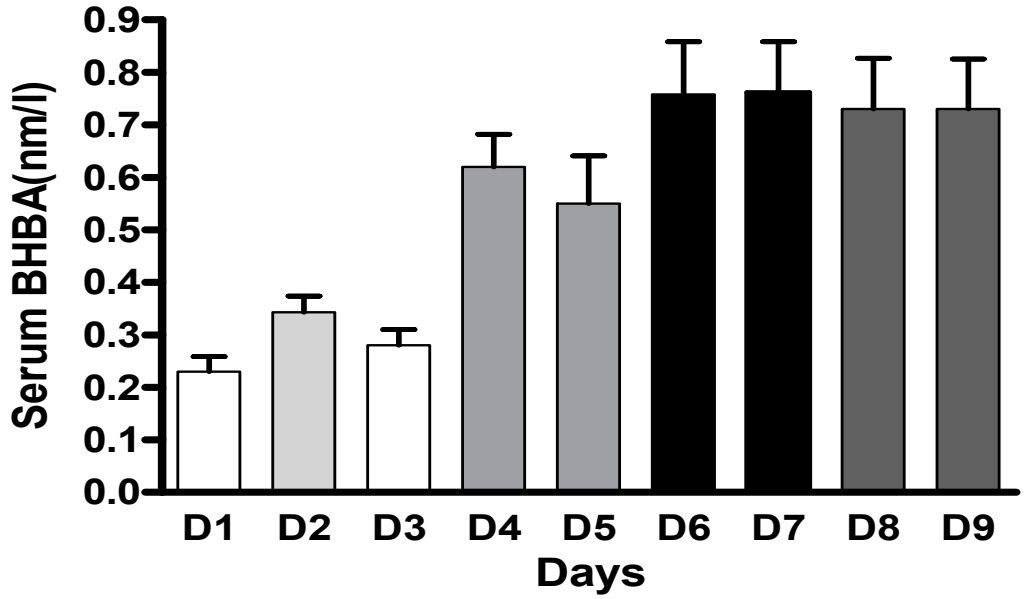

Figure 2: The concentration of NEFA at the day before (1) and duration of fasting

DISCUSSION

In this study only the concentrations of the NEFA and ß-hydroxybutyrate (BHBA) increased significantly at the end of fasting. In the study of Oikawa and Oetzel (2006), fasted cows had increased plasma nonesterified fatty acid (NEFA) concentrations and increased plasma beta-hydroxybutyrate (BHBA) concentrations at the end of the fasting period. Plasma NEFA, and plasma BHB A in fasted cows returned to prefasting concentrations by the end of the experiment. Plasma glucose concentrations were not affected by fasting. Plasma insulin concentrations were decreased and insulin-stimulated blood glucose reduction was decreased in the fasted cows compared with control cows at the end of the fast, indicating reduced insulin response. They concluded that insulin response was negatively correlated with plasma NEFA and liver triglycerides(1).

Non- esterified fatty acids (NEFA) are sensitive indicators of energy balancey. They are useful for monitoring energy status of dry cows in the last month of gestation, when rapid changes in energy balance status may not be detectable from changes in body condition score. High values of NEFAs indicate negative energy balance which occurs in animals which are inappetent for any illness(2).

Serum BHBA concentration are affected by energy and glucose balance and are a less specific indicator of energy balance than plasma NEFA. High values are associated with reduced milk production, increased clinical ketosis and LDA and reduced fertility. The gold standard test for subclinical ketosis is blood BHBA which is more stable ketone body than acetone or acetoacetate(2).

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EFFECTS OF COLD TEMPERATURES ON PRODUCTIVE PARAMETERS AT MANGALICA AND LARGE WHITE PIGS

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SUMMARY

Although the fattening pigs tolerate variations temperature quite well, the excessively cold or excessively hot temperatures will cause stress, poor growth and more health problems. The studies aimed the productive effects of cold temperature on Mangalica and Large White breeds (males castrated). The experiment was conducted on each 25 pigs of Mangalica and Large White breeds reared in alternative system, exposed at 11-8°C and 22°C. Experimental period was 23 days. The animals had free access to standard, isoprotein and isocalory diets, with 13.5% crude protein (CP) and 3100 kcal/kg metabolizable energy. Feed intake was measured daily. Body weight was determined individually, twice of month. Fat thickness was determined using ultrasound device. In cold temperatures conditions, Mangalica pigs have made the following productive parameters: 5.87 kg feed intake; 385.4 g daily weight gain; 67.8 mm fat thickness. White Large pigs have achieved: 4.93 kg feed intake; 527 g daily weight game; 35.2 mm fat thickness. The results between breeds were statistically significant ($p \leq 0.05$). Compared with Large White breed, Mangalica adapted better to expose at cold temperatures.

INTRODUCTION

Mangalica is a breed with a valuable genetic background which can be used in the current context of organic livestock, considering the natural resistance and adaptation to growing conditions in intensive, extensive and alternatives systems.
Mangalica is one of the most popular breeds of pigs in Europe, because meat has superior properties, such as taste, marbling and low cholesterol content. Many Americans Farmers have imported the Mangalica breed and the technology of its raising.

The environmental factors have major influence on productive performances and feed efficiency. The negative implications of long-term exposure are often obvious in the critical production stages, as they are threatening and negatively impact the animal's welfare [1]. The climate conditions have a major role in expressing the genetic potential of races and productive parameters [2].
The studies aimed the effect of cold temperatures on productive parameters at Mangalica pigs, compared with Large White pigs.

MATERIALS AND METHODS

The experiments were conducted on each of 25 pigs (males castrated) each of Mangalica (group 1) and Large White (group 2) breeds, raised in alternative system, exposed to cold temperatures, ranging between 11-8°C. Data were compared with those obtained from about 25 pigs exposed to 20°C, raised in controlled room (group 3 Mangalica, group 4 large White).
Experimental period was 23 days.

The animals had free access to standard, isoprotein and isocalory diets, with 13.5% crude protein (CP) and 3100 kcal/kg metabolizable energy. Feed intake was measured daily. Body weight was determined individually, twice a month. Fat thickness was determined using the ultrasound device.
The data were ANOVA statistically processed.

RESULTS

The productive performances are presented in Table 1.

Table 1. The productive performances of pigs

Specification	G1 M	G2 LW	G3 M	G4 LW
Initial weight, kg	100.50	100.58	100.56	100.23
Final live weight, kg	109.36	112.72	110.21	117.38
Daily weight gain, g	385.4	527	419.56	745.65
Feed intake, kg	5.87	4.93	4.48	3.25
Fat thickness, mm	67.8	35.2	41.2	13.4

After 23 experimental days, the final live weight was 109.36 kg at G1, 112.72kg at G2, 110.21 kg at G3 and 117.38 at G4 (Table 1). The daily weight gain was 385.4 at G1, 527 g at G2, 419.56 g at G3 and 745.65 at G4 (figure 1). The average daily weight gain decreased with 26.8% in group 1 compared to group 2, the differences being significant ($p \leq 0.05$).

The feed intake was 5.87 kg at G1, 4.93 kg at G2, 4.48 g at G3 and 3.25 g at G4 (figure 2). The differences were significant ($p \leq 0.05$).

The fat thickness was 67.8 mm at G1, 35.2 mm at G2, 41.2 mm at G3 and 13.4 mm at G4, the differences between groups being significant ($p \leq 0.05$).

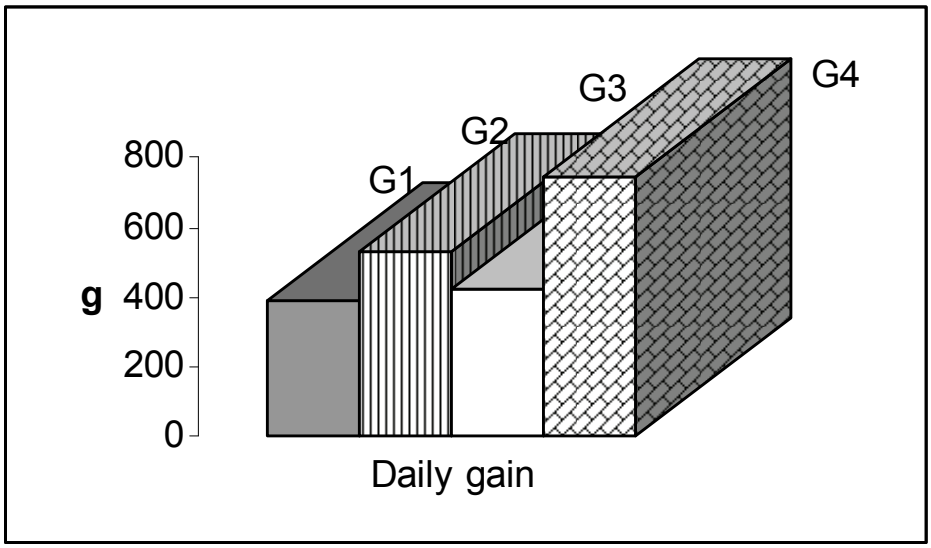

Figure 1. Daily weight gain

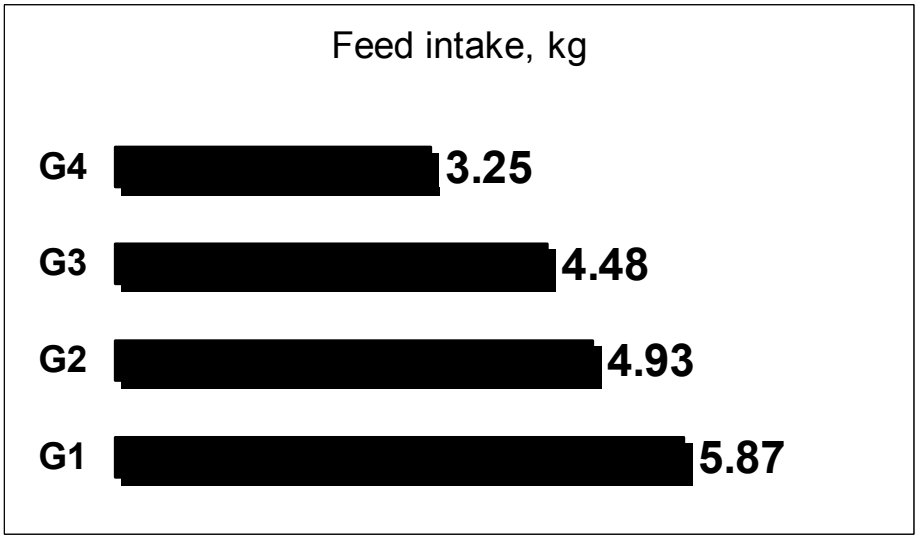

Figure 2. Feed intake

DISCUSSION

In terms of daily weight gain, the results are determined by the physiological particularity of Mangalica breed, which is a late race, the literature data showing that the weight of 140-180 kg is achieved at the age of 12-15 months, ensuring an average daily weight of 450-500 g [4]. Compared with the results obtained at neutral temperature (G3 and G4), the daily weight gain decreased with 8.1% at Mangalica (G1) and 29.3% at Large White (G2). Exposure to cold temperatures was better tolerated by Mangalica, which decreased average daily gain was less drastic.

Because Mangalica is a rustic breed, it is characterized by high specific consumption, even if the diet is made up of compound feed, when the feed intake is 5.2 kg [3]. In the UK, the breed is kept free-range, fed on standard sow and weaner pellets. The higher quality and protein levels of this food results in a slightly larger stockier pig [5]. Compared with the results obtained at neutral temperature (G3, G4), the feed intake increased with 31% at Mangalica (G1) and 51.6% at Large White (G2). Exposure to cold temperatures was better tolerated by Mangalica, considering physiological peculiarities of this race in providing thermal homeostasis.

By exposure to cold temperatures, the fat thickness increased with 1.65 times at Mangalica and 2.62 times at Large White, compared with exposure to neutral temperatures

CONCLUSIONS

Results of this research confirmed that animals of Mangalica breed is better adapted to expose at cold temperatures, compared with Large White race.

ACKNOWLEDGEMENT

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BIOSECURITY, HEALTH CONTROL, FARMING CONCEPTION AND MANAGEMENT FACTORS : IMPACT ON TECHNICAL AND ECONOMIC PERFORMANCES

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SUMMARY

This study is based on an analysis of relationships between the characteristics of farms with a regard to biosecurity, health control, farming conception, management factors and technical and economic performances. Questionnaires were used to gather information about farming characteristics and practices. Seven techno-economic indices from the technical and economic database (GTE) have been identified: sow productivity, average daily gain, feed conversion ratio, mortality rate, medication costs, lean meat percentage and percentage of pigs within optimum carcass weight range. A standardized margin was defined in order to summarize the economic effect.

The influence of some farming practices and characteristics on the technical and economic performances was demonstrated: the technical and economic results were lowered by diseases with clinical signs. Good building conception, strict management with a rigorous batch farrowing, a complete protocol of cleaning and disinfection and correct sanitary practices were linked to good technical and economic performances. The difference of the margin between farms with and without favourable practices is estimated at around 180 €/sow/year.

INTRODUCTION

Numerous recommendations concerning biosecurity, hygiene and management factors are given to farmers through the Good Hygiene Practices Guide or other improvement initiatives. In a difficult economic environment, these advances may appear as additional obligations that require investments or changes in practices without necessarily favourable effects on technical and economic performances of farm.

The purpose of this study is to analyze the relationships between the characteristics of farms with regard to biosecurity, conception and management and their technical and economic performances.

MATERIAL AND METHODS

In 166 farrow-to-finish pig herds, questionnaires were used to gather information on farming characteristics and practices. The questionnaire, with a little over 400 points, focused on biosecurity, quarantine, feed and farming management, types of rooms for each physiological stage, health control, protocol of cleaning and disinfection [2].
The following seven techno-economic indices from the French technical and economic database (GTE) were selected:

- sow productivity: number of pigs produced per present sow and per year (Productivity),
- average daily gain (8-115kg), in g per day (ADG),
- feed conversion ratio (8-115kg), in kg of feed consumed per kg of growth (FCR),
- mortality, from weaning to sale, in percentage (Death rate),
- medication costs, in € for 100kg of carcass weight (Health cost),
- lean meat percentage (carcass leanness),
- percentage of pigs within optimum carcass weight range (% of range).

The standardized margin (in €/present sow/year) was calculated to have an economical estimation [2].

Standardized margin = product – feed cost – replacement cost With: the technical indices are those of GTE results from each farm. The economical indices (pork and feed prices) are the average values over 5 years.

In a first step, the 8 technical and economic indices have each been submitted to a statistical analysis independent from the others. The relationship between farming practices (descriptive and explicative variables) and each of the 8 technical and economic indices (quantitative variables that we want to explain) has been studied in 2 stages: an ANOVA with one factor followed by a multivarious analysis with a multiple linear regression model (GLM-SAS).

In a second step, the impact of good practices on technical and economic performances was assessed. For each variable included in the 8 multivariate models, a score of 10 is attributed to farms complying with the favourable modality of this variable. By adding these individual scores, a global index is calculated by farm. The farms are grouped in 3 profiles (Profile 1: unfavourable practices, Index ≤ 150 - Profile 2: average practices, 150 < Index ≤ 200 - Profile 3: favourable practices, Index >

200) and the impact of these profiles on the 8 technical and economic indices is analyzed (GLM -SAS).

RESULTS

Farming characteristics linked to technical and economic performances

For each 8 technical and economic indices, the results of analysis of variance with one factor and multiple linear regression models are presented in Table 1. The number of significant variables at 5% of the univariate analysis varies from one variable to another: some explanatory variables are linked to medication costs and percentage of pigs within optimum carcass weight range, respectively 14 and 18. On the contrary, for the daily gain and the standardized margin, respectively 47 and 53 variables are emphasized.

Table 1: Results of statistical analysis

	Sow Productivity	ADG (g/d)	FCR (kg/kg)	Death Rate (%)	Health cost (€/100 kg)	Carcass leanness	% of range	Margin (€/ sow/ Year)
Univariate analysis: N*	41	47	37	38	14	34	18	53
Regression model : N	8	6	6	5	5	4	5	6
Regression model : R^2	0,33	0,32	0,24	0,20	0,21	0,20	0,16	0,31
* number of significant variables								

The regression models emphasize four to eight variables related to each indice. Practices which have a positive effect for each of these 8 models are:

- **sow productivity:** age of weaning at 21 days, no change of pens and presence of large rooms in post-weaning, over 50% of partition walls between the pens in the fattening unit, on farm mixed-diet, antibiotic treatment in fattening, footbath in front of rooms and in corridors.
- **average daily gain**: farm geographical localization, over 80% of slatted floors in fattening, all in-all out management in fattening, no clinical PMWS in post-weaning, manure spreading equipment in common, correct protocol of cleaning/disinfection in farrowing units.
- **feed conversion ratio**: dead animals stocked in a closed container, systematic disinfection in post-weaning unit; in fattening unit, all in-all out management, less than 24 pigs per pen, no clinical PRRS and use of a detergent.
- **mortality**: dead animals stocked in a closed container, no change of pens in post-weaning; in fattening, less than 24 pigs per pen, no clinical PRRS and systematic disinfection.
- **medication costs**: no ileitis vaccination and no antibiotic treatment in post-weaning, correct management of manure, automatic soaking in farrowing units, Cleandown time more than 48 hours in post-weaning units.
- **lean meat percentage:** farm geographical localization, no heat-treated feed, washing pits in farrowing unit and corridors cleaning and disinfection.
- **percentage of pigs within optimum carcass weight range:** low rate of piglets adoption, no pre-fattening unit, no clinical signs in farrowing unit and cleaning and disinfection of the loading area.
- **standardized margin:** more than 20% of piglets adoption, no change of pens and presence of large rooms in post-weaning unit, over 50% of partition walls between the pens in fattening, no clinical PRRS in pregnant sows and a favorable salmonella serological status.

Impact of farming conditions and practices on technical and economic performances

Apart from the medication costs and the percentage of pigs within optimum carcass weight range, there is an effect of the farming profile on technical and economic indices (Table 2). For the standardized margin, the 3 profiles are significantly different from each other, with values of profile 1, linked to unfavourable practices, lower than those of profile 2, itself lower than those of profile 3. For the productivity, results of profile 3 farms (with favourable practices) are significantly higher than those of profiles 1 and 2. For other indices, the averages of farms in profile 1 are lower than those of profiles 2 and 3 which are not significantly different. Finally, difference of technical performances between these 3 profiles are important, concerning productivity, average daily gain, feed conversion ratio and mortality. The economic impact, estimated from the standardized margin, shows a difference in margin of 83 €/present sow/year between profiles 1 and 2 and 85 € between profiles 2 and 3. This difference reaches 182 €/present sow/year in favor of farms with favourable practices (profile 3) compared to farms with unfavourable practices (profile 1).

Table 2: Average results of farms from the 3 profiles

Technical and economic indices	p	Practices			△ Profile 3 – profile 1
		Unfavourable Profile 1	Average Profile 2	Favourable Profile 3	
Number of farms		43	90	33	
Sow Productivity	<0.001	20.3 a*	21.0 a	22.2 b	+1.9
ADG (g/d)	<0,005	654 a	676 b	682 b	+ 28
FCR (kg/kg)	<0,0001	2.73 a	2.61 b	2.59 b	– 0.14
Death Rate (%)	<0,001	7.94 a	6.64 b	5.73 b	– 2.21
Health cost (€/100 kg)	ns	6.33	6.20	6.61	nc
Carcass leanness	<0,005	61.35 a	61.76 b	61.89 b	+ 0.54
% of range	ns	84.0	84.0	84.6	nc
Standardized margin (€/sow/year)	<0,0001	863 a	960 b	1045 c	+ 182

* Different letters in a row mean a significant difference at 5% level, ns not significant, nc not calculated

DISCUSSION

The epidemiological questionnaire used was developed for a risk-factors study of the contamination of pigs by salmonella and not especially for this analysis [2]. Also, some variables that could have an impact on technical and economical performances were not taken into account: for example, some information on the health status, on the use of drugs or even on the feed and the reproduction management. This can be why, only a low part of the variation of technical and economic indices (R^2 from 0.16 to 0.33) is explained by the models obtained. Despite this, this study allows us to show the influence of health control, some biosecurity measures and farm management on technical and economic performances.

In this study, although the medication costs are not linked to the farm profile, some farm practices are linked to drugs cost and also previous studies have demonstrated the relationship between this cost and the sanitary status of farms and some farm conditions such as the respect of all in- all out management, of density or of some measures of bio-security [4].

The calculation of index which describes practices may be questionable: it doesn't consider all farming practices which influence performances and it doesn't prioritize them. It shows however an aspect of the bio-security level and health as well as conception and farming management. It also emphasizes important differences in farming performances.

As we know, considering the number of farms taken in account as well as the methodology applied, this is the first study of that size used to estimate technical and economic consequences of good farming practices.

Indeed, few studies have examined the relationships between performances and farming practices. Cariolet et al. [1] used an evaluation grid of health to highlight differences between sow productivity and feed efficiency. Laanen et al. [3] also underline a relationship between the level of external biosecurity and the average daily gain, but not between internal biosecurity and the average daily gain, neither between biosecurity levels and mortalities. These different studies are based on index calculation to characterize farms, but the lack of harmonization between them makes hard results comparison.

CONCLUSION

An important number of factors influencing technical and economic performances are highlighted. These results help to argue in favour of respect of recommendations with regard to biosecurity, health control, conception and farming management. Farms implementing favourable practices have a higher margin. This should motivate farmers to apply strict policies of health control but also to think about modifications in practice or in farming conceptions which make for an optimization of technical and economic results. This study constitutes also a strong argument to the implementation of the Good Hygiene Practice Guide in pig farms.

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DOES BIOSECURITY HAVE ANY INFLUENCE ON THE HEALTH AND PROFITABILITY IN PIG FARM?

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SUMMARY

The general requirements for farming practice are to determine the optimal conditions that allow maximum efficiency and health of the herd. The aim of our work was to express the effect of Biosecurity precautions in a pig farm with the average monthly number of fattening pigs around 4,000 pieces after implementation the TEKROCID system and after repopulation of the pig farm from the animal health and economical points of view. The results showed the positive effect of implementation of the TEKROCID Biosecurity system in the farm for fattening pigs. The average monthly number of dead pigs decreased after the introduction of the system to 69.4[%]. Final reduction to 44.4[%], compared with values before implementation of the TEKROCID system, was reached after repopulation of the herd. After restocking of livestock, in accordance with the new animal hygiene practices, no therapeutic medication was necessary.

INTRODUCTION

The causation of illness involves a complex relationship of three factors: host resistance, microbial agents and the environment. The influence of the environment on the interaction between the agent and the host can determine the level of contact and greatly affect the development of the disease [1]. Farm animal Biosecurity represents a complex of preventive arrangements to reduce risk by ensuring the absolute health of livestock [2]. In comparison to Biosecurity, therapeutic arrangement is very expensive. Using good breeding practices creates the premises to prevent the herd from health disorders. Biosecurity is an integral part of health herd management. Animal health status depends on the capability of animals to resist the infection pressure of microorganisms in animal houses. The general requirements for farming practice are to determine the optimal conditions that allow maximum efficiency and health of the herd. If the environmental conditions fall below or above this optimum range, the growth of the organism is reduced with the negative effect on health, production and reproduction parameters. It is necessary to assess all options, because the maximum yield does not always mean economic efficiency. Therefore it is necessary to manage for criteria, production efficiency and animal well-being and health [3].

ANIMALS, MATERIALS AND METHODS

The aim of our work was to express the effect of Biosecurity precautions in pig farm with the average monthly number of fattening pigs around 4,000 pieces after implementation the TEKROCID system and after repopulation of the pig farm from the animal health and economical point of view. The study was carried out in stables with total slotted floor with dry feed to the group feeders. The stable was thermal insulated with regulated forced ventilation system. The TEKROCID system was created in the company TEKRO Ltd. and is available in the Proceedings of the XIV ISAH Congres2009 XIV.ISAH Congress 2009 in Vechta, Germany [4, 5]

RESULTS

The results are summarised in following tables.

Table 1: The evaluation of sanitation precautions in pig fattening farm (expressed as monthly average values)

	The average number	Mortality		Medication costs [EUR]		Vaccination costs [EUR]	All medication costs [EUR]
		Pcs	[%]	Preventive	Therapeutic		
2008 Before Tekrocid	4,184	36	0,860	1,517.84	1,828.61	1,626.29	6,104.49
2009 After Tekrocid	2,984	25	0,840	543.20	997.59	93.58	1,906.21
2010 After repopulation	3,161	16	0,506	797.21	0	0	1,103.48

Table 2: The comparison of economic effect of implementation of the system Biosecurity - TEKROCID and repopulation YTD

	Mortality	Medication costs [EUR]		Vaccination costs [EUR]	All medicationcosts [EUR]
	[%]	Preventive	Therapeutic		
Before and after implementation of the system Biosecurity - TEKROCID					
2008 - 2009	- 0.02	- 11,695.68	- 9,972.24	-18,392.52	- 50,379.36
After implementation of the system Biosecurity - TEKROCID and repopulation					
2009 - 2010	- 0.33	3,048.12	- 11,971.08	-1,122.96	-9,632.76

The achieved results showed the positive effect of implementation of the TEKROCID Biosecurity system in the farm for fattening pigs. The average monthly number of dead pigs decreased after the introduction of TEKROCID to 69.4[%] and to the 44.4[%] after repopulation of the farm compared to the values before the start of observation. Preventive medication costs after the introduction of the Biosecurity system significantly decreased to 35.8[%], whereas the repopulation declined only to 52.5[%] from its initial state. Interesting results were achieved in the section of the therapeutic medication costs which decreased to 54.6[%] after the introduction of the Biosecurity system. After the eradication of the farm the therapeutic medication was not necessary at all. Decrease of the all pharmaceutical costs as the indicator of health was more significant after the introduction of the Biosecurity and reached 31.2[%] of the original values. After the repopulation the all medication costs decreased even to only 0.6[%] of the costs paid by the farmer before the start of our field trial.

DISCUSSION

Biosecurity programme TEKROCID operates in conjunction with a material prepared by Fotheringham [6, 7]. Dirty, less hygienic environment increases the level of immunological stress and depresses growth and performance of pigs [8]. The sources of disease agents of growing pigs are related to moving the groups of pigs from farrowing to nursery and then to finishing as each subsequent environment must be free of potential pathogens that might infect the high health status [9]. Microbial contamination of the environment in stables develops to an important factor affecting the picture of infections in breeds. Animal health status depends on the capability of the animals to resist the infection pressure of microorganisms in the environment [10]. Disease control is only one part of a successful management program. Treatment of disease is not as effective or as economical as prevention. Many outbreaks of disease in swine herds can be avoided by using management practices that include strict sanitation and immunization programs [2]. It is possible to agree with the fact, that when a group is moved from any production facility, area should be cleaned thoroughly by power washing and then disinfected. Disinfection should be attempted only after thorough cleaning [10]. With a good Biosecurity program optimal growth can be reached by minimizing the negative effects of subclinical illness. To keep pigs performing up to their genetic potential, their exposal to pathogens must be minimized. Minimum pathogen exposure is the objective of a complete Biosecurity program. Traditional disease-prevention techniques, including immunization and controlled biofeedback practices, are only a small part of an overall Biosecurity plan. Today's plans are intended to reduce the use of antibiotics in farms [11]. This goal was achieved by implementation of the TEKROCID program followed by restocking of the animals.

CONCLUSIONS

This study proved that good environmental conditions for farm animals in stables depend not only on barns management, but also on the sanitary care. Our results imply that the maintenance of adequate hygienic level ensures high level of observed parameters of the fattening pigs and the economical profit for the stockman. A good Biosecurity program helps to decrease the risk of pathogens being transferred from farm to farm and simultaneously increase the economic efficiency of the farmer.

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A LABORATORY STUDY TO DETERMINE THE EFFECT OF CLEANING AND DISINFECTION TO PREVENT THE SPREAD OF *CLOSTRIDIUM DIFFICILE* IN PIG FARMS (Abstract)

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SUMMARY

Cleaning and disinfection in pig farms to diminish pathogens can be a problem if these pathogens are spore forming. In this research several cleaning and disinfection strategies were tested to determine a best practice to prevent the spread of *Clostridium difficile*.

INTRODUCTION

It is well known that cleaning and disinfection play an important role in an approach to eliminate infectious diseases. However, cleaning and disinfecting agents can be corrosive to materials, hazardous to the health of animal caretakers and animals and instead of eradicating the pathogens making them more viable. One of these pathogens is *Clostridium difficile*. In unfavorable conditions, e.g. air exposure, the cell creates a spore to increase the survivability. Spores can survive for many years on surfaces and are protected against almost all disinfectants including ethanol.

A good cleaning and disinfection protocol is necessary to decrease the prevalence of *C. difficile* infection and prevent the spread of *C. difficile* in the environment. Therefore a study was done to investigate which cleaning and disinfection procedure is most effective against *C. difficile* in the laboratory.

The goal of this study was to improve the cleaning and disinfection methods used in pig farms to prevent the spread of *C. difficile*.

ANIMALS, MATERIAL AND METHODS

The effect of the different disinfection agents (BIO CID-S (CIDLINES), Halamid® (Axcentive sarl), and other disinfectant commonly used in the pig farm industry) was measured in a controlled environment using a *C. difficile* isolate derived from a Dutch pig farm De Tolakker. A solution with the concentration of about 1 x 10^6 cfu/ml Clostridium difficile was used. 10 ml of the solution was inoculated on surfaces (tiles) of 10cm^2 and allowed to dry 24 hours at room temperature.

The experiment was performed on four types of surfaces to mimic the original flooring used in the pig farm.

- Concrete tile without plastic coating
- Concrete tile with plastic coating
- Tile with coating
- Plastic grid

After C&D was done, the tiles were placed in a stomacher for 2 minutes with infusion broth. 100µl of the broth was plated out on CLO plates. After incubation colonies were counted.

RESULTS AND DISCUSSION

Results will be presented and discussed on the poster at the congress

IMPROVING WORKING CONDITIONS BY USING MEDIUM PRESSURE (40 BARS) DURING CLEANING IN PIG FARMS

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SUMMARY

Cleaning with a medium-pressure nozzle (Fitjet® nozzle, 40 bars) was compared to conventional cleaning at high pressure with a rotary nozzle (160 bars), based on criteria such as working time, water consumption, cost, difficulty of work and cleaning efficiency.

The results suggest that the cost is similar and the cleaning score with contact plates is correct for both nozzles. Concerning the difficulty of work, the results favour the Fitjet® nozzle: the noise level is significantly lower, the visibility during washing is significantly higher, musculoskeletal disorders are lessened, projections towards the operator's face are significantly reduced and cleaning is considered less difficult and less tiring for the operator.

INTRODUCTION

Cleaning rooms represents significant physical constraints for the operator: a risk of developing musculoskeletal disorders and other difficulties such as: noise, vibration, splashes of water and organic matter in eyes.

The objective of this study is to compare cleaning with a medium-pressure nozzle (Fitjet® nozzle) to a conventional cleaning at high pressure with a rotary nozzle, based on criteria such as working time, water consumption, cost, difficulty of work and cleaning efficiency.

MATERIAL AND METHODS

Two modalities of cleaning, high pressure (160 bars) with a rotary nozzle and medium-pressure (40 bars) with a Fitjet® nozzle, are compared for eight observations: 3 in farrowing, 3 in post-weaning and 2 in fattening. For each repetition, the modalities are applied by the same operator, previously trained, in the same room. The protocol is identical, only the nozzle type, used during the prewash, washing and rinsing, changed.

The work time and water consumption allows to calcultate the cost of the operations from a method developed in a previous publication [1].

The effectiveness of the cleaning and disinfection is assessed by visual scoring, visual semi-quantitative scoring with paper roll and Total bacteria counts in Petri dishes, according to the method previously described by Corrégé et al. [2], [3].

Noise is measured with a sound level meter, placed near the shoulder of the operator, on different materials (plastic, stainless steel, galvanized steel, brick, concrete and cast iron) and distances (20, 30, 40 and 70 cm).

The importance of mist generated during the washing is measured too. Adhesives marked with squares are installed in different parts of the room: the number of adhesives and squares, that we can observe during the washing are regularly recorded.

The hardness of the cleaning for the operators is described by a specific questionnaire developed by IFIP, which takes into account the constraints of posture, noise, visibility, splashing and the overall difficulties. Finally, an ergonomist from the Agricultural Social Mutual Insurance (MSA) noted the number of postural constraints (curvature of the back above 30° and upper limbs above the heart) and stop-start motions.

The Student t test, the Wilcoxon nonparametric test and the Anova (SAS) are used for data processing.

RESULTS

The working times are higher in farrowing with the Fitjet® nozzle, but lower in post-weaning and fattening (Table 1). Water consumption is also lower in post-weaning but higher in farrowing and fattening. Economically, the use of the Fitjet® nozzle involved an additional cost in farrowing, but a reduction in post-weaning and fattening. Per sow and per year, with the Fitjet® nozzle, there is a profit of 0.49 € per productive sow, for a 168 sows farrow-to-finish farm (Table 1).

Table 1: Comparison of the Fitjet® and rotary nozzle results

Difference Fitjet® - Rotary nozzle	**Water- l**	**Time-min**	**Cost- €**
Farrowing– 100 m² Post-weaning – 100 m² Fattening – 100 m² Per sow/year (2)	509/+12% (1) -1153/-21% 140/+3% 4/+0%	19/+10% -35/-12% -11/-5% -3/-1%	11.23/+12% -20.58/-15% -1.43/-1% -0.49/-1%
	Fitjet®	**Rotary nozzle**	**Stat**
Noise in dB	86.6	93.3	p<0,001
Visibility : % of visible squares	96.0	97.7	p<0.01
Visual semi-quantitative scoring	1.76	1.83	ns (3)
Total bacteria counts	1.93	2.10	ns
(1) Value / percentage of the difference / (2) Calculation for a 168 sows farrow-to-finish farm (3) ns : no significant at the threshold of 5%			

Concerning the cleaning efficiency, the results obtained with the visual semi-quantitative scoring by paper roll and with the Total bacteria counts in Petri dishes are not significantly different between the two nozzles (Table 1).

Table 2: Noise measurement (in decibels)

Distances	**Rotary nozzle**	**Fitjet®**	**Difference Fitjet® - Rotary nozzle nozzle**
20 cm	96.4	90.1	- 6.3
30 cm	96.5	89.7	- 6.8
40 cm	93.6	87.0	- 6.6
70 cm	88.8	82.2	- 6.6
No impact	86.1	77.7	- 8.4
Materials	**Rotary nozzle**	**Fitjet®**	**Difference Fitjet® - Rotary nozzle**
Plastic	96.2	89.5	- 6.7
Stainless steel	94.3	87.4	- 6.9
Cast iron	94.0	86.8	- 7.2
Concrete	93.8	86.4	- 7.4
Brick	92.8	90.7	- 2.1
Galvanized steel	91.8	82.9	- 9.8
Ceiling	87.4	78.9	- 8.5

Sound intensity decreases when the distance between the nozzle and the surface to clean increases (Table 2). It also varies with the type of materials, galvanised steel generates the least noise and plastic the most (Table 2). For all distances and materials, the noise level is significantly lower with the Fitjet® nozzle: the difference between the nozzles varies from 2.1 dB (brick) to 9.8 dB (galvanized steel) and the average difference is about 6.7 dB. The decibel scale is logarithmic; these differences equal a noise level that is respectively 1.6, 9.8 and 4.7 times lower. In addition, 99% of the noise measured with the rotary nozzle exceed the risk threshold for hearing (85 dB) cons 60 % with the Fitjet® nozzle.

Regarding the visibility tests, all adhesives were observed with the two nozzles. However, the number of squares observed is significantly higher with the Fitjet® nozzle.

About the hardness of cleaning, the operators are in favour of the Fitjet® nozzle. In fact, the pains are significantly smaller with the Fitjet® nozzle for the arms and shoulders the day of washing and for the fingers, wrists, arms and shoulders the day after washing (Figure 1).

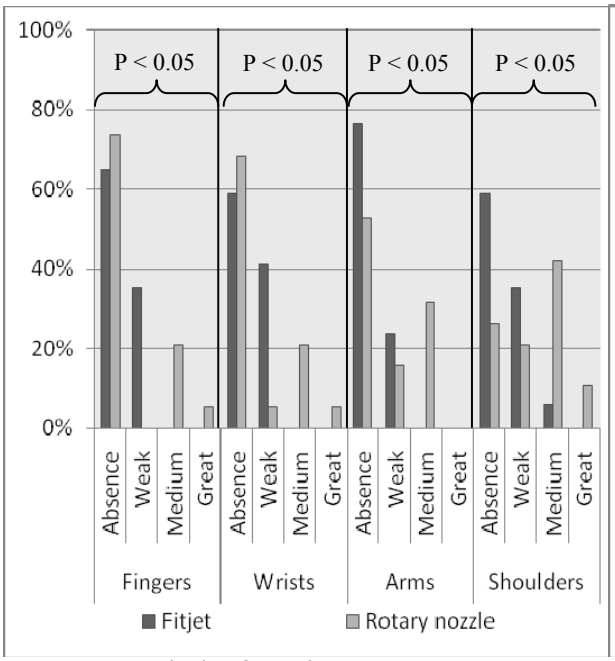

Figure 1 - Pains the day after washing

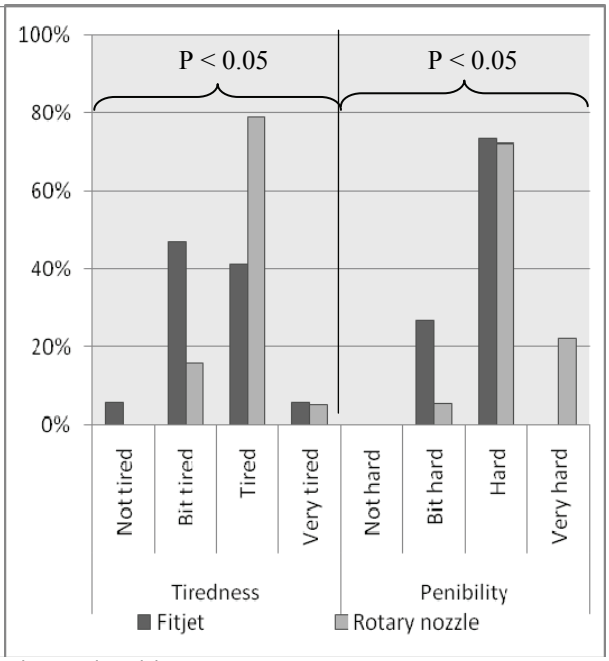

Figure 2: Tiredness and penibility

There is no difference concerning the noise but the visibility is perceived as worse with the rotary nozzle. The face projections are significantly higher with the rotary nozzle.

The state of tiredness and difficulty are considered significantly lower with the medium-pressure. (Figure 2).

Finally, the ergonomist of the MSA noticed a decrease of the mist produced, the vibration effects and jerks experienced by the operators with the Fitjet® nozzle. The number of stop-start motion is substantially equivalent between the two nozzles.

DISCUSSION

The cost is similar between the two nozzles, for the same cleaning efficiency. However the Fitjet® nozzle requires a prior soaking phase and a change in work habits, with a time of adaptation different according for the operator.

Concerning the noise, the recommended working distance with the Fitjet® nozzle is 70 cm whilst it is 30 cm for the rotary nozzle. This accentuates the noise decrease level. The difference between the Fitjet® nozzle at 70 cm and the rotary nozzle at 30 cm is about 14 dB or 25 times less noisy. The operators do not notice any difference in noise disturbance, the hearing protection they wear probably reduces the difference between the nozzles.

The evaluation of the hardness confirms the results of the study on the painfulness during cleaning operations [4]: the rotary nozzle shows projection of organic matter in eyes, poor visibility in saturated atmosphere with water and shoulders and wrists pains.
The Fitjet® nozzle has a greater manoeuvrability and allows less pressure so we can hold it in just one hand, which facilitates the access to some areas. In addition, it offers the possibility to work with a rotary or fixed position, so it's possible to perform various stages of washing with the same nozzle.

The impact of the medium and high pressure on the wear of materials is not included in this study. The wear of materials has consequences in the medium and long term on the cost, the effectiveness and the difficulty of cleaning: indeed, materials with many asperities because of erosion are more difficult to clean and to disinfect.

CONCLUSIONS

With the Fitjet® nozzle the water consumption, the working time and the cost are similar to those generated by high pressure (rotary nozzle) for the same washing efficiency. Concerning the hardness of work, it leads to improved visibility, reductions of the noise, the projections, the postural constraints and the perceived pains. However, it needs a prior soaking phase and a change in work habits, with a time of adaptation for the operator, necessary to optimize the working time, the painfulness and the costs.

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DEVELOPMENT OF A CARRIER TEST TO DETERMINE THE VIRUCIDAL EFFICACY OF DISINFECTANTS

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SUMMARY

Because of a revision of the DVG-guidelines in the field of veterinary medicine a carrier test to determine the virucidal activity of disinfectants was developed with focus on food production and veterinary practices. Two potential test viruses [murine norovirus (MNV) and canine parvovirus (CPV)] were tested for their suitability. Different biocidal agents were used in the carrier test to evaluate the stability of both viruses. In addition, a variety of interfering substances and temperatures of 10°C and 20°C were involved into the testing to simulate practical conditions. All tested disinfectants, except ethanol, showed a virucidal activitiy against the two test viruses. Noticeable was the resistance of CPV against the tested concentration of ethanol. The use of interfering substances and temperatures showed different influence on the efficacy of the disinfectants. Thus, adapting of these factors is recommended for revising the DVG-guidelines.

INTRODUCTION

In Germany, testing of disinfectants for application in veterinary medicine is performed according to the guidelines of the German Veterinary Medical Society (DVG). Actually, the testing for virucidal efficacy has only been performed for animal husbandry by these guidelines. Thus, the aim of the study was to develop a quantitative carrier test to investigate the virucidal activity of disinfectants with focus on food production and veterinary practices.

MATERIAL AND METHODS

Two different viruses [murine norovirus (MNV) and canine parvovirus (CPV)] were used to investigate the practicability of this carrier test. MNV was cultivated by using a Mouse leukaemic monocyte macrophage cell line (RAW 264.7) and CPV by using a Crandell-Reese feline kidney cell line (CRFK). Various parameters had to be investigated by the reference to MNV and CPV (i. effect of drying in a desiccator on the infectivity of these test viruses, ii. optimisation of the coating volume and iii. the analysis of two desorption processes from the carrier surface).

For the quantitative carrier test the test virus suspension was mixed with interfering substances according to EN 14675 [1]. In addition to a low contamination with a final concentration of 3g/l bovine albumin, a high contamination with a final concentration of 10g/l bovine albumin and yeast extract was used. A volume of 50 µl of this suspension was pipetted on each carrier (stainless steel discs, GK Formblech, Berlin), which were placed in a 6-well plate. The carriers were dried in a desiccator for 15-20 minutes with a vacuum of 900-1000 mbar. After drying, 100 µl of the biocidal agent and 100 µl of hard water as control carrier were pipetted on the discs and incubated for 5 and 30 minutes, respectively. Dilutions of five different biocidal agents (sodium hypochlorite, sodium hydroxide, ethanol, peracetic acid and glutaraldehyde) were tested. After the exposure time, hard water was added to collect the solution. Each solution was diluted immediately. 25 µl of each dilution were placed in eight wells of a 96-well microtiter plate with the CRFK or RAW 264.7 cells. Referring to practical conditions the carrier experiments were performed at temperatures of 10°C and 20°C. An efficient inactivation was defined by a 3 $\log_{10}$ reduction of the virus infectivity.

RESULTS

Both test viruses were highly resistant during the drying process in the desiccator. The loss of infectivity was ≤0,25 $\log_{10}$ for MNV and ≤0,225 $\log_{10}$ for CPV. In addition, the drying time proved to be dependent on the volume applied. A coating volume of 50 µl was most appropriate for coating the carriers. Comparing the different desorption processes, no significant difference was observed regarding the recovery rates of the test virus. Therefore the more practicable method was chosen.

All tested biocidal agents, except ethanol, sufficiently inactivated CPV and MNV on the carriers by ≥3 $\log_{10}$. The results of the carrier tests at a temperature of 20°C are shown in table 1. The highest concentration of ethanol (80%) achieved no sufficient reduction of CPV. In contrast to CPV, a concentration of 50% ethanol led to an efficient inactivation of MNV independently of the amount of interfering substances. Furthermore, the testing with

ethanol and sodium hydroxide showed no differences in virus reduction, independently of the interfering substances or temperatures applied. Especially the virucidal activity of sodium hypochlorite and glutaraldehyde was influenced by the presence of interfering substances (high contamination).

Table 1: minimal concentration and exposure time of the disinfectants at 20°C to inactivate the virus infectivity by 3 $\log_{10}$ reduction

virus	conta-mina-tion	disinfectant (concentration [%] / exposure time [min])				
		sodium hypochlorite	sodium hydroxide	ethanol	peracetic acid	glutar-aldehyde
MNV	l	1,5/5 1,0/30	0,5/5 0,5/30	50/5 50/30	0,05/5 0,05/30	0,25/5 0,1/30
CPV	l	2,0/5 2,0/30	0,5/5 0,5/30	-	1,0/5 0,5/30	0,5/5 0,25/30
MNV	h	2,0/5 2,0/30	0,5/5 0,5/30	50/5 50/30	0,05/5 0,05/30	0,5/5 0,25/30
CPV	h	3,5/5 3,5/30	0,5/5 0,5/30	-	1,0/5 0,5/30	1,0/5 0,5/30

l low contamination h high contamination

DISCUSSION

The testing of the virucidal activity of disinfectants is important for their effective and reliable application. The most simple disinfectant test is the suspension test, in which the test virus suspension was added to a mixture of interfering substance and disinfectant [2]. However, to simulate practical conditions, the use of carrier tests is essential. Based on this model, several biocides were evaluated for their virucidal activity against two non-enveloped viruses. The test viruses (MNV, CPV) are relevant viruses for the food producing industry and the veterinary practice. MNV was culturable to high titres with a clearly cytopathogenic effect (cpE). For the food production it could be a possible test virus for revising guidelines. CPV was a less suitable model virus. The slow replication, the development of a morphologically weak cythopathic effect, the performance of an indirect immunofluorescence-test and the moderate titres were disadvantageous characteristics of the virus. Thus, another test virus should be taken into consideration for the veterinary practice.

With exception of ethanol, all tested biocidal agents showed a virucidal efficacy in this carrier test. It was obvious that CPV was resistant against the tested concentration of ethanol. Also several studies have shown that parvoviruses are more resistant to disinfection than other non-enveloped viruses [3]. In contrast, ethanol led to a complete inactivation of MNV independently of the amount of interfering substances. The study of MAGULSKI et al. showed several results of the inactivation with ethanol [4].

CONCLUSIONS

The results of the carrier tests are helpful in predicting antiviral properties of disinfectants under field conditions. The adaption of the temperature and application of interfering substances is recommended for testing models referring to conditions in food production and veterinary practices. These results should be taken into consideration for revising the DVG-guidelines.

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THE IMPORTANCE OF SURFACE MATERIAL AND METHOD OF APPLICATION IN DISINFECTION

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SUMMARY

Introduction: Implementation of disinfection is an important measure in outbreaks of contagious diseases. The method of applying a disinfectant to a surface and the surface material and location have a significant impact on the effect of disinfection, Means of transport can be the cause of rapid spread of infectious material. In our experiment, the effect of a commonly used antiseptic was assessed after treating materials typically used in means of transport.

Materials and Methods: For our experiment, we prepared a test area of pine wood, rubberized canvas, glass, and metal. We first deposited on the test area the test strain ATCC 25923 Staphilococcus aureus in a concentration of 1.5-5x109 CFU/ml. The test area was then dried and installed into a vehicle in places where the materials are usually present. We chose the horizontal, vertical and overhead positions of surfaces. We carried out disinfection with a disinfectant composed of peroxygen compounds, surfactant, organic acids and an inorganic buffer system. We used a 1% solution in quantities of 0.4 l/m2. We used a low-pressure method of spreading the disinfectant.

Results: We found that both the location and the material have an impact on the performance of disinfection. In particular, we found that metal reduced the performance.

Conclusions: We noted that the location of an area, and in particular the type of material to be disinfected significantly affect the success of disinfection. This should be considered in the implementation of disinfection measures during outbreaks of infectious diseases, as inappropriate disinfection may result in an increase in the survivability of microorganisms.

INTRODUCTION

Considering the specific conditions and, in particular, quick actions of the veterinary service of the Slovenian army, required in biological decontamination in emergency situation and in situations where larger quantities of equipment of means of transport have to be disinfected, we decided to test the effect of disinfection after a shorter contact time compared to the usual contact time required for most disinfectants. In line with the regulatory requirement for testing of biocidal activity on carriers under laboratory conditions (SIST EN 14349, 2004) and following the requirements of the veterinary service of the Slovenian army, we decided to test the activity of a biocide at a 1% concentration using a contact time of 5 or 15 minutes, which is a contact time still acceptable for decontamination of vehicles and equipment when the army units return from their missions or in other emergency situations. The method of application of the biocidal solution with a high-pressure machine and with a motor sprinkler was tested.

MATERIALS AND METHODS

The experiment was done with materials that are frequently used in military forces. We used rubber, wood, tarpaulin, and sheet metal. The microbial culture of Staphylococcus aureus ATCC 25923 was placed on carriers and 5 ml of bacterial suspension at a concentration of 5x108 were dispersed in 195 ml of sterile physiological solution and evenly sprayed over the carriers. The carriers were then dried. A biocidal preparation containing the active substance potassium peroximonosulfate (Ecocid® S, Krka) was used as a model in the experiment.

After drying the carriers were fastened on a vehicle made of the same materials as were used as test carriers. The carriers were fastened in horizontal and vertical position and to the ceiling. We distributed them evenly over the interior and the exterior of the vehicle. After fastening the test carriers with microbial cultures to the vehicle, we applied 300 ml/m2 area of the biocidal preparation at a 1% concentration. Spraying was carried out with a high-pressure machine and a motor sprinkler. The selected contact times were 5 minutes ± 10s and 15 minutes ± 10s.

Immediately after the end of the contact time of the biocide, sterilised water was gently poured over the carriers to inactivate the biocide. Then, the water was removed and samples were taken from each carrier of a size of 4x5 cm or 20 cm2 with sterile swabs. The samples were inoculated into a nutrient medium for the assessment of the total number of microorganisms.

RESULTS

Application of the biocidal solution with the high-pressure machine using a 5-minute contact time on carriers

A comparison of mean values obtained on test carriers showed the following mean reductions: 3.08 log10CFU/cm2 on wood, followed by 4.14 log10CFU/cm2 on metal, 5.82 log10CFU/cm2 on tarpaulin, and 6.05 log10CFU/cm2 on rubber. The statistical analysis showed that there was a modest difference between the horizontal and ceiling position in the effect on tarpaulin (P=0,086) and between the horizontal and ceiling position in the effect on wood (P=0.052). A comparison of mean values also showed a modest difference between the effects on sheet metal and rubber (P=0.052).

Application of the biocidal solution with the high-pressure machine using a 15-minute contact time on carriers

A comparison of mean values obtained on test carriers (all positions of test carriers together) showed the following average reductions: 2.24 log10CFU/cm2 on wood, followed by 5.09 log10CFU/cm2 on metal sheet, 5.46 log10CFU/cm2 on tarpaulin, and 6.20 log10CFU/cm2 on rubber. The statistical analysis of data showed that there was a modest difference in the effect on rubber carriers between the horizontal and vertical position (P=0.077) and in the effect on tarpaulin between the horizontal and vertical position (P=0.059), and a significant difference in the effect between the horizontal and ceiling position (P<0.05). A comparison of the mean values obtained for different materials demonstrated significant differences between the sheet metal and wooden carriers (P<0.05), between the rubber carrier and the tarpaulin (P<0.05), and between the rubber carrier and the wooden carrier (P<0.05).

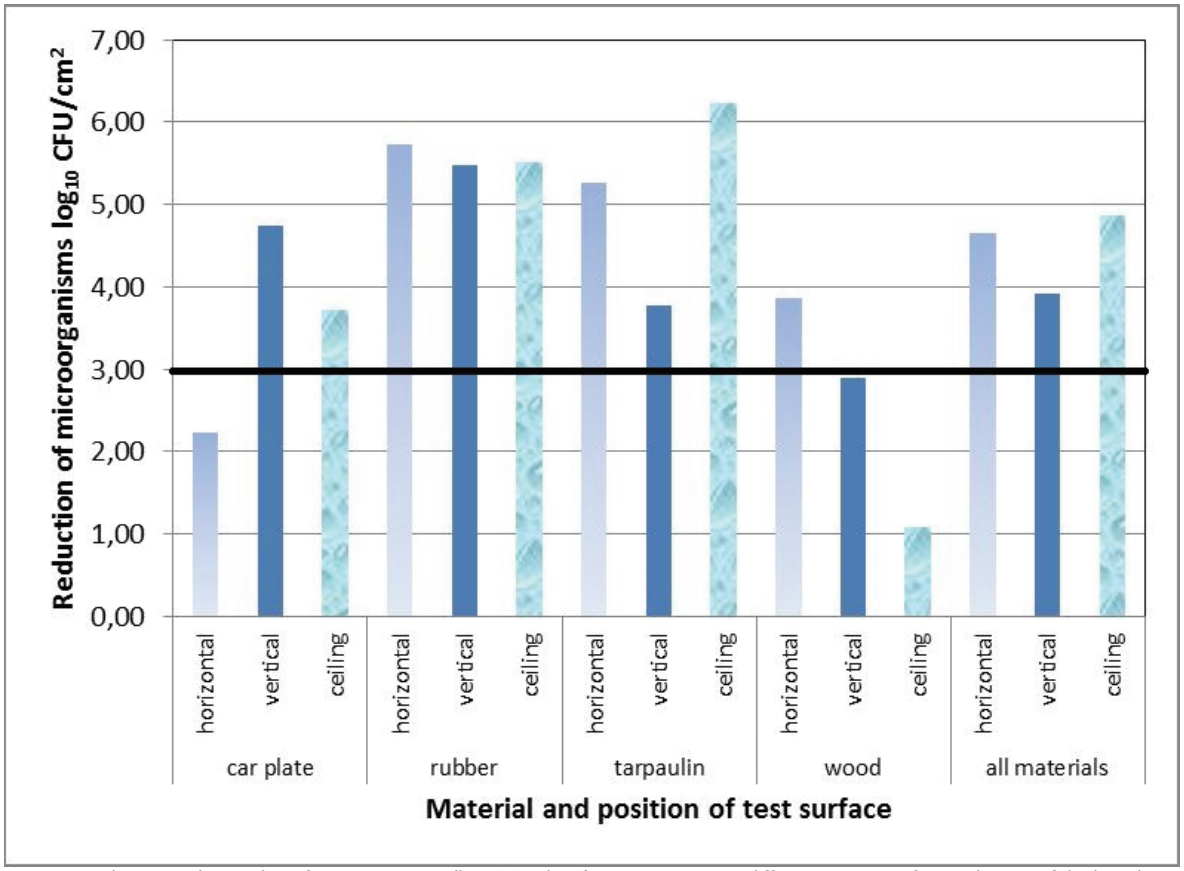

Figure 1: Reduction in the number of microorganisms (log10 CFU/cm2) on test carriers in different positions after application of the biocide (application with high-pressure machine, 5-minute contact time)

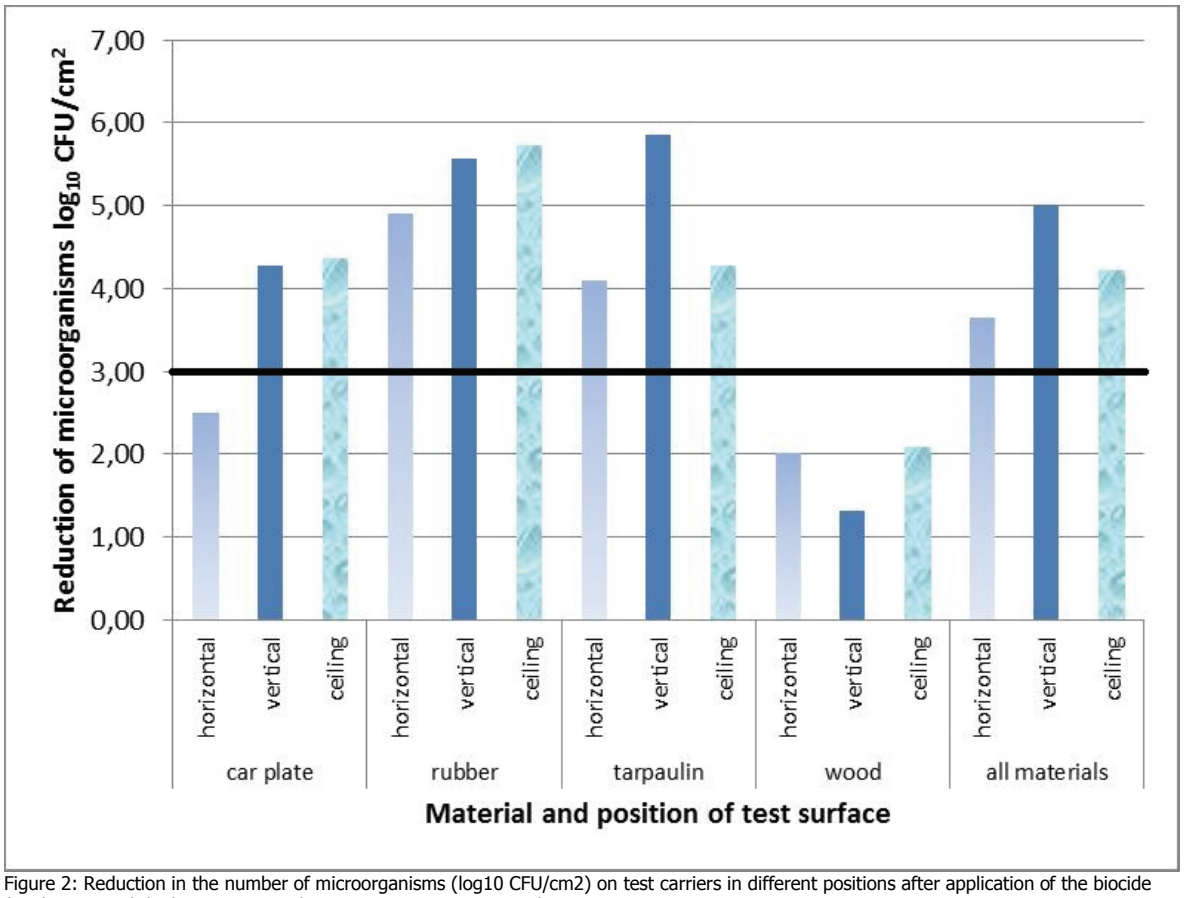

Figure 2: Reduction in the number of microorganisms (log10 CFU/cm2) on test carriers in different positions after application of the biocide (application with high-pressure machine, 15-minute contact time)

Application of the biocidal solution with the motor sprinkler using a 5-minute contact time

A comparison of mean values obtained on test carriers (all positions of test carriers together) showed the following average reductions: 2.90 log10CFU/cm2 on wood, followed by 5.46 log10CFU/cm2 on sheet metal, 5.66 log10CFU/cm2 on tarpaulin, and 5.94 log10CFU/cm2 in rubber.

The statistical analysis of data comparison showed that there was a modest difference in the effect on sheet metal carriers between horizontal and ceiling position (P=0.059) and a significant difference between the vertical and ceiling position (P<0.05). A modest difference was observed in the effect on tarpaulin between horizontal and vertical position (P=0,053) and a significant difference between horizontal and ceiling position (P<0.05). A comparison of mean values obtained on single test carriers showed a modest difference between the sheet metal carrier and the tarpaulin (p=0.068) and between the rubber carrier and the tarpaulin (P=0.060).

Application of the biocidal solution with the motor sprinkler using the 15-minute contact time

The following reductions were observed when comparing mean values obtained on test carriers (all positions of the test carriers together): 3.70 log10CFU/cm2 on wood, followed by 3.75 log10CFU/cm2 on sheet metal, 6.58 log10CFU/cm2on rubber, and 6.86 log10CFU/cm2 on tarpaulin.

No significant differences were found by the statistical analysis of data between test carriers and their different positions or between mean values.

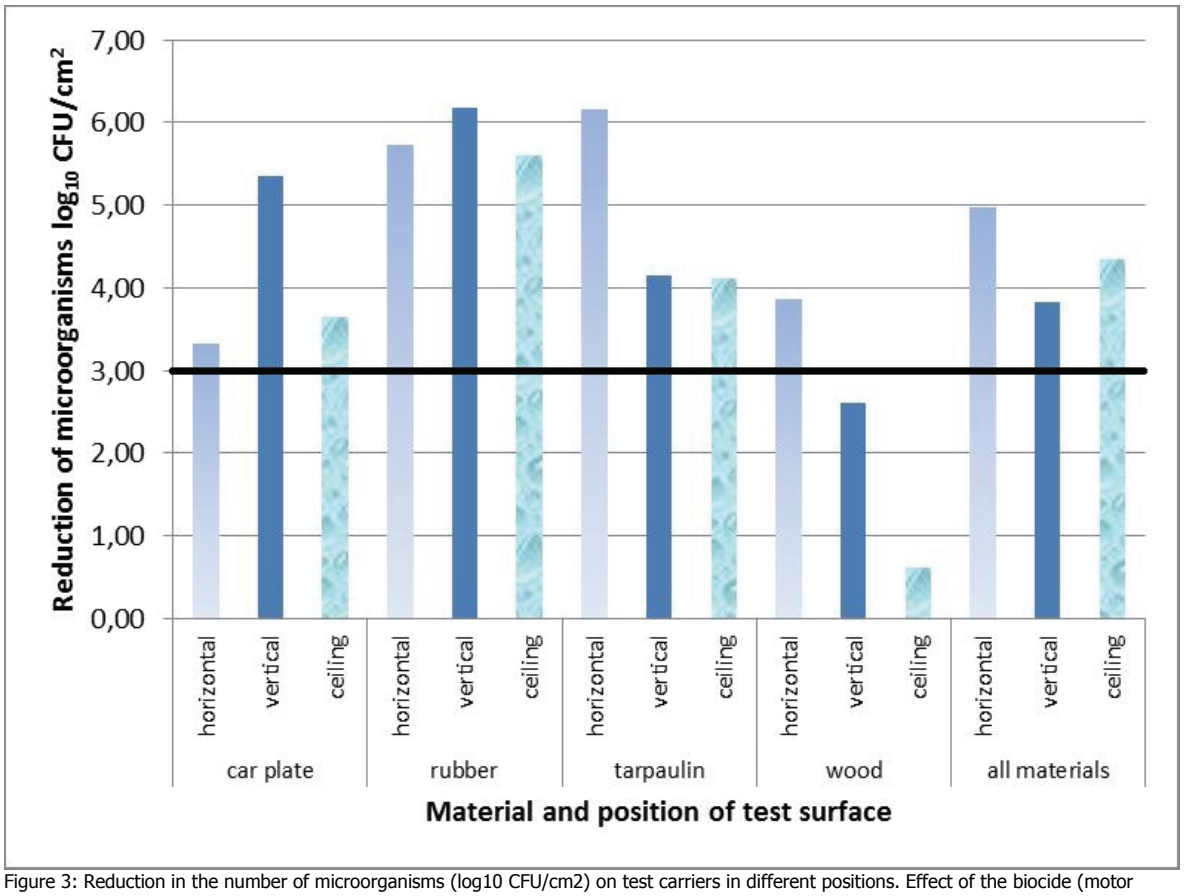

Figure 3: Reduction in the number of microorganisms (log10 CFU/cm2) on test carriers in different positions. Effect of the biocide (motor sprinkler, 5-minute contact time)

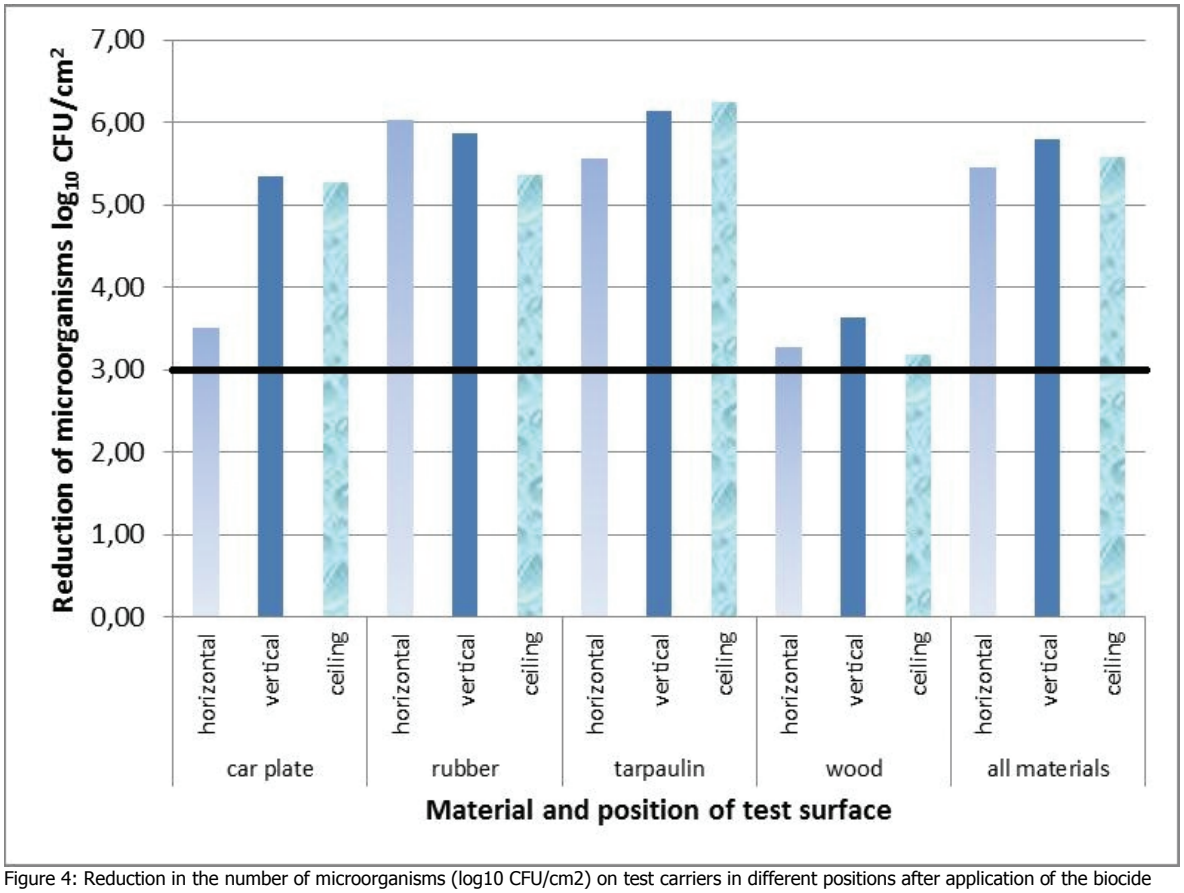

Figure 4: Reduction in the number of microorganisms (log10 CFU/cm2) on test carriers in different positions after application of the biocide (motor sprinkler, 15-minute contact time)

DISCUSSION AND CONCLUSION

Summarising, the data allow for the following conclusions:

1. The efficacy of disinfection depends on the material on which it is applied as well as its position.
2. High-pressure spraying of a biocidal preparation is unreliable with both contact times, 5 and 15 minutes, in particular on wooden surfaces. It would be necessary to test a high-pressure machine at a reduced working pressure (3-30 bar), using a wide-angle flat hose with a jet angle at least 70-80°, or carry out a test with a disinfection hose. In this way the required quantity of the disinfectant for effective disinfection would be applied to the surfaces.
3. The most reliable method of disinfection is with the mounted knapsack motor sprayer using a 15-minute contact time, a 1% concentration of the disinfectant and a quantity of 300 ml/m2 area.
4. When applying a biocidal solution, special attention should be paid to wooden surfaces and horizontal metal surfaces. These areas should be thoroughly cleaned before the biocidal solution is applied.
5. Due to its good adhesive properties, the biocide used in the test had a good effect on both vertical and ceiling areas.

LITERATURE

1. **SIST EN 1656:2010** Chemical disinfectants and antiseptic quantitative.suspension test
2. **SIST EN 14349: 2004** Chemical disinfectatns and antiseptics . qualitative surface test
3. **SIST EN 1656:2001** Chemical disinfectants and antiseptic quantitative.suspension test
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EFFECT OF FERMENTED WHEAT GERM EXTRACT (FWGE) ON SHEDDING SALMONELLA INFANTIS AND IMMUNREACTIONS OF BROILERS

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SUMMARY

170 broilers have been infected with *Salmonella infantis* and vaccinated against IBD,(infectious bursal disease) IB, (infectious bronchitis) ND. (Newcastle disease)Feed of 85 broilers has been completed with *FWGE* (fermented wheat germ extract) Shedding-characteristics of *Salmonella infantis* and immune reactions after vaccinations were investigated with both broiler groups. Intensity and dynamics of shedding of bacteria by cloacal swabs proved to be equal with both groups and stopped between 18-19th day after infection, but cecal content remained positive even on 31-33 days after infection.

FWGE increased the IBD VN GMT (virusneutralization geometric mean titers) 7 days after vaccination by 26%, but 21 days after immunization there was no difference in the titres. IB ELISA MMT (mathematical mean titers) 28 and 42 days after vaccination have been increased with the FWGE treated group by 820 and 180% compared to the control. ND HI GMT (haemagglutination inhibition geometric mean titers) proved to be 100% higher with the *FWGE* treated group on the 42nd day of rearing.

INTRODUCTION

EU has banned AGP-s (antibiotic growth promoters) in 2006 and researches has been carried out to find alternatives to control and prevent colonization of pathogen bacteria e.g. *Salmonellas* in the intestines modulating gut microbiota [Ribeiro et al, 2007]. Broilers produced in EU, must be free of *Salmonellas* since 2003. *Salmonella infantis* infection of broiler flocks and contamination of chicken meat came to the front and became almost exclusive.

Beneficial effect of *FWGE* has been verified in Hungarian poultry production among others with the production parameters, immune reactions, mycoplasmosis and coccidiosis of broilers [Kósa et al, 2003, Stipkovits et al, 2004] production level and vaccination reactions with parent flocks of table egg layers [Kósa,E., Bajcsy.E., 2008] and histo-morphology of intestinal mucosa with broilers [Ózsvári et al, 2010].

MATERIAL AND METHODS

In two fully separated and climatized compartments each of them 14 m^2, 85 (group No I, experimental group) 85 (Group No II, control group) Ross 308 day old broilers originated from Salmonella free parent stock have been settled. Before settling floor, walls and all equipment including feeders, drinkers, door, etc. of the premises have been thoroughly cleaned first mechanically and by high pressure water secondly. After drying up of the premises all of the surfaces and equipment have been disinfected by 200 ml/m^2 of 1% Virocid solution with high pressure spray. After complete drying up floor of the premises has been bedded by 10 cm thickness with heat treated, chopped straw closed into plastic sacks at the producer.

Each compartment has been equipped by 3 small drinkers till the end of 2nd week and 2 bell drinkers till the end of rearing. In the first second weeks broilers were fed from the chicken boxes placed on the litter, and after 2 of 1,5 m long trough feeders have been placed in the compartments. Microclimate of the premises has been fitted by the usual broiler technology and controlled automatically. 1 hour light and 23 hours dark period have been used as lighting program with 20 lux. Both of the experimental and control flocks have been fed by a commercial broiler starter feed till the 14th day and grower feed till the end of rearing to the 42nd day, ad libitum. Starter feed of the experimental group have been completed by 3 g/kg, grower feed by 2 g/kg of *FWGE.* Feed of the control group did not contain *FWGE.*

Each broilers of experimental and control groups has been orally infected by culture of live *Salmonella infantis* containing 10 000 000 bacteria/individual dose on the 4th day of rearing.

Both groups has been vaccinated by coarse spray against ND and IB by Nobilis Vaccine Ma5+Clone 30 on the 1st day, against IBD by drinking with Nobilis Gumboro D78 vaccine on the 21st day and against ND by coarse spray with Nobilis vaccine Clone 30 on the 28th day.

Environmental samples were taken by agar sausage method for Salmonella investigation before settling of birds from the surfaces of compartments, and from the covering papers of chicken boxes by the regulation of

home veterinary authority180/2009.(XII.29.) FVM, based on Commission regulation (EC) No.1003/2005.

*Bloodsamples (*10/groups) were taken by puncturing from cubital vein for determining of humoral antibody level of ND and IB on the 14th day, and determining of ND, IB, IBD humoral antibody level on the 28th day, finally ND, IB and IBD humoral antibody level on the 42nd day. Antibodies of ND have been tested by HI, IB by ELISA, IBD by VN.

Cloaca tampon samples (10/groups) were taken on the 5, 6, 7, 9, 10, 15, 17, 22, 23, 29th day of rearing for Salmonella cultivation by enrichment method.

Cecal samples were taken from 5 sacrificed birds/ group on the 35th and 37th day of rearing. Cecum has been removed after double ligation of the organ at the ileum avoiding contamination of its content.

Drinking water samples, 1 l were taken in both compartments for Salmonellas before settling of flocks from the water supplying tap by the hygienic rule of sampling.

Feed samples, 1 kg were taken for presence of Salmonellas from the starter feed on the 1st day of rearing and from the grower feed 16th and 30th day of the rearing with the experimental and control flock.

Mortality has been noticed and the cause of it has been diagnosed.

RESULTS

Environmental samples

a) 32 agar sausage and 11 drag swab samples taken from the surfaces of the two compartments proved to be negative for Salmonellas as well as the 500 g of bedding material (chopped straw).

b) 5x5 g of covering papers spoiled by droppings (meconium) proved also to be negative for Salmonellas in the bacteriological investigation.

Serological investigations

a) IBD VN geometrical mean titres are shown in the Table 1.

Table 1:

Age at the blood sampling/day	*FWGE+*	*FWGE-*
28	*317*	*251*
42	*141*	*158*

b) IB ELISA mathematical mean titres are shown in the Table 2.

Table 2:

Age at the blood sampling/day	*FWGE+*	*FWGE-*
14	*32*	*470*
28	*559*	*68*
42	*493*	*176*

c) ND HI geometrical mean titres are shown in the Table 3.

Table 3:

Age at the blood sampling/day	*FWGE+*	*% negative*	*FWGE-*	*% negative*
14	*12*		*18*	
28	*8*		*8*	
42	*549*	*0*	*274*	*0*

Bacteriological investigation

a) Cloaca-tampon samples. Percentage of positive samples is shown in the Table 4.

Table 4.

	Days of sampling										
	5	6	7	9	10	15	16	17	22	23	29
FWGE+	*90*	*10*	*70*	*10*	*90*	*50*	*40*	*50*	*30*	*0*	*0*
FWGE-	*50*	*50*	*90*	*10*	*90*	*30*	*70*	*60*	*0*	*0*	*0*

b) Cecal samples

The cecal samples taken on the 35th and 37th day proved to be positive for Salmonella infantis with both flocks.

c) Drinking water was Salmonella negative on the day of settling of the birds as well as the *feed samples taken* with both flocks on the 1st, 16th and 30th day of rearing.

Mortality

In the group fed by *FWGE* supplementation died 5 birds (5.9 %) because of omphalitis,1, mechanical suffocation 1, polyserositis 1, pneumonia 2 birds. On the group fed without FWGE supplementation died 4 birds (4,7%), because of omphalitis 1, pericarditis 1, polyserositis 1, and pneumonia 1 broiler.

DISCUSSIONS

Prebiotics are non-digestible carbohydrates containing feed ingredient a substrate for multiplication of useful bacteria in microbiota, prevent or at least suppress colonization of pathogen microflora including *Salmonellas* [Patterson,J.A., Burkholder,K.M., 2003] and influence the immune status of farm animals beneficially [Soltan,M.A., 2009].

The colonization of *Salmonellas* has been decreased with poultry fed by a diet completed with *prebiotics* by decreasing harmful effects of stress factors and decrease shedding of pathogens [Line, J.E., Bailey, J. S., Cox, N.A., 1997, Patterson,J.A., Burkholder,K.M,.2003]. In our investigation could not been demonstrated any difference neither in intensity nor dynamics in shedding *Salmonella infantis* between the FWGE+ and FWGE-groups but from the 23rd day both flocks proved to be negative by the cloaca tampon test (Table 4) though the cecal content remained positive with both flocks even on the 35th and 37th day of rearing.

As far as the immune responses are concerned it has been stated, that inclusion of MOS (mannan oligosaccharide) in broiler diets increased the immune response to vaccination against IBDV and NDV. It has also been determined that PKC (palm kernel cake) containing NSP (non starch polysccharid) added to the broiler feed increased HI antibody level to ND vaccine and relative weight of immune organs [Soltan,M.A., 2009] *FWGE* increased humoral antibody levels after ND, IBD, EDS and AE immunisation with egg layer parents [Kósa,E., Bajcsy.E., 2008]. *FWGE* influenced IBD VN antibody level of broilers, because on the 28th day, 7 days after vaccination it proved to be 26% higher compared to the control., but 21 days after vaccination this difference could not be shown out. (Table 1)

Beneficial effect of *FWGE* on the IB ELISA titres can be seen in t he Table 2, which proves that the titres on the 28th day (28 days after vaccination) 820% and on the 42nd day (42 days after vaccination) 180% higher antibody titres. (Table 2)
As far as the ND HI titres are concerned as result of two vaccinations (1st day, 28th day) titres of blood samples taken on the 42nd day proved to be 100% higher with the FWGE + (Table 3) group compared to the controls and all samples proved to be positive.

CONCLUSIONS

Bacteriological investigation of cloaca swabs proves, that FWGE in the feed did not influence neither of dynamics nor intensity shedding of *Salmonella infantis,* but it can be stated, that while the cloaca-swabs became negative on the 23rd day of rearing (19 days after infection) cecal content proved to be positive even on the 37th day, so the flock remained salmonella positive in spite of negative tampon-results.

Humoral immune response after vaccination has been positively influenced by *FWGE* in case of IBD vaccination till the 28th, in case of IB and ND till the 42nd day of rearing (slaughtering time). On the basis of the results of our study the conclusion may be drawn that *FWGE* has no positive effect in prevention of Salmonella infantis infection, but increases the immune responses after vaccination in broilers remarkably.

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THE CONTAMINATION MODEL OF SALMONELLA ALBANY IN BROILER CHICKEN FARMS IN TAIWAN

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SUMMARY

This paper describes the presence situation of *S.* Albany in broiler chicken farms and to determine the contamination model of the broiler during the rearing period and characterization of *S.* Albany isolates. The result showed the contamination may caused by feed-borne outbreak and vertical transmission by broiler breeder.

INTRODUCTION

Salmonella Albany was the fifth most common serovar that caused human salmonellosis in Taiwan from 2004 to 2010. Previous studies showed *S.* Albany to be the predominant serovar in poultry source, especially from chicken [1-3]. Although previous studies showed *S.* Albany to be the most predominant serovar in chicken meat in Taiwan, the contamination model of the broiler during the rearing period was still unknown.

MATERIAL AND METHODS

Fifty-two broiler chicken farms in northern Taiwan were sampled from April 2008 to March 2010. The visceral organs of broilers and their feed were collected at three consecutive time-points in each farm during the rearing period. Feed samples were collected from three different sampling sites including feed barrel, feed conveyers and feed through. Isolation of *Salmonella* was performed separately by ISO 6579: 2002/Amd.1:2007(E) and ISO 6579:2002. All strains were serotyped according to the Kauffmann-White scheme. Moreover, a total of 16 *S.* Albany isolates from feeds were collected between 2008 and 2009 from seven poultry farms. A total of 40 *S.* Albany isolates underwent pulsed-field gel electrophoresis (PFGE), which were carried out according to the standardized *Salmonella* protocol of the CDC PulseNet.

RESULTS

Among 52 total broiler farms, 28 were *Salmonellae* positive, and the most frequent serovar was Albany (15/28, 53.57%). *S.* Albany was isolated from the visceral organs of broiler chickens at eleven broiler farms (11/15, 73.33%), with ill or dead day-old chicks being most susceptible (8/11, 72.73%). *S.* Albany was isolated from feed in six broiler farms, and the day-old chicks' feeds were most contaminated with *S.* Albany (5/6, 83.33 %), which was frequently isolated from the feed trough (5/6, 83.33 %). All isolates were macrorestricted with *Xba*I and yielded 18 different patterns consisting of 12 to 16 fragments. The results showed that four isolates from ill or dead day-old chicks from four different broiler farms shared the same PFGE type. Five feed samples yielded *S.* Albany with PFGE type matching the dead day-old chick isolates, and three isolates were from three different sampling sites of feed in one broiler farm and showed the same PFGE type.

DISCUSSION

Although there is no reports of *S.* Albany in association with clinical disease in animals have been found. However, this serovar was frequently isolated from ill or dead day-old chicks in this study that may caused highly ecolomic loss in poultry industry. In addition, the contamination in feed play an important risk factor to transmission S. Albany too. The result showed *S.* Albany was frequently isolated from feed trough and may contamination through the feed transport line in chicken house. Feeds may contamination by direct contact with sick birds in feed trough, but the contamination of feeds from feed barrel and feed conveyers may caused by the raw materials of feed. In previrus study, feed-borne outbreak of S. Cubana in Swedish pig farm that having received possibly contaminated feed plant [4]. In Taiwan, previous studies showed *S.* Albany to be the most predominant serovar in chicken meat and caused average 4 % infection of human Salmonellosis in recent years. The prevalence was remains a stable status showed the continued presence of contamination source.

CONCLUSIONS

Although the evidence for vertical transmission of *S.* Albany still is exiguous, there is no doubt that we must draw attention to broiler breeder and feed for possible cause of *S.* Albany infection.

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MOLECULAR CHARACTERIZATION OF ISOLATED SALMONELLA TYPHIMURIUM FROM CASPIAN PONY

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INTRODUCTION

Typhoid disease or salmonellosis is a common sickness in horses. In several epidemiological studies in hospitalized horses, several serotypes of *Salmonella* often predominant in nosocomial infections. Acute form is characterized by fever, depression, anorexia and diarrhea containing blood, mucus and epithelial casts. Transportation, overcrowding, dehydration, oral antimicrobial therapy and infections are the risk factors which may activate latent or subclinical salmonellosis. In this study, the occurrence of typhoid due to *Salmonella* serogroup B were considered in a Caspian ponieś flock kept in a husbandry center of ponies around Tehran.

MATERIALS AND METHODS

In the present study, 19 ponies were transported. During transportation, two pregnant ponies from 19 ponies aborted and four cases died because of acute septicemia. Pathological, bacteriological and laboratical follow up showed salmonellosis. A multiplex polymerase chain reaction (m-PCR) assay was used for detection and identification of *Salmonella* to confirm pathological and bacteriological studies. For m-PCR assay, four set primers were selected: 139-141, specific for *inv*A gene of *Salmonella* genus and the Rfbj, FliC and FljB, specific for the *rfb*J, *fli*C and *flj*B genes of *Salmonella* Typhimurium or other *Salmonella* serovars with similar antigenic properties. Pulsed Field Gel Electrophoresis (PFGE) and antibiotic susceptibility test were also performed.

RESULTS

Salmonella Typhimurium were isolated from bone marrow, mesenteric lymph nodes, liver and intestinal contents of died pony. *Salmonella* was not isolated from stools of other ponies. PFGE pattern was simillar to the other collected isolates which have existed since more than 30 years ago in Iran.

CONCLUSIONS

Because of importance of salmonellosis in ponies, Using of rapid methods are recommended to confirm the presence of *Salmonella*. Results showed that m-PCR permit to evaluate samples more rapidly than other methods and also can detect multiple genes simultaneously like virulence factors which declare virulence of the isolates and have surveillance significance.

Keywords: Caspian Pony; *Salmonella* typhimurium; Transportation; Multiplex PCR; PFGE.

INTRODUCTION

In several epidemiological studies in hospitalized horses, several *Salmonella* serotypes such as *Salmonella* Typhimurium, S. Newport, S. Anatum and S. Agona have been isolated and one or two serotypes often are predominant in nosocomial infections. *Salmonella enterica* serovar Typhimurium is the most frequently isolated serovar worldwide (Gay, 1995). Typhoid disease or salmonellosis is a common disease of hospitalized horses. The spectrum of disease associated with *Salmonella* infections ranges from fecal shedding without clinical signs to septic shock and death. Serotypes of salmonella commonly isolated from horses include *S*. Typhimurium. *S*. Newport, *S* Anatum, *S*. Agona, *S*. Heidelberg, *S*. Ohio etc (Walker et al., 1995; Mainar-Jaime et al., 1998). *S*. Typhimurium is the most common serotype identified and is considered as one of the most virulent serotypes, affecting horses of all ages. Many of the risk factors for salmonellosis in horses have been elucidated through descriptive studies of outbreaks on breeding farms and in hospitalized horses. In addition, a limited number of case-control studies have compared affected horses with unaffected horses to investigate potential risk factors. Stress factors that have been associated with salmonellosis in horses include transportation, antimicrobial administration, intestinal surgery, changes in diet, parturition, anesthesia and antihelmintic treatment (House et al., 1999).

In addition, dosage of bacteria and virulence are important to incidence of disease. Diagnosis and isolation

of bacteria is very difficult in subclinical cases because of a few and or alternative fecal shedding. For laboratorial diagnosis the amount of bacteria must be at least 100 particles/1gr. However not all patients shedding *Salmonella* may be detected by bacterial culture of fecal samples. Recently, advanced PCR based on oligonucleotide primers called m-PCR has been developed. This technique results can be obtained more quickly and sensitive than bacterial culture (Aabo et al., 1993). This study showed that results from m-PCR agreed with biochemical and serological tests. Four different primer sets, inv-A, RfbJ, FljB and FliC were chosen for testing these PCR assays, were reportedly successful at detecting *Salmonella* Typhimurium (Zahraei Salehi et al., 2007). The aim of this study was molecular characterization of *Salmonella enterica* serovar Typhimurium isolated from Caspian ponies.

MATERIALS AND METHODS

Samples

Samples included intestinal content, mesenteric lymph node, liver and bone marrow which were collected from died Caspian pony. Samples freshly placed into plastic bag with ice pack and quickly were transported to Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran. Samples were inoculated into selenite-cystein broth (Merck, Darmstadt, Germany) for overnight enrichment at 37ºC, and later were plated on MacConkey agar (Merck, Darmstadt, Germany) for primary selection. Presumptive *Salmonella* isolates were confirmed using conventional biochemical tests [triple sugar iron (TSI), urease test, MR-VP, Indole product and citrate utilization test] and serological agglutination (Bacto-*Salmonella* O and H antisera; DifcoTM; Becton Dickinson and Company, Franklin Lakes, MI, USA).

Oligonucleotide primers

For m-PCR assay, four primer sets were selected: 139-141, specific for *Salmonella* genus (Rahn et al., 1992) and the RfbJ, FliC and FljB, specific for the *rfb*J, *fli*C and *flj*B genes of *Salmonella* Typhimurium or other *Salmonella* serovars with similar antigenic properties (Lim et al., 2003). The primers sequences and their corresponding genes are shown in the Table 1.

DNA amplification

Polymerase chain reaction was performed with 10µl of DNA sample, 5mM KCl, 1.5 mM MgCl2, 10 mM Tris-HCl pH 8.5, 1 µM of each primer, 200 µM dNTPs (Fermentas, Latvia) and 1 U of Taq DNA polymerase (Fermentas, Latvia) in a final volume of 25 µl. Amplifications were performed in a DNA thermocycler (Techne, TC-512, Cambridge, UK). The m-PCR protocol consisted of the following steps: The initial denaturation step of 5 min at 95ºc; 30 cycles, with considering of 1 min at 95ºc, 1min at 65ºc and 30 s 72ºc; and a final extension step of 7 min at 72ºc. The PCR products were electrophoresed in 1.2% (w/v) agarose gel, stained with ethidium bromide and photographed under UV transilluminator. In each PCR run, a negative control (distilled water) and a positive control tube (*Salmonella* Typhimurium ATCC14028) were included.

Pulsed Field Gel Electrophoresis (PFGE)

Pulsed-field gel electrophoresis was performed according to the procedures developed by the Centers for disease Control and Prevention (CDC) for molecular subtyping of *Escherichia coli* O157:H7, non-typhoidal *Salmonella* serovars and *Shigella sonnei* and as previously described (Centers for Disease Control and Prevention, 2004). Briefly, agarose-embedded DNA was digested with 50 U of *Xba*I (Fermentas, Latvia) overnight in a water bath at 37°C. The restriction fragments were separated by electrophoresis in 0.5X Tris-borate-EDTA (TBE) buffer at 14°C for 20 h in 6 V/Cm using a CHEFF DR II electrophoresis system (Gene Navigator, Pharmacia, Sweden) with pulse times of 2.2 to 63.8 s. The gels were stained with ethidium bromide (1 µg/ml) and destained with the buffer remained in the electrophoresis apparatus for 60-90 min. A Gel Doc 2000 equipped with the software (Bio-Rad, Hercules, CA, USA) was used for image capture and conversion of gel images to a Tiff file. Also isolates presenting DNA smear patterns were retested. The size standard used for all gels was *Xba*I-digested DNA from *Salmonella* Braenderup strain H9812 (ATCC no BAA-664), the universal size standard used by all PulseNet laboratories.

Antibiotic susceptibility

Antibiotic susceptibility test was performed by the standard disk diffusion method in Mueller-Hinton agar, and the results were interpreted in accordance to the criteria of the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 2001). The strain was screened for resistance to the following antibiotics: cephalexin (CN, 30 µg), oxytetracycline (T, 30 µg), trimethoprim (TMP, 5 µg), lincospectin (LP, lincomycin/spectinomycin 15/ 200), enrofloxacin (NFX, 5 µg), trimethoprim sulfamethoxazole (SXT), nalidixic acid (NA, 30 µg), nitrofurantoin (FM, 300 µg), ampicillin (AM,10 µg), chloramphenicol (C: 30 µg), kanamycin (K, 30 µg), streptomycin (S, 10 µg) and ceftiofur (CFTIO, 30 µg).

RESULTS

Isolation and identification of *Salmonella* Typhimurium

Samples were culture positive for *Salmonella enterica* serovar Typhimurium. Also the samples were positive with serotyping (1,4,5,12:i:1,2). Four amplified product (663, 526, 284 and 183 bp) were found in all specimens that had serovar Typhimurium, they corresponded, respectively, to the *rfb*J*, fli*B*, inv*A and *fli*C genes of this serovar. In *Salmonella* Enteritidis (1,9,12;g,m:-) only one PCR product (284 bp) was amplified from the *inv*A gene. In *Salmonella enterica* serovar paratyphi B (1,4,5,12:b:1,2) three positive bands (284, 526 and 663 bp) were amplified corresponding to the *inv*A*, fli*B and *rfb*J genes respectively (Fig1).

Pulsed Field Gel Electrophoresis (PFGE)

Eleven bands were presented in PFGE pattern of the isolate. Bands sizes were almost 40, 70, 90, 230, 260, 300, 380, 550, 670, 730 and 780 kb. This pattern was like to the other PFGE patterns isolated from cat (1979), cat (2006), sparrow (2005) and parrot (2005) in Iran which their information was documented in surveillance system of our laboratory. Comparison between PFGE pattern of the isolate with the standard size and *S*. Typhimurium with ATCC 14028 was presented in figure 2.

Antibiotic susceptibility

The isolates presented resistance against cephalexin, oxytetracycline and streptomycin but were sensitive to oxytetracycline (T, 30 µg), trimethoprim (TMP, 5 µg), lincospectin (LP, lincomycin/spectinomycin 15/ 200), enrofloxacin (NFX, 5 µg), trimethoprim sulfamethoxazole (SXT), nalidixic acid (NA, 30 µg), ampicillin (AM,10 µg), chloramphenicol (C: 30 µg), kanamycin (K, 30 µg) and ceftiofur (CFTIO, 30 µg).

DISCUSSION

Salmonellosis is a commonly encountered infectious disease of horses (Walker et al., 1995). *Salmonella* are among the most frequent causes of acute diarrhea in horses and the incidence seems to be increasing (van-Duijkeran et al., 1995). *Salmonella* Typhimurium is the most common serotype identified and is considered as one of the most virulent serotypes affecting horses of all ages. A number of other serotypes with apparent varying degrees of virulence have also been reported to cause salmonellosis in the horses (Walker et al., 1995). such as *S*. Enteritidis, *S*. Newport, *S*. Anatum, *S*. Java, *S*. Saintpaul, *S*. Kerfeld, *S*. Thompson, *S*. Heidelberg, *S*. Hadar, *S*. Infantis, *S*. Derby, *S*. Oranienburg, *S*. Hindmarsh (Mainar-Jaime et al., 1998; van-Duijkeran et al., 1995; Traub-Dargatz et al., 1990; Daniel et al., 1997; van Duijkeren et al., 2002; Ernst et al., 2004). Recently, the multiresistant *S*. Typhimurium phage type DT104 was the most common phage type isolated from horses correspond with those found in human, pigs and cattle and have highly zoonotic significance (van Duijkeren et al., 2002).

The predominance of one or two *Salmonella* serotypes in the most outbreaks of salmonellosis involving horses herd and hospitals suggests that many of these outbreaks reflect nosocomial infections. In addition, a limited number of case-control studies have compared affected with unaffected horses to investigate potential risk factors. Stress factors that have been associated with salmonellosis in horses include transportation, antimicrobial administration, intestinal surgery, changes in diet, food deprivation, dehydration, colic, gastrointestinal tract disease, anesthesia and antihelmintic treatment (Mainar-Jaime et al., 1998; House et al., 1999; Ernst et al., 2004).

Owen *et al*. 1983 that Transportation has a major role in reactivating the *Salmonella* infection in ponies. Diarrhea due to a reactivation of the *Salmonella* infection occurred greater than 3 days after stress, although maximal shedding of organisms occurred within 24 hours (Owen et al., 1983).

In this study, it seems that transportation of ponies, changing in diet and probably deprivation during transportation, have been the most important risk factors and in accordance to predominance of *S*. Typhimurium, the outbreak has had nosocomial identity. Any results were not obtained following of source of the infection in examination of water supplies and environments of the herd. There were not any food sources for examination but it seems that only food materials could be the source of initial infection. PFGE pattern of the isolate revealed similarity with the other collected isolates which have existed since more than 30 years ago in Iran and indicated the probable presence of this strain for several years in this region. Antibiotic susceptibility test indicated that the isolate dose not have any noticeable antibiotic resistance pattern. So, it seems that pathogenesis of this strain is related to risk factors or susceptibility of pony (as host) to *S*. Typhimurium.

The greatest difficulty in identifying of the outbreaks is detection of the first source of infection for determination and separation of nosocomial infection with one source from those acquired prior to admission (House et al., 1999). Therefore, sensitivity and rapidity of the methods which are applied for *Salmonella* detection are important. The limit of detection for most culture techniques is around 100 *Salmonella* organism/g of the feces. Subclinical infections in horses are more difficult to detect

because less organism are shed and shedding may be intermittent (Mainar-Jaime et al., 1998).

Advanced PCR based techniques such as multiplex PCR are logically the most sensitive methods which could be applied for detection of theoritically even one organism/g of feces as Amavisit *et al*. 2001 indicated While the sensitivity of the PCR assay was less than culture of feces for *Salmonella*, its sensitivity on fecal samples obtained from horses, was much greater than culture method. Also they detected *Salmonella* DNA in 40% of fecal samples using the PCR assay while *Salmonella* was isolated from only 2% of the samples. Also, this method performs in less than 5-7 hours and actually decreases the time of identification (Amavisit et al., 2001). With due attention to correlation between *inv*-A virulent gene and clinical signs such as fever, bloody diarrhea and prolaps of rectum in ponies, it seems M-PCR by presenting multiple virulent genes, confirms the virulent properties of an isolate and is useful for molecular characterization of the isolate which causes an epidemic. So, finally in accordance to importance of disease of horses especially ponies and low prevalence of *Salmonella* shedding in horses about 1/7% and low drainage of *Salmonella* bacteria from feces (Mainar-Jaime et al., 1998), m-PCR method was suggested to be employed as rapid and sensitive method for identifying the source of salmonellosis especially outbreaks of *S*. Typhimurium in carrier status for it's virulence for equidae and zoonotic importance and molecular characterization of an isolate which causes S. Typhimurium epidemics.

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Table 1: Primers sets used for detection of *Salmonella* serovars and identification of *Salmonella* Typhimurium

Primers sets and sequences	Taget gene	Amplified fragment size	Reference
ST139-s: 5′-GTGAAATTATCGCCACGTTCGGGCAA-3′	invA	284	Rahn et al., 1992
ST141-as: 5′-TCATCGCACCGTCAAAGGGAACC-3′			
Rfbj-s: CCAGCACCAGTTCCAACTTGATAC-3′	rfbJ	663	Lim et al., 2003
Rfbj-as: 5′-GGCTTCCGGCTTTATTGGTAAGCA-3′			
Flic-s: 5′-ATAGCCATCTTTACCAGTTCCCCC-3′	fliC	183	Lim et al., 2003
Flic-as: 5′-GCTGCAACTGTTACAGGATATGCC-3′			
Fljb-s: 5′-ACGAATGGTACGGCTTCTGTAACC-3′	fiB	526	Lim et al., 2003
Fljb-as: 5′-TACCGTCGATAGTAACGACTTCGG-3′			

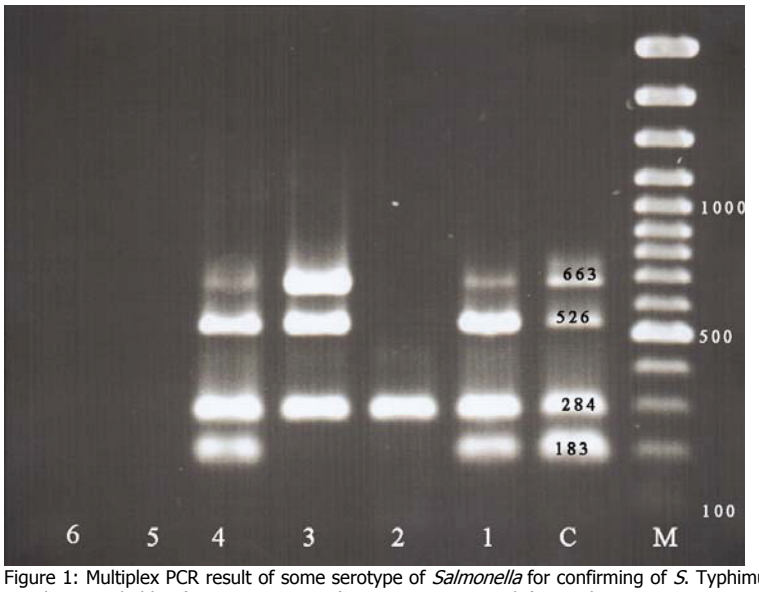

Figure 1: Multiplex PCR result of some serotype of *Salmonella* for confirming of *S.* Typhimurium. 183 bp: *fliC*; 284bp: *invA*; 526bp: *fljB*: 663bp: *rfbJ*; M: 100 bp DNA ladder (Fermentas, Latvia); C: Positive control (*S.* Typhimurium ATCC14028); 1: *S.* Typhimurium isolated from pony (samples); 2: *S.* Enteritidis (wild type); 3: S. Paratyphi B (wild type); 4: *S.* Typhimurium (wild type); 5: *E. coli* (wild type); 6: negative control. Numbers have been explained on the basis of bp.

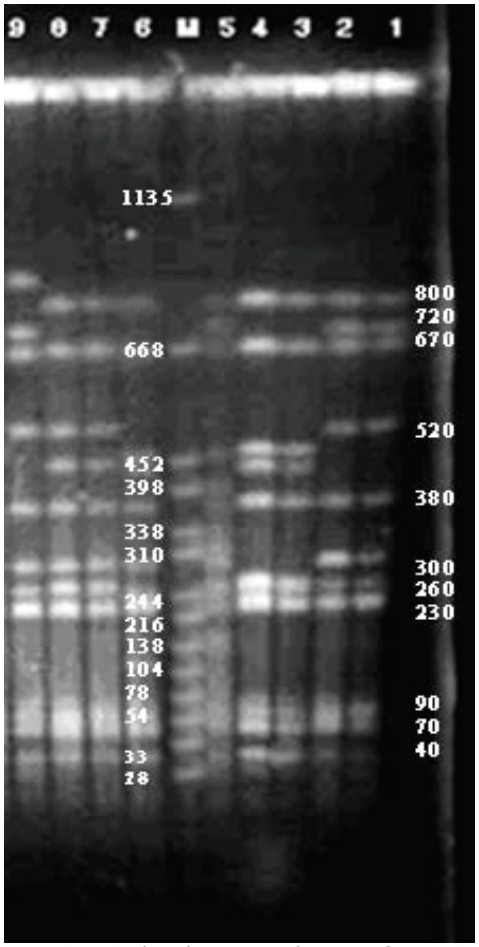

Figure 2: PFGE by *Xba*I enzyme digestion of some *S.* Typhimurium isolates. 1: ponie's isolate and 9: *S. Typhimurium* with ATCC 14028 and M: *S.* Braenderup H9812 Marker

MINOR SALMONELLA: POTENTIAL PATHOGENS IN CONSUMPTION EGGS

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SUMMARY

Salmonelloses are one of the major food borne diseases known to be in close relationship with the consumption of contaminated eggs, infected chickens and poultry products. Control and survey of the poultry chain are the key elements and the most critical steps in the prevention of human transmission.

This study was carried out in the East of Algeria on 150 consumption eggs, collected from mini-markets and immediately processed to seek for salmonella using standard methods (ISO AFNOR 6579 modified in 2002 [1]), briefly the shell surfaces were carefully wiped using sterile appropriated tissues while the white and yellow yolks are separated one from the other. Each 10 samples were pooled together and a total of 45 samples were carefully analyzed.

Our study showed a contamination rate of 4.4% and two strains of *Salmonella bradford* were isolated from white and yellow yolks. The results showed that XLT4 was the best media for salmonella isolation from yolks.
Occurrence of salmonella in yolks at this high level is a serious and potential danger to the public health. Radical and preventive measures must be taken at the critical points to control and to avoid the human transmission; these measures must be installed to all levels of the consumption eggs through application of appropriate and strict regulations, respect of the good practices of transportation, storage and food preparation.

INTRODUCTION

Salmonelloses are one of the major food borne diseases (FBD) known to be in close relationship with the consumption of contaminated eggs and Salmonella Enteritidis phage PT6 is suspected to be the cause of epidemics FBD in many countries [2].

Among all food products, eggs have physical and chemical properties that give them the best weapons for antimicrobial defense. Yet, within the chain of egg consumption, eggs contamination can be performed by vertical transmission for *Salmonella Enteritidis* that have an invader character which lead to ovarian follicles infection without apparent symptoms. The role of virulence factors is mainly important in contamination it's starts at the level of pre-ovulatory follicles and go towards contamination of internal fields

MATERIAL AND METHODS

Five egg trays pack (30 eggs each) have been obtained from retail sellers characterized by lack of air-conditioning facilities of their small markets. These consumption eggs were transported as soon as possible to the laboratory and quickly analyzed to search for salmonella according to ISO AFNOR 6579 method modified in 2002 [1]. Briefly, this method consists in shell surface cleaning of 10 eggs with sterile wet tissues and constitutes the equivalent of one sample. A number of 10 yellow yolks aseptically sampled and pooled together then mixed using stomacher to get best homogenization; the same was done for the albumin and 10 were pooled as one sample. Each sample has been inoculated on 225 ml of buffered peptone water (BPW), after 16h of incubation at 35°C 0.1 ml and 10 ml of BPW have been transferred on 10 ml tetrathionate broth and 100 ml of SB respectively. Then spreaded on XLD Agar, XLT4 Agar and Hektoen Agar.

The suspected colonies appeared on TSI and related to salmonella (Gaz (+), H_2S (+), Glucose (+), lactose (-), sucrose (-) and urea indole (-)) were confirmed on API-10S strips. Serotyping is achieved by polyclonal *Salmonella* antisera O and H as well as an inversion of phase WHO CCRRS (2007). An antibiogram for 14 antibiotics; the more used in human and veterinary medicine, was achieved using the Kirby-Bauer NCCLS method [3].

RESULTS

Two strains of *Salmonella bradford* (*S. bradford*) have been isolated from the tray pack 5 and on the 3rd pool of sample.

Table 1: Samples, serotypes and number of isolated salmonella

	Tray pack 1	Tray pack 2	Tray pack 3	Tray pack 4	Tray pack 5
Number of samples	9 (3+3+3) Shell + white + yellow	9 Shell + white + yellow	9 Shell + white + yellow	9 Shell + white + yellow	9 Shell + white + yellow
Number of isolated Salmonella	0	0	0	0	02 (White + yellow)
Serotype	-	-	-	-	*S. bradford*

Among the used isolation media of salmonellas, only the XLT4 permitted to isolate salmonella strains from the white and yellow yolks but not from shell (table 2)!!

Table 2: Isolation of Salmonella on selective media

	Hektoen	DCLS	XLT4	XLD
Shell	-	-	-	-
White	-	-	+	-
Yellow	-	-	+	-

DISCUSSION

The prevalence of *Salmonella* in our commercial eggs samples is 4.44% (two samples positive from 45 samples). This rate is higher than those reported by Radkowski [4] and Poppe [5] with 0.4%; the cracked and dirty eggs are the more incriminated by the *Salmonella* contamination.

While Suresh and his coworkers [6] have found that contamination of trade eggs by salmonella is about 7.7% and egg surface shell contamination is 5.9% while contamination of its content is around 1.8% the most incriminated serotype is *S. typhimurium*.

Existence of *Salmonella* on the shell surface and contamination of the egg content represents a potential threat for the public health. The surface can be contaminated either in the distal part of oviduct or by fecal contamination [7]. That correspond to the contaminations problems of our retailers of consumption eggs.

The prevalence in enterobacteria of the egg components is generally more important than those recovered on the loaded shell with or without feces or blood or cracked compared with the unscathed shells, but the differences are non significant [8].

The microbial load of egg components is generally significant when related to the cages type of laying hens [9] as well as the source of food, the use of drugs and exposition to temperature.

It is important to consider the protective effects of eggs albumin which inhibit the SE growth in a dependent mode of time and temperature but when the white and the yellow are mixed together the protective effects of lysozymes contained in the white are inhibited what influence bacteria contained in the egg can proliferate and SE with more vivacity especially if the iron sulphate is added in the pre-enrichment broth [10].

The sun exposition and ionizing radiations of commercial consumption eggs and lack of storage conditions have direct consequence on the egg quality but have direct effect on the microbial charge of the shell surface [11]. This may explain lack of salmonella serotypes in the shell surface in our study.

CONCLUSIONS

The microbes present in our eggs is incontestably excessive added to the egg tray pack exhibition without respect of storage conditions what makes consumption of eggs from small markets a source of contamination for human even the rate of contamination by the salmonellas is not very higher (4.4%) and without isolation of salmonellas responsible for zoonosis but this doesn't mean that the risk is faraway.

Preventive measures must be installed to all levels of the consumption eggs through application of appropriate and strict regulations, respect of the good practices of transportation, storage and food preparation.

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BASELINE SURVEYS ON THE PREVLENCE OF *SALMONELLA* SPP IN AUSTRIAN POULTRY FARMS: AN OVERVIEW

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SUMMARY

Within the EU, including Austria, EU-wide baseline surveys organized by the commission on the prevalence of *Salmonella* spp. in poultry and pigs were conducted from October 2004 to September 2006. The aim of the studies was to identify risk factors for the infection of layers, broilers, with *Salmonella* spp.

A randomized sampling plan was designed according to EU-commission parameters (p= 50%; CI 95%, α = 5%). Sampling was carried out regularly throughout the whole year. In the lab samples were analyzed according to annex D of ISO 6579(2002). All isolates were serotyped according to Kauffmann-White-scheme and all isolates of S. Enteritidis (SE) and S. Typhimurium (ST) were phage typed in line with the guidelines provided by the HPA Colindale. In laying flocks 15,4% were positive on *Salmonella* spp., 10,7 % were positive on *S*. Enteritidis/*S*. Typhimurium. In broiler flocks 7,7% were positive on *Salmonella* spp, 2,2% were positive on *S*. Enteritidis/*S*. Typhimurium. Data show that the risk for human infection is mainly coming from laying hens. Goals where set by the EU to reduce the prevalence of *S*. Enteritidis/*S*. Typhimurium in Austria by 10% each year in layers, in broilers to reach below 1%. Measures to reduce the prevalence of *S*. Enteritidis and *S*. Typhimurium on poultry farms significantly reduced the number of Salmonellae infections in humans

INTRODUCTION

Within the EU, including Austria, EU-wide baseline surveys on the prevalence of *Salmonella* spp. in poultry were conducted from October 2004 to September 2006. The aim of this studies was to identify risk factors for the infection of layers and broilers with *Salmonella* spp.

MATERIAL AND METHODS

A randomized sampling plan was designed according to EU-commission parameters (p= 50%; CI 95%, α = 5%). Sampling was carried out regularly throughout the whole year. One flock in each selected farm was sampled. In Laying hen cage flocks 5 bpooled samples with a total of 200-300 g for each sample were collected, in barn or free range laying houses 5 pairs of boot swabs were used for sampling. Additional 2 samples of dust were collected, each weighing about 200 ml. In poultry flocks 5 pairs of boot swabs were collected. At the IVET samples were analyzed according to annex D of ISO 6579(2002), a method recommended by the Community Reference Laboratory for Salmonella in Bilthoven, Netherlands. In this method a modified semisolid Rappaport-Vassiliadis medium (MSRV) is used as a single selective enrichment medium after pre-enrichment in buffered peptone water. At the IMED all isolates were serotyped according to Kauffmann-White-scheme and all isolates of *S*. Enteritidis (SE) and *S*. Typhimurium (ST) were phage typed in line with the guidelines provided by the Health Protection Agency. Analysis and evaluation of data were carried out by the CC INFE together with QGV by means of Microsoft ® Office Excel 2003 and EpiInfo TM Version 3.5.1.

RESULTS

In laying flocks out of 337 flocks 52 (15,4 %) were positive on *Salmonella* spp., 32 flocks (9,5 %) were positive on SE, 4 flocks (1,2 %) on ST. Depending on the flock production type 96 cage, 72 barn, 100 free range standard and 69 free range organic flocks were sampled (Table 1). 33 flocks positive for Salmnella spp. revealed being infected with one strain, 13 flocks (all cage flocks) with 2 strains, either 2 different serotypes or 2 different SE phage types and 2 flocks (both cage flocks) with 3 different strains (3 different SE phage types each). The comparison of the prevalence of Salmonella spp. and SE in different holding size categories demonstrated higher

rates in larger flocks. Salmonella spp,. Or SE could be detected in 5,9 % of holdings of the smallest size category 1,000 to 2,999 hens (SE in 3,2 %), in 16,7 % of holdings of the category 3,000 to 4,999 (SE in 8,3 %), in 28,3 % of holdings of category 5,000 to 9,999 (SE in 13,2 %), in 45,0 % of holdings of category 10,000 to 30,000 hens (SE in 45,0 %) and in 83,3 % of holdings on the largest category, $\geq$ 30,000 chicken (SE in 66,7 %).

Altogether 223 different *Salmonella* spp. isolates were typed at the NRC. 70,4 % of all serovars were SE (n=157), 5,4 % ST (n=12) and 24,2 % other serovars like *S*. Infantis 6,7 %, *S*. Montevideo 5,4 %. In one samples 2 different serotypes (*S*. Agona, *S*. Mbandaka) in 27 samples 2 different SE phage types (PT) could be found. The most frequently found phage type was PT 4 (30,6 %) PT 8 (27,4 %) PT 7 (19,1 %) and PT21 (11,5 %). ST definitive types (DT) were DT104H (58,3 %), DT193 (25 %) and RDNC (16,7 %). The seven phage types PT4, PT8, PT7, PT21, PT correspond to 96 % of al SE phage types identified in laying hens in the course of this survey. The same phage types caused 84 % of all human SE cases in 2005 in Austria. The three ST definitive types RDNC, DT104H and DT193 found in this survey could also be isolated from humans although these DT only correspond to 22 % of all human ST isolates in 2005. These facts affirm the relevance of laying hens as a source for disease in humans in terms f SE but the minor role of ST.

The antimicrobial resistance testing revealed very low resistance rates, out of 78 strains tested only two strains showed resistance, one against Spectinomycin, one against Ampicillin.

Table 1: Findings of *Salmonella* spp., *S*. Enteritidis (SE), *S*. Typhimurium (ST) and other serotypes (non SE and ST) according to flock production types

Flock Production Type	Flocks tested N	*Salmonella* spp.		SE		ST		Non SE and ST	
		n	%	n	%	n	%	n	%
Cage	96	33	34.4	21	21.9	2	2.1	12	12.5
Barn	72	11	15.3	6	8.3	1	1.4	4	5.6
Free range standard	100	7	7.0	4	4.0	1	1.0	2	2.0
Free range organic	69	1	1.4	1	1.4	0	0.0	0	0.0
Total	337	52	15.4	32	9.5	4	1.2	18	5.3

In broilers 363 flocks consisting of at least 5000 broilers each were tested. Of these, 28 flocks (7.7%) were positive for *Salmonella* spp., eight flocks (2.2%) showed either SE (six flocks, 1,6 %) or ST (two flocks, 0,5 %). None of the flocks showed prevalence of both serotypes. Additional serotypes found were *S*. Montevideo (15 flocks, 4,1 %), *S*. Infantis (2 flocks, 0,5 %), and *S*. Senftenberg, *S*. Tennessee and *S*. Virchow (each in 1 flock, 0,3 %).

Of all isolated field strains (n = 29, Table 2) four showed resistance patterns: one isolate of SE and S. Montevideo each was resistant to ampicillin, another S. Montevideo was resistant to ampicillin and sulfmethoxazol, and one ST was resistant to sulfmethoxazol, spectinomycin and streptomycin.

DISCUSSION

The higher infection rate of layers in comparison with broilers is linked to the combat system in Austria. The Austrian programme on Salmonella spp. started in 1991 with the poultry hygiene regulation coming into effect which was focusing on breeding flocks and broiler flocks. Laying hens were only controlled before slaughter. With the poultry hygiene regulation 2007 the Salmonellae control programme was extended to laying hens as well. In broiler flocks due to the control programme the risk of vertical transmission of Salmonella spp. has been reduced significantly, but the risk of horizontal transmission is there. The high number of S. Montevideo which are linked to a feed mill is one indicator for this.

The antimicrobial resistance rates found in the baseline studies are in line with data derived from the harmonised monitoring of antimicrobial resistance in Salmonellae according to EU commission decision 2007/407/EC (resistance rates 2009: broiler flocks 12.2%, laying hens 10%). Since resistance rates mainly depend on the serotypes found differences of resistance rates are due to a different or changed distribution of serotypes within the tested flocks. Low numbers of isolated strains make it difficult to statistically evaluate the development of resistance patterns.

CONCLUSIONS

Data showed that in Austria laying hens showed a higher prevalence of Salmonella spp. as well as of SE/ST. Goals where set by the EU to reduce the prevalence of SE/ST in Austria by 10 % each year in layers, in broilers to reach below 1 %. Measures in layers and in broilers reduced the prevalence accordingly. Also the infection rate in humans dropped significantly during the same time.

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COMPARATIVE INVESTIGATION OF VACCINATION STRATEGIES FOR PREVENTION OF *SALMONELLA ENTERITIDIS* IN LAYING HENS

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SUMMARY

The aim of this present study was to determine the efficacy of five different vaccination programmes, commonly applied in Saxony, Germany, including inactivated and live vaccines against *S*. Enteritidis (SE). On the 39th, 54th and 69th week of age, five groups of 12 birds originating from five farms, each on which different vaccination programmes were applied, were housed separately in the isolation facility of the Institute. One week after housing, animals of each group were orally challenged with a nalidixic acid resistant SE strain. Faecal shedding was controlled by taking cloacal swabs of each chicken on day one, three and five post challenge. From hens at the age of 54 and 69 weeks, eggs were collected on day two and seven post challenge and egg shell, yolk and albumen were examined for SE. Two days and seven days post challenge, six randomly selected chickens from each group were euthanized. The bacterial load (CFU) of liver, ovary, oviduct, caecal wall and content, as well as the proportion of *Salmonella*-positive samples, were determined. The quantitative and qualitative investigations revealed no important differences between the different vaccination programmes.

When taking into consideration the efficacy of the vaccination at different points during the laying period, groups B-E demonstrated a lower level of protection in week 54 of age, and group A was less protected in week 69 compared to other time points during the laying period. However, these differences were slight and only represented tendencies. In the cloacal swabs, SE could be most frequently isolated three days post challenge and at the age of 54 weeks. In all groups, shedding was intermittent. Egg shells were only positive in week 54 of age, and the internal egg was completely SE-negative. No important differences in shedding and egg contamination between the groups were revealed. In conclusion, the animals treated with a double protection of both killed and live vaccines were not more protected than the animals treated merely with live vaccines in this study.

INTRODUCTION

Human Salmonellosis, particularly caused by Salmonella Enteritidis (SE), remains since years one of the most important food borne diseases in Europe [1]. As contaminated poultry egg products are supposed to be the most important source of human SE infection [2], the control usually focus on laying hens. In spite of different national efforts to control Salmonella (improved management and biosecurity, vaccination, cleaning and disinfection, rodent control) [3], it has not yet been possible to reduce SE in laying hen flocks in Germany as demanded by the European Regulation No. 1168/2006 [4;5]. Hence, there is a general interest in the revaluation and, if necessary, improvement of strategies to reduce Salmonella in laying hen flocks.

Several studies regarding the efficacy of live vaccines (LV) and inactivated Salmonella vaccines (KV) were carried out, but only a few focussed on the combined use of live and inactivated Salmonella vaccines [6;7]. In addition, there only exists a sparse amount of data that focuses on the efficacy of the different vaccination programmes actually used in the field.

For reasons such as the capacity of inducing humoral and cell-mediated immune response as well as the colonization-inhibiting activity [8], it is generally accepted that LV are more effective against Salmonella than KV. Furthermore, the application of LV via drinking water is cheaper than the application of KV via injection. In terms of consumer safety, inactivated vaccine preparations are considered to be the safest. Overall, there is no risk of causing illness in humans, widespread dissemination or reversion to virulence by horizontal gene transfer [9]. Concerning the efficacy of several Salmonella vaccines under field conditions, scientific data demonstrating the level and duration of protective effects is incomplete [2].

The purpose of this semi-field study was to assess the efficacy of five different vaccination programmes involving live and inactivated vaccines actually used in Saxony, Germany. This was tested in hens that were between the 39th and the 69th week of age, reared up and vaccinated under field conditions.

MATERIAL AND METHODS

Five groups consisting of 12 laying hens A-E (Lohmann Brown layers excl. group B: white Lohmann Selected Leghorn layers) acquired from different commercial layer farms were used following different vaccination programmes (exclusive LV or the combined use of LV and KV against SE and SE and STM respectively). Given the fact that all layer hens have to be vaccinated against SE in Germany, unvaccinated control groups were excluded. At about 39, 54 and 69 weeks of age, the hens were orally challenged with approx. $1.0x10^9$ cfu of a nalidixic acid resistant strain of *S.* Enteritidis (*S.* Enteritidis 147Nal^r) which had been kindly provided by Dr U. Methner, *Institute of Bacterial Infections and Zoonoses* at the *Friedrich Loeffler Institute*, Jena, Germany. On day one, three and five post infection, cloacal swabs of each bird were collected and examined. On day two and seven post infection, the animals were euthanized and dissected, and eggs were collected (on week 54 and 69). Caecal wall and content, ovary, oviduct and liver were withdrawn for bacteriological examination. Each organ sample was weighed and homogenized. Serial 10-fold dilutions were made and spread-plated onto modified XLD- and BPLS agar (supplemented with 50 µg/ml sodium nalidixate). After incubation for 24 h (37 °C), SE colonies (confirmed by agglutination) were enumerated in the different organ samples. In addition, the organ samples analogous to the cloacal, egg shell, albumen and yolk samples were enriched in BPW (37 °C, 24 h) and Rappaport-Vassiliadis broth (42 °C, 24 h), spread-plated onto a modified BPLS and XLD agar, incubated (24 h, 37°C) and examined for SE. The quantitative and qualitative results of the groups were statistically computed.

RESULTS

The highest SE recovery rate (4.0 $\log_{10}$) was observed in the caecal wall and content. The second highest rate (2 $\log_{10}$) was observed in the liver. Generally more SE was found in the Caeca two days post infection than five days later. The examination of the liver samples revealed the reverse results. The reproductive tissue was rarely contaminated (ovary: 6,67 %, oviduct: 0,56 % positive results). In groups B-E, the SE recovery rate in the organ samples was slightly higher in week 54 than in week 39 and 69 of age. In contrast in group A, the highest amount of SE was found in the 69th week of age, but this was not more than determined for the organ samples of groups B-E in the 54th week of age.

Comparing the results of the SE detection in the cloacal swabs at the different points of age, the SE was most frequently reisolated in the 54th week of age (groups B-E).When comparing the results on the different days post challenge, SE was most often found three days post challenge. Faecal excretion was intermittent and differed at the different days post-challenge from negative to 100 % SE-positive cloacal swabs. The examined egg contents (on week 54 and 69) were completely negative. Only a few egg shells in week 54 (2 d.p.inf: 33,33 %, 7 d.p.inf.: 6,67 %) were contaminated with SE. In week 69, there was no SE recovered. The comparative analysis of these results considering SE in organ samples, cloacal swabs and eggs between the five groups only revealed tendencies but no severe and statistically proved differences.

DISCUSSION

The aim of this study was to compare the protective effect during and at the end of the laying period of five different vaccination programmes against SE, involving LV exclusively and the combined use of LV and KV, as are currently applied in Saxony, Germany.

Our investigation revealed no important differences between the groups A-E in regards to the faecal excretion, organ colonisation and egg contamination. Only when comparing the efficacy of the vaccination at different points in the laying period had groups B-E demonstrated a slightly lower level of protection in week 54 of age and group A a slightly lower protection in week 69 of age. These differences only illustrate tendencies and no severe differences.

In conclusion, the combined use of KV and LV provides no more protection against SE than the exclusive use of LV. These findings are consistent with the results of SPRINGER et al. (2011), and the fact that KV is not more efficient than LV [7]. HAFEZ et al. (2001) carried out field trials in hens vaccinated by a programme correlating with group B (LV and KV) of the present study. They only noted a limited success in the decline of *Salmonella* [6].

The application efficacy of the vaccines used in our study has been approved by the manufacturing pharmaceutical company and the Paul-Ehrlich-Institut, Langen, Germany (institution for approval and marketing authorization of vaccines). The application of KV in the field, i.e. one dose of KV does not correspond to the vaccination recommendation of the manufacturer. Consequentially the protective effect needs to be revaluated. As the present study, a so called semi-field-study, involves groups of animals originating from different farms, the age, race, behavior, physical and immunological constitution differ more or less between the groups. Although these naturally given factors and the former mentioned factor might influence the outcome of *Salmonella* infection in our study [10;11], the aim of the study was to reflect the situation in the field and to investigate the efficacy of vaccination programmes under field-conditions. The results also highlight that vaccination is not suitable to eradicate SE and, consequently, has to be integrated in to the good hygiene and husbandry management [6].

Despite the contamination of the reproductive tissue, no internal egg contamination could be found. This correlates with previous investigations demonstrating that whilst many reproductive tissue of artificially infected hens samples are positive, SE is only rarely recovered from the egg contents [12;13]. The higher egg shell contamination rate in week 54 correlates, as in other studies, with the higher rate of positive cloacal swab samples, indicating the cloaca as infection site of the eggshell.

CONCLUSIONS

The results indicate that layers vaccinated following a protocol including both live and inactivated vaccines are not more protected against SE under the given conditions of the present study than hens which were merely vaccinated with live vaccines. The tendencies found might to be examined in further investigations with a higher number of animals. The live vaccines provide layer hens with an adequate protection against SE infection during and at the end of the laying period. Results indicate that hens are more susceptible for SE at the middle time point of the laying period than at the end.

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PROTECTIVE EFFECT OF VACCINATION STRATEGIES FOR PREVENTION OF SALMONELLA INFECTION IN LAYING HENS DURING LAYING PERIOD

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SUMMARY

The aim of this study was to examine the duration of immunity of different vaccination schemes using inactivated and live vaccines against *Salmonella* Typhimurium (STM) currently used in Saxony, Germany. Five groups (A-E) of 12 birds from five commercial farms each applying different vaccination strategies the during rearing period, were housed separately in isolation facilities in 39th, 54th and 69th week of age. One week after housing hens of each group were orally challenged with a nalidixic acid resistant STM strain. One, three and five days after challenge faecal shedding was controlled by taking cloacal swabs of each chicken. Two and seven days after challenge six randomly selected hens of each group were euthanized and the number of challenge strains (CFU) in liver, ovary, oviduct, caecal wall and caecal content was enumerated. In addition the proportion of *Salmonella*- positive samples was determined. In all groups shedding was intermittent and no important differences between the groups were found. The vaccination programs in groups B-E demonstrated lower level of protection in week 39 and 54 of age, than at the end of laying period. Only group A demonstrated the lowest level of protection in the 69th week of age two days after challenge. In conclusion in this study the hens with the protection of live and inactivated vaccines were not more protected than the animals vaccinated with only live vaccines.

INTRODUCTION

Acute bacterial enteritis caused by *Salmonella* species is a major public health burden internationally. Contaminated poultry products are widely accepted as a primary source of human *Salmonella* infection and most cases are attributed to the consumption of contaminated eggs or egg products [4; 5]. Improved management, including better biosecurity, cleaning and disinfection and rodent control in combination with vaccination are implemented to reduce the prevalence of *Salmonella* on laying henfarms [3].

Nevertheless, some *Salmonella*- positive samples were detected at laying farms at the end of the laying period [9] and in the last few years the rate of *Salmonella* Enteritidis continuously decreased and the rate of STM advanced slightly [7].

A beneficial effect of vaccination against *Salmonella* has been shown in both experimental work and field studies. However, there are only a few data on the efficacy of combined use of LV and KV [1; 6] till the end of the laying period under field conditions [13].

It is generally accepted, that live vaccines (LV) are more effective against *Salmonella* than inactivated vaccines (KV) [10; 13; 14], but in terms of consumer safety, KV preparations are considered to be the safest [2].

The present semi- field study compared the efficacy of five different vaccination protocols including combinations of three different live vaccines and two different inactivated vaccines currently used in Germany.

MATERIAL AND METHODS

Altogether for this study 180 laying hens from five different companies were used. The animals were vaccinated according to five different vaccination strategies during the rearing period. On the 39th, 54th and 69th week of age five groups (A-E) of 12 laying hens (Lohmann Brown layers, excl. group B white Lohmann Selected Leghorn layers) were housed in isolation facilities of the Institute for Animal Hygiene and Veterinary Public Health, Leipzig. The animals were challenged orally with $1x10^9$ cfu of a nalidixic acid resistant STM strain (*S.* Typhimurium 9098 Nal^r) kindly provided by Dr U. Methner, *Institute of Bacterial Infections and Zoonoses* at the *Friedrich Loeffler Institute*, Jena, Germany. Cloacal swabs from each bird were collected on day one, three and five after challenge and examined for the challenge strain. Two and seven days post infection six randomly selected hens from each group were euthanized and dissected. Colonization of caecal wall and content as well as bacterial load of liver, ovary and oviduct were assessed. Each organ sample was weighed and homogenized. Serial 10-fold dilutions were made and spread-plated onto modified XLD- and BPLS agar (supplemented with 50 µg/ml sodium nalidixate). After incubation for 24 h (37 °C), STM colonies confirmed by agglutination were enumerated in the different organs. In addition, the organ samples and the cloacal swabs were enriched in BPW (37 °C, 24 h) and Rappaport-Vassiliadis broth (42 °C, 24 h), spread-plated onto modified BPLS

and XLD agar (supplemented with 50 µg/ml sodium nalidixate), incubated (24 h, 37°C) and examined for STM. The quantitative and qualitative results of the groups were statistically computed and differences were reported.

RESULTS

Faecal excretion: STM was detected after enrichment of cloacal swabs in all groups and investigated points of time. Although percentage of positive animals ranged from 0 to 100%, no severe differences between the vaccination strategies could be identified.

Caecal colonization: Quantitative examination of caecum revealed bacterial loads between 0 and 10^5 CFU/g. However, severe differences concerning the vaccination strategies were not present.

Bacterial load of liver, ovary and oviduct: Low numbers of bacteria could be detected in liver, ovaries (max. 10^3 CFU/g) and oviducts (max. 10^2 CFU/g). The enrichment of liver, ovaries and oviducts revealed presence of STM (in mean 9.86% of organ samples). The vaccination programs in groups B-E demonstrated lower level of protection on 39^{th} and 54^{th} week of age, than at the end of laying period. Only group A demonstrated the lowest level of protection in 69^{th} week of age two days after challenge. However, no severe differences between the vaccination strategies were elevated.

DISCUSSION

The aim of this study was to examine the protective effects of vaccination with either *Salmonella* live vaccines or the combined use of live with inactivated vaccine against STM during the entire laying period.

The present study involved groups of animals originating from different farms. The age, race behaviour, physical and immunological constitution differed more or less between the groups. These factors can highly influence the outcome of *Salmonella* infections [14]**.**

Furthermore, the use of KV in the field does not correspond to the vaccination recommendation of the manufacture and in consequence, the protective effect has to be revaluated.

Given the fact that all layer hens have to be vaccinated against SE in Germany an unvaccinated control groups could not be included.

There are no severe differences present between the vaccinated groups. For this reason it is assumed that both, the exclusive use of live vaccines and the combined use with inactivated vaccines in a vaccination program were able to confer protective effects against STM up to the end of the laying period. These results correspond with results of other studies [1; 13].

Furthermore, in this study a cross immunity against STM was shown for live and inactivated vaccines against *S.* Enteritidis. This also correlates with results reported in other studies [8]. On the other hand some studies demonstrated no cross protection against *Salmonella* Typhimurium [11; 12].

CONCLUSIONS

According to our results the vaccination with live and inactivated vaccines was not more protective than an exclusive use of live vaccines. Live vaccines are able to protect the laying hens until the end of laying period. Nonetheless, none of these vaccination strategies was able to prevent colonisation of caecum and internal organs. In summary, the use of *Salmonella* vaccines should always be combined with other animal health measures including cleaning and disinfection, effective rodent control and biosecurity.

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DUST CONCENTRATION IN VARIOUS LAYING HEN HOUSING SYSTEMS

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SUMMARY

The aim of this epidemiological study was to measure and to compare the concentrations in ambient dusts in the different French housing systems for layers in France.

Sixty six commercial buildings distributed for 30 in cage system without litter (conventional cages=22 and furnished cages=8) and for 37 on on-floor system with litter (free range=20, organic=13, aviaries=3 and on-floor=1) were investigated. From June, 2006 to September, 2007, 130 dust samples were collected using a stationary captor (CAP 10, ARELCO).

The average concentration of ambient dusts (≤ 4 µm) was respectively 0.12 mg/m³ (IC95% [0.10-0.14]) in cage systems and 0.46 mg/m³ (IC95% [0.35-0.57]) in alternative systems (P<0.01). The highest concentration of dusts was observed in the aviary system: 1.53 mg/m³ (IC95% [0.86-2.21]).

This study underlines the impact of the housing system on the indoor air quality and the need for information and prevention towards the poultry workers particularly exposed to air dusts.

INTRODUCTION

Organic dusts are one of the most widely recognized airborne pollutants in intensive pig and poultry buildings (Cambra-Lopez, 2010). In poultry production, dust is almost composed of litter, but also of feathers, skin, mineral crystals from urine, feces and feed (Ellen*, et al.*, 2000). Bioaerosols or organic dust are usually defined as aerosols of microbial, plant or animal origin (Douwes*,* et al., 2003). They include airborne bacteria, fungi, viruses and their by-products, endotoxins and mycotoxins. The levels of endotoxins in henhouses are higher than in cattle and pig buildings (Seedorf*,* et al., 1998). Chronic exposure to these pollutants, particularly endotoxins, may impair health, performances and welfare of animals and humans (Al Homidan and Robertson, 2003; Donham et al, 1984, Andersen et al, 2004).

The evolution of the European regulation on hen housing systems (European Directive 1999/74/EC) which promotes welfare, requires the ban of conventional cage system and the development of furnished cages and alternatives systems such aviary system, where animals have more space and are provided with litter. It is well known that the housing system greatly influences the airborne dust concentration. But few comparative data on air quality between housing systems are available. An epidemiological study was carried out in Western France in various laying hen housing systems, to measure the concentrations of ambient dusts, to estimate the workers' exposure to dusts and to measure the impact on the respiratory health of the poultry farmers. Only measures of ambient dust are reported here.

MATERIAL AND METHODS

The study took place from June 2006 to September 2007. The farm sample was stratified according to the housing system and the presence of bedding (litter) or not: half buildings where hens were kept in cages (conventional or furnished) and half where birds were housed in an alternative rearing system (including barn, free range, organic and aviary). Farm selection was based on the willingness of the owners to participate in the study**.** Dust samples were taken within each animal house in a point located in the middle of the henhouse at approximately one meter above floor level and without allowing the birds to interfere with the measure. Respirable particle concentration was measured using a stationary captor (CAP 10, ARELCO) equipped with a pre-weighted filter with a pore size of 4 µm. One sample was taken during the day time (about 8h period) for each sampling site, based on previous studies (Pedersen and Pedersen, 1995). Two dust samples were collected per farm, one in April to September period (warm period) and one in October to March period (cold period). After sampling, the exposed filters were weighed (AG 104, Mettler Toledo,) after desiccation for 12 hours at 37 °C. The respirable particle concentration in each building was calculated and given as mg/m^3air. In addition and for each flock, data were collected concerning farm and house characteristics (ventilation, dimensions, management and hygiene practices). The concentrations of dust were compared among rearing systems using non-parametric tests of Kruskal-Wallis.

RESULTS

Caged buildings were characterized by a largest size (38, 656 ± 15, 488 hens/building) and a mechanical ventilation. The number of hens per cage varied from 5 to 9 hens in standard cages versus 20 to 60 hens in furnished cages equipped with a nest box and perches. On the contrary, alternative buildings were smaller (5 788 ± 1 869 hens/building) with a natural system of ventilation and an open-air run except for one flock. The aviaries were intermediate between the caged and the on-floor systems because they consisted in claustration building with mechanical ventilation system, but the volume of air available per hen and the capacity building was similar to those in alternative systems (8000 ± 4597 hens/building).

A total of 130 respirable dust measurements were obtained including 70 measures during the warm season and 60 during the cold season. The average ambient dust concentrations were higher ($P<0.01$) in the alternative houses than in the caged houses: respectively 0.46 mg/m³ (IC95% [0.35-0.57]) versus 0.12 mg/m³ (IC95% [0.10-0.14]) ($P<0.01$) (Figure 1). Dust concentrations in alternative systems showed a great variability compared to the caged system, and still remained higher than that observed in cages. The highest concentration of dusts was observed in the aviary system: 1.53 mg/m³ (IC95 % [0.86-2.21]). Excluding aviaries, the average concentration of ambient dusts in alternative systems was equal to 0.36 mg/m³ (IC95% [0.30-0.42]) and remained significantly higher than in cage systems. The average concentrations of dusts were similar in conventional cage building (0.11 mg / m³ (IC95 % [0.10-0.14])) than in furnished cage buildings (0.14 mg/m³ (IC95 % [0.10-0.18])).

No seasonal effect was observed on respirable dust concentrations (0.23 mg/m³ (IC95 % [0.17-0.29]) during the cold period vs. 0.26 mg/m³ (IC95 % [0.20-0.32]) during the warm period).

DISCUSSION

The dust levels observed in the ambient air of henhouses are in accordance with other studies and included within the range presented by Ellen et al (2000) in a review about dust levels in poultry productions: from 0.01 to 6.5 mg/m³ of respirable dust. Our study confirms the higher rates in alternative housing systems observed in previous studies. A degradation of air quality in alternative systems compared to caged systems was reported for dust concentrations (Takai et al., 1998; Ellen et al., 2000) but also for endotoxins concentrations (Seedorf et al., 1998). The degradation of the air quality in alternative systems may be due to the presence of litter and to the greater activity of the hens in the on-floor buildings (walking, flying, foraging…). In addition the natural ventilation systems in the alternative henhouses, in contrast to the dynamic ventilation systems in the caged buildings, could lead to a lower ventilation rate and thus a higher rate of dust concentration (Takai, et al., 1998; Radon, et al., 2001).

If in our study the average concentrations of dusts were similar in conventional cage building than in furnished cage buildings, the highest concentrations of dusts were observed in the aviary system. Too little data are available to conclude, however it seems that this housing system promotes animal activity and high concentrations of dust. Larsson et al. (1999) reported concentrations of dust 2 times higher in aviaries than in cages.

No seasonal effect on dust levels was demonstrated whatever the housing system. According to Donham et al. (2000), the exposure concentration associated with significant pulmonary function decrements in poultry workers is 2.4 mg/m^3 for total dust and 0.16 mg/m^3 for respirable dust. In our study, the mean exposures are higher than these thresholds in alternative systems. Various methods of air treatment (water fogging, electrical filtration…) have been tested with success under experimental conditions to reduce aerial dust concentration in the air (Ellen 2010). But prevention towards poultry workers is also necessary and the development of personal protection measures such as wearing a mask is also necessary especially for farming activities particularly exposed to dusts.

CONCLUSIONS

The present study confirms that high dust concentrations could occur in the air of commercial henhouses. It underlines the higher rates recorded in alternative housing systems than in caged systems already obtained in previous studies and gives us data for new systems complying with the European Directive 1999/74/EC, the enriched cages and the aviaries. For an occupational point of view, the poultry workers are particularly exposed to air dusts with a potential health impact. Information and prevention towards the workers and effective methods to reduce dust concentrations in the air need to be developed.

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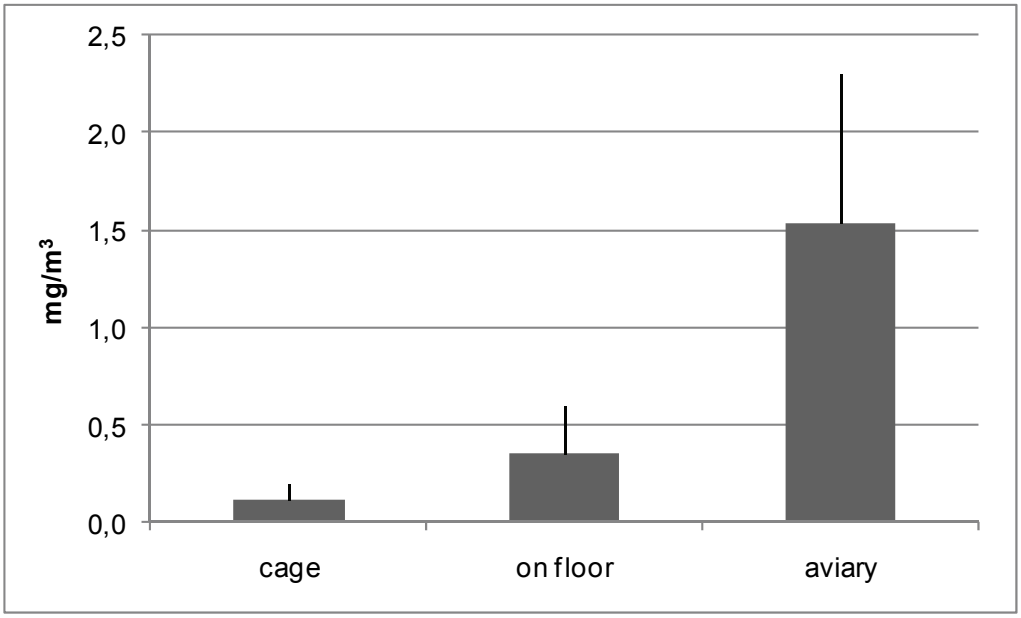

Figure 1: Respirable dust concentrations (mean + STD) in ambient air according to housing system.

MICROBIOLOGICAL ANALYSES OF DRINKING WATER AND WATER SUPPLY SYSTEMS IN POULTRY HUSBANDRY

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SUMMARY

Water is important for the healthy growth of chicken. Therefore proper cleaning and disinfection of water supply systems must be proven and adapted to ensure the application of drinking water in hygienic quality. Different methods to analyse water and water supply systems in poultry husbandry, such as the total bacteria count at 22°C and 36°C, *Enterobacteriacea* count and *E.* coli count were performed. Results showed an effect of sampling time and sampling place on the microbiological results which even differed extremely within the same holding. From the results it could be concluded that it is necessary to analyse water and the water supply system in order to estimate the health risk for poultry, to evaluate the effectiveness of disinfection processes of water systems and to judge the hygienic composition of drinking water.

INTRODUCTION

Water is considered as a feed according to the EU regulation 178/2002. No legal requirements for the quality of drinking water in animal husbandry exist. Unique quality assurance systems do not exist within the EU as well as no regulations about the sampling and analyses of animal drinking water are in evidence. In Germany there is only a framework on the legal evaluation of water as feedstuff. Drinking water for animals should be free from pathogens and from harmful substances in toxic concentrations. Pathogens and harmful substances do not only affect the health of the animals but also the sanitary harmlessness of the food produced from them. Water is important for the healthy growth of chicken and is used very often for drug application, vaccination and the application of feed additives. Water can contain high numbers of microbes and these are able to contribute to and further develop biofilms in water supply systems. Cleaning and disinfection of water supply systems must be proven and adapted to ensure the application of drinking water in hygienic quality.

MATERIALS AND METHODS

The authors used different methods to analyse water and water supply systems in poultry husbandry, such as the total bacteria count at 22°C and 36°C, *Enterobacteriacea* count and *E.* coli count. At least 100 ml water was taken from different parts of drinking water supply systems in poultry husbandry. Depending on the size of the husbandry 4-10 water samples were taken. Fast (less than 24 hours) and cooled (4°C-8°C) transport of the samples to the lab was ensured.

Water samples were analysed after log10 -dilution in physiological NaCl peptone water with the spread plate method on CASO agar. 0.1 ml of the dilution was used for analyses. Additionally the water samples were analysed without dilution by using 0.1 ml and 1 ml for direct plating. The plates were incubated for 48h at 22°C and 36°C. Considering the dilution and the size of the analysed volume, the amount of total bacteria was calculated. In parallel 0.1 ml of the diluted water sample was plated on Chromocult coliform agar for analysing the amount of *Enterobacteriacea* and *E.* coli. The CCA plates were incubated for 24 h at 36°C.

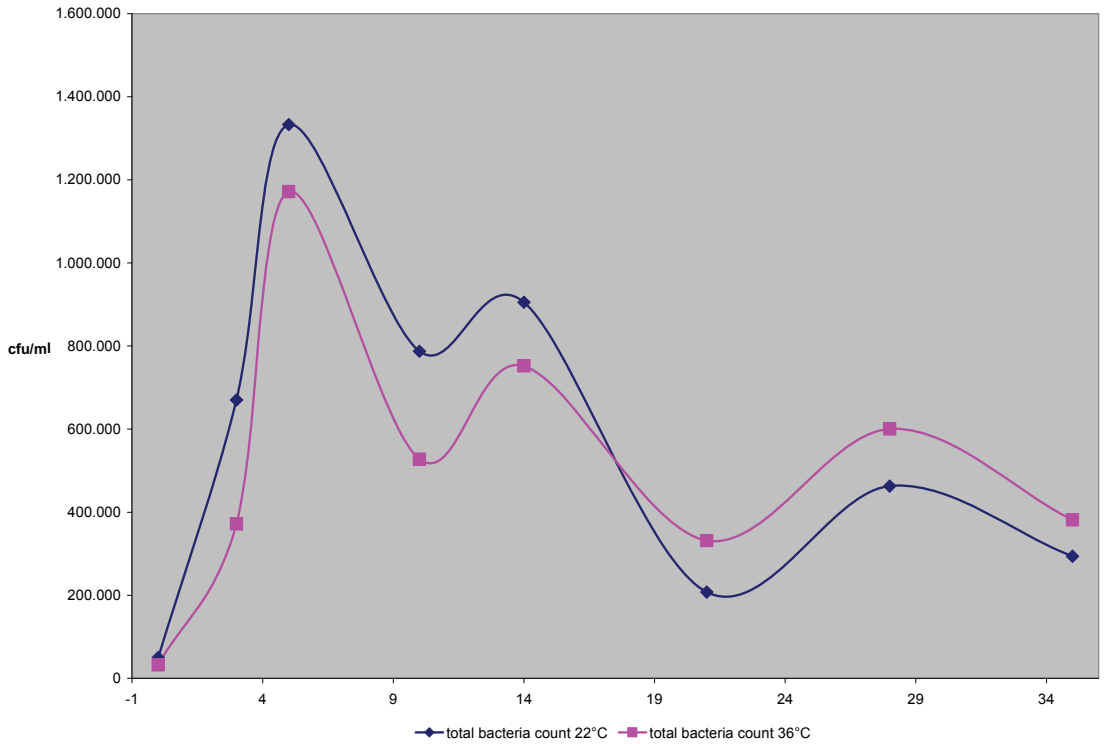
Additionally, swabs were taken from internal surfaces of water supply systems and analysed for the same parameters. The internal surface was wiped off with AMIES swabs on one circle. The swabs were transported fast and cool in the transport medium of the AMIES system. The swabs were shaken in 5 ml physiological NaCl peptone water directly after arrival in the lab. After that the NaCl peptone water was log 10 diluted. 0,1 ml of the adequate dilution was plated out on CASO agar or on CCA agar. The incubation and calculation was carried out the same way as for the water samples.

E. coli strains were analysed additionally for their antibiotic resistance profile by using the AVIPro plate. This method is based on measuring the OD value after incubation of the strains to be tested with the antibiotics to be tested in a 96-well format.

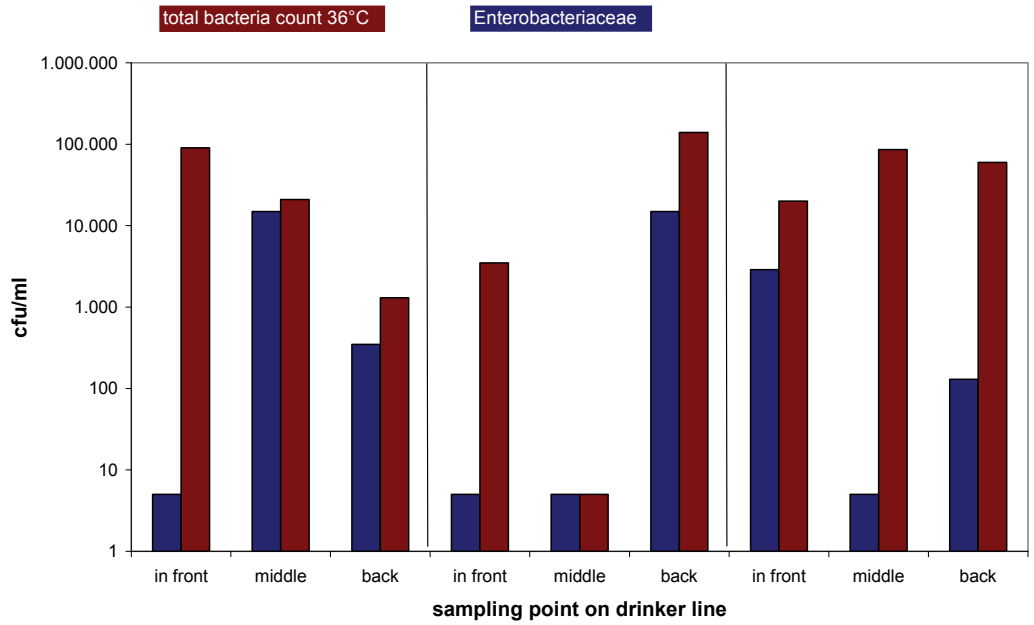
RESULTS

The results show that the sampling time (picture 1) and the sampling place (picture 2) have an impact on the microbiological results and may even differ extremely within the same holding. The amount of total bacteria in water ranges from 10^1 to 10^7cfu/ml or for *E. coli* from 10^1 to 10^3 cfu/ml. Even the swab results in table 1 show the range of microorganisms in water and water supply systems between 10^1 to 10^3cfu/ml or 10^1 to 10^5cfu/cm². It

is shown that the cleaning process is one of the most important weak points in the hygienic treatment of water for animal husbandry (table 2) and *E. coli* present in water is often resistant to antibiotics (table 3).



Picture 1: development of bacteria in broiler drinking water over the production days



Picture 2: amount of microorganism in drinking water (3 different barns)

Table 1: microbiological results of swabs and water samples in poultry husbandry

farm	Sampling point	Swab (cfu/cm²)	Water (cfu/ml)
X	Drinker line 1	<50	<10
	Drinker line 2	<50	<10
	Drinker line 3	4000	<10
	Drinker line 4	2000	<10
Y	Drinker line 1	1250	<10
	Drinker line 2	180000	5400
	Drinker line 7	750000	<10
	Drinker line 8	175000	<10

Table 2: amount of microorganism in water samples after cleaning and disinfection

Sampling point	After standard cleaning and disinfection		After improved cleaning and disinfection	
	Total bacteria count 22°C	Total bacteria count 36°C	Total bacteria count 22°C	Total bacteria count 36°C
End of drinker line house 1	1000	1000	<10	<10
Water from nippel house 1	2000000	2000000	240000	190000
End of drinker line, house 2	2000000	1200000	<10	<10
Water from nippel house 2	1000	1000	<10	<10

Table 3: resistance analyses of E.coli present in drinking water of poultry

	strain A	Strain B	Strain C	Strain D
n	8	8	10	9
N	19	19	19	19
%	42,11	42,11	52,63	47,37

DISCUSSION

The reason for our investigation was the holistic approach on water hygiene. Within a framework on the legal evaluation of water as feedstuff in Germany there are recommendations for microbiological quality of water. We could show that on different parts of the water line we found different amounts of bacteria. Other authors detected high amounts of bacteria in water as well without any detail explanation on the sampling point. To ensure that animals get always water of high quality it is necessary to define the time of sampling, the point of sampling, the number and frequency of sampling and the method of sampling. Depending on these parameters, we could show that the result and the conclusion based on these results could be different resulting in different effects for the animals. The analyses of strains present in water show the importance of more detail investigations on this topic.

The different methods of analyses explain the importance of multivariable analyses of drinking water systems and the risk for animal health and performance. There are different steps which have to be considered in ensuring a high water quality for the animals. One of the most important facts is to clean and disinfect the water supply system effectively.

CONCLUSION

It is necessary to analyse water and the water supply system in order to estimate the health risk for poultry, to evaluate the effectiveness of disinfection processes of water systems and to judge the hygienic composition of drinking water. Only with all these analysis methods a risk assessment is possible.

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from the author on request

INFRARED THERMOGRAPHY AS AN ALTERNATIVE MEASUREMENT OF THERMAL COMFORT IN DAIRY HEIFERS

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ABSTRACT

There is a demand on animal welfare research for indicators of heat stress non-invasive, that could be good predictors of heat stress condition. The impact of thermal load on animal body can be determined by observing the distribution of surface temperature using infrared thermography as an alternative measurement to investigate the environment and physiological processes associated with thermal comfort. This study was conducted to determine the relationship among temperatures measured at different anatomical sites of the animal body and physiological and environment parameters of dairy heifers kept in confinement. For twenty-four days the dry bulb temperature (DBT), black globe-humidity index (BGHI), rectal temperature (RT), respiratory frequency (RF), surface temperature (ST) of rump, barrel, flank and foot were collected from 16 dairy heifers crossbreds at 0700, 1000, 1300, 1600 e 1900 hours. Environment parameters (DBT and BGHI) showed high and positive correlations with all body site temperatures. Correlations among RT and rump (r=0.53), barrel (r=0.57) and flank (r=0.54) were moderate, however were observed high correlation with foot (r=0.72). Correlations among rump, barrel, flank and foot were 0.68, 0.70, 0.66 e 0.81, respectively. The Pearson's correlations demonstrated that all body site temperatures were positively associated with RT and RF, with highlight for foot, indicating that increases in these temperatures are linked to the increase of RT and RF. The foot was higher and positive correlation than others anatomical sites, can be used as a good indicator of thermal comfort.

INTRODUCTION

The outer shell of cattle, consisting of the coat is very important for heat exchange between the organism and the environment. Differences in metabolic activities of tissues causes the surface temperature (ST) is not homogeneous, and present variations according to the anatomical regions [10]. The pattern of variation of ST in different anatomical regions and its thermoregulatory significance in cattle was studied by [6]. The use of infrared thermography allows one to know the thermal profile of the animal and may be useful in evaluating the thermal conditions of housing environment, as well as being an easily accessible, without need to tie up the animal. Linked to the RF, RT and thermal comfort indices ST is being used as an indicator of thermal stress [8]. This study aimed to determine the relationship between the surface temperature of different anatomical regions, with the environmental variables involved in the authoring environment and physiological traits of dairy heifers.

MATERIAL AND METHODS

We used 16 crossbred Holstein x Mantiqueira featuring predominantly black coat with a mean age of 240 days and average weight of 115 kg during the month of December 2009 to March 2010. The animals were randomly divided into four covered pens (4.0 x 7.9 m) with ad libitum access to diet (hay of Cynodon dactylon cv coastcross and concentrate), water and minerals. The experiment consisted of eight periods of 12 days. Data collection occurred interchangeably in three days (6th, 8th and 10th days) within each period, totaling 24 days of collection. The collections of physiological variables occurred at 7h00min, 10:00, 13:00, 16:00 and 19:00. The respiratory frequency (RF) was obtained by counting the flank movements and rectal temperature (RT) was measured with the aid of a digital thermometer, inserting it approximately 5 cm into the rectum. The ST of each animal was obtained by thermographic camera (Fluke ® Ti 25) with automatic calibration. The sites of ST were the rump, flank, barrel and foot, all performed on the right side of the animal [9]. Each thermogram generated was analyzed by the software SmartView ®, where average temperatures were obtained from each anatomical region, considering the emissivity of 0.95. The dry bulb temperature, relative humidity, black globe temperature were recorded at intervals of 15 minutes, with the aid of dataloggers (Lufft ® Opus 10 and Hobo ® U12) installed in the center of each bay to 1.8 m high floor [1].The association of different anatomical regions was evaluated by Pearson correlation using the statistical software Minitab ® v. 1.5.

RESULTS

The barrel had the highest average of ST, with the lowest mean on the rump (Table 1). The largest increases of ST throughout the day were on the rump (9 ° C) and foot (8.28°C), while the flank and barrel had 7.6 ° C and 6.73 ° C, respectively.

Table 1 – Descriptive statistics of infrared measurements (°C), physiology and environment data.

Traits	Rump	Barrel	Flank	Foot	RT	RF	DBT	BGHI
Mean	34.16	35.26	34.88	34.73	39.05	60.66	26.73	76.48
Standard deviation	2.07	1.56	1.78	1.68	0.33	14.42	3.39	3.54
Minimum	29.45	31.67	31.27	29.37	38.22	34.5	20.10	69.70
Maximum	38.45	38.40	38.87	37.65	39.87	107.25	33.58	83.00

RT = Rectal temperature (°C); RF = respiratory frequency (rpm); DBT dry bulb temperature (°C); BGHI= Black globe-humidity index.

The DBT ranged between 20.1 ° C and 33.6 ° C, observing a largest increase in RF from 28 ° C, while the response of the environmental conditions of RT were observed from 31 ° C. The BGHI values varied throughout the day between 69.7 to 83, respectively to 7h00min and 13h:00min. Moderate to high positive correlations (0.53 - 0.86) were found between the anatomical regions and the environmental and physiological variables (Table 2). Environmental variables (DBT and BGHI) had high and positive correlations with the anatomical regions. Similar correlations were observed in the rump, flank, barrel and foot with respect to DBT.Significant correlation coefficients were found in relation to BGHI. This is because of consider BGHI beyond the DBT, the effects of radiation, convection and relative humidity.

Table 2 – Pearson correlation coefficients between measures of body temperature in different anatomical regions (P<0.0001).

Body sites	Variables[a]			
	RT	RF	DBT	BGHI
Rump	0.53	0.68	0.79	0.84
Barrel	0.57	0.70	0.82	0.85
Flank	0.54	0.66	0.80	0.84
Foot	0.72	0.81	0.86	0.84

[a]RT = Rectal temperature; RF = respiratory frequency; DBT dry bulb temperature; BGHI= Black globe-humidity index.

Moderate correlations were obtained between RT and rump, barrel and flank, and high correlation with foot (r = 0.72). The same pattern was observed for the RF, but the coefficients obtained were higher in all regions compared to RT, highlighting the foot, which made the most significant correlation coefficient (r = 0.81). The Pearson correlation analysis showed that all regions were positively associated with RT and RF, especially the region of the foot, indicating that increased TS is linked to increased RT and RF.

DISCUSSION

Under the conditions of this study, it was found that the values of BGHI that the animals were submitted throughout the day between 69.7 and 83, show the alert condition (between 75 to 78) and at times the dangerous condition (between 79 to 84) [2]. The BGHI considers DBT the also effects of radiation, convection and relative humidity. These results demonstrate the influence of the environment around the animal, directly interfering in the absorption of heat by animals. We found moderate to high correlations between the anatomical regions and the environmental and physiological variables. Positive correlation between RF and ST (r = 0.73), were also reported in literature [4]. The high correlation coefficient as the ST feels good indicator of the microenvironment around the animal. Different anatomical regions have different relevance in the dissipation of heat [3]. Peripheral regions of cattle are very important in heat loss, because their greater surface / volume ratio, lose proportionately more heat due to increased blood flow and the large number of venous branches [10]. The effect of environmental indices in the peripheral (leg and foot) was higher than the more protected areas such as udder [11]. These evidences are involved in the mechanism of thermoregulation, as the thermal conditions increase, at

first activate the thermoregulatory system by the mechanism of vasodilation, increasing blood flow to tissue. If the temperatures continue to rise the animal activates the mechanisms of evaporative heat exchange, increasing its rate of sweating and RF in order to maintain body temperature [5]. The choice of an anatomical region as an indicator of physiological condition and environmental variables showed the possibility of electing the ends of the animal as it is closest to an environmental and physiological indicator in dairy heifers. Using the same anatomical region in another animal category, as milking cows can result in different results from those presented here, as have the animals in metabolic heat production of lactation as aggravation of the mechanisms of heat loss [7].

CONCLUSIONS

The evaluation by thermographic camera showed that this region of the foot was the most appropriate measure to predict the thermal comfort condition of dairy heifers.

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LABELLING OF VIDEO IMAGES: THE FIRST STEP TO DEVELOP AN AUTOMATIC MONITORING TOOL OF PIG AGGRESSION

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SUMMARY

Aggressive behaviour in pigs is a serious issue in pig farming since it poses enormous welfare and economic problems in livestock management. Mixing animals usually results in aggression among the group members because the animals have to establish a social hierarchy. At times, this aggressive behaviour can become so intense that animals seriously injure themselves or their pen mates. To immediately identify aggression is therefore extremely important for the farmer in order to limit the economical losses and to increase the welfare of the animals. By using video analysis, it is possible to develop an automatic monitoring technology that is able to detect aggression in pigs. Applying a non-invasive technology such as cameras is a very powerful approach since it does not intrude into the animals' hierarchy and is at the same time highly cost efficient. To develop such a product, it is first required that ethologists observe video images and label the behaviour of the animals in order to define the Golden Standard. This step is very crucial, but time-consuming since it demands hours and hours of observation. This paper describes a tool for helping experts in the observation and manual labelling of specific behaviours, for example aggression.

INTRODUCTION

The welfare of an individual is its state as regards to its attempts to cope with its environment [3]. During their life animals adjust to their environment and cope with difficulties by using physiological and behavioural methods. Therefore, pigs' comportments have been used as an indicator of their welfare. Aggressive behaviour, that is one expression of social behaviour amongst others, increases when the welfare of the animals is poor.

Aggressive behaviour in pigs is not only a serious welfare issue; it also poses enormous economic problems in livestock farming management. Many studies have tried to reduce the levels of aggression in pigs by using sedatives and masking odours [7, 10], by enriching the environment [1], by applying pre-exposure techniques [5] or by giving high tryptophan diet [9]. However, no suitable method to prevent augmented agonistic behaviour has been found up to now. A different approach consists in using the aid of modern technology in order to develop online tools that can monitor the behaviours of animals in a fully automatic way without causing stress for the animals. Information science and technology have rapidly given an important impact on the methods used in livestock production.

Precision Livestock Farming (PLF) is a particular way of livestock farming that is based on measuring animal-based variables, modelling these data to select information, and then using these models in real time for monitoring and control purpose [2].

Image analysis is one of the tools used in PLF for monitoring with two major advantages. First, it is relatively inexpensive nowadays since it requires only some cameras and computers. Second and importantly, it does not interfere with the animals' environment and therefore does not changes the animal's behaviour.

In order to develop an automatic model of pigs' behaviour it is first necessary to define a Golden Standard (GS). The GS is a theoretical test or procedure that is absolutely valid and reliable [4] and that is necessary to develop and validate an automatic monitor tool. The automatic labelling can be defined as the process of assigning equivalences between the output data of an automatic monitor and the GS. A mathematical predictive model could not be more accurate and precise than its Golden Standard, thus the election of a suitable method is one of the crucial points in developing a PLF product. In the specific case of an automatic monitor of pig aggression, a valid and reliable Golden Standard is the observation by human ethologists. No image processing algorithms and mathematical model can outperform the ability of the human eye and the knowledge of the experts.

In order to identify manually in recorded videos the behaviours of animals it is necessary to develop a labelling tool that can guide the user to this really time-consuming task.

MATERIAL AND METHODS

The labelling tool was developed in MATLAB© 2010b. The output of the program can be used for statistical analysis and for developing a behavioural model - for example, a model able to detect pigs' aggression. The User Interface was designed as simple as possible, with an immediate usage for the end user. The tool is also highly configurable and customisable in order to be reused for different experiments. A configuration file stores all the parameters of the program, such as the list of behaviours to be labelled.

The tool allows defining zones of interest inside a pen. This is particularly useful for studying the occurrence of behaviours in a particular area. For each region the activity and occupational index are measured from the video in order to speed up the manual labelling process. The normalised *activity index* is a measurement that quantifies the activity of animals in practical field condition such a barn [6]. The idea behind is that the change in intensity of the pixels in consecutive frames provides a good estimation of the activity of the animals. This information can be used for skipping part of the videos. In fact, if the activity is close to zero, the animals are not moving in the particular zone and therefore the experts can leave out these intervals. Since pigs spend most of their time in complete inactivity, the time needed for labelling is drastically reduced. In addition, the activity index is a good estimator to identify aggression because it is often associated with an increase in the activity level in pigs [8].

The *occupational index* is a measurement that calculates the fraction of the area occupied by the animals [6]. In the images, the pixels representing the pigs have a different intensity than the ones of the background. By applying a threshold value to the intensity, it is calculated whether each pixel is considered foreground (pigs) or background. The occupational index can also support the manual labelling process because an index value of zero means that no pigs are present in the zone and that it is therefore not necessary to look at these parts of the recordings.

RESULTS

The resulting labelling tool consists of two modules. The first one (Figure 1) is necessary to configure and to initialise the videos. In this phase the zones of interest are defined (rectangles in figure 1) and the relation between square centimetres and pixels is calibrated. The linear factor that measures the distance in the video pixels is calculated by knowing the dimension of a specific object or the dimension of the pen itself.

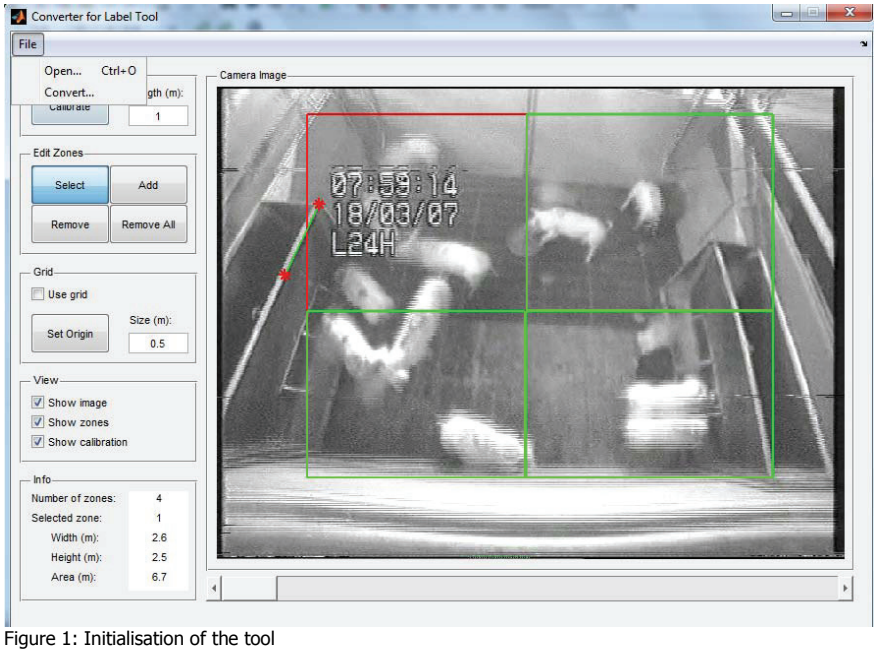

Figure 1: Initialisation of the tool

In the initialization step, all the behaviours to identify are defined. For each entry a specific button is created in the second module (Figure 2).

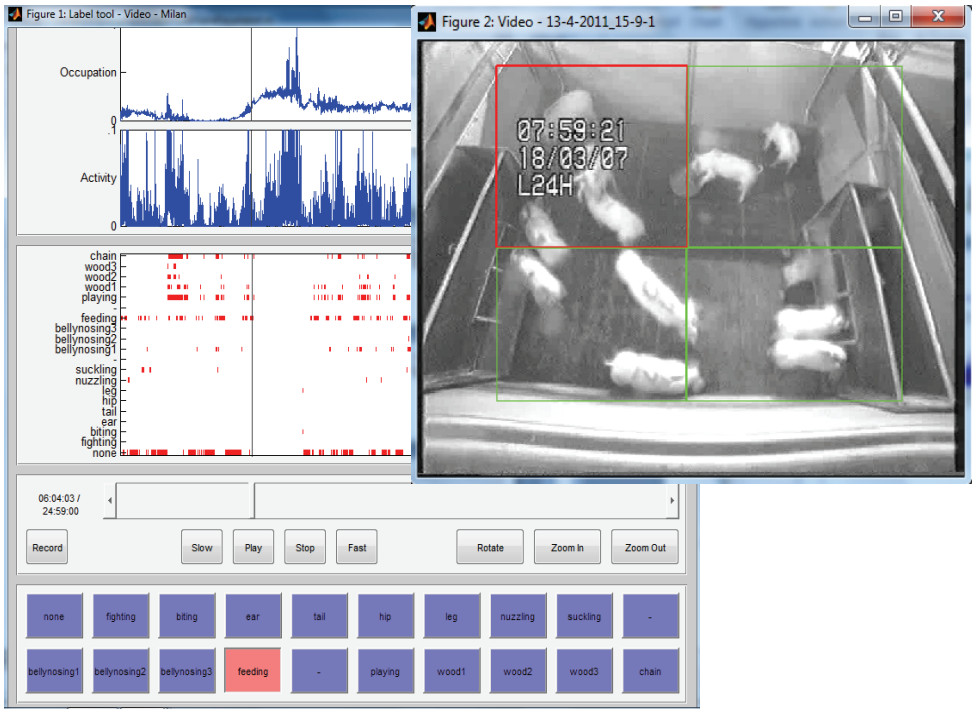

Figure 2: Labelling tool

The labelling tool allows to play the video or to slide the images frame by frame. The speed of the video can also be adjusted individually. When a specific behaviour is observed in the image, the matching button is selected and its colour turns from blue to red. At the same time, the panel containing the list of behaviours becomes red. It is, of course, possible to press multiple buttons in case different behaviours occur at the same time. If the same behaviours take place in consecutive images, the start and the end of these comportments can be registered by pressing the "record" button.

DISCUSSION

The manual labelling is a fundamental step in order to gather data for analysis and model development. Since the observation of the animal is the most crucial part in the biological process, many hours of observation are needed to get an accurate result.

The experts can observe and label the animals' behaviour in the barn house or remotely by video camera. An advantage of being in the barn is that some behaviour is only recognisable by being present on site. For example it is difficult from the video to distinguish between tail suckling and tail biting. However, to use video records for labelling has different advantages. First of all, humans do not interfere in the behaviour of the pigs by modifying their environment. Second, the videos can be played an infinitely number of times and may reveal particulars that cannot be discovered in real time. Some pre-processing of the data can also be made in order to speed up the manual labelling process.

Since the aim is to develop an automatic tool that is able to recognise automatically the behaviours of the pigs by using cameras, video labelling is necessary and it can also be important in order to force the observer to focus only on the variables available in the images. In order to fasten the manual process of labelling a tool is convenient and can save a lot of time in contrast to using a normal spreadsheet, for example.

CONCLUSIONS

This paper describes a tool to label the pigs' behaviour. Since the animals' observation is the first step to develop an automatic monitoring model of pig aggression, it requires configurable, fast and easy software in order to facilitate and to speed up the process as much as possible. The tool described allows defining different zones in order to understand better where specific behaviours take place. It also includes the activity and occupational index in order to skip the parts of the video where the animals are not active or not present in a specific zone. The monitoring of the activity level and occupation index of a pig group can also be used as a first step to recognise patterns of aggressive behaviours and therefore to have an indicator of animal welfare.

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EVALUATION OF AN IMMUNOLOGICAL RAPID-TEST FOR CAMPYLOBACTER DIAGNOSIS IN CHICKEN FAECES

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SUMMARY

Human Campylobacteriosis shows the highest incidence of notifiable bacterial gastro-intestinal diseases in developed countries. Broilers excreting *Campylobacter spp*. in high concentrations (> 7 $\log_{10}$ CFU/g of faeces) have been described as the most important source of carcass contamination by the Dutch "Campylobacter Risk Management and Assessment" (CARMA) project study. During the" Base-line study *Campylobacter* for broilers" launched by the European Commission 2008 pooled faecal samples from 187 broiler flocks (April - November 2008) were analysed with a rapid test (Lateral-flow-test), the standard quantitative cultural method ISO 7218:2007 and ISO 272-2 (2006) and a modified quantitative real time-PCR (La Gier et al., 2004). Out of these 102 samples delivered positive results with both the lateral-flow test and the reference method, another 69 flocks were tested negatively with both methods. The test yielded 4 false positive and 12 false negative result (11 below the detection limit) so sensitivity was 89.5% with a confidence interval of 95% [82.5%; 93.8%] and specificity was 94.5% with a confidence interval of [86.7%; 97.8%].

Results obtained during the period of investigation indicated a *Campylobacter spp.* prevalence from 61% (quantitative culture) to 71.7% (real time-PCR) in Austria.

INTRODUCTION

Human campylobacteriosis is the leading cause of acute food-borne bacterial gastroenteritidis in industrial nations since the year 2005 (1). Primary sources of human infections are poultry and poultry products. Broilers excreting Campylobacter spp. in high concentrations (> 7 log 10 CFU/g of faeces) have been described as the most important source of carcass contamination by the Dutch "Campylobacter Risk Management and Assessment" (CARMA) project study (3). One potential way of minimising risk of infection for humans is therefore eliminating high shedder flocks from the production chain of fresh broiler meat. Wadl et al. (4) developed a rapid test for the identification of high shedder flocks, producing results within two hours. This test enables experienced people to examine flocks even directly before slaughtering. In this study, the lateral-flow test was evaluated for practicability by using it within the frame of the "Base-line study *Campylobacter* for broilers" commissioned by the 2007/516/EG committee.

MATERIAL AND METHODS

The so called Lateral-flow-test or rapid test (Gold Labeled Immuno Sorbant Assay - GLISA) is a special form of immune electrophoresis whereas detection of antigens is achieved via gold-labelled, monoclonal antibodies. The assembly of the test parts was carried out manually, as described by Wadl (21).

As reference method quantitative cultural identification was performed true to ISO 7218:2007 and ISO/TS 10 272-2 (2006).

A modified quantitative real-time PCR (2) was used as additional detection and quantificationmethod to confirm the results of the rapid test and the reference method.

RESULTS

From April until November 2008, 187 Austrian chicken flocks were analysed. Out of these, 102 flocks (54.54%) showed positive results when examined with the lateral-flow test as wells as the cultural ISO reference method. Another 69 flocks (36.89%) were tested negative both with the Lateral-flow test and the reference method. Only 53 flocks (28.3%) had a negative result in real-time PCR, the other 16 flocks (8.56%) showed low concentrations from 5.31 to 6.55 log10 CFU per g of faeces. The lateral-flow test achieved 4 false positive and 12 false negative results (11 below the detection limit of the rapid test). As the rapid test detected 102 of 114 positive samples and 69 of 73 negative samples, its sensitivity was 89.5% with a confidence interval of 95% [82.5%; 93.8%]. Specificity

was at 94.5% with a confidence interval of [86.7%; 97.8%].

Results obtained during the period of investigation indicated a *Campylobacter spp.* prevalence from 61% (quantitative culture) to 71.7% (real time-PCR) in Austria.

DISCUSSION

Of 102 positive flocks examined with the lateral-flow test, 88 (86.27%) exceeded the detection limit of ≥ 7.3 log10 CFU/g of faeces, as described by Wadl (4). The remaining 14 flocks (13.73%) showed low concentrations. Therefore, nearly all flocks tested positively were high-shedder flocks.

The flock prevalence of 61% that had been detected by culture from April to November 2008 is consistent with recently published data of 64.5% (2004) and 61.4% (2005) in Austria (1).

Due to the results of real time-PCR the proportion of *C.jejuni* positive flocks equalled 94.8%, the proportion of *C.coli* positive flocks 36.6%. Therefore, on basis of molecular data, 32.8% of positive flocks had been infected with both strains of *Campylobacter*.

CONCLUSIONS

Our results highlight the applicability of the lateral-flow test for fast identification of broiler flocks shedding *Campylobacter spp.* at high levels. Given that nearly all positively tested flocks were high shedders, reducing *Camplyobacter* prevalence by eliminating high shedder flocks from fresh meat production is doomed to failure as long as no adequate decontamination strategy for carcasses is developed.

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INVESTIGATIONS ON THE STRESS RESPONSE OF C. JEJUNI

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INTRODUCTION

Human campylobacteriosis is caused by thermotolerant *Campylobacter (C.) spp.* The infective dose of these bacteria is generally low. The species commonly associated with human infection are *C. jejuni* followed by *C. coli*, and *C. lari*, but other Campylobacter species are also known to cause gastroenteritis. Campylobacter is the most frequently reported bacterial pathogen of gastroenteritis infection in humans and thus, constitutes a major public health problem. In 2007, more than 190.000 cases of campylobacteriosis were confirmed in the European Union [1].

The bacteria can readily contaminate various foodstuffs, including meat, raw milk and dairy products, and less frequently fish and fishery products, mussels and fresh vegetables. Among sporadic human cases, contact with live poultry, consumption of poultry meat, drinking water from untreated water sources, and contact with pets and other animals have been identified as the major sources of infection. Raw milk and contaminated drinking water have been responsible for larger outbreaks [1].

Under environmental stress and unfavorable growth conditions that are potentially lethal, *C. jejuni* has been proposed to enter a viable but nonculturable (VBNC) state [2]. Bacteria in VBNC state no longer grow on conventional media, but remain intact and retain viability. The question of alive or dead is important because the answer is basis for decisions regarding safety of food and drinking water and the sterility of pharmaceuticals.

Therefore, aim of the study was to develop a method to induce VBNC and to identify genes that are involved in the introduction and the maintenance of VBNC.

MATERIAL AND METHODS

In the present study *Campylobacter jejuni* type strain NCTC 11168 was used. *C. jejuni* was routinely maintained on Columbia blood agar plates at 42°C under microaerophilic conditions. In order to induce the VBNC state, bacteria were grown microaerobically at 42°C in Bolton broth. The *campylobacter* broth was centrifuged (5 min, 4000 x g) at 4°C, and the pallet was resuspended in pre-chilled medium. Three different media were used in our study including artificial sea water (ASW) [3], phosphate buffered saline (PBS), and distilled water. *Campylobacter* containing media were kept at 4°C up to 5 weeks. RNA samples collected after 10, 30 and 60 minutes; 2, 4, 8 and 24 hours; 1, 2, 3, 4, and 5 weeks of exposure to the media. For RNA sampling the cells were centrifuged and the total RNA was purified using TriFast® (PeqLab, Germany) according to the manufacturer's instructions. The total RNA was resuspended in RNase free water, and remaining traces of genomic DNA were removed by treatment with DNase I (Fermentas, Germany). First strand synthesis was performed using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Germany). The quantitative RT-PCR was performed in a Mx3000P RT-PCR cycler (Agilent, CA, USA) using the ABsolute™ QPCR SYBR® Green ROX Mix (Thermo Scientific, Germany) according to the manufacturer's instructions. Expression levels of 14 target genes were analyzed in duplicate applying the $\Delta\Delta C_t$-method (housekeeping gene: *rpoA*).

The target genes are known to be part of the response of heat shock stress (*groEL*, *groES*, *grpE*, *htrA*, *racR*, *hspR*), oxidative stress (*sodB*, *ahpC*), starvation stress (*ppk1*) or are genes with unknown/putative function (*cj1168, cj1208, trmD, rim, wlaJ*).

RESULTS

Depending on the media used in this study *C. jejuni* was exposed to different kinds of stress including cold stress, oxidative stress (all media), starvation stress (PBS, distilled water) and osmotic stress (distilled water).

The investigation of the RNA samples revealed that the expression level of the target genes was almost constant, irrespective of the time points and the media. The expression level of each target gene (all media and time points) was not exceeding a 2 fold increase or decrease, respectively, compared with RNA samples derived from non stressed bacteria.

DISCUSSION

Our finding that the target genes involved in heat stress response were not significantly up or down regulated by means of the experimental design could be expected. Interestingly, genes that are part of the specific response to oxidative stress (*sodB*, *ahpC*) and starvation stress (*ppk1*) are also more or less constantly expressed. Also, *cj1168, cj1208, trmD, rim, wlaJ* lack changes of the expression level. Our results contradict previous investigations which identified these genes as being part of the specific stress response. Especially, the results for *cj1168, cj1208, trmD, rim, wlaJ* were in contrast to a study performed by Stintzi et al. dealing with cold shock response of *C. jejuni* [4].

Further investigations are needed, especially the inclusion of more target genes and the optimization of the experimental design.

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CAMPYLOBACTER AND *ARCOBACTER* SPP. IN DAIRY CATTLE FARMS IN GALICIA (SPAIN)

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SUMMARY

Bacteria from genera *Campylobacter* and *Arcobacter* are potentially zoonotic pathogens for human beings and they may be detected in cattle farms. In this study the presence of these bacteria was considered in dairy cattle farms in Galicia (northwest of Spain). In total, 254 faecal samples were gathered on 89 dairy farms. *Campylobacter* spp. was found with a herd prevalence of 36% and it was detected in 20.5% of faecal samples. *Arcobacter* spp. was isolated in 68.5% of farms and in 41.7% of faecal samples, with *A. cryaerophilus* being the most frequently identified species. The results showed in this study prove their presence in high number in different dairy farms, and suggests that more epidemiological studies regarding this bacteria need to be performed.

INTRODUCTION

Thermotolerant *Campylobacter* spp. are widespread in nature. The main reservoirs are the digestive tract of wild and domestic animals such as poultry, cattle, pigs, dogs and wild birds. For this reason, this bacterium can easily contaminate food, including dairy products. Campylobacteriosis was the most frequent reported gastrointestinal zoonotic disease in humans in the European Union in 2009 with 198,252 confirmed human cases, being raw milk one of the food vehicles causing large outbreaks. *Campylobacter* spp. was detected in 0-5.2% of cow's milk samples but with data from four member states (1). Notwithstanding, *Campylobacter* spp. presence in raw milk is commonly consequence of secondary faecal contamination during milking process.

Arcobacters differ from campylobacters by their ability to grow at lower temperatures an under aerobic conditions. Currently, the genus includes 12 species, and seven may be considered emerging food-borne pathogens and a serious hazard to human health. However, it significance in human infections may be underestimated as routinely clinical samples are not tested for *Arcobacter* spp. as it is done for *Campylobacter* spp. Consumption of contaminated food or water is considered the main route of transmission. Some studies have reported occurrence of *Arcobacter* ranging from 3.18% to 46% in milk samples and 3.6 to 41% from faeces and rectal swabs of cows without symptoms (2).

The environmental presence of campylobacters and arcobacters in cattle manure may be a significant factor in the transmission of infections to human through contaminated milk when milking practices are not hygienic. The objective of the present study was to investigate the role of Galician dairy clinically healthy cattle as a potential faecal reservoir for *Campylobacter* and *Arcobacter* species.

MATERIALS AND METHODS

The study was performed in 89 dairy farms from Galicia (northwest of Spain), a region accounting for 40% of Spain´s total milk production. The mean number of lactating cows per herd was 40.4 (13-179).

Faecal samples were obtained directly from the recta of 254 lactating cows representing approximately the 10% of the total of milking cows of each farm. Animals did not show clinical symptoms compatible with *Campylobacter* spp. nor *Arcobacter* spp. Samples were transported to the laboratory under refrigeration and were processed individually within 24 hours.

Bacteria isolations were carried out using several enrichment broths and selective agar plates. *Campylobacter* species were identified first by biochemical and morphological characteristics and subsequently they were confirmed by PCR technique, and *Arcobacter* species identification was performed by multiplex PCR (3).

RESULTS

Farms were considered positive when at least one animal that proved positive for *Campylobacter* or *Arcobacter* spp. was detected.

In total, 36.0% (32/89) of the farms were *Campylobacter* positive, and the 20.5% of clinically healthy cows presented this bacterium in their faeces. *Arcobacter* spp. was isolated in 61 (68.5%) farms and in 41.7% of individual faecal samples, being *A. cryaerophilus* the most abundant species. Table 1 shows the percentage of *Arcobacter* species distribution in animals. Animals co-infected by more than one *Arcobacter* spp. were identified in 15.3% of the sampled lactating cows within 30 farms, and represented the 36.8% of all positive samples.

Table 1. *Arcobacter* spp. presence in feces samples from Galician dairy cattle farms.

	No. samples	No. positives (%)	A. cryaerophilus (%)	A. skirrowii (%)	A. butzleri (%)	No identified (%)
Feces	254	106 (41.7)	71 (27.9)	51 (20.1)	19 (7.5)	7 (2.8)

DISCUSSION

The percentage of farms and individual faecal samples positives to *Campylobacter* spp. described in this study was within the range of prevalence rates reported by other authors involving faecal samples of dairy cattle. However, it is demonstrated that prevalence of *Campylobacter* spp. in cattle varies from very low to very high, depending on several factors such as geographical locations, seasonality, different diagnostic procedures, etc.

Comparing prevalence data from several studies, we could observe that there are significant differences in prevalence of *Arcobacter* spp. in cattle maybe due to farming practices, herd size, feeding, etc. but also to design, sample size, sampling and isolation methods; and even geographical and seasonal variations could have affected the occurrence of this bacterium. Besides, the absence of an optimal isolation method is one of the major problems to estimate the accurate prevalence (2). In fact, some studies have reported occurrence of *Arcobacter* spp. ranging from 3.6% to 39.2% clinically healthy cattle, values lower than the ones presented by us both to cattle level and to individual samples. By contrast, many studies have observed *A. butzleri* as the most commonly isolated, followed by *A. cryaerophilus*, while our study identified *A. cryaerophilus* as the most frequent.

CONCLUSIONS

This study is the first description of the presence of *Campylobacter* and *Acrobat* spp. in dairy cattle farms in Spain. More studies not only in Spain but in other European countries to determine prevalence of and risk factors associated with their presence in cattle herds are needed. This is of great significance, given the important role of these bacteria as potential zoonotic pathogens, through consumption of contaminated milk. For this reason, applying good hygiene procedures during milking it is essential to produce milk with absence of intestinal pathogens.

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TOWARDS OPTIMAL DETECTION OF CAMPYLOBACTER SPP. IN POULTRY MEAT AND WATER SAMPLES

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SUMMARY

Naturally and artificially contaminated samples of fresh and frozen chicken meat and marinated meat products (N=48) and drinking and environmental surface water (N=35) samples were analyzed using conventional method (ISO 10272-1 and 17995) and real-time PCR. Bolton, Preston, blood free Bolton and Bolton base broth with changed supplements (CAT) were compared for enrichment, using the enrichment ratio of 1:4 and 1:9. The results showed that the ratio of 1:4 is as effective as the ratio of 1:9, which is important for cost rationalization of materials and labor. However, different optimal enrichments were confirmed for each type of the samples. Regardless to the matrix effect, Preston broth gave optimal results in culture detection, but Bolton+CAT enrichment resulted in optimal real-time PCR detection. Detection limit for spiked samples of chicken meat and drinking water samples was in all combinations 1 CFU/ml, but most reliably with PCR detection after enrichment in blood free Bolton medium. In general, the blood in the medium did not inhibit PCR amplification neither promote *Campylobacter* growth.

INTRODUCTION

Thermotolerant bacteria of the genus *Campylobacter* are the leading agents of human intestinal infections in the developed world. They are relatively slow-growing microorganisms with inert biochemical characteristics, resulting in long and difficult conventional identification. In addition, the cells are normally present in very small numbers, and may be injured in food and water samples, consequently transforming in the viable-but-non-culturable state. The standard enrichment methods use *Campylobacter*-specific broths supplemented with blood or other protective compounds under microaerophilic conditions. In addition, the methods based on the detection of nucleic acids have become an important alternative for detection of *Campylobacter* (6).

The aim of this study was to determine detection limit in artificialy contaminated meat and water samples for detection of thermotolerant *Campylobacter* by ISO and real-time PCR, to confirm it in natural samples after the optimal enrichment for different types of meat and water samples and to examine the efficiency of 1:4 (25 g sample : 100 ml of enrichment broth) versus 1:9 enrichment ratio (ISO 10272-1).

MATERIAL AND METHODS

Samples

Naturally contaminated samples were collected during official sampling in the frame of the state monitoring of surface water quality and monitoring of zoonosis in Slovenia for 2009. The spiked samples of chicken meat and drinking water samples were prepared by serial dilution in the range 10^0 -10^3 CFU/ml in three independent repetitions.

Cultivation methods

The analyses were based on a modified method of ISO 17995 for water and ISO 10272-1 for meat samples (1, 2), using four different enrichments: i) Bolton broth ii) Bolton broth with CAT (cephoperazon, amphotericin B, teicoplanin), iii) Blood free Bolton broth and iv) Preston broth. After enrichment, mCCDA medium was used for isolation of *Campylobacter* culture.

Real-time PCR

Detection from the enrichment broth was performed according to Josefsen et al. (3). Briefly, 1 ml of enriched sample after 48 h incubation was centrifuged at 10000 rpm for 10 min at room temperature. DNA was isolated with QIAamp Mini Kit considering the manufacturer instructions. The amplification of the 16S rRNA gene fragment was done with the primers 5' CTG CTT AAC ACA AGT TGA GTA GG 3' and 5' TTC CTT AGG TAC CGT CAG AA 3'. The PCR conditions used are described by Lund et al. (4) with a few modifications. The PCR amplification was performed in 20 µl containing 5 µl of the DNA and 15 µl of a PCR master mix, consisting of 4 µl Taqman reaction mixture, 0,8 µl of a 400 nM of each of the primers, 0,6 µl of a 300 nM probe, 0,3 µl of a 10mg/ml BSA solution and PCR water. Amplification was performed with 95 ºC for 10 min, 45 cycles of 95 ºC for 10 s, 58 ºC for 20 s, 72 ºC for 1 s and one cycle of 40 ºC for 30 s, with the last elongation of 5 min. The result was evaluated as positive when the signal was reached before 35 cycles, otherwise it was considered as negative.

RESULTS

Detection limit in artificially contaminated meat and water samples

We determined the detection limit for thermotolerant *Campylobacter* spp. in artificially contaminated samples of marinated fresh and frozen chicken meat samples and drinking water samples by conventional and real-time PCR methodology. It was in all combinations 1 CFU/ml, but most reliably with PCR detection after enrichment in blood free Bolton medium.

Optimal enrichment

The results clearly presented that each type of the samples had its optimal enrichment medium, but PCR detection was in all types more efficient as the conventional cultivation method (Tab.1).
Bolton + CAT has proven to be the most effective enrichment medium for the detection of *Campylobacter* spp. in surface water samples, but Preston broth gave the worst results, if ISO and PCR detections were considered. Thus, Preston is not a suitable choice for enrichment of water samples, probably due to too selective effect and/or consequent *Campylobacter* growth inhibition (Tab.1).
However, we found Preston as the optimal enrichment broth for poultry samples. With the classical method this enrichment was the most selective one, consequently we got and confirm the greatest number of *Campylobacter* isolates in this way. Although all enrichments followed by molecular detection method gave the same number of positive samples, the minimum average value of Ct was confirmed in Preston medium.

Table 1: The number of positive and negative results of *Campylobacter* detection in different types of meat and water samples according to the enrichment and detection method used.

TYPE OF SAMPLE	ENVIROMENTAL SURFACE WATER				FRESH CHICKEN MEAT				MARINATED CHICKEN MEAT				FROZEN CHICKEN MEAT			
ISO / PCR / ENRICHEMENT	+/+	-/+	+/-	-/-	+/+	-/+	+/-	-/-	+/+	-/+	+/-	-/-	+/+	-/+	+/-	-/-
BOLTON	4	9	0	18	4	5	0	1	6	1	0	3	5	1	0	4
BOLTON + CAT	4	11	0	16	0	9	0	1	4	2	0	4	2	6	0	2
BLOOD FREE BOLTON	1	7	0	23	4	9	0	1	7	0	0	3	5	1	0	4
PRESTON	3	4	0	24	6	3	0	1	4	1	0	5	6	1	0	3

The best medium for marinated meat products has proven to be Blood free Bolton broth, closely followed by Bolton broth. We assumed that blood did not affect the regeneration of *Campylobacter.* The medium Preston and Bolton + CAT were in this case too selective and they most likely inhibited the growth of *Campylobacter.*

DISCUSSION

Efficiency of enrichment depending on the type of sample and detection method

The medium Bolton + CAT has proven to be the most efficient after detection of *Campylobacter* by molecular method, but the least effective with the classical method. In the latter case, much more efficient was Bolton medium, closely followed by Preston and blood free Bolton. We must comply with the universality to evaluate the optimal enrichment, considering the results of both methods. On this basis we would determine the Bolton medium as the most selective enrichment.

The medium Bolton + CAT indicates potential for efficient enrichment, but it would be necessary to explore the most effective ratio of antibiotics, which would contribute to a higher percentage of *Campylobacter* isolation after growth on mCCDA agar plates.

The problem with *Campylobacter* detection by PCR in naturally contaminated samples could represent false-positive results because of the free DNA presence, dead cells or VBNC forms. However, in our study we confirmed the problem of false-negative samples in the classical method because with different enrichments we encountered varying number of positive samples.

Comparison of the enrichment ratio

The study conducted by Oyarzabal et al. (6) by the method of determining the most probable number of bacteria showed even better efficiency ratio of 1:4 for the detection with cultivation of bacteria. In order to confirm their results we tested the effectiveness of the both ISO standard methods and real-time PCR after the enrichment. We confirmed our prediction that it is better to use a smaller volume or lower enrichment ratio, as we have more opportunities to capture a larger amount of cells when the sample is transferred to the plate or to the microcentrifuge tube.

Effects of blood in enrichment broths

Analyzing the results, we concluded that the blood did not affect better regeneration of *Campylobacter*. The only exception is evident in surface waters where blood free Bolton medium presented the least effective results. But these results should be investigated and confirmed by a systematic contamination of surface water samples. It was also not observed that the blood would inhibit PCR reaction. Bolton and blood free Bolton enrichment broths gave very comparable values of Ct values in real-time PCR detection.

CONCLUSIONS

We concluded that the efficiency of detecting thermotolerant *Campylobacter* depended on the type of sample and enrichment method applied. The reason for this outcome is probably in different microbiota compositions of different types of food and water samples. Blood in the enrichment medium has not inhibited the PCR reaction neither promoted bacterial growth. Practical consideration of this work points out the fact, that smaller ratio of enrichment gave more positive results and lower Ct values in real-time PCR procedure. Respecting our findings, it would make sense to transfer this protocol to routine laboratories to economize current practice, since this approach would reduce material costs and work investments as well.

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STATISTICAL ANALYSIS OF RISK FACTORS FOR CAMPYLOBACTER COLONIZATION AT THE FARM LEVEL

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SUMMARY

The *Campylobacter* status of 53 broiler-flocks (Styria, 2010), determined by caecal samples at slaughter, was related to specific information of flocks and farm management obtained by audits of official veterinaries. This data set was investigated with two different approaches: Spearman's rank correlation coefficients and odds ratios were calculated for each of the 18 hygiene characteristics according to the *Campylobacter* status of the farm. Harvest crews visiting more than one farm a day increases the risk of positive *Campylobacter* batches (OR=15.8) significantly. As well poor pest security (OR=10.6), untidy premises (OR=10.6), more than one depopulation (OR=5.1), and moist environment of drinking facilities (OR=4.7), has been identified as significant risk factors.

Another approach intended to model the influence of the 18 hygiene characteristics on the *Campylobacter* status with ordinal logistic regression. As the 18 predictor variables were correlated among each other first a principal component analysis was performed to identify groups of related variables. Three bunches of variables have been identified: F1 = stable condition, ventilation system, pest security, chicken watering systems, feeding, hygiene sluice and litter storage; F2 = stable cloth, cleaning & disinfection equipment, collection system; F3 = number of stables, thinning frequency. Subsequently logistic regression was performed with the sums of the recognized variable bunches as predictors. The obtained model forms the base for predictive tool development (estimating the *Campylobacter* status on the observation of specific criteria).

INTRODUCTION

Campylobacter is an important zoonotic pathogen related to poultry - however no symptoms are caused in birds. Several risk factors are discussed contributing the entry of *Campylobacter* in broiler flocks. Due to the high diversity in structure, management and hygiene compliance of broiler farms international studies detect various risk factors [1], [5] and rank them differently (depending on the country, study design, etc.). Data for this investigation were provided by the official veterinary administration laboratory and the official veterinarian. In this study current statistics are applied to identify recent risk factors for *Campylobacter* colonization in Austrian broiler farms.

MATERIAL AND METHODS

At the official veterinary administration laboratory caecal samples were examined on *Campylobacter spp*. in 2010 qualitatively (May-November). As the sampling lasted more than one rearing period, categories for the *Campylobacter* status of the farms were established retrospectively (negative/positive or 1-5).

Simultaneously hygiene criteria were examined at the same farms. The data of these audits were investigated in two different ways:

1. The hygiene parameters are weighted as described by [8]. This dataset is used for calculating Spearman's rank correlation coefficients, conducting principle components analysis and performing ordinal logistic regression. The dependent, categorical variable is the faecal category and the above mentioned set of hygiene criteria serves as independent variables. Auxiliary correlation coefficients between the hygiene criteria are investigated extensively.
2. To estimate the specific contribution of single risk factors on *Campylobacter* colonization the odd ratio was measured. For this purpose the categorical distinction of hygiene criteria and faeces was modified to binary variables.

The data are analyzed with SPSS software, Version 19.0.0 (Copyright © 1989, 2010 SPSS Inc, an IBM Company, USA). Principal components analysis and logistic regression is performed with SAS/STAT software, Version [9.2] of the SAS System for Windows (Copyright © 2002-2008 SAS Institute Inc. Cary, NC, USA).

RESULTS

There is no single factor contributing to a *Campylobacter* free flock exclusively, which is in concordance with previous international and national studies ([1], [4], [6], [9]). Nevertheless, the Spearman's rank correlation coefficient calculated for each hygiene criterion in relation to the faecal category of the farm separately indicates significant values ($\alpha<0.01$) for stable environment (r_s = 0.69), hygiene slice (r_s = 0.61), pest security (r_s = 0.60), cleaning & disinfection of the stable (r_s = 0.58), structural and technical stable condition (r_s = 0.57), other animals at the farm (r_s = 0.55).

Another approach for testing single factors is the calculation of odds ratios. The odds ratio measures the strength of the relationship between two binary variables. One variable is represented by the *Campylobacter* status of a farm (negative/positive) and the second variable is each hygiene parameter. Therefore the raw data of 5 categories has been adjusted into two mutually exclusive characteristics. The results for significant values ($\alpha<0.05$) with a confidence interval not including 1 are presented in Table 1.

Table 1: Significant OR-values related to single risk factors

risk factor	OR	Confidence interval	significance
harvesting crew visits more than one farm per day	15,8	3,5-70,9	Fisher: 0
poor or missing pest security	10,6	2,5 - 46	P-value: 0,001
concrete as surface layer on the premises: < than 30% (stable environment: untidy)	10,6	2,5 - 46	P-value: 0,001
more than one depopulation	5,1	1,2 - 21	Fisher: 0,049
moist environment of drinking facilities	4,7	1,1 - 19,7	P-value: 0,025

Principal component analysis was used to characterize relations within the set of predictor variables. For an easier interpretation the components were rotated with varimax rotation. The first principle component retains most of the variation of the data set. The seven variables stable condition, ventilation system, pest security, chicken watering systems, feeding, hygiene sluice and litter storage load highly on the first component. The second component has high loadings in the three variables stable cloth, cleaning & disinfection equipment and collection system. Finally the two variables number of stables and thinning frequency show high loadings on the third component.

In the ordinal logistic regression model sums of identified variable bunches were used instead of the principle components themselves for the purpose of a simpler model and easier interpretation. The ordinal logistic regression model is used to determine the relationship between a categorical response variable (here the *Campylobacter* status of the herd) and a set of predictor variables. The estimated regression parameters of the ordinal logistic regression are presented at the conference.

DISCUSSION

Several international studies investigated various risk factors for *Campylobacter* colonization of broilers ([1], [2], [3], [6], [9]). Horizontal transmission has been identified as a very important route [7] therefore everything slightly related to a hygiene barrier and the condition of the premises is relevant. This is consistent to our results, as e. g. tidy stable environment (concrete as surface layer on the premises: more than 30%) and efficient pest control has been identified as important hygiene criterion with two different approaches. A general impression of the complex situation is revealed by the two different methods. The Spearman's correlation coefficient is related to the used model [8], whereas the odds ratio concentrates on factors with two mutual exclusive characteristics.

It is reasonable in a non statistical perspective that a relation between stable conditions, hygiene slice and pest security exists. Subsequently ordinal logistic regression was performed with the recognized components. This statistical analysis measures the association of the *Campylobacter* status of the herd and different risk factors. The interpretation of logistic regression results is not straightforward and requires specific knowledge. The ordinal logistic regression forms the basis for a predictive tool development (estimating the *Campylobacter* status on the observation of specific criteria). Such a predictive model will be generated and validation with new data of other farms is planned.

CONCLUSIONS

In general, a high biosecurity standard is recommended for the prevention of *Campylobacter* entering a broiler flock. Additional some factors may increase the chance of producing a *Campylobacter* free broiler batches. The results emphasize the crucial interaction between different factors and the hygiene compliance to avoid *Campylobacter* colonization at broiler farms. Finally a predictive tool may help – at least in educatory sense – to combat *Campylobacter* colonization of broilers and hence reduce the risk of Campylobacteriosis of humans.

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COMPARISON OF *CAMPYLOBACTER COLI* ISOLATED FROM PIGS AND HUMANS IN THE CZECH REPUBLIC

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SUMMARY

The aim of this study is to compare antimicrobial resistance and pulsed field gel electrophoresis (PFGE - *Sma*I) subtypes of *Campylobacter coli* isolated from human patients and slaughtered pigs. The MIC values and presence of genes associated with resistance to ampicillin, ciprophloxacine, nalidixic acid, erythromycine, gentamycine, chloramphenicol and tetracycline for *C. coli* from humans (55) and pigs (188) were determined. Isolates from pigs were maily resistant to erythromycine (63%) and mutation A2075G in *23S rRNA* gene was also detected by PCR. Resistance to tetracycline was found in 48% and presence of *tet*O gene discovered. Resistance to fluoroquinolones was low (27%). Only *C. coli* isolates from pigs (7,6%) were confirmed as multiresistant. On the other hand isolates from human patients were highly resistant to nalidixic acid (49%), to ciprofloxacine (46%) with detected Thr86Ile mutation in *gyr*A gene; to erythromycin (24%) and tetracycline (6%). All strains were sensitive to chloramphenicol and gentamycine. *Campylobacter* population was genetically highly diverse. The PFGE analysis revealed 147 genotypes (50 for humans and 97 for pigs). The PFGE patterns were grouped into clusters of at least 80% genetic similarity. Among human isolates 80% had unique genotypes; 20% represent five clusters of 2 to 3 isolates each. Among pig isolates 36% had unique genotypes; 19% represent three clusters of 2 to 4 isolates and 45% fourteen clusters of 5 to 11 isolates. Each one of the 14 different clusters was composed of isolates only from one farm. In all clusters only genotypes of one species (pig or human) were present.

INTRODUCTION

Campylobacter spp. is the second leading bacterial cause of foodborne gastroenteritis in the Czech Republic. In the most cases of human campylobacteriosis *C. jejuni* was found but up to 20% *C. coli* is associated with this gastroenteritis. *C.coli* has been suggested to be particularly suited to the swine production environment and has been isolated from pigs on farms up to 100% of the samples collected. This has been viewed as of minor importance to human health, however, recent publication has suggested that healthburden may be considerable and greater than previously thought. The data about increasing resistance of *Campylobacter* isolates, particularly to fluoroquinolones, macrolides and tetracyclines as well as to other antimicrobial agents raised concerns that the treatment of human infections might be complicated. Molecular typing techniques can allow isolates to be grouped on the basis of genotype, potentially enabling the identification of host-associated lineages trom possible food chain sources. The pulsed field gel electrophoresis (PFGE) method, which comprises agarose gel separation of large endonuclease-digested fragments, has proven to be both discriminatory and reproducible. PFGE has proven useful for epidemiologic characterization of *Campylobacter* isolates [6]. The aim of the study was to compare human and pig isolates to assess possible epidemiological links.

MATERIAL AND METHODS

The isolates in this study originated from gastrointestinal tract of slaughtered pigs (n=188) fattened on 28 commercial farms in the Czech republic. Human *C. coli* strains (n =55) were isolated from swaps of human patients with campylobacteriosis. For antimicrobial resistance profile characterization the minimum inhibitory concentration (MIC) against a panel of seven antimicrobials was determined using the agar dilution method as recommended by the Clinical and Laboratory Standards Institute. These antimicrobials were representative of drugs used in humans or in swine industry and medical area: ampicilin (range of concentrations 0.25-128 µg/ml; breakpoint levels 32 µg/ml), chloramphenicol (0.25–128; 32), ciprofloxacin (0.063–32; 4), erythromycin (0.063–32; 8), gentamicin (0.063–32; 8), nalidixic acid (0.25–128; 32) and tetracykline (0.06–32;16). Multidrug resistance was defined as isolates with resistance to three or more antimicrobials. Genes *tet*(O) and *tet*(M) associated with tetracyclin resistance were detected by mPCR [2]. Single base mutations in *23S rRNA* gene associated with macrolide resistance [1] and Thr86Ile mutation in *gyr*A gene associated with fluoroquinolone resistance [11] were detected by MAMA PCR. Chromosomal DNA for PFGE analysis was isolated from *Campylobacter* isolates cultivated on Blood agar Base no.2. Lysis of harvested

bacteria was performed in 1% agarose blocks with 1mg/ml proteinase K 15 min at 54°C. The consequent DNA digestion with *Sma*I was performed for 5 h at 30°C. For PFGE, Bio-Rad CHEF-DR III apparatus was used with pulses increasing from 5 to 10 sec for 4 h, from 10 to 40 sec for 14 h and from 50 to 60 sec for 4 h at 200 V and 9°C. The gels were stained with ethidium bromide and photographed under UV illumination. A gel image was then constructed by BioNumerics software (Applied Maths, Belgium).

RESULTS

Overall, *C. coli* isolates from pigs (n=188) exhibited highest frequency of resistance against erythromycin (n=63%) followed by tetracycline (48%). Resistant isolates were divided to the subgroups according to their level of resistance. Erythromycine high level resistant *C. coli* isolates (MIC≥128 µg/ml) were tested for the presence of the mutations A2075G and A2074C in the gene *23S rRNA*. The mutation A2075G were confirmed in all of the high level resistant isolates; mutation A2074C was not found. From *tet* genes t*et*(O) gene was detected in all *C. coli* isolates with MIC level 8–128 µg/ml and in more than 10% of isolates with MIC level 1–8 µg/ml. Eleven *C. coli* isolates (MIC 128 µg/ml) were also positive for *tet*(M) gene but these results must be confirmed by sequencing analysis. No other *tet* genes - *tet*(A), *tet*(B), *tet*(E) were detected. Resistance against nalidixic acid, ciprofloxacin, and ampicillin was detected in 36%; 27% and 21% of isolates, respectively. Only one isolate was resistant to gentamycine. All *C. coli* isolates from pigs were sensitive to chloramphenicol. More than 23% of *C. coli* isolates from pigs were sensitive to all antimicrobial agents tested; 76.6% of isolates were resistant at least to one antimicrobial agent and 7.45% isolates were confirmed as multidrug resistant.

C. coli isolates from human patients (n=55) were mainly resistant to nalidixic acid (49%) and ciprofloxacin (46%). All *C. coli* isolates with high level resistance to NAL (MIC≥128 µg/ml) and to CIP (MIC≥4 µg/ml) were positive for point mutation Thr86Ile in *gyr*A gene associated with resistance to fluoroquinolones. More than 15 % of low level resistant isolates (MIC≤32 for NAL and MIC<1 µg/ml for CIP) were negative for this modification. Resistance of *C. coli* human isolates against erythromycin, ampicillin, and tetracykline was detected in 24%, 15% and 6%, respectively. 21% *C. coli* human isolates were resistant at least to one antibiotics but non of them was multidrug resistant. All isolates were sensitive to chloramphenicol and gentamycine. More than 33% isolates were sensitive to all antibiotics.

243 samples of *C. coli* human and pig isolates were typed by PFGE analysis. Some of the PFGE patterns were similar with one or two bands absent or present or size-shifted but 147 unique patterns (50 for humans and 97 for pigs) were found. Clustering of all isolates with the unweighted paired group method with arithmetic means (UPGMA) revealed 22 clusters of at least 80% genetic similarity. Among 55 human isolates 44 (80%) genotypes were found once and 11 (20%) belong to five clusters with 2 or 3 isolates. Among 188 pig isolates 67 (36%) genotypes were found once, 36 (19%) formed three clusters of 2 to 4 isolates and 85 (45%) represent fourteen clusters of 5 to 11 isolates. Each of the 14 clusters comprised isolates from only one farm. In all clusters only genotypes of one species (pig or human) were present.

DISCUSSION

Most studies dealing with campylobacteriosis is focused solely on the characterization of the dominant species *C. jejuni*. Studies of other thermophilic *Campylobacter* species identified in human patients to a lesser extent were largely neglected until the beginning of the 21 century. Recently, the research also focused on chareacterization of *C. coli*, which is the second most frequently isolated species associated with human campylobacteriosis.

C. coli isolates from pigs were high resistant to erythromycin (63%) and tetracycline (48%). The same trends of *C. coli* resistance to erythromycine were declared by other studies worldwide: 43% in Italy [9], 46.5% 55% in France [8] and 61% in Canada [5]. This high frequency may be explained by the fact that macrolides (tylosin in particular) were still in the last decade of the last century used as growth promoters in pigs in Europe. 48.4% of *C. coli* isolates demonstrated resistance to tetracycline. A higher number of TET-resistant *C. coli* isolates (94%) were detected in Spain [10], 79% in France and 77% in Italy [9,8]. High resistance appears to be related to the frequent use of this group of antibiotics in veterinary practice and the earlier use of tetracyclines as growth promoters and prophylactic.

Human *C. coli* isolates exhibited the highest frequency of resistance against chinolones and fluorochinolones (NAL-49%; CIP-46%) followed by erythromycine (24%). It can be concluded that the antibiotic resistance in human isolates in the Czech republic is the same or lower if compared with other EU countries. Higher incidence of isolates *C. coli* resistant to chinolones, fluoroquinolones and macrolides is associated both with the policy implemented in human and veterinary practice and to some extent by the fact that these drugs are antibiotics of first choice in the treatment of gastroenteritis [7]. The detection of the *tet*(O) gene can be used for the testing of TET-resistance of pathogen before the medical treatment and similarly detection of point mutation in *gyr*A seems to be effective method for the quick detection of resistance to fluoroquinolones.

The PFGE genotyping demonstrated that *Campylobacter* population was genetically highly diverse. In human isolates larger variability in PFGE polymorphisms was found. This corresponds with the relatively greater

number of human samples. While in the sets of pig samples from a limited number of farms related clones mostly originated in the same farm, human samples represent individual samples taken from a geographically extensive area. 22 clusters of at least 80% similarity were revealed. Clusters comprised genotypes only of one species (human or pig). Denis at al. [3] found that isolates from pigs never clustered with human isolates, and are not genetically related to those from humans. Moreover authors concluded that the analysis of the genetic profiles of *Campylobacter* from humans showed that there were few identical or genetically close isolates between the human cases declared in the year 2003 or 2004. This highlighted a great genetic diversity in the isolates and indicated that it could be difficult to bind the human infections with groups of *Campylobacter* isolates presenting particular genetic profiles. Also Guévremont et al. [4] used PFGE to compare isolates from pig caeca with isolates from cases of human diarrhea. No genetically identical isolates common to the two sources was found.

CONCLUSIONS

The monitoring of the resistance rates and enquiry of resistance mechanisms of *C. coli* seems to be very important for the public health risk assesment and risk of treatment failures. Different resistance profiles and genotypes of *C. coli* of human and pig origin suggested that sources other than pigs are more important in human campylobacteriosis. *Study was supported by the project MSM621571240.*

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SURVIVAL OF *CAMPYLOBACTER JEJUNI* IN BROILER FAECES

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SUMMARY

Broiler meat is known as a major source of *Campylobacter* causing disease in humans. It is assumed that broiler flocks become infected in the course of the fattening period from the contaminated and insufficiently cleaned and disinfected housing environment. In particular, contaminated faeces are supposed to form the most predominant reservoir in the animal environment. However, little is known about the survival times of *Campylobacter* spp. in broiler faeces. Samples of fresh faeces were collected from a *Campylobacter* free SPF broiler herd. Samples were divided into two equal parts. One part was inoculated and mixed with a freshly prepared *C. jejuni* B5 solution with concentrations of $1x10^8$ CFU/ml and stored at room temperature for 10 days. Every day a sample was investigated by microaerophiloc cultivation on selective media. The other part of the *Campylobacter* free faeces was inoculated 14 days later and also sampled daily. Furthermore, naturally infected faeces from poultry flock were collected and investigated daily for 10 days. Inoculated *C. jejuni* was recovered for 3 and 2 days from fresh faeces and 14 day stored faeces, respectinely. However, it was cultivated for 5 days from the naturally infected faeces. The isolates from both inoculated trials at all sampling days showed the same band pattern by restriction fragment length polymorphism using *Dde I*. Moreover, the naturally infected faeces show two band patterns. The survival times of *C. jejuni* indicate that fresh faeces can play a considerable role in spreading *Campylobacter* within a herd and if applied to land also between poultry farms.

INTRODUCTION

Campylobacteriosis is the most reported gastrointestinal bacterial pathogen in humans world wide. In 2008 the total number of confirmed cases was 190,566 in Europe and 64,731 in Germany (2). It colonizes the alimentary tracts of most warm blooded animals and humans. However, the most preferential environment appears to be the intestine of poultry. Poultry meat especially fresh broiler meat is considered to be the major vehicle of *Campylobacter* causing disease in humans (5). It is assumed that broiler flocks become infected in the course of the fattening period from the contaminated and insufficiently cleaned and disinfected housing environment. Many investigations indicate that horizontal transmission is the primary route of *Campylobacter spp.* within flocks or even during transportation (1). In particular, contaminated faeces are supposed to form the most predominant reservoir in the animal environment. Therefore, investigations were carried out to estimate the survival time of *C. jejuni* in poultry faeces.

MATERIAL AND METHODS

Sampling Methods

Faeces from *Campylobacter* free SPF poultry were collected. One half was stored at 4°C. From the other half 10 samples were taken and mixed with 18 h old *C. jejuni* B5 culture suspened in PBS solution at concentrations of $1x10^8$ CFU/ml and stored at room temperature. Over a time period of 10 days, one sample was investigated using microaerophilic incubation on selective media. The trail was repeated with the stored faeces after 14 days. To study the survival of *C. jejuni* in naturally infected faeces, 8 samples of faeces from *Campylobacter* infected poultry were stored at room temperature. Over a period of 8 days, one sample was examined as described before.

Isolation and identifications

Isolation of *Campylobacter* from both types of faeces was carried out according to ISO 10272-1:2006 by microaerophilic cultivation at 42ºC for 48 h in Bolton Broth (Oxoid, Germany) and direct streaking onto modefied Charcoal Cefoperazone Desoxycholate Agar (mCCDA, Oxoid, Germany). Suspected *Campylobacter* colonies were confirmed by microscopic observation of characteristic spiral shape and corkscrew-like motility using wet mount. Pure cultures were obtained by cultivation on Columbia blood agar (Oxoid) and then tested for Gram Staining, hippurate hydrolysis, catalase test, oxidase test, microaerobic growth at 25°C and aerobic growth at 42°C. Initially positive isolates were further identified biochemically using API Campy test (BioMerieux,

Germany). All *C. jejuni* positive isolates were stored in FBP medium (Oxoid) at -80°C until confirmion by PCR. The DNA from all *C. jejuni* isolates were extracted from 48 h cell cultures on Columbia Agar using peqGOLD bacterial DNA Kit (PeQlab Biotechnologie, Germany) following the manufacturer instructions. The *mapA* gene was used for the identification of *C. jejuni*. Amplification reactions were carried out using Mastercycler (Eppendorf AG.Germany) using FastStart Taq DNA Polymerase kit (Roche, Germany). The PCR product was separated by electrophoresis using 1.5 % agarose gel (Application AG, Germany) containing ethidium bromide. The gel was visualized with an ultraviolet transilluminator (Biometra, Germany) and photographed. The amplification of the *fla A* gene of *C. jejuni* isolates was performed) using peqGOLd Taq-DNA-polymerase (PeQlab Biotechnologie, Germany). The PCR products were purified using MinElute PCR Purification kit (QIAGEN, Germany) following the manufacture's instruction. The purified PCR products were analyzed by restriction fragment length polymorphism (RFLP) using *DdeI* (Roche, Germany). The digested DNA was subjected to electrophoresis in 2.5 % agarose gel with ethidium bromide and proceeds as described above.

RESULTS

C. jejuni B5 was isolated 3 and 2 days after inoculation from the artificially infected faeces of first and second trial, respectively. The isolates from both trials at all sampling days have the same bands by RFLP as the original challenge strain (Figure 1). However, regarding the naturally infected faeces *C. jejuni* was isolated up to five days after storage and RFLP revealed two different isolates were obtained from the pooled faeces (Figure 2).

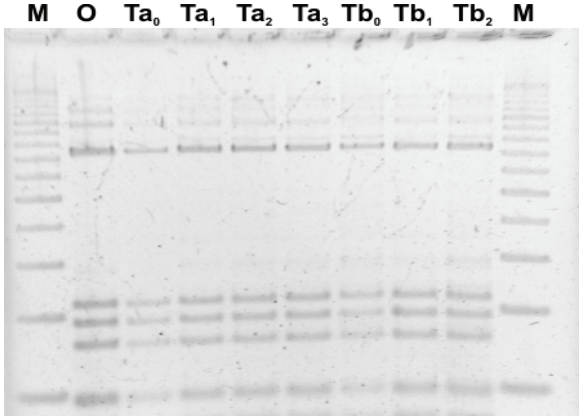

Figure 1: RFLP of *C. jejuni* B5 isolated from two trails artificially infected faeces M: 100 bp DNA marker, O: he original isolate Ta $_{0-3}$: first Trial isolates till 3 days Tb $_{0-2}$: Second trial isolates till 2 days.

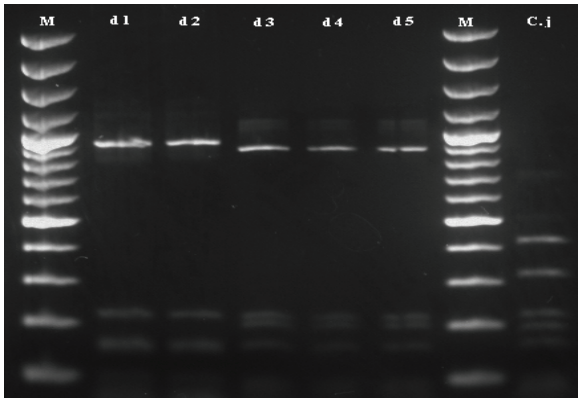

Figure 2: RFLP of C. jejuni isolated in naturally infected faeces from first till fifth day d1-d5: M: 100bp DNA marker

DISCUSSION

This study shows that *C. jejuni* can survive 2-5 days in artificially and naturally contaminated poultry faeces. In infected faeces *C. jejuni* survived for 3 days after infection. Relatively short survival times were in agreement with (1) where, *Campylobacter* was isolated from broiler faeces in transport cages few days later. However, the survival times of *C. jejuni* in our experiment clearly shorter than observed by (4) who isolated *Campylobacter* from cattle faeces under field conditions after 7 days and (3) were able to find *Campylobacter* after 14 days in 4 out of 7 cattle pats. The longer survival times may be due to the higher moisture content of cattle faeces (82%) compared to poultry faeces (68%) which may be enable *Campylobacter* to survive longer and duo to different composition of cattle and poultry faeces.. Interestingly in the case of the naturally infected faeces which originated from pool sampling from different birds two RFLP were recovered. It is not clear if these strains originated from hens or their environment.

CONCLUSIONS

The survival times of *C. jejuni* indicate that fresh faeces can play a considerable role in spreading *Campylobacter* within a herd and if applied to land also between poultry farms.

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EFFECT OF USING ACTIVE EFFECTIVE MICROORGANISMS AS AN ALTERNATIVE ANTIBIOTICS ON IMMUNITY IN LOCAL DOMESTIC FOWLS NUTRITION

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The main object of this work was to investigate the effect of adding two probiotics, (effective microorganism (EM) as a live microbes and Zinc bacitracin as antibiotic) to the diets of Inshas chickens (a local Egyptian chicken strain) on chickens immunity. The experimental design consisted of six experimental groups: control and 5 dietary treatments as follows; (T1) Basal diet (control), (T2) Basal diet + EM (2.5 ml/kg diet), (T3) Basal diet + EM (5.0 ml/kg diet), (T4) Basal diet + EM (7.5 ml/kg diet), (T5) Basal diet + EM (10.0 ml/kg diet) and (T6) Basal diet + Zinc bactracin (500 mg/kg). Feeding treatment started at 4 wk of age and lasted at 41 wk. 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 (T2 to T6 diets). Also, The addition of EM with different levels and Zinc bactracin to the chicks diet had significant increased in CMI response as compared with control , although in many cases Zinc bactracin seemed to be less effective than the EM.

INTRODUCTION

The first goal of the livestock production is the delivery of safe foods for human consumption taking into account the welfare of the animal and respect for the environment. In the past, antibiotics have been included in animal feed at sub-therapeutic levels, acting as growth promoters (Antibiotic Growth Promoters; AGPs);. In this context probiotics, prebiotics and synbiotics could be possible solutions. The main effects of these feed additives are the improved resistance to pathogenic bacteria colonization and enhanced host mucosa immunity; thus resulting in a reduced pathogen load, an improved health status of the animals [1] and a reduced risk of food-borne pathogens in foods.

Effective Micro-organisms (EM) is a microbial preparation developed by Professor T. Higa of University Of The Ryukyus in Japan. The EM is composed of different microbes that include bacteria, yeasts and/or fungi. Some of the benefits claimed to accrue from the use of EM include improved animal health, reduction of foul smells and absence of toxic effects on bird growth [2]. Use of EM in Africa is a new innovation and novel idea. There is no available literature regarding use of microbial preparations in broiler production. Therefore, this experiments was designed to investigate the possibility of using probiotic namely, (EM) effective microorganism (instead of using antibiotics) to Inshas chickens (Egyptian local strain) , and to evaluate its effects on immunity.

MATERIAL AND METHODS

A total number of 540 unsexed vaccinated Inshas (local Egyptian chicken strain) one day-old-chicks were weighed , wing banded and randomly divided into six experimental groups (three replicates each group) . The birds were placed in a room (floor pens) maintained at a constant temperature of 28+/-3 oC and a relative humidity of 70+/-3% .Food and water were always available *ad libtum* . The basal diet was formulated to meet the nutrient needs suggested by the NRC, 1994[9]. The beta-procedure of the (Haemagglutination-Inhibition) HI was employed as a micro-test in plastic plates as outlined in "Methods for Examining Poultry Biologies and for Identifying and Quantifying Avian Pathologens [3]. Cutaneous basophil hypersensitivity (CBH) response, elicited by an intradermal injection of a T-cell mitogen, provides an *in vivo* evaluation of cell-mediated immunity [4]. The EM used in these studies was the commercial product produced by Ministry of Agriculture, Egypt. containing different types of micro-organisms *Lactobacillus* (*Lactobacillus plantarum* (ATCC8014), *Lactobacillus casei* (ATCC7469); *Streptococcus*: *Streptococcus lactis* (IFO12007), *Streptomyces albus* (ATCC3004), *Streptomyces griseus* (IFO3358), *Rhodopseudomonas*: *Rhodopseudomonas palustris* (ATCC17001), *Rhodobacter sphaeroides* (ATCC17023), Yeast: *Saccharomyces cerevisiae* (IFO0203), *Candida utilis* (IFO 0619), Fungi: *Aspergillus oryzae* (IFO 5770) and *Mucor hiemalis* (IFO 8567), was mixed with the experimental diets at the levels of 2.5, 5, 7.5 and 10 ml/kg diet.The experimental design consisted of six dietary treatments as follows; (T1) Basal diet (control), (T2) Basal diet + EM (2.5 ml/kg diet), (T3) Basal diet + EM (5.0 ml/kg diet), (T4) Basal diet + EM (7.5 ml/kg diet), (T5) Basal diet + EM (10.0 ml/kg diet) and (T6) Basal diet + Zinc bactracin (500 mg/kg).. The results obtained were statistically analyzed using

Duncan's Multiple Range Test {17}. Statements of statistical significance are based on $P<0.05$.

RESULTS AND DISCUSSION

Immune response: 1- Effect of different dietary treatments on humoral immune response

Results concerning immune response of birds against Lasota as influenced by the different dietary treatments are given in Fig (1) **.**It is clearly evident that feed additives significantly improved the natural immunity of birds against viral invasions. An average antibody titer record in control diets was always significantly less than those found in all feed additives treatments (T2 to T6 diets).The addition of EM with different levels and Zinc bactracin to the chicks diets had significant increased in HI antibody response.In previous studies, probiotic has been shown to have a positive influence on humoral immunity and immunoglobulin status.EM diets improvement was also agree with [2].

EM improvement immunity this could be attributed to the high performance of birds induced by the probiotic used which possibly indirectly improved the immunity status of the treated chickens. [5] reported that the use of competitive exclusion products can protect newly hatched highly susceptible chicks or poults being placed into commercial production system and could be of great benefit in reducing colonization and disease caused by Paratyphoid salmonella . He added also that a similar protective effect has been demonstrated in controlled studies against *E.coli*, *complobacter jejuni* , *clostridium botulinum* and *clostridium prefringens* .

Effect of different dietary treatments on cell mediated immunity (CMI)

Results regarding (CMI) to different dietary treatments investigated are shown in Fig(2).

It is clearly evident that feed additives significantly improved the cell mediated immune of birds. An average (CMI) record in control group (T1) was always significantly less than those for all feed additives treatments (T2-T6).The addition of EM with different levels and Zinc bactracin to the chicks diets had significant increased in CMI response.The better values were 51.59, 37.30, 32.68, 24.71 and 15.85 in chick fed T5, T4, T3, T2 and T6 diets, respectively, as compared with control group (T1) . These is agree with obtained by [6] who observed that probiotic improvement the immune response .

In a different experimental system in which monocyte-derived DC were cultured with the probiotic L. rhamnosus and the subsequent effect on T cells was assessed, decreased T-cell proliferation and T-cell cytokine production, particularly IL-2, IL-4, and IL-10, was demonstrated [7]. This *in vitro* effect of *L. rhamnosus* on DC and subsequent T-cell hyporesponsiveness was reflected in *in vivo* studies in which healthy controls and patients with CD were fed *L. rhamnosus* for 2 weeks. Ingestion of L. rhamnosus reduced IFNy and IL-2 production by peripheral T cells in CD patients and also reduced IL-4 production in healthy controls. Probiotic bacteria influence the generation of regulatory T cells in a murine model of contact dermatitis. Daily oral administration of fermented milk containing the probiotic *L. casei* DN-114 001 reduced antigen-specific skin inflammation by controlling the antigen-specific T cell response in hapten 2,4-dinitrofluorobenzene, a model of allergic contact dermatitis mediated by CD8+ CTL and controlled by CD4+ regulatory T cells. The alleviation of contact hypersensitivity by prior feeding with L. casei was due to downregulation of the hapten-specific CD8+ T-cell response as indicated by a decrease in expansion of hapten-specific IFNy producing CD8+ effectors [8].

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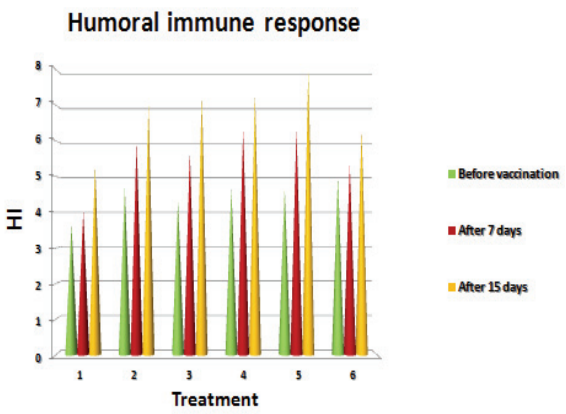

Fig (1): Effect of different levels of EM and Zinc bactracin on the immune response antibody titre against Lasota of birds.

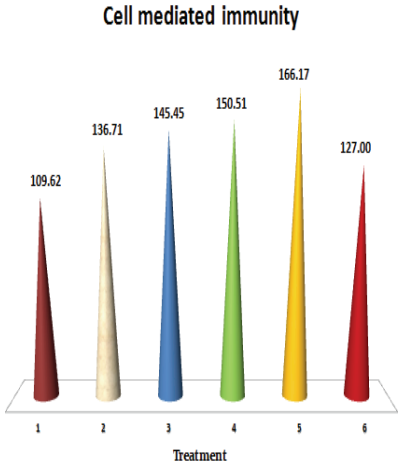

Fig (2): Effect of different dietary treatments on cell mediated immunity of birds.

HAEMATOLOGICAL AND ENZYME BIOCHEMICAL STUDIES ON THE EFFECT OF PROBIOTCS IN DOMESTIC FOWLS RATION

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SUMMARY

The purpose of this study was to determine the effect of probiotics on hematology and enzyme biochemistry of Inshas chickens (a local Egyptian chicken strain) . The experimental design was consisted of six experimental groups: control and 5 dietary treatments as follows; (T1) Basal diet (control), (T2) Basal diet + EM (2.5 ml/kg diet), (T3) Basal diet + EM (5.0 ml/kg diet), (T4) Basal diet + EM (7.5 ml/kg diet), (T5) Basal diet + EM (10.0 ml/kg diet) and (T6) Basal diet + Zinc bactracin (500 mg/kg). Feeding treatment was started at 4 wks of age and lasted at 41 wks of age. The Characteristic investigations were : serum biochemical estimates as (Total protein, Albumin , globulin, , Uric acid , Creatinine) and Hematological Parameters (Total RBC's and WBC's counts, differential leukocytic count, hematocrite %, H / L ratio). The obtianed results can be summarized as following ; 1) all studied traits were affected by feed additives treatment; 2) the studied feed additives showed significant beneficial effects almost in all studied traits; 3) The most effective improvment was obtained with the basal diet + EM (10.0 ml/kg diet); 4) in many cases ,Zinc bactracin trait seemed to be less effective than the EM trait.

INTRODUCTION

The impact of biotechnology in poultry nutrition is of significant importance. Biotechnology plays a vital role in the poultry feed industry. Nutritionists are continually putting their efforts into producing better and more economical feed. Good feed alone will not serve the purpose but its better utilization is also essential. Dietary changes as well as lack of a healthy diet can influence the balance of the microflora in the gut thus predisposing to digestion upsets. A well-balanced ration sufficient in energy and nutrients is also of great importance in maintaining a healthy gut. A great deal of attention has recently been received from nutritionists and veterinary experts for proper utilization of nutrients and the use of probiotics for growth promotion of poultry.

In poultry nutrition, probiotic species belonging to Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida, and Saccharomyces have a beneficial effect on poultry performance [1], modulation of intestinal microflora and pathogen inhibition [2], intestinal histological changes [3], immunomodulation [4], certain haematobiochemical parameters [5], improving sensory characteristics of dressed broiler meat [6] and promoting microbiological meat quality of broilers[7].

Effective Micro-organisms (EM) is a microbial preparation developed by Professor T. Higa of University Of The Ryukyus in Japan. The EM is composed of different microbes that include bacteria, yeasts and/or fungi. Some of the benefits claimed to accrue from the use of EM include improved meat and manure quality, improved animal health, reduction of foul smells and absence of toxic effects on bird growth [8]. Use of EM in Africa is a new innovation and novel idea. There is no available literature regarding use of microbial preparations in broiler production. Therefore, this experiments was designed to investigate the possibility of using probiotic namely, (EM) effective microorganism (instead of using antibiotics) to Inshas chickens (Egyptian local strain) , and to evaluate its effects on hematology and enzyme biochemistry.

MATERIAL AND METHODS

A total number of 540 unsexed vaccinated Inshas (local Egyptian chicken strain) one day-old-chicks were weighed , wing banded and randomly divided into six experimental groups (three replicates each group) . The birds were placed in a room (floor pens) maintained at a constant temperature of 28+/-3 oC and a relative humidity of 70+/-3% .Food and water were always available ad libtum . The basal diet was formulated to meet the nutrient needs suggested by the NRC, 1994. At 40 weeks of age, 2 birds from each replicate (6 birds/treatment) were chosen randomly. Then slaughtered , blood samples were collected and divided into two halfs . The first half was used for determining the hematological parameters. The second half of each blood sample was centrifuged at 3000 rpm for 15 minutes, to separate the serum. Serum samples were stored at -20 °C for biochemical analysis. Colorimetric methods using commercial kits which were purchased from Diamond Diagnostic, Dokki, Giza, Egypt.
The experimental design consisted of six dietary treatments as follows; (T1) Basal diet (control), (T2) Basal diet + EM (2.5 ml/kg diet), (T3) Basal diet + EM (5.0 ml/kg diet), (T4) Basal diet + EM (7.5 ml/kg diet),

(T5) Basal diet + EM (10.0 ml/kg diet) and (T6) Basal diet + Zinc bactracin (500 mg/kg).. The results obtained were statistically analyzed using Duncan's Multiple Range Test {17}. Statements of statistical significance are based on $P<0.05$.

RESULTS AND DISCUSSION

Total protein, Albumin and globulin

Serum total protein was significantly ($p \leq 0.001$) increased in chickens fed EM and Zinc bactracin diets as compared with those fed control diet Fig (1). This might be due the higher digestibility of CP in there diets. These results are in agreement with those obtained by [9]. Both protein fractions were increased in dietary treatments (EM and Zinc bacitracin) as compared with control diet, but albumin was insignificantly. These results are in agreement with those obtained by [10] .

The increases in the previous parameters may indicate that an enhancement of immunity occurred corresponding to feeding either probiotics, prebiotics or both synbiotics as a result of improving feed conversion, absorption and utilization of nutrients. Similarly, [11] with Gimmizah and Matourh strains and [12]with Japanese quail reported that addition of microbial probiotic caused higher level of plasma total protein as well as albumin and globulin fractions than those of control group.

Uric acid and Creatinine

No differences in uric acid and Creatinine were observed among dietary treatments (Fig 2). the similitude of uric acid concentration in supplemented or not groups in exhibit healthy, non-pathological or non-toxic effects of EM with different levels and Zinc bactracin on kidney functions. Similarly, [9] concluded that creatinine levels was insignificantly difference in broiler chicks fed microbial diets. These results agree with [10].

Hematological Parameters

Results concerning total red (RBC's) and white blood cells (WBC's) and hematocrete values as influenced by the different dietary EM and Zinc bactracin treatments are presented in Table (1).

Total RBC's count and hematocrete value: Results obtained clearly indicated that feeding birds EM and Zinc bactracin diets significantly increased erythropoiesis. The total number of RBC's was significantly enhanced due to EM and Zinc bactracin dietary treatments. The increase ranged between 8.54 and 14.35 %. The RBC'S count was increased ($p \leq 0.05$) by 14.35, 12.81 and 12.34 % in chicks fed T5, T4 and T3 diets, respectively, as compared with those fed control diets. It was generally noticed that hematocrete values nearly followed the same trend was observed in RBC's counts.These results agreed with [10]. The most likely explanation are improvement of bioavailability of essential nutrients [13] and enhancing vitamin B absorption resulted from increased small intestinal absorption [14].

Total WBC's count and differential leukoytic counts :

The same trend to what has been found with RBC's counts; WBC's were significantly increased due to consuming all feed additives to basal diets. WBC's count in T5 and T4 diets reached about 125 and 119% ,respectively, as compared with control count. All feed additives effectively improved the WBC's count in all dieatry treatments as compared with control diet. Statistical analysis proved that the significant increase in the total count of WBC's was not equally distributed among the different types of leukocytes which were also found to vary in their response to the different treatments. Percentage of lymphocytes was significantly increased while that of heterophiles markedly ($p<0.05$) decreased due to feed additives of treatment leading to a significantly higher H/ L ratio, meanwhile monocytes, basophiles and eosinophiles were slightly (insignificantly) changed. All the feed additives used significantly enhanced the rate of H/ L values which became significantly lower than control- group. In this respect the best values were obtained with those fed T5 and T4 diets.These results indicated that EM have an enhancement effect to the humoral and cell mediated immune response which agreed with that reported by [15] . Come to similar results, [10] who indicated that an enhancement of immunity might be expected corresponding to adding probiotic.

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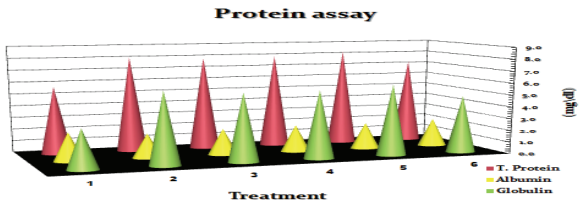
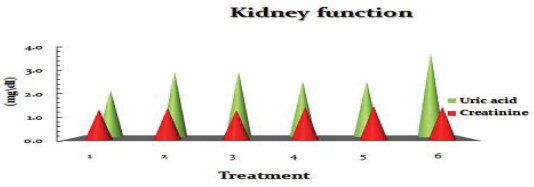


Fig(1): Protein assay as affected by different levels of EM and Zinc bacitracin.



Fig(2): Kidney function as affected by different levels of EM and Zinc bacitracin.

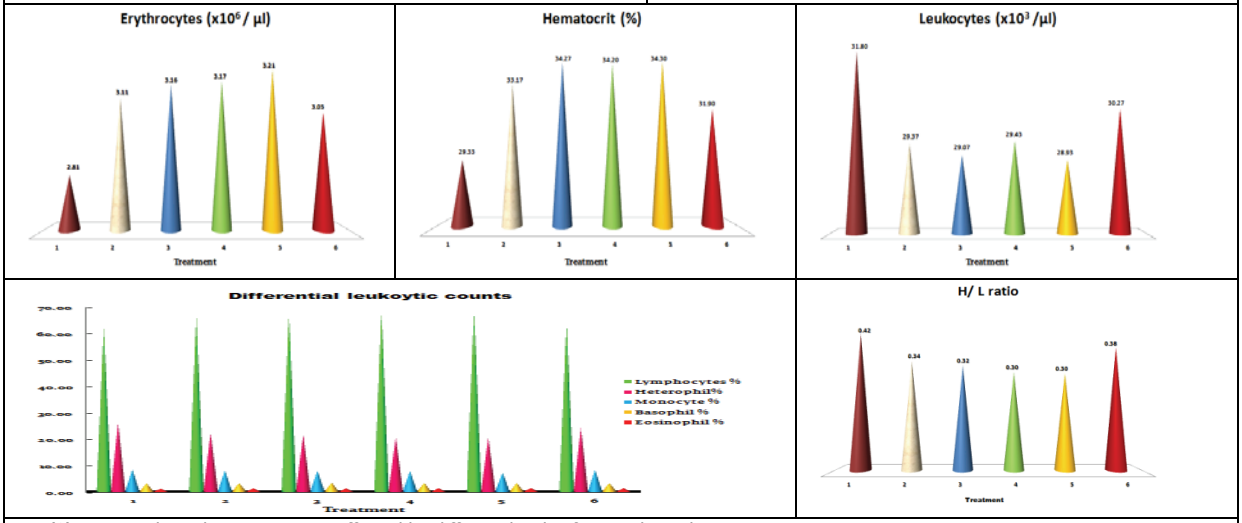


Fig (3) : Hematological parameters as affected by different levels of EM and Zinc bacitracin.

USE OF CENTRIFUGAL SAMPLERS FOR DETECTION OF MICROORGANISMS IN THE AIR

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SUMMARY

Introduction: The most commonly used method for demonstrating microorganisms in the air are sedimentation, pumping air through a liquid medium, impaction on solid medium, using Andersen sampler, and others methods. These methods are, in practice they have some shortcomings that affect the accuracy of the microorganisms in the air. We tried the sampler air which sampling air in liquid medium, using the principle of centrifugal force.

Materials and Methods: In the experiment we used the method of impaction the sampler Merck, pumping through the liquid medium through a system of Impinger and sampling the air using centrifugal force in a liquid medium.

Results: The results of extensive research, which has not yet completed. Previous results indicate a more reliable demonstration of microorganisms in the air for the study of air into the liquid medium by centrifugal force. During the course of research on this method we can prove in the air for the presence of *L. inocua* and *L. monocytogenes* in meat plants, by using other methods of sampling could not be demonstrated.

Conclusions: The method of sampling the air in the liquid medium by centrifugal force enables reliable sampling. Turbulence of centrifugal force presses the microorganisms present in liquid medium (media). This microbial suspension can be easily used in modern microbiological diagnosis (ELISA, PCR, counting on plates).

INTRODUCTION

Animal breeders and animal husbandry facilities are exposed to increasing concentrations of bioaerosol, which are a part of micro-organisms (1, 2). Microbial flora in the air was seen in animal breeding barns. The maximum density of microorganisms is determined in poultry plants and the lowest in cattle (3). Animal health is important to determine the number of microorganisms or to determine their abundance in the CFU liter of air. There are many methods, but also have their disadvantages (4). Sedimentation have been caught in a specific lighter particles, electrostatic precipitation produced ozone which destroys micro-organisms, also has disadvantages filtration, impaction on the medium, and systems based on the principle of Andersen sampler operating in a relatively small number of microorganisms in the air. As the most appropriate method of sampling the air in livestock buildings has so far proved catching micro-organisms in the fluid (impinger) (5).

On the market there has been a cyclone sampler air sampling as a method of micro-organisms from the air using the centrifugal force and pushing the micro-organisms in liquid medium where they accumulate. The survey was to determine the reliability and practicality for the study of air samples and the possibility of working in the investigations.

MATERIAL AND METHODS

For the study we selected three different methods of sampling:

- impaction air medium 90 mm diameter (MAS 100Ex, Merck) (The principle is based on the Andersen Air Sampler)
- impinger (SAS pcr)
- cyclone sampler (Coriolis Delta Air sampler)

Sampling site was chosen and cattle breeding barn. With all the equipment's we also conducted air sampling volume of 100 l., 300 l. and 1000 l. Merck and the apparatus SAS velocity could not be changed. Airflows in the apparatus were different. The air flow rate in MAS 100Ex was 100 l/min in SAS PCR and 35 l / min. After the air sample is taken under the scheme by Merck, were placed in incubation medium, liquid samples taken by the SAS system and Coriolis, we prediluted and then applicate to the medium. To determine the count of microorganisms, we used by our examination the medium yeast agar.

RESULTS

In the apparatus, the Merck MAS 100 and SAS pcr we encounter problems. Despite the low flow value count at the withdrawal of Merck under the system, because of high densities could not be counted.
In the apparatus pcr SAS we have encountered another problem. The system consists of a number of silicone and glass tubes. Despite the attempt disinfection system, the latter has remained contaminated, which is not acceptable in determining the presence of micro-organisms and their count in the air.

For the system of Coriolis Air sampler to the problems we had. Air sampling system can be disinfected with a disinfectant. You can also wash disinfectant residues from surfaces. The resulting pattern is in a liquid medium, which can be used for further work. Also note proportional increase in the number of microorganisms in the sample by increasing air flow.

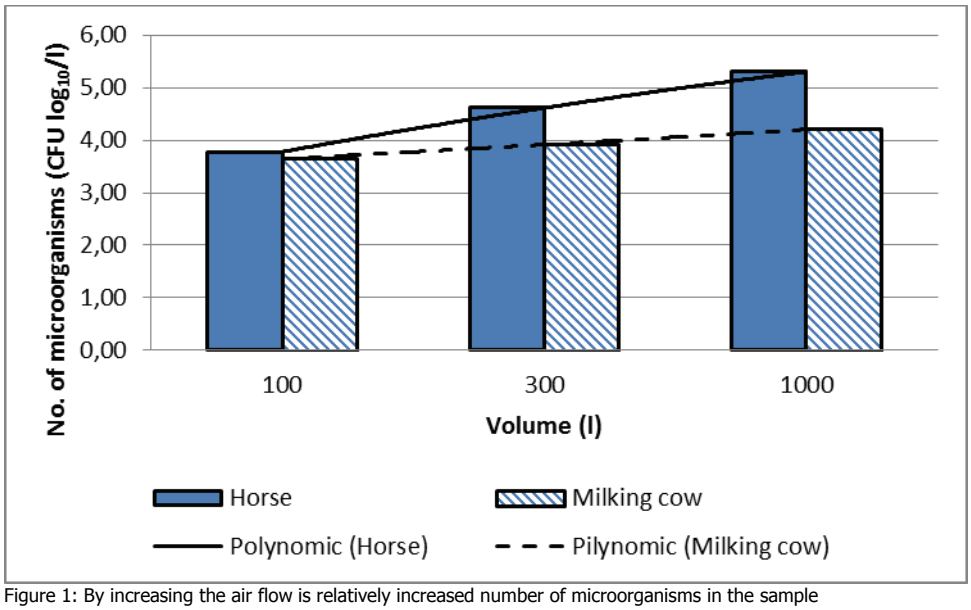

Figure 1: By increasing the air flow is relatively increased number of microorganisms in the sample

For the study, we also increased the flow rate and compared the number of microorganisms in the samples. We find that by increasing the flow increases the number of captured microorganisms, if the values are calculated per liter of air. This means that we calculated per liter of air cover over the micro-organisms as the higher the air flow. If the same microbial suspension sampled at 100, 200 and 300 l / min, will be at 200 l / min cover 2.44%, at 300 l / min and 4.40% more germs than the flow of 100 l / min.

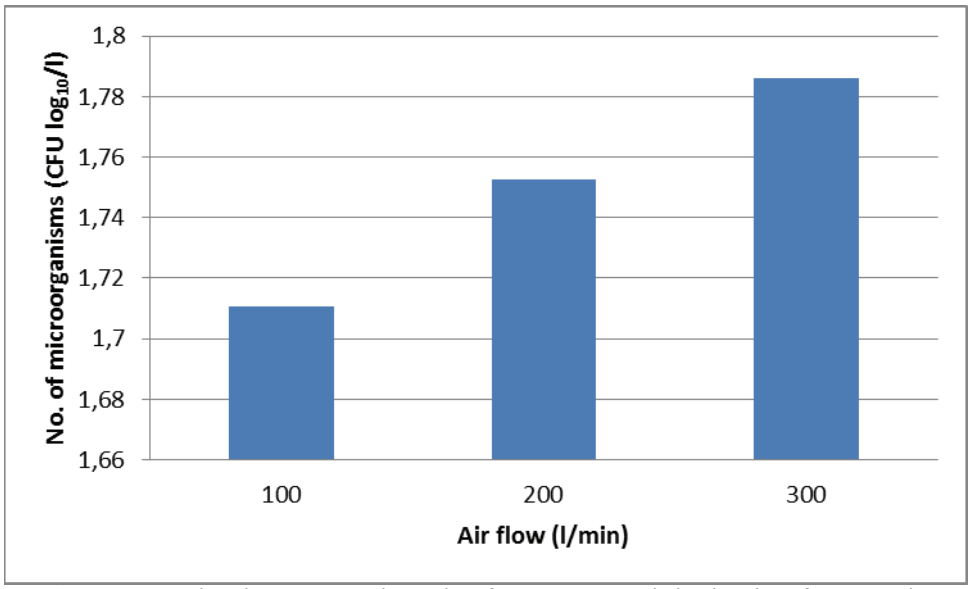

Figure 2: By increasing the velocity increases the number of microorganisms calculated per liter of air captured

CONCLUSIONS

Due to the high density of microorganisms and the possibility of simple sample treatment, the cyclone system in the liquid sampling system (Coriolis Air sampler) proved more reliable. According to other systems, a liquid sampling more appropriate medium for manipulation in future research. Coriolis Air sampler allows pumping of large volumes of air (up to 100 m^3) in a relatively small volume of liquid medium (5-15 ml of liquid). The system can be disinfected on site immediately before re-use and allows the automatic dosing of liquid medium through a pericyclic pump.

Because of the possibility to transfer large amounts of air is especially suitable in cases where the air looking for the presence of particular pathogens (eg Listeria). Since their density is low, it is necessary to pump a large amount of air that can prove their presence.

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MICROSCOPIC ANALYSIS OF SIZE, STRUCTURE AND AMOUNT OF PARTICULATE BIO-AEROSOLS DIRECTLY SAMPLED FROM RAW AND CLEAN GAS OF AN EXHAUST AIR BIO-WASHER IN A PIG FATTENING UNIT

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SUMMARY

Air emissions from swine housing include a complex mixture microbial particulates and there is increasing use of abatement techniques in order to reduce these emissions. Recent reports show that reduction efficiencies of up to 90 % can be reached when combinations of bio-washers and bio-filters are applied. However little is known about the amount, size, structure and composition of bio-aerosols which pass the washer and filtration systems or are emitted by these devices themselves. A novel impaction method on silicone surfaces and fluorescence microscopic image analysis allow the investigation of airborne microbial particles in their original shape as well as counting of total bacteria and mould spores. The number of particles collected in raw gas and clean gas was very similar. The number of the total bacteria counted under the microscope was higher than the number of culturable bacteria (cfu). Most micro-organisms were in aggregates or attached to other particles. Most of the bacteria in raw gas were found in the particle fraction 80 to 100 µm, whereas in clean gas most of the bacteria were found in the particle fraction 10 to 20 µm. In addition, the clean gas contained a high amount of small organic particles (<5 µm) with many carrying bacteria which can be inhaled deep into the thorakal or even alveolar region of the lung where they may cause respiratory health problems.

INTRODUCTION

Air emissions from swine housing include a complex mixture of inorganic, organic and microbial particulates. Little is known about the exact composition of microbial particles and if they exist as aggregates of micro-organisms or single cells or are attached to other particles. This lack of information is mainly due to the usually applied sampling methods such as impingement, filtration and impaction on nutrient plates. Most airborne particles are disintegrating during sampling and probe processing and only the numbers of culturable micro-organisms which form colonies on agar plates can be quantified. Some investigators found that the amount of culturable micro-organisms from the exhaust air of swine barns can be reduced by biofilters to approximately 90 % when they are designed correctly (e. g. Seedorf & Hartung 1998). The influence of such devices on the total amount of micro-organisms as well as on particle composition is not known.

In this investigation a novel method according to Clauß et al. (2010) was tested for this purpose and compared to impingement. Airborne particles were gently impacted on special silicone surfaces with over 95 % sampling efficiency, stained and then investigated under a fluorescent microscope. The method provides discrimination between inorganic and organic particles and different particle size fractions. In addition micro-organisms, single cells and aggregates, separated or attached to other particles, can be stained and counted.

MATERIAL AND METHODS

The sampling was performed on a MagixX exhaust air washer (Big Dutchman, Germany) installed in a pig fattening house with 2000 fattening places. The measurements took place at one day in summer around 12:00 o'clock when ventilation rates were high.

Impingement was performed in raw gas and clean gas parallel with three AGI-30 impingers filled with 30 mL PBS to determine colony forming units. The impinger flow rate was 12.5 L/min, sampling time was 30 minutes. After sampling aliquots from the sampling liquid were plated on blood-basis agar and incubated 48 h at 30°C.

The sampling of airborne particles by impaction onto silicone coated microscope slides for determination of total bacteria in different particle size fractions took place at the same sampling positions as impingement. Sampling time was 1 min. with a flow rate of 1.2 L/min. Collected particles were stained directly on the silicone surface with 10 µL SYBR®Safe and washed after 5 minutes with distilled water. Pictures were taken under a Primostar fluorescence microscope (Zeiss, Germany) equipped with a C5 CCD-camera (Jenoptik, Germany) for the latter analysis. Inorganic and organic particles (incl. micro-organisms) were discriminated in the particle size fractions < 5, < 10, < 20, < 40, < 60, < 80, < 100, and < 200 µm. In addition, all detected microbial cells were counted. For

reasons of better comparison, all values were converted into particles/m³ and microorganisms/m³ respectively (total count and cfu).

RESULTS

Figure 1 shows the number of different particle fractions in raw gas and clean gas of the bio-washer. The total particle number in raw gas (1.15 x 10^6 m-³) was remarkably close to clean gas (1.14 x 10^6 m-³). The fraction of inorganic particles was in clean gas twice as high as in raw gas but with less than 1 % very low. The number of organic particles without bacteria (9.11 x 10^5 m-³) was in clean gas slightly higher than in raw gas (7.69 x 10^5 m-³). The number of organic particles containing bacteria in raw gas (3.37 x 10^5 m-³) was more than twice as high as in clean gas (1,20 x 10^5 m-³). Concentration of mould spores and yeasts in raw gas (3.00 x 10^3 m-³) was 10 times higher than in clean gas (3.60 x 10^4 m-³). The total bacterial count in raw gas (5.17 x 10^6 m-³) was only 30% higher than the number of cfu obtained with impingement (3.29 x 10^6 m-³). The total bacterial count in clean gas (4.82 x 10^5 m-³) obtained when using the new sampler was 5 times higher than the culturable fraction (9.68 x 10^4 m-³) obtained by impingement.

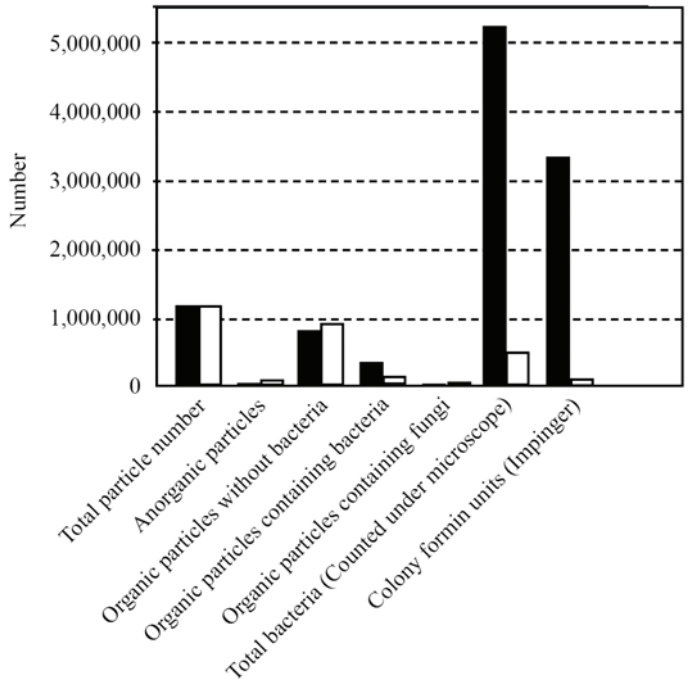

Figure 1: Different particle fractions in 1 m³ raw air ■ compared to clean gas

The different ratios between total bacterial count and cfu in raw gas and clean gas can be explained by the assumption, that bacteria in larger aggregates or on larger particles are better protected from drying or other negative environmental conditions and may be longer culturable. Most of the bacteria in raw gas were found in the particle fraction 80 to 100 µm (Figure 2). Only 40 % of the particles are smaller than 10 µm and the biggest are above 100 µm (Figure 3). In contrast in clean gas most of the bacteria were found in the particle fraction 10 -20 µm. Over 90 % of the particles are smaller than 10 µm and no particles could be found bigger than 40 µm.

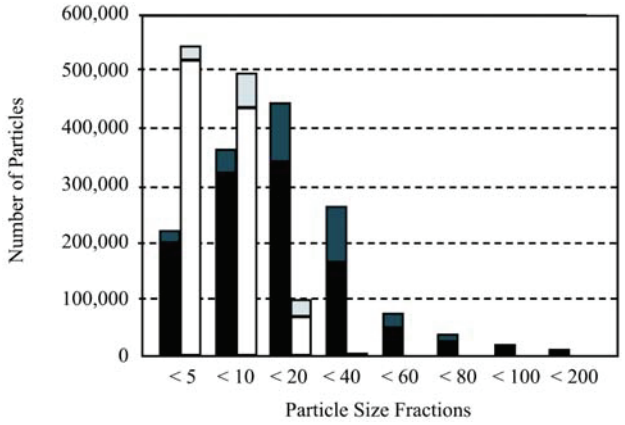

Figure 2: Number of particles with (gray scales) and without (black/white) bacteria in different particle size fractions in 1 m³ raw air ■ compared to clean air □

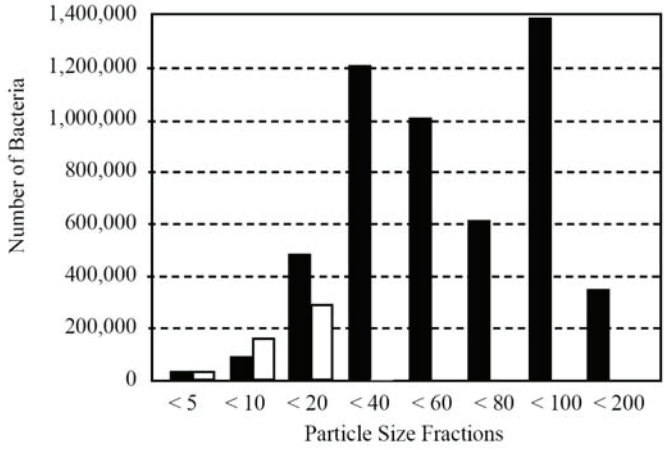

Figure 3: Total bacteria in 1 m³ of air found in the different particle size fractions in raw gas ■ and clean gas □.

Another important point is that the number of particles in raw gas and clean gas is the same (Figure 1), but in clean gas a high amount of small organic particles containing bacteria was found (Figure 2, 3) which have the potential to be inhaled deep into the thorakal or even alveolar region of the lung where they may cause respiratory health problems.

CONCLUSIONS

The number of particles collected in raw gas and clean gas was very similar. The number of the total bacteria counted under the microscope was higher than the number of culturable bacteria (cfu). Most micro-organisms were in aggregates or attached to other particles. The clean gas contained a high amount of small organic particles (<5 µm) with many carrying bacteria which can be inhaled deep into the thorakal or even alveolar region of the lung where they may cause respiratory health problems.

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AIRBORNE DISTRIBUTION OF BIO-AEROSOLS OF DIFFERENT SIZE AND COMPOSITION AFTER PASSING A BIO-SCRUBBER

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INTRODUCTION

Rising concerns about detrimental emissions from animal housings, such as dust particles, odours, gases and micro-organisms led to an increasing enforcement to install abatement techniques, especially exhaust filtration systems, both in existing and new-built animal houses. A promising method is the combination of bio-washers and bio-filters: after a washing step where larger particles, some gases and odours are partially removed from of the exhaust air, the air flows through a packed layer of irrigated materials (e.g. wood scrubs or ceramic pellets) where the remaining pollutants are absorbed by a thin bio-film on the surface of the packing material. Micro-organisms, including bacteria and fungi are immobilized in this bio-film and degrade the transferred pollutants. Reduction efficiencies of up to 90 % are reported. However, recent studies show that particles in the air stream before and behind such a bio-scrubber not only differ in their bacterial load but also in their size. Little is known about the distribution of such small particles leaving a bio-filter system in the ambient air. Therefore, a model was applied to compare the dispersion of of raw and clean gas particulates. an atmospheric dispersion modelling was conducted to evaluate the differences in the dispersion of raw and clean gas particulates.

MATERIAL AND METHODS

Particle counts of different sizes per litre unfiltered and filtered air were taken from an assessment study of an exhaust air washer installed in a conventional pig fattening unit [(1)]. Emission rates were calculated by using the found particle concentrations and the mean ventilation rate of the fattening unit for 2000 pigs.

The total particle concentrations of both investigated exhaust air types were very similar, with 1.2 x 10^7 particles per second in the raw exhaust air and 1.1 x 10^7 particles per second in the filtered exhaust air respectively. But the shares in particle fractions differed between the two types: the majority of particles in the filtered air was found to be sized smaller than 5 µm, whereas the particles in untreated air were larger and even particle sizes ranging between 100 and 200 µm were found (table 1).

The data were calculated and transferred to the dataset for dispersion modelling with the particle model LaSAT (Lagrange Simulation of Aerosol Transport)[(2)] . Source type (point source) and height (7 m) were set as typical for a fattening unit for 2000 pigs with and without a filter system, and weather conditions were set as found typical for northern Germany (wind speed 4 m/s, wind direction 290°), dispersion category and roughness length were chosen to be 3.1 (stable to neutral with only slight turbulences) and z0=0.1 (open landscape with low crops, cultivated area).

After calculation of the wind fields over a period of 12 hours and calculation of the particle concentrations in a 5000 x 5000 m grid the emission plumes were plotted in two-dimensional graphics for comparison.

Table 1: Emission rates dataset for the dispersion modelling of airborne particles in untreated exhaust air and exhaust air after passing a bio-scrubber in a conventional pig fattening unit.

	particle emission rates (particles/s)							
	total particles emitted	Particle fraction, aerodynamic diameter (µm)						
		0-5	5-10	10-20	20-40	40-60	60-80	80-100	100-200
untreated exhaust air	1.2 x 10^7	2.0 x 10^6	3.3 x 10^6	3.6 x 10^6	1.7 x 10^6	4.9 x 10^5	2.5 x 10^5	1.5 x 10^5	3.1 x 10^4
% of total particles	100	18	29	31	15	4	2	1	<1
exhaust air after bio-scrubber	1.1 x 10^7	5.4 x 10^6	4.5 x 10^6	7.4 x 10^5	4.2 x 10^4				
% of total particles	100	50	42	7	1				

Tab. 2: Deposition and sedimentation velocities in cm/s (vd and vs) after TA Luft (2002) [3] and total bacteria cell counts in different particle fractions emitted from a pig fattening unit without and with exhaust air treatment.

			total bacteria counts (cells/s)	
particle fraction, aerodynamic diameter	vd (cm/s)	vs (cm/s)	untreated exhaust air	exhaust air after bio-scrubber
total	-	-	5.4×10^7	5.0×10^6
0-20 µm	0.01	0.00	6.4×10^6	5.0×10^6
20-40 µm	0.05	0.04	1.3×10^7	4.2×10^4
40-60 µm	0.2	0.15	1.1×10^7	0

RESULTS AND DISCUSSION

After emission the concentrations of particles are diluted constantly by aerial transportation, deposition and sedimentation. According to their aerodynamic properties, smaller particles can be transported over longer distances than larger ones which deposit quickly after emission. The total particle emissions of both untreated and filtered exhaust air are in the same range (Tab. 1), but since the majority of airborne particles in exhaust air after passing a bio-scrubber is smaller than 5 µm, the aerial distribution of particles from raw and filtered exhaust air show considerable differences. Figure 1 gives the calculated concentration plumes of the particle fraction up to 5 µm in raw and filtered exhaust air. While particles of this size from raw exhaust air are still found (at least 2 per m²) at a distance of 2000 m, the maximum distance is calculated to be nearly 5000 m when dealing with the fine particles from filtered exhaust air.

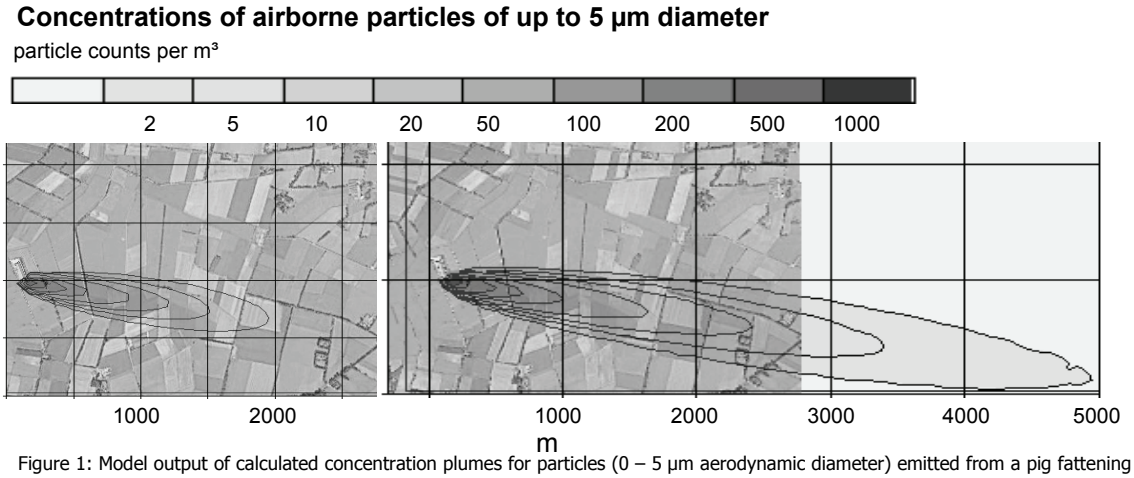

Figure 1: Model output of calculated concentration plumes for particles (0 – 5 µm aerodynamic diameter) emitted from a pig fattening unit with exhaust air filtration (bio-scrubber) (right) and without (left) under the same weather conditions.

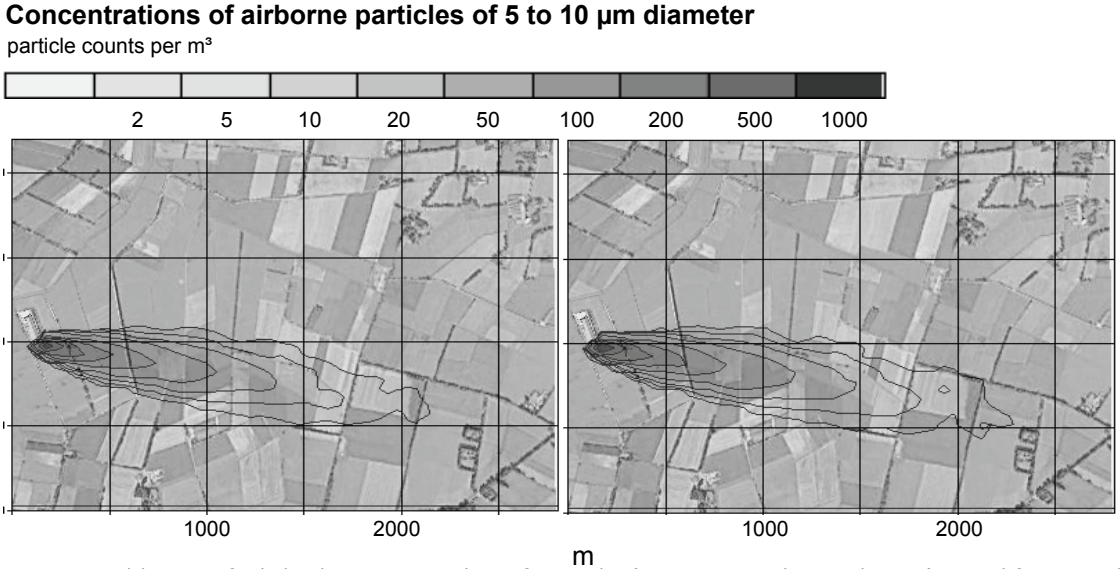

Figure 2: Model output of calculated concentration plumes for particles (5 – 10 µm aerodynamic diameter) emitted from a pig fattening unit with exhaust air filtration (bio-scrubber) (right) and without (left) under the same weather conditions.

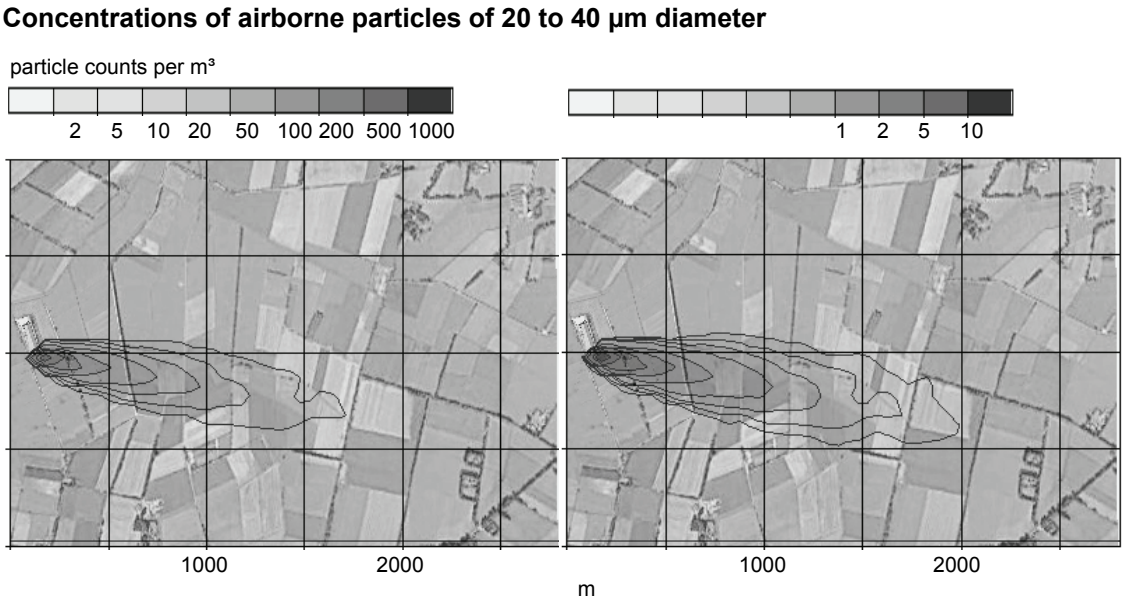

Figure 3: Model output of calculated concentration plumes for particles (20 – 40 µm aerodynamic diameter) emitted from a pig fattening unit with exhaust air filtration (bio-scrubber) (right) and without (left) under the same weather conditions.

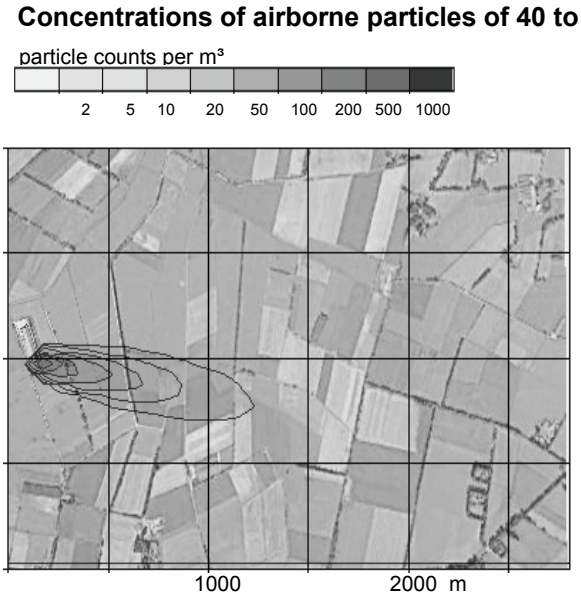

Figure 4: Model output of calculated concentration plume for particles (40 – 200 µm aerodynamic diameter) emitted from a pig fattening unit without exhaust air filtration (bio-scrubber).

When looking at other particle fractions the maximum distance lengths, where particles from the pig fattening unit can be found in the ambient air, change: While the concentration plumes for the particle fractions of 5 to 10 µm diameter in raw and filtered exhaust air are congruent nearly (figure 2), the maximum distance lengths, where at least 2 particles/m³ can be found, measures about 400 m when calculating particles from filtered air and is still about 1600 m for untreated exhaust air (figure 3). Larger particles (< 40 µm aerodynamic diameter) can only be found in unfiltered exhaust air and deposit quickly due to their aerodynamic properties, so that their transportation distance is rather short (figure 4).

CONCLUSIONS AND OUTLOOK

Presumably due to the higher share of particles smaller than 5 µm, particles emitted from a pig fattening unit after passing a bio-scrubber system can be transported over longer distances than particles in unfiltered exhaust air. Neighbouring animal housings and nearby residents may be exposed to fewer pollutants (especially when looking at bacteria cell counts, table 2) than without a filter systems, because of both the reduction of pollutants of up to 90 % and the slower decline of particles during aerial transportation. But the radius of potential influence of particles has to be drawn wider than without filtration. Further investigations are necessary to support this calculation results by measurements in the field. There should be also more research in order to understand the nature of the particles emitted from the bio-system and the bio-films themselves.

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SIZE AND COMPOSITION OF AIRBORNE BACTERIA AGGREGATES COLLECTED IN ANIMAL HOUSE AIR BY A NOVEL IMPACTOR SYSTEM

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SUMMARY

To evaluate shape and composition of airborne particulate matter from animal houses, dust-particles and micro-organisms were collected in chicken and pig houses as well as in "clean" ambient air by a novel bioaerosol impactor using special silicone surfaces. Analysis took place under a fluorescence microscope in the lab after staining with SYBR®Safe fluorescence dye. More than 90 % of particles in ambient air were of inorganic nature. In contrast more than 90 % of particles in animal houses were of organic nature and half of them contain bacteria. Bacterial cells in animal house air mostly appear in large aggregates whereas mould spores can be found predominantly as single cells. The lowest number of bacterial cells was found in ambient air followed by distinctly higher numbers in pig houses and highest numbers in chicken houses. The number of mold spores didn't differ much at the sampling places.

INTRODUCTION

The exhaust air of animal houses contains high amounts of particulates like dust-particles and micro-organisms which originate from feed, litter, faeces and skin and hair or feathers of the animals (Seedorf & Hartung 2002). Little is known about shape and composition of these viable and non-viable particles and whether they mainly appear as single cells or larger aggregates in an airborne state which is of high interest e.g. for designers of abatement technologies such as air scrubbers and for risk assessors in human and veterinary medicine. Therefore it is important to have a tool at hand for the fast analysis of shape and composition of such airborne particles. This can help quickly to assess risks for animal and human health in the field. In this regard, sampling of airborne particles by impaction on special silicone surfaces and fluorescence microscopic image analysis provides a fast and reliable solution.

MATERIAL AND METHODS

Airborne particles were sampled in three different chicken houses and three different pig houses and compared to three samples of "clean" ambient air. All samples were taken from February to April. The particles were collected by impaction on novel adherent silicone surfaces and treated with SYBR®Safe fluorescence dye to distinguish bacterial cells from background debris as recommended in Clauß et al. (2011). Analysis of collected and stained particles took place under a fluorescence microscope in the lab. For pig houses, chicken houses and ambient air, ratios between inorganic and organic particles were calculated as well as the number of particles containing micro-organisms and the number of total microbial cells and mold spores. In each case, the appearance of particles that contain micro-organisms was observed.

RESULTS AND DISCUSSION

The average total bacterial count was clearly lower in ambient air (4,620 bacteria/m³) compared to pig house air (1,724,000 bacteria/m³) and chicken houses (2,801,200 bacteria/m³) (Table 1). In contrast, the average number of mold spores was with about 1,000 spores/m³ similar at each sampling place.

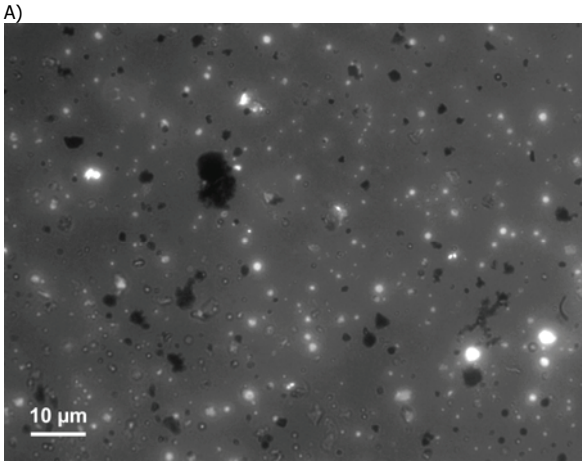
Table 1: Concentrations of different particle fractions in ambient and animal house air (means of n=3)

Particle fractions in %	Ambient Air	Pig House	Chicken House
Inorganics particles	90.6 (+/- 50.1)	4.2 (+/- 2.3)	3.3 (+/- 1.5)
Organic particles without bacteria	7.3 (+/- 12.3)	54.9 (+/-35.69)	31.4 (+/- 41)
Organic particles containing bacteria	0.1 (+/- 0.4)	40.6 (+/- 34.02)	65.0 (+/- 40,4)
Mold spores	2.0 (+/- 2.9)	0.3 (+/. 0.2)	0.3 (+/- 0.3)
Total number of countable cells			
Total bacterial count	4,620 (+/- 2,310)	1,724,000 (+/- 99,470)	2,801,200 (+/- 800,500)
Mold spores	980 (+/- 120)	804 (+/- 86)	1,160 (+/- 408)

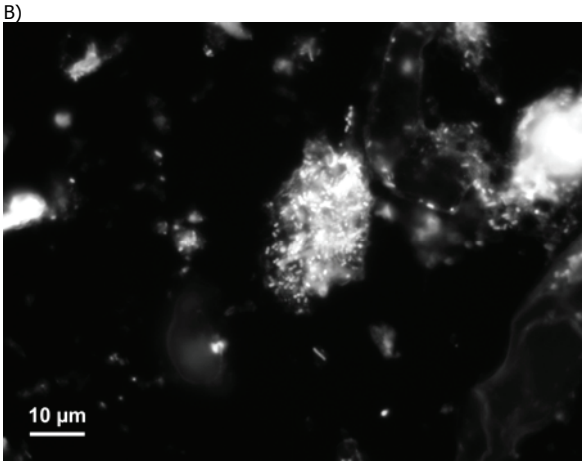
More than 90 % of particles collected in ambient air were of inorganic nature. About 2 % of the collected particles were mold spores and only 0.1 % of the organic particle fraction contained bacteria. In contrast, in pig and chicken houses 95 % of particles were organic and about half of them contained bacteria. The percentage of mold spores was with 0.3 % lower than in ambient air, but the total concentration of dust particles and microorganisms was much higher in animal housings. Therefore, a sampling time of 30 seconds was sufficient to collect sufficient particles for later analysis, whereas in ambient air a minimum of 5 to 10 minutes was necessary. Generally, it has to be taken into account that standard deviations for the presented data were very high. Concentrations of dust particles and micro-organisms in the air are depending on many factors like season, temperature, humidity and UV-radiation (Jones & Harrison 2004) as well as in animal houses age of the animals, day-time or litter (Seedorf & Hartung 2002).

It was observed that airborne bacteria from animal housings almost always appeared as large aggregates or were attached to other particles (Figure 1). Very rarely they appeared as single cells. The microscopic view showed that a lot of the collected micro-organisms were embedded in kind of an "organic matrix" or in small liquid droplets. Strong staining results indicated intact DNA and active cells. In contrast, most bacteria cells from ambient air appeared in small aggregates or sometimes as single cells. Most of them were stained poorly. The used fluorescence dye intercalates with the DNA so that poor staining results indicate damaged DNA, probably harmed due to solar UV-radiation. Most of the mold spores were – in contrast to the bacterial aggregates – found as single cells. Most of the inorganic particles appeared as opaque black sooty particles or round "pebbles" with typical diameters of 1 – 3 µm.

A)



B)



C)

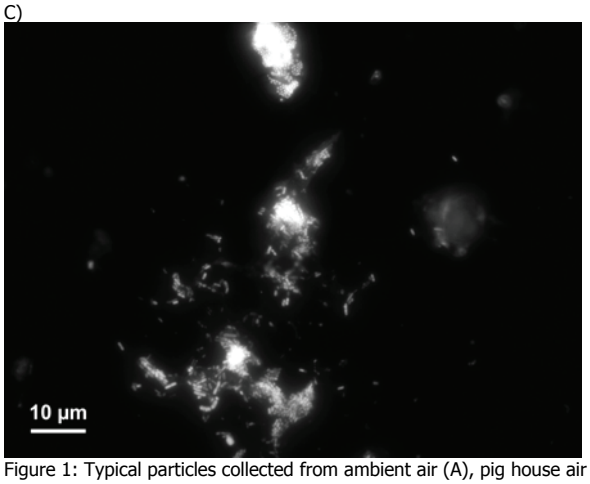


Figure 1: Typical particles collected from ambient air (A), pig house air (B) and chicken house air (C).

CONCLUSIONS

More than 90 % of particles in ambient air were of inorganic nature. In contrast more than 90 % of particles in animal houses were organic,half of them containing bacteria. Bacterial cells in animal house air mostly appear in large aggregates, mold spores can be found predominantly as single cells. This should be taken into consideration in various fields such as bio-aerosol sampling, constructing of abatement techniques or when assessing the dispersion of bio-aerosol plumes from animal houses.

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THE GAS POLLUTION OF THE AIR IN THE STABLE DEPENDING ON THE ABSORB HEIGHT OF THE SAMPLE

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SUMMARY

The investigation of nine chemical compounds in the air of a stable, affecting harmfully both animals and their caretakers (NH_3, CO_2, CO, NO_2, SO_2, COS, HCN, C_8H_8) and also appearing as greenhouse gases (CO_2, COS, CH_4) affecting the natural environment was conducted. For the investigation the mobile gas pollution analyzer Gasmet DX – 4030, functioning on the base of modern method FT-IR (Fourier Transform InfraRed Spectroscopy) was used. It was stated that all of chemical compounds were fund in the stable air in at least trace amounts. For some gas chemical compounds the tendency of concentration of gases in the stable air depending on the height from which they were collected was noticeable.

INTRODUCTION

The domestication of horses and their use in sport and leisure activities are closely linked with housing, especially the utilization of box stalls. High sport class horses spend most of their lives – up to 23 hours per day – in their stalls. Therefore, the quality of the surrounding horse stables air is an important factor in maintaining the good health of horse, because the equine respiratory tract is sensitive to noxious gases [2]. Besides that horse breeding centers keep numerous horse herds, which nevertheless affect the natural environment. The gas compounds, which were the theme of own analysis, represent a serious danger for both animals and people staying in stable and also for the environment. Ammonia (NH_3) is one of the most import and noxious gases present in stable air and one that can damage the respiratory tract [2]. At greater than 100 ppm, NH_3 irritates eyes and respiratory membranes. NH_3 affects the course and intensity of microbial or parasitic infections in animals in these facilities [7]. Carbon dioxide (CO_2) belongs to the group of greenhouse gases, but in high concentration (over 4 – 5%) it may be harmful for animals and humans. Although that in practice, the concentration of CO_2 in the livestock facilities is never that high to affect animal physiological adverse, exceeding the allowable level of the gas (3000 ppm) is an index of faulty ventilation system [4]. Sulfur dioxide (SO_2) is a chemical compound with pungent and irritant smell as well it lowers the health and comfort of living, both humans and animals. Its odor threshold values amount to 0,009 ppm [8]. Furthermore it is the cause of acid rain and a compound of smog. Carbon monoxide (CO) is strongly toxic because it binds about 250 times as tenaciously to hemoglobin as oxygen, forming carboxyhemoglobin (COHb). The concentration in the air above 1600 ppm may be the cause of death by suffocation [4]. Hydrogen cyanide (HCN) is a highly toxic gas, because it blocks molecular oxygen transfer in cytochrome oxidase systems in mitochondria causing tissue anoxia, which in effect may cause poisoning [7]. Inhaled nitrogen dioxide (NO_2) can pass through the upper airways and permanently damage pulmonary parenchyma. At higher levels, e.g. 20 ppm, NO_2 animals have coughing, some fluid in the lungs. Animals that die at varying times after exposure have evidence of pulmonary edema and emphysema [7]. Fumes of styrene (C_8H_8) have negative influence on air – passages and irritate eyes. In humans body it has depressive effect on central nervous system and it irritates mucous membrane [10]. Inhalation of carbonyl sulfide (COS) causes irritation of mucous membrane. It was shown, that if there was a mucous membrane irritation in rabbits exposed at 1343 ppm, and at very high exposure concentrations (> 3053 ppm) evidence of irritation was observed at necropsy and consisted of hyperaemia of the tracheal mucosa and the peritoneal cavity, and blood pooled in the lungs [1]. COS also belongs to the group of greenhouse gases. The significance of presence of methane (CH_4), as one of greenhouse gases, consists in its important role in the global warming. Agricultural emissions of methane in the EU have recently been estimated at over 10 million tones per year and represents the greatest source. Of these, one – third come from livestock manure [5]. To develop environmentally sound, sustainable agricultural operations it is necessary to integrate research that focuses on modern analytical techniques and latest sensory technology of measurement, together with fundamental knowledge of factors that are the basic units contributing to the production of odour and pollutants [6]. Among methods used for measuring gas pollution can be distinguished analytical methods e.g. gas chromatography and methods which use multidetectors devices with selective detectors, sensitive to different air – polluting compounds such as fotoionisation detectors [8]. The mobile gas – meter Gasmet DX – 4030 employing FTIR (Fourier Transform InfraRed Spectroscopy) analysis producing almost intstantaneous readings for multiple compounds across a wide measurement range that extends to sub – ppm levels, brings new possibilities in gas pollution investigations. The aim of this study was a quantitative identification of nine noxious chemical compounds present

in the stable air (including 3 greenhouse gases) in samples collected from three different heights (20 cm, 150 cm, 170 cm) according to the bedding level.

MATERIAL AND METHODS

The study was carried out in a stable keeping recreational horses. The length of the building was 52 m, width 11 m, height 4 m. The area of usable facilities (72 m^2) which was walled – off from the rest of the building and was not taken under consideration during enumerating the cubature (2000 m^3) and cubic capacity (100 m^3) per horse. The usable area of stable measured 500 m^2. In the centre of the building there was a corridor. On its side there were 8 box – stalls (4 m x 3 m), usable facilities, grooming stands and on the other side there were 6 box – stalls (4 m x 4 m) and 6 box – stalls (4 m x 3 m). In both gable walls there were entrance gates, divided into four sections that opened individually to the outside. The manure was removed from the stalls couple times a day, and a complete removal of used straw was carried every fourth day. Besides that the building was regularly aired during the day. For measuring the air gas pollution the mobile gas analyzer Gasmet DX – 4030 employing FTIR was used. Samples were collected during 7 days, 3 times a day (at 4:00, 13:00 and 21:00). Measurement was conducted on two levels representing the height of horse's head during resting in a lying position (20 cm) and during standing (150 cm) and one measurement representing the height of a human (170 cm). The investigation was conducted in 6 stall – boxes and in 3 points in the corridor (two points near the door and in the middle of a building). To characterize chemical compounds, from collected air samples a computer program Calcmet Pro, containing a library enabling identification of presence and concentration of 9 chosen chemical compounds: oxides (CO_2, CO, NO_2, SO_2, COS), ammonia (NH_3), hydrogen cyanide (HCN), styrene (C_8H_8) and methane (CH_4).

RESULTS

All groups of investigated gas compounds were found in the stable air.

Table 1. Air pollution in stable buildings (±SD, max)

Gas Component	Height (cm)	$\overline{x}$ (ppm)	±SD (ppm)	Maximum (ppm)
Carbon dioxide CO_2	20	1778.8	1250.4	2350
	150	1944.4	1450.4	3700
	170	2000.0	1850.7	3800
Carbon monoxide CO	20	0.24	0.30	0.99
	150	0.24	0.30	1.09
	170	0.24	0.29	1.03
Nitrogen dioxide NO_2	20	0.05	0.14	0.99
	150	0.06	0.14	1.09
	170	0.08	0.16	1.03
Sulfur dioxide SO_2	20	0.63	0.62	2.26
	150	0.75	0.69	2.43
	170	0.79	0.70	2.60
Carbonyl sulfide COS	20	0.03	0.04	0.30
	150	0.03	0.03	0.12
	170	0.03	0.03	0.12
Ammonia NH_3	20	1.19	1.36	7.13
	150	1.10	1.32	6.92
	170	0.96	1.17	5.49
Hydrogen cyanide HCN	20	0.28	0.26	1.23
	150	0.24	0.27	1.06
	170	0.25	0.26	0.95
Styrene C_8H_8	20	0.09	0.23	1.31
	150	0.07	0.16	0.95
	170	0.10	0.22	1.31
Methane CH_4	20	7.07	6.39	23.39
	150	8.41	7.62	38.16
	170	8.18	7.62	34.28

DISCUSSION

The adaptation of a very sensitive device for livestock environment allowed to define the concentration of all requested gas compounds, which, in a farm conditions appear in threshold values. The received results of gas pollution compounds in the stable air (Table 1) are incomparable, due to the lack of data in the literature considering detected in own investigation gas compounds. Available data considers only NH_3 and CO_2. Comparing received results of the NH_3 and CO_2 level in own investigation (Table 1) to the allowable level 20 ppm for NH_3 and 3000 ppm for CO_2 [9] it needs to be stated that the received result show a very good care for the condition of bedding and a proper air exchange in the stable. Continuous exposure of rabbits to 0.5 ppm HCN produced no microscopically detectable morphological changes of the lungs, pulmonary arteries, coronary arteries or aorta, however exposing dogs to 45 ppm HCN demonstrated extensive CNS toxicity, including dyspnea and vomiting, with vascular and cellular CNS lesions identified post-mortem [11]. It is possible to assume that the concentration of HCN revealed in own investigation did not constitute hazard for horses. Sensitivity of the used method also allowed to display insignificant tendency to different concentration of gases depending on the height from which the air samples were collected. Concentration increase together with the height from the bedding were observed in case of CO_2, NO_2, SO_2, C_8H_8 and CH_4. Refering to ammonia the opposite tendency has occurred, whereas the height from which the samples were collected did not differentiate concentrations of CO and COS.

CONCLUSIONS

Received results indicate that the mobile gas analyzer Gasmet DX – 4030 is very useful for the identification of gas compounds, which appear in livestock facilities in threshold values, but which are toxic and characterize by bothersome smell or belong to a group of greenhouse gases. Sensitivity of the method also allowed to display the differences, although insignificant in the concentration of gases depending on the collecting air samples height.

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SIZE DISTRIBUTION OF AIRBORNE PARTICLES IN ANIMAL HOUSES

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SUMMARY

The objective of this study was to determine concentration and size distribution of airborne particles inside and outside animal houses for broilers, broiler breeder (with bedding); layers (floor or aviary housing system); turkeys (with bedding), pigs: fatteners (traditional house, low emission houses (dry feed, or wet feed), piglets, sows (individual or group housing); cattle (cubicle house), and mink (cages). Dust concentrations, both in counts and mass, in the different particle size ranges were highest in poultry houses. The concentrations in pig houses were higher than those in cattle and mink houses. The count particle size ranges < 1.0 µm was highest with average of 95%, while mass particle was highest in size ranges > 2.5 µm (on average 95%). Most count particles outside were in the size range < 1.0 µm (99%).
Keywords: Particle size distribution, animal houses, dust concentration

INTRODUCTION

In animal houses, especially those for pigs and poultry, air quality can be seriously impaired by high dust concentrations (Wathes, Holden et al. 1997; Takai, Pedersen et al. 1998). These cause health problems for humans working in this environment (Donham, Reynolds et al. 1995; Herr, Bittighofer et al. 1999; Pope, Burnett et al. 2002; Andersen, Von Essen et al. 2004), and probably also for the animals living in these houses (Al Homidan and Robertson 2003). Also, airborne particles from inside the animal houses can escape the building via exhausted air and contribute to particle concentrations in the ambient air (Takai, Pedersen et al. 1998; Seedorf and Hartung 2000). One of the most important characteristics of dust is its size and its related size distribution, because this influences the behaviour and transport of the particles in the air and the choice of control technology (Zhang 2004). Particle size determines the impact of dust on human and animal health, as well (Mercer 1978). Particles size PM10, PM2.5, and PM1 are mainly responsible for health problems because they can travel into the respiratory system (Collins and Algers. 1986). The smaller the particles are, the deeper they can penetrate into the respiratory system and the greater their impact is on animal and human health.

Despite of many efforts, knowledge on particle size distribution (PSD) is still limited, for example particle size distributions in a wide range of animal houses and categories. The objective of this study was therefore to determine the particle size distribution, counts and mass, in different commercial animal houses in the Netherlands.

MATERIAL AND METHODS

Animal houses

PM10 mass and PSD were determined in houses of 13 different combinations of animal species/housing types, located in the Netherlands. Each species/housing combination was measured at two farms (replicates) at two time points in spring and summer 2009. The following animal species/housing combinations were studied: broilers, layers housed in floor system (layer_floor), layers in aviary system (layer_aviary), broiler breeders, turkeys, piglets, fattening pigs in traditional houses (fat_pig_trad), fattening pigs in modern low-emission housing with dry feed (fat_pig_mod_dry), fattening pigs in modern low-emission housing with wet feed (fat_pig_mod_wet), sows in individual housing (sow_individual), sows in group housing (sow_group)), dairy cattle (cattle), and mink.

Dust sampling

PM10 mass concentrations and PSD in counts were both measured using aerosol spectrometers based on the light-scattering principle. PM10 mass concentrations were measured with a DustTrak monitor (TSI inc., 500 Cardigan road Shoreview, MN 55126-3996, USA) **,** whereas PSD in counts was measured with a Grimm instrument model number 1.109 (Grimm Aerosol Technik GmbH & Co., Ainring, Germany)

RESULTS

PM10 mass concentration

PM10 mass concentrations were highest in layer_floor (3.78 mg m^{-3}) followed by layer_aviary (2.81 mg m^{-3}), turkey (1.87 mg m^{-3}), broiler (1.42 mg m^{-3}), piglet (1.15 mg m^{-3}), broiler_breeder (0.89 mg m^{-3}), fat_pig_trad (0.87 mg m^{-3}), fat_pig_mod_dry (0.65 mg m^{-3}), fat_pig_mod_wet (0.47 mg m^{-3}), sow_group (0.30 mg m^{-3}), sow_individual (0.18 mg m^{-3}), mink (0.07 mg m^{-3}) and cattle (0.07 mg m^{-3}).

Particle size and size distribution

Most particles inside the animal houses were found in the size ranges smaller than 1 µm: on average, 87.0% of total number of particles; 5.5% were in the size range 1 – 2.5 µm; 7.3% in the size range 2.5 – 10 µm and 0.24% in the size range 10 – 32 µm. In the outside air, 99.2% of the particles were smaller than 1.0 µm; 0.7% was in the size range 1.0 – 2.5 µm; 0.1% in the size ranges 2.5 – 10 µm and 0.005% in the size range 10 – 32 µm.

Figure 2 shows the standardized number fraction of particles in poultry, pig, cattle, and mink houses. The standardized number fraction for outdoor particles is given in each sub-figure, for comparison. For all animal house categories and also for outside samples, the highest fraction of particles was in the size range 0.25 – 0.30 µm. Number fractions decreased sharply with increasing particle size. For pig and poultry houses, two small peaks were observed: one between 0.65 to 0.70 µm, and one between 2.5 to 3.7 µm. It is obvious from Figure 2 that within the animal houses, especially those for poultry and pigs, the number fractions of the larger particles were much higher than outside.

Mass distribution

Particle size distribution in mass is dominated by particles in the size range > 2.5 µm. On average, 0.5% of particle mass was smaller than 1.0 µm, 2.0% of particle mass was in the size range 1 – 2.5 µm, 50.3% of particle mass in the size range 2.5 – 10 µm, and 47.3% of particle mass was in the size range 10 – 32 µm. For outside air, 11.0% of particle mass was smaller than 1.0 µm, 5.9% of particle mass was in the size range 1.0 – 2.5 µm, 17.1% of particle mass was in the size range 2.5 – 10 µm, and 66.0% of particle mass was in the size range 10 – 32 µm.

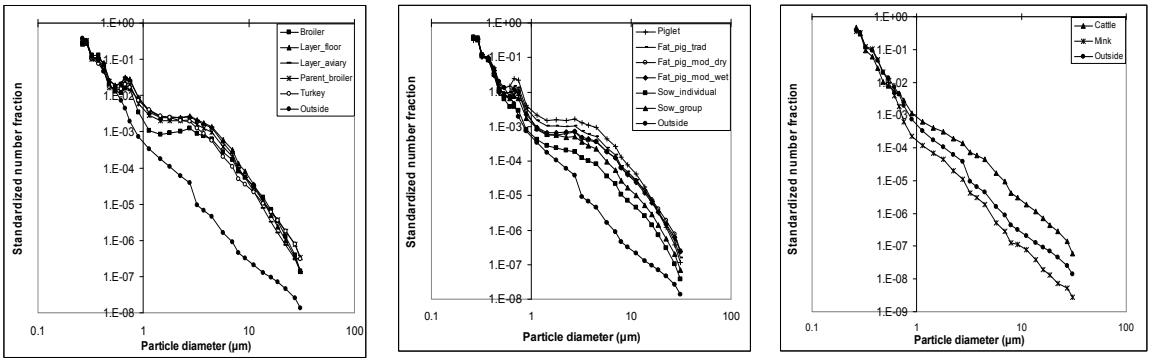

Figure 1. Standardized number fraction (at log-scale) of particles in the different size ranges (at log-scale) in 5 species/housing combination for poultry (left), 6 for pigs (middle) and 1 cattle house and 1 mink house.

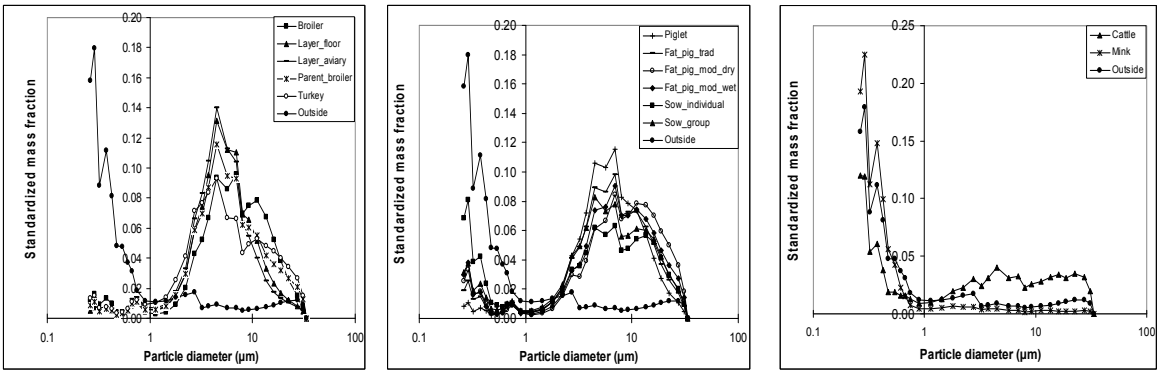

Figure 2. Standardized mass fraction of particles in the different size ranges (at log-scale) in 5 species/housing combinations for poultry (left), 6 for pigs (middle) and 1 cattle house and 1 mink house.

DISCUSSION

The high PM10 concentrations in poultry houses were most probably related to the presence and use of bedding by the hens for scratching and dust-bathing. The litter contains the main dust sources i.e. manure and feathers (Cambra-Lopez, Torres et al. 2010). In layer houses with battery cages a lot lower dust concentrations were reported (Takai et al., 1998). The low dust concentrations in cattle and mink houses are probably the result of a low dust production in combination with a high ventilation rate in the open naturally ventilated buildings. The number of particles smaller than 1.0 µm in pigs, cattle and mink houses did not differ much from the number of particles in this size range measured outside. This corroborates the hypothesis that the small particles in animal houses mainly come from outside (Zhang, Tanaka et al. 1998).

High variations in particle concentrations occurred not only between animal species/housing combinations, but also between farms within the same category, as shown by the relatively high standard error of means (Figure 2). This is in agreement with the findings of Martin et al. (1996) who also reported high variations in dust particle concentrations between animal houses. This is caused by the fact that each animal farm has its own control and managing practices and its own details in housing design, for the variations within farms of the same category in that study(Martin, Zhang et al. 1996) was the fact that farms were not sampled on the same day and at the same moment in the production cycle (Martin, Zhang et al. 1996).

The standardized mass distribution for the different animal species/housing combinations is contrary to the standardized count distribution. The standardized mass distribution of particles inside had a very different pattern than the pattern outside. Because of the relatively high numbers of small particles and very few big particles outside, the contribution of the small particles to mass was relatively large, while the mass of inside particles was dominated by the bigger particles. The standardized mass distributions of particles inside cattle and mink houses were very similar to those outside. As mentioned earlier, a possible explanation is the low particle production inside these houses and the high ventilation rate in these open naturally ventilated buildings.

CONCLUSIONS

- In terms of counts and mass, the dust concentrations in the different particle size ranges are generally higher in poultry houses than in pig houses, and are generally higher in pig houses than in cattle houses and mink houses.
- Particle counts in mink and cattle houses are more or less similar to the particle counts in outside air for all particle size ranges.
- Particle counts in animal houses are highest in the size range < 1.0 µm (87%), while particle mass is highest in size ranges > 2.5 µm (95%). Most particles outside are in the size range < 1.0 µm (99% in counts).

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For the reference list contact the corresponding author.

AIRBORNE BACTERIA IN FREE-STALL DAIRY BARNS

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SUMMARY

The aim of this study was to assess the microbiological quality of the indoor air in free-stall dairy cattle barns, by identification of bacteria and determination of their numbers. We investigated 24 dairy barns with free-stalls, in Transylvania, in winter period. The number of bacteria varied in the investigated barns and it was slightly higher in the evening as opposed to the morning, but the differences were statistically insignificant (Mann-Witney Test, $p>0.05$). The mean number of bacteria in the morning and in the evening was: 7.60 – 8.82 x 10^4 cfu/m^3 for mesophilic bacteria, 1.99 – 2.49 x 10^4 cfu/m^3 for staphylococci, 1.15 – 1.49 x 10^4 cfu/m^3 for streptococci and 5.99 – 7.62 x 10^3 cfu/m^3 for gram negative bacteria. The isolated microbial flora with pathogenic and conditional pathogenic potential was represented mainly by gram positive species. The isolated and identified bacterial species in the air samples were: *Staphylococcus xylosus, Staphylococcus simulans, Staphylococcus lentus, Staphylococcus sciuri, Aerococcus viridans, Aerococcus viridans 2, Aerococcus viridans 3, Escherichia coli and Citrobacter freundii*. The results showed that the numbers of bacteria found in the morning and in the evening are variable and high in many cases in the indoors air of dairy cattle barns with free-stalls. The identification of microbial species with pathogenic risk imposes unconditional conformation to hygienic measures which can lead to the elimination of the microbial flora, at least temporarily.

INTRODUCTION

The inside air of dairy cattle houses usually harbours quite variable numbers of germs, depending on different parameters, such as the size of the livestock, breeding and production technologies, the floor and bedding types, the air temperature and humidity and, especially, the ventilation level [8, 13]. Among different saprophytic species which are numerically very well represented, there are pathogenic microorganisms as well, such as viruses, different bacteria and fungi. In dairy housing, the sources of bioaerosols include food, manure, litter and the animals themselves. As the level of microbial pollution is higher, there is a proportionally growing risk of pathogenic microbial contamination of the animals. Therefore, the density and quality of airborne microflora in the barns' air influences the health of animals, human workers and not lastly the degree of bacterial pollution of the close environment of the barns [10, 12]. Considering all of these, monitoring the microbiologic quality of the inside air in dairy cattle barns became a necessity. The aim of this study was to assess the microbiological quality of the indoor air in free-stall dairy cattle barns, by identification of bacteria and determination of their numbers.

MATERIAL AND METHODS

We investigated 24 dairy barns with free-stalls, in Transylvania, between December 2009 and January 2010. Each barn was visited twice during the study. The air sampling was made early in the morning (5-6 a.m.) and in the evening (6-7 p.m.) in three different locations of the barns (at the extremities and in the centre). The total number of samples/barn was 24. Air samples were taken using a MAS-100 air sampler (Merck, Germany) based on the principle of the Andersen air sampler. Bacteria were collected in Petri dishes on different standard culture mediums: Columbia agar for mesophilic bacteria, Chapmann agar for staphylococci, Endo agar for Gram negative bacteria and blood agar for hemolytic bacteria. Air was sampled in a volume of 10 L because preliminary studies showed it to be optimal for the subsequent plate analysis and type of agar. Plates with the usual bacterial nutrient Columbia agar and with selective culture mediums were then incubated for 24 h in an incubator at a working temperature of 37 C°. The grown colonies were calculated by a mechanical optic colony counter, and the results were corrected using the conversion formula devised by Feller [Feller, 1950]. The mean number of bacteria and fungi was calculated for each barn and the mean number for all of the 24 barns, both for the morning and for the evening samples. The average number of bacteria was calculated as colony-forming units in one cubic metre (cfu/m^3). The identification of bacterial colonies was carried out according to API system (bio-Mérieux, Marcy-l'Etoile, France). The obtained data were statistically processed using the SPSS version 17 software.

RESULTS

The tables 1 and 2 present the values of descriptive statistical parameters (mean, standard deviation, median, minimum, maximum) for the numbers of bacteria determined in the 24 dairy cattle barns in the morning (Table 1) and in the evening (Table 2). It can be observed that the values are slightly higher for the evening determination in comparison with the morning samples, for all the groups of bacteria. In order to compare the values of determinations made in the morning and those made in the evening the Mann-Whitney Test was used, which showed no significant differences between the two determinations (in the morning and in the evening) for the numbers of bacteria ($p>0.05$).

Table 1. Descriptive statistical indicators for the numbers of bacteria determined in the barns in the morning

cfu/m^3	n	Media	Minimum	Maximum	Median	Standard deviation
Mesophilic bacteria	24	7.60×10^4	5.79×10^4	1.00×10^4	2.22×10^5	6.35×10^4
Staphylococci		1.99×10^4	2.04×10^4	5.42×10^2	9.77×10^4	1.42×10^4
Streptococci	24	1.15×10^4	1.52×10^4	1.4×10^3	7.49×10^4	6.94×10^3
Gram-negatives	24	5.99×10^3	6.95×10^3	3.67×10^2	2.94×10^4	3.35×10^3

n = number of barns
cfu/m^3 = colony-forming units in one cubic metre

Table 2. Descriptive statistical indicators for the numbers of bacteria determined in the barns in the evening

cfu/m^3	n	Media	Minimum	Maximum	Median	Standard deviation
Mesophilic bacteria	24	8.82×10^4	6.79×10^4	9.52×10^3	2.42×10^5	6.96×10^4
Staphylococci	24	2.49×10^4	2.35×10^4	1.10×10^3	1.10×10^5	2.08×10^4
Streptococci	24	1.49×10^4	1.83×10^4	3.14×10^3	9.08×10^4	8.03×10^3
Gram-negatives	24	7.62×10^3	7.84×10^3	5.76×10^2	3.34×10^4	5.33×10^3

n = number of barns
cfu/m^3 = colony-forming units in one cubic metre

The isolated and identified bacterial species in the air samples were: *Staphylococcus xylosus, Staphylococcus simulans, Staphylococcus lentus, Staphylococcus sciuri, Aerococcus viridans, Aerococcus viridans 2, Aerococcus viridans 3, Escherichia coli and Citrobacter freundii*.

DISCUSSION

The total numbers of bacteria varied in the investigated barns, with slightly elevated values in the morning. However, from the statistical point of view the differences between the two determinations (in the morning, in the evening) were insignificant (Mann Whitney test, $P>0.05$).

The total numbers of mesophilic bacteria were different in the 24 assessed barns; the determined values were similar to those in the scientific literature. Several researches showed that the total number of mesophilic bacteria in cattle houses range from 10^4 to 10^6 cfu/m^3 [2, 3, 6]. More recent studies showed that the mean values of total bacterial count in the dairy cows' barn ranged from 1.7×10^3 to 8.8×10^4 [7] or from 2.82×10^4 cfu/m^3 to 7.76×10^4 cfu/m^3 as it was found by Matković et al. [9] in their research. The great variability of the mesophilic bacterial count in the air of the barns is the reason for which a compulsory hygienic standard for the acceptable number of airborne bacteria is not yet established on an international level. The total number of mesophilic bacteria represents a basic assessment criteria of air hygiene quality. The microbial load of the air indicated through the total number of mesophilic bacteria is influenced by several factors, such as the number of sheltered animals, the breeding technologies, the flooring type, the bedding materials, the quality of microclimate, the concentration of dusts, the ventilation level and so one. As a cause of high air contamination levels, Lange [8] indicated an improper functioning of ventilation systems, storage moisture of feed rations, kinds of work practice and climatic conditions.

The values obtained in the evening determinations were slightly higher than those determined in the morning, agreeing with the results of other studies [2, 12]. Matković et al. [9] in their research made in a Croatian dairy cattle barn showed that the total bacterial count measured in the barn's air according to time of day was 1.40×10^4-1.20×10^5 cfu/m^3 in the morning and 2.38×10^4-2.11×10^5 cfu/m^3 in the evening. These results could be explained as a consequence of diurnal animal and barn activities.

Hartung [5] indicate that in the barns' air predominates the gram-positive bacteria, like the staphylococci and streptococci, which may be explained by their high resistance in the environment. Our results reaffirm this

finding, in the studied barns the gram-positive bacteria represented up to 50%. Moreover, it is asserted that the gram-negative bacteria isolated from the air of the barns represents a minor proportion within the totality of bacteria [9, 14], as it was ascertained in our study also. As a potential endotoxin carrier, gram-negative bacteria represent a very important microorganism group, which may negatively affect animal health. Thus, it is assumed that their numbers in the air should not exceed 10^3 cfu/m^3 [1].

Regarding the quality of the aero-microflora similar results were obtained in other studies [14]. Many of the identified species have pathogenic potential, their presence in the air indicating an increased risk of disease for animals and humans.

CONCLUSIONS

The results showed that the numbers of bacteria found in the morning and in the evening are variable and high in many cases in the indoor air of dairy cattle barns with free-stalls. The identification of microbial species with pathogenic risk imposes unconditional conformation to hygienic measures which can lead to the elimination of the microbial flora, at least temporarily.

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DETECTION OF AIRBORNE MICROORGANISMS AND ANTIBIOTIC RESISTANCE FROM ANIMAL HOUSING FACILITIES

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SUMMARY

Agricultural animal production is increasingly regarded as a source of airborne pollutants, such as bioaerosols, dust and gases, which are both aggravating and ecologically harmful. In stalls are good conditions (increased temperature, humidity and presence of organic material in the air) for creation of bioaerosol that contain many microorganisms.

Repeated exposure of bacteria to antimicrobial agents and access of bacteria to increasingly large pools of antimicrobial resistance genes in mixed bacterial populations are the primary driving forces for emerging antimicrobial resistance (Schwartz and Chaslus-Dancla, 2001).

The aim of the study was to compare concentration of airborne microorganisms and presence of antibiotic resistance in pig and cattle farm.

INTRODUCTION

Generaly are in bioaerosol detected saprophitic bacteria, but can be occured patogenic bacteria, which comes from sick animals, feed, liter and manure. Concentration of pathogenic bacteria mostly depends on health status of stabled animals (Matkovič et al., 2006).

Microorganisms present in the air in animal houses may affect negatively the health, growth and productivity of animals. Bacteria levels are influenced by the density of stocking, the age of the animals, the ventilation system, the microclimate in the animal houses and the amount of dust in the air and deposited on the surfaces. Intestinal microflora, in the manure can contaminate food chain, water, vegetables, vegetable organic wastes, straw, sawdust, flies and is a potential source of resistant coliform bacteria and introduction of antibiotic residues which might exert a selection pressure (Ondrašovič et al., 1997).

Animal workers are exposed to a range of organic and inorganic dusts, endotoxins, fungi and bacteria which have been implicated in health outcomes, concluding respiratory illness, allergies and infections. The health impact of working in the animal industry may also extend beyond the exposed workers.

MATERIAL AND METHODS

In the experiment, samples were collected by means of a sampler MAS-100 Eco from pig, cattle and layer farm. Susceptibility (MIC) was determined by colorimetric broth micro-dilution method.

In the experiment, samples were collected by means of a sampler MAS-100 Eco. Petri dishes with respective nutrient media (Meat-pepton agar, Endo agar, Sabouraud agar, MacConkey agar) are placed on top of the dish support of the sampler and after aspiration of a preset volume of air, they are incubated at appropriate temperatures (37°C meatpepton agar, Endo agar for one day and 24°C Sabouraud agar for four days and room temperature). The plate counts were recalculated per 1 m^3 of air.

Susceptibility (MIC) was determined by colorimetric broth microdilution method according to CLSI guidelines using ampicillin, ampicillin and sulbactam, ceftiofur, ceftriaxon, ceftazidime, ceftazidime and clavulanic acid, gentamicin, streptomycin, neomycin, spectinomycin, nalidixic acid, enrofloxacin, ciprofloxacin, chloramphenicol, florfenicol, tetracycline and cotrimoxazol.

RESULTS

The number of microorganisms in the air in animal houses varies. Bacteria levels are influenced by the density of stocking, the age of the animals, the ventilation system, the microclimate in the animal houses and the amount of dust in the air and deposited on the surfaces. They also depend on the housing technology (litterless or with litter), system of stocking (continual stocking or the all in all out system) and feeding (dry or wet feed).

Samples of bioaerosol from different locations of pig farm and cattle farm were analyzed in spring seasons. Simultaneously we measured relative humidity and temperature of the air (table 1, 2).

Housing system for cattle was mostly arranged free on straw litter. They have sufficient place for physiological movement and behavior needs. Concentration of total count of microorganisms was in the range from $1{,}02 \cdot 10^3$ (milk calves in hutches) to $>10^6$ CFU·m^3 (heifers). Concentration of coliform bacteria is not so high neither in cattle farm nor in pig farm. In heifers housing facility was found the highest concentration total count bacteria which is influenced by insufficient ventilation system.

Table 1: Average concentration of airborne microorganisms in cattle farm

Place of sampling	T	RH%	TCB	CB	Moulds
Maternity	10°C	74,3%	$5{,}2 \cdot 10^5$	$2{,}2 \cdot 10^3$	$21{,}5 \cdot 10^3$
Gravid cows	9,7°C	68,9%	$16{,}4 \cdot 10^3$	$0{,}15 \cdot 10^3$	$10{,}9 \cdot 10^3$
Dairycows	6,3°C	89,5%	$80{,}5 \cdot 10^3$	$1{,}4 \cdot 10^3$	$11{,}3 \cdot 10^3$
Calves in vegetable diet	8,6°C	75,3%	$13{,}4 \cdot 10^3$	$0{,}6 \cdot 10^3$	$14{,}6 \cdot 10^3$
Milk calves	7,4°C	87,3%	$1{,}02 \cdot 10^3$	$0{,}15 \cdot 10^3$	$1 \cdot 10^3$
Heifers	7,6°C	86,7%	$>10^6$	$1{,}2 \cdot 10^3$	$37{,}8 \cdot 10^3$

Table 2: Average concentration of airborne microorganisms in pig farm

	T	RH%	TCB	CB	Moulds
Lactating sows	18,3	75	$9{,}3 \cdot 10^5$	$9 \cdot 10^2$	$36 \cdot 10^3$
Weaned pigs	16,4	67,8	$8 \cdot 10^5$	$5{,}6 \cdot 10^3$	$68 \cdot 10^3$
Farrowing swine	17,1	71,1	$1{,}5 \cdot 10^5$	$0{,}45 \cdot 10^2$	$23 \cdot 10^3$
Growing pigs	17,6	73	$9{,}6 \cdot 10^4$	$5{,}6 \cdot 10^3$	$49 \cdot 10^3$
Finnishing pigs	17,5	71,5	$9{,}4 \cdot 10^4$	$2{,}3 \cdot 10^3$	$14 \cdot 10^3$
Vicinity of farm	20,1	65,3	$5{,}6 \cdot 10^3$	$0{,}06 \cdot 10^3$	$0{,}72 \cdot 10^2$

Efficient ventilation is one of the principal tenets of intensive animal production, that mean ensure an aerial environment in which can be maintained the animal health and can be sustained their productivity, the employment accomplish his tasks in comfort and without risk to his health and the building and its equipment are protected from corrosion or physical damage (Wathes and Charles, 1994).

In pig farm was found permanently higher concentration of microorganisms (10^3 - 10^6 CFU·m^3). In weaning, farrowing and growing stage was observed the highest concentration total count of microorganisms as well as coliform and moulds(see Table 2). That relates with increasing temperature, relative humidity and insufficient ventilation. In gestation stage with straw bedded was found after exchange of bedding the concentration of microorganisms decreased mostly by 20 orders. Increasing of microorganisms in weaning due to problems with wit belt manure scraper conveyer. Relative humidity is probably the most crucial factor controlling bacterial aerosol stability. The recommended maximum acceptable concentration of microorganisms in the air in animal houses is 250 000 in 1 m^3 (Ondrašovič et al., 1997).

Although in some cases was concentration of airborne microorganisms in cattle farm higher we didn't find multidrug resistance. In pig farm we found high levels of multiple resistance *Escherichia coli*. In this farm were detected dangerous ESBLs and high level of quinolone resistance with a potential health risk for animal and employees.

DISCUSSION

Antimicrobials are used in animal production for therapeutic, metaphylactic and prophylactic purposes as well as for growth promotion, although the latter use is banned in EU countries.

The most common antimicrobial drugs used presently or in the past as growth promoters include macrolides (tylosin and spiramycin), polypeptides (bacitracin), glycolipids (bambermycin), streptogramins (virginiamycin), glycopeptides (avoparcin), quinoxalines (carbadox and olaquindox), everninomycins (avilamycin) and ionophores (monensin and salinomycin). The distinction between growth promotion and prophylactic use is not always clear since growth promoters also contribute to the prevention of certain diseases and can be administered for this purpose. Some countries allow antimicrobials that are used therapeutically to also be used as growth promoters in sub-therapeutic doses. In the USA, antimicrobial agents such as penicillin, erythromycin, tylosin and tetracycline

are approved for both growth promotion and therapeutic use (Guardabassi and Kruse, 2008).

Our results refers that the greatest bacterial level was in weaning stage that is critical phase of life, because pigs are moving and mixing. They can be hit by post-weaning diarrhoea and are treated by antibiotics.

Kmet and Kmetova (2010) found high levels of multiple resistance in calf and poultry commensal faecal *Escherichia coli* on farms in Slovakia.

E. coli isolates from livestock showed resistance to the largest number of antimicrobials and multidrug resistance was most common in swine fecal samples. Resistance was demonstrated most frequently to tetracycline, cephalothin, sulfisoxazole, and streptomycin (Sayah et al., 2005).

In Denmark, an increase in the use of antimicrobials used for therapy in recently weaned and grower pigs due to *Escherichia coli* and *Lawsonia intracellularis* respectively, was observed shortly after the ban of AGPs. However, the situation has stabilised following changes in management practices by farmers and veterinarians (Burch et al., 2008).

CONCLUSIONS

Hygiene of the air and of building surfaces is often less than satisfactory and is potentially a serious limitation to high efficiency of production and good health in those intensive systems of animal husbandry which involve housing at the stage of production cycle. The air and surfaces of buildings may act as reservoirs of primary and opportunistic pathogens. (Wathes and Charles, 1994).

Antimicrobial resistance is a global public health problem, and growing scientific evidence indicates that it is negatively impacted by both human and animal antimicrobial usage. Resistant microorganisms that exist in bioaerosols in different environments pose hazard to animals and people, particularly through exposure to infective antibiotic resistant pathogens or commensals and related potential mortality and failure of therapy.

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FIRST DETECTION OF ATYPICAL SCRAPIE IN AUSTRIA

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SUMMARY

After the first and only case of Classical Scrapie in the year 2000 Austria has performed extensiv Scrapie screening from the year 2001 till december 2010 using different methodes without finding a second case.

In January 2011 brain samples from two old fallen stock sheep reacted positive in a Scrapie rapid test. One sample was weak positive in the cerebellum sample with elevated but negative signal in the obex sample. A second sample tested strongly positive in the obex but even stronger in the cerebellum.

Both samples showed positive results in confirmatory immunoblot. Sequencing revealed the genotype ALHQ/ALHQ for both animals.

Discriminatory tests at the EURL Weybridge excluded BSE and Classical Scrapie but confirmed both cases to be Atypical Scrapie.

All other sheep from the same herds gave a negative rapid test result.

In March 2011 a third but rather young sheep tested positiv for Atypical Scrapie. It was also showing higher signals in Cerebellum than in obex region and also had the Genotype ALHQ/ALHQ.

INTRODUCTION

Atypical Scrapie is of increasing importance in European countries performing extensive Scrapie screening programs. During this screening from january 2001 to december 2010 eight sheep and seven goats were found reactive in Austria using different rapid tests. All these „laboratory suspects" and additional eight clinical suspects (five sheep and 3 goats) turned out negative in confirmatory testing.

According to national law all fallen stock and healthy slaughtered sheep and goats from herds that have imported animals from possibly Scrapie infected herds or countries have to be tested if they are at least 18 months old.

In order to optimize sensitivity of Scrapie rapid tests in Austria, from august 2007 on obex (or brainstem) and cerebellum of sheep and goats aged more than 18 months have been tested separately.

Austria had a single Scrapie outbreak in the year 2000 on a farm that had imported Texel sheep from the Netherlands. The European Community grants Austria additional guarantees regarding Scrapie since March 2006.

MATERIAL AND METHODS

The recent cases have been detected at the AGES IVET Mödling using Idexx HerdCheck BSE Scrapie Antigen Testkit, EIA (Idexx Laboratories).

Confirmatory testing at the Austrian NRL for TSE at AGES IVET Mödling was done by using Bio-Rad TeSeE Western Blot assey (Bio-Rad Laboratories).

Genotyping of PrP gene was perfomed using a modification of the Test procedure LT4001 (CRL Weybridge, UK) based on SYBR Green real-time PCR detection and direct DNA sequencing in a 3130xl Genetic Analyzer (Applied Biosystems). Sequencing analysis and alignment was done with SeqScape Software v2.5 (Applied Biosystems).The EURL at VLA Weybridge UK performed immunohistochemistry using anti PrP mab 2G11 (Institut Pourquier) and the Bio-Rad TeSeE Hybrid Western immunoblot protocol using mabs SHA31 and P4 according the "Technical handbook for TSE strain characterisation in small ruminants for NRLs in the EU".

RESULTS

Table 1: Rapid test signals

case identification	age (years)	Cut off	first rapid test obex signal	first rapid test cerebellum signal
Atypical Scrapie 1	>9	0,210	0,159	0,241
Atypical Scrapie 2	5,5	0,212	1,792	3,304
Atypical Scrapie 3	2,5	0,203	1,710	3,294

The confirmatory WB for cases „Atypical Scrapie 2" and „Atypical Scrapie 3" had a stronger result on cerebellum than on obex.

Brain material from „Atypical Scrapie 1" was rather autolytic but clearly positive in confirmatory testing.

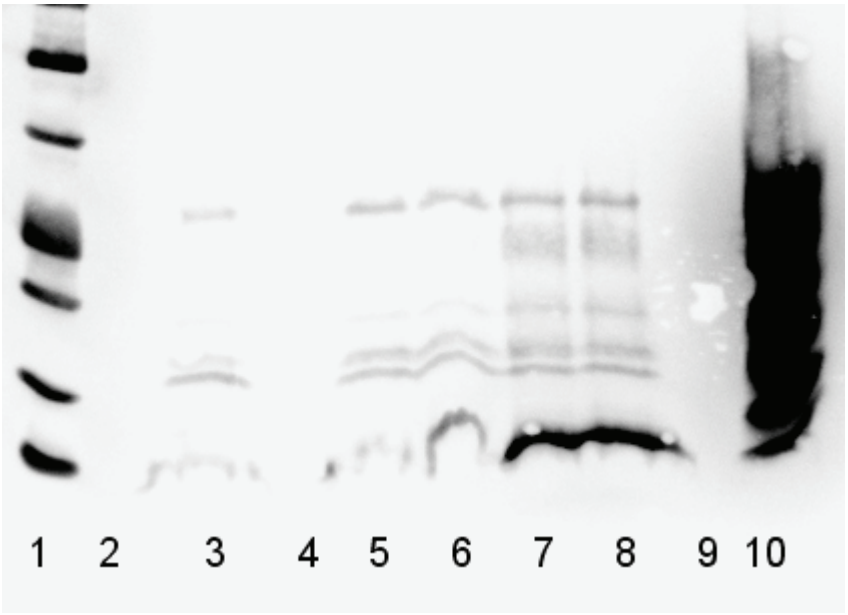

Figure 1: confirmatory western blot

Discriminatory testing at the EURL for TSE diagnosed Atypical Scrapie for all three cases and excluded BSE and Classical Scrapie. (Figure 2)

lane	material
1	marker
2	empty
3	Scrapie neg.
4	empty
5	obex
6	obex
7	cerebellum
8	cerebellum
9	empty
10	Scrapie pos.

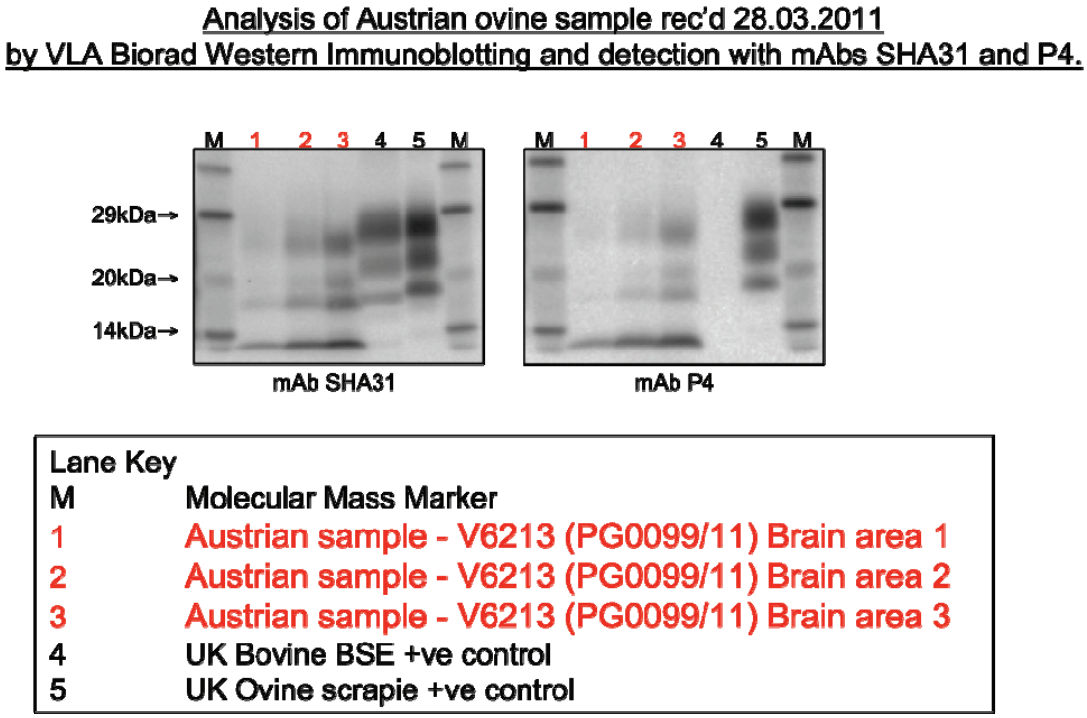

Figure 2: Result of discriminatory western blot of case "Atypical Scrapie 3"

DISCUSSION

Atypical Scrapie cases have been detected in several countries worldwide during the last years. After more than 10 years of screening nearly all fallen stock of Austrian sheep and goats, three cases of Atypical Scrapie have been detected.

This gives rise to the idea that Atypical Scrapie could be found in any small ruminant population if it is screened extensively and long enough with sensitive rapid tests.

The first Austrian case of Atypical Scrapie would not have been found if only Obex would have been tested.

This shows that if it is the intention to find all atypical Scrapie cases not only obex but also other promising brain areas have to be tested.

In the year 2000 the last Austrian C-type BSE case was born and the sole Austrian case of classical Scrapie happened. Two of the recent cases of Atypical Scrapie were born 4.5 and 7.5 years later.

CONCLUSIONS

The finding of atypical Scrapie in animals born many years after a single case of classical Scrapie happened and after the last C-type BSE-case has been born support the thesis that cases of Atypical Scrapie happen spontaneously and independent of other prion diseases.

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CASEOUS LYMPHADENITIS CONTROL PROGRAM IN UPPER AUSTRIA (Abstract)

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INTRODUCTION

Caseous lymphadenitis (CLA) caused by *Corynebacterium (C.) pseudotuberculosis* has become a significant disease in sheep and goat in many parts of the world. The increase of herd size and international trading of infected animals are the main reasons for a continuous spreading of CLA. Objective of the Upper Austrian voluntary CLA control program is the reduction of financial losses caused by infections with *C. pseudotuberculosis*. The main targets are the elimination of infected animals and the prevention of further spreading within the herds as well as in between herds.

ANIMALS, MATERIALS AND METHODS

Participating farms undergo a risk based periodical serological testing for the detection of CLA infected herds and are classified positive, suspicious or unsuspicious. A CLA eradication program by segregation and culling of infected animals in combination with management and hygienic measures is conducted in serologically positive tested farms. In 2010 939 sheep and 956 goats kept on 75 farms were tested by Elitest CLA (Hyphen Biomed, France) for *C. pseudotuberculosis* specific antibodies in serum.

RESULTS

The results of the 31 goat, 34 sheep and 10 mixed farms are listed in table 1.

Table 1: Elitest CLA ELISA results in 2010

Species	ELISA result	Samples (%)
Sheep (n=939)	Suspicious	17 (1,81)
	Negative	896 (95,42)
	positive	26 (2,77)
Goat (n=956)	Suspicious	35 (3,66)
	Negative	568 (59,41)
	positive	353 (36,92)

CONCLUSIONS

CLA seems to be a major problem in goat farms with an intra-herd prevalence of more than 50% infected goats in 11 of 20 positive tested farms, whereas not more than 3 infected sheep were detected in the 12 positive tested sheep farms. The introduction of the Upper Austrian CLA control program started in 2010, therefore an evaluation of the eradication measures is not possible at this point of time.

NEW PPV ISOLATES AND EVIDENCE OF A HIGH RATE OF VIRAL EVOLUTION

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SUMMARY

In the last years, it has been shown that some parvoviruses exhibit high substitution rates, close to those of RNA viruses. In order to monitor and determine new mutations of the porcine parvovirus (PPV), recent PPV field isolates from Austria, Brazil, Germany and Switzerland were sequenced and analyzed. These samples, together with sequences retrieved from GenBank, were included in three datasets (consisting of VP2 complete gene, VP2 partial gene and NS1 complete gene). For each dataset, nucleotide substitution rate and molecular clock were determined. The analysis of the PPV field isolates revealed that a recently described amino acid substitution S-436-T in the VP2 protein appears to be common in the Austrian, Brazilian and German virus population. Furthermore, new amino acid substitutions were identified, mainly located in the viral capsid loops. By inferring the evolutionary dynamics of the PPV sequences, a nucleotide substitution rate of approximately 10^{-5} nucleotide substitutions per site per year for the non-structural protein gene and 10^{-4} for the capsid protein gene (for both viral protein datasets) were found. The latter rate is similar to that commonly found in RNA viruses. An association of the phylogenetic tree with the molecular clock analysis revealed, that the mutations, on which the divergence for both capsid proteins is based, occurred in the last 30 years. Based on these findings, can be conclude that PPV variants are continuously evolving, and that vaccines, which are mainly based on strains isolated about thirty years ago, should possibly be updated.

INTRODUCTION

Porcine parvovirus (PPV) is a small, single-stranded (ss) DNA virus. The genome has a length of about 5000 nucleotides encoding four proteins transcribed from two promoters and the coding capacity is extended through alternative splicing. Two nonstructural proteins, NS1 and NS2, are transcribed and translated from the 5´ ORF and are important for DNA replication. Additionally, two structural proteins are transcribed and translated from the 3´ ORF (VP1 and VP2). The smaller protein, VP2, is produced by splicing from the same RNA template as the larger protein (VP1). The whole VP2 sequence is therefore present in the VP1,which has a unique amino terminus of about 120 amino acids. A third protein, VP3, is a post-translational modification product of VP2 [5]. Together, these three viral proteins assemble to form the icosaedral capsid [1]. Various biotypes of PPV are known with completely different pathogenic properties. The genetic basis of the pathogenicity has not yet been defined, but the structural protein appears to play a major role. Recently, genetic variation and the possible emergence of a new antigenic type of PPV has been described [11], although its importance in the field is not yet clear.

As PPV replicates by using the host DNA replication machinery, it is generally assumed that the virus has a low rate of nucleotide substitution, close to that found in its mammalian host. In the last years, it was shown that canine parvoviruses and human B19 erythrovirus, both autonomous parvoviruses, showed a nucleotide substitution rate at approximately 1×10^{-4} substitution per site per year [6, 7], a rate similar to that known from RNA viruses.

The continuous use of an inactivated vaccine in swine herds in the last three decades, and the remaining occurrences and reports of reproductive failures caused by PPV, highlights the importance of a continuous monitoring of PPV isolates. To address these questions, recent PPV were analyzed isolates by determining nucleotide substitutions, by phylogenetic analysis and by estimation of the molecular evolutionary rate of PPV.

MATERIAL AND METHODS

A total of fifteen recent isolates from Austrian, Brazil, Germany and Switzerland were analyzed. The Austrian (693a, 694a and 695a), German (7a, 8a, 9a and 14a), Swiss (15411, 15421 and 15425) and the Brazilian strains (PA and PB) were originated from clinical cases involving reproductive losses. The Brazilian strains (S30 and S31) were obtained from 55-day-old (mean age) wasting piglets, and one (Embrapa) was isolated from an unknown source in Brazil. Total DNA was purified from clinical samples and cell culture supernatant using the QIAamp

DNA Mini kit (Qiagen, Germany), according to the manufacturer's instructions. The NS1 and VP1/VP2 genes amplification were performed as previously described [8, 11]. The assembly of the obtained sequences to a full length sequence was performed using the SeqMan program of the Lasergene software (DNASTAR, USA). All nucleotide numbers used in the present study refer to the Kresse strain (GenBank accession number U44978). The amino acids numbers are according to the VP2 protein of the same strain.

All NS1 and VP gene sequences deposited in GenBank (up to September 2010) containing the isolation year, were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/). These sequences, taken together with the new sequences described here totaled 34 complete sequences of the NS1 gene and 35 complete sequences of the VP2 gene, which were analyzed separately. In order to increase the number of sequences in the analysis, a third dataset containing 70 partial sequences of VP2 gene was also constructed. The latter dataset consisted of 739 nucleotides (between nucleotide position 3701 and 4439). The datasets were aligned applying the ClustalW method using MEGA 4 software [10].

The rates of nucleotide substitution per site per year were estimated with a Bayesian Markov chain Monte Carlo method, using the software BEAST v.1.5.4 [2]. The resulting data were visually analyzed using Tracer software (http://tree.bio.ed.ac.uk/software/tracer/) after removing a 10% "burn in" for each data. A "consensus" tree for each dataset was generated by the software TreeAnnotator v.1.5.4 (BEAST package). Phylogenetic trees were visualized with the software FigTree v.1.3.1 (http://tree.bio.ed.ac.uk/). A more detailed version of the methodology can be found in Streck *et al.* [9].

RESULTS

The sequence analysis of the structural gene (VP2) of these isolates (n=13) revealed nucleotide substitutions at 32 sites. Seventeen substitutions were synonymous and fifteen substitutions were non-synonymous. The analysis of the nonstructural genes (NS1) of the new isolates (n=10) revealed nucleotide substitutions in 44 sites. Twenty-seven substitutions were synonymous and seventeen substitutions were non-synonymous.

Unique amino acids substitutions could be identified in the sites 436 and 565. In all the new German and Austrian strains, substitutions were located in sites 228, 414 and 419. In the site 436, all German strains contain the amino acid Threonine, what also occurred in two Brazilian strains (S30 and S31). A higher number of amino acids substitutions in comparison to the other isolates could be shown in strain 15425 with six unique amino acids changes.

The mean evolutionary rate estimated for the three data sets using the BEAST approach ranged from 10^{-5} to 10^{-4}. According to the best model fitting the data, the nonstructural genes dataset presented a mean rate (substitution/site/year) of 5.39×10^{-5}, the VP2 complete gene dataset a mean rate of 3.57×10^{-4} and the VP2 partial dataset a mean rate of 4.64×10^{-4}. According to the methodology, a mean variation between 4.00 - 7.73 x 10^5 was found for the nonstructural dataset and a variations between 2.39 - 3.58 x 10^4 for the two structural datasets. The chronological analysis of the NS and VP2 datasets demonstrated two distinct temporal periods. In the structural genes, the main divergences occurred in the last 30 years, for both datasets and the events resulting in the outcome of new strains were dated in the last 20 years. In the nonstructural dataset, the divergences were estimated to be formed in a former time range, between 10 to 125 years ago, mainly concentrated in the last 30-60 years.

DISCUSSION

Here, we observed that the substitutions in the new strains were mainly located in the protein loops. The only exception was the amino acid 82 (R → K) substitution at the site number 82 in the Swiss strain 15425. Several substitutions in these loops could also be observed in the canine parvovirus and are reported to be involved in cell specificity and hemagglutination activity [1]. The substitutions 226 (Q → E), 228 (Q → E), 320 (I → T), 419 (E → Q) and 423 (N → K) are located near to the 3-fold shoulder of the capsid subunit. This location was considered to be a common antigenic surface region in distinct parvoviruses [1]. These sites also present a higher distance to the center of the PPV capsid in comparison with other capsids protein amino acids [5], suggesting that these sites interact directly with the host immune system.

In all new German and Austrian strains and in two Brazilian strains (S30 and S31) the amino acid Threonine was found in position 436, an amino acid site located right in the 3 fold spike center of the capsid subunit. The higher incidence of a Threonine in the recent samples suggests that this amino acid is providing some adaptive advantage to the virus.

In this study, a high evolutionary rate was found for PPV capsid genes (~3-4 x 10^{-4}) and only a moderate evolution rate was found for PPV non-structural genes (~5 x 10^{-5}). Rapid evolution is already known among ss DNA viruses, including canine parvovirus and human B19 erythrovirus [6, 7]. For the canine parvovirus, a rapid evolution was demonstrated in one specific branch that separates the feline panleukopenia parvovirus and canine parvovirus clades.

Reasons for the observed high substitution rates in parvoviruses and other ss DNA viruses remain unclear. Unlike RNA viruses, which use their own error-prone polymerases, PPV replicates using the cellular DNA polymerase of the swine host, implying that they should

have the same replication fidelity as its host. Previous studies suggest that the proofreading or repair mechanism may be not efficient or accurate in these genomes or in cells with an active viral infection [6, 7]. Alternatively, processes like the ss DNA deamination due to the lack of the double helix or non-functional host exonucleases due to absence of proper methylation patterns, could also lead to a mutational vulnerability [3, 4].

The phylogenetic tree associated with a molecular clock analysis revealed that the divergences between the main isolates were introduced in the last 100 years for the nonstructural gene dataset and in the last 10-30 years for both structural gene datasets. This last date agrees with the beginning of the use of inactive vaccines against PPV (~30 years ago) and support that currently vaccines may be overcome.

CONCLUSIONS

Our data suggest that the currently used immunization schedules that are based on regular revaccination of breeding sows in four to six months intervals with inactivated whole-virus vaccines may need to be remodeled.

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STUDY OF AN UNUSUAL PARATYPHOID EPORNITIC IN CANARIES (*SERINUS CANARIA*)

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SUMMARY

High mortalities in 17 canary flocks from different regions of Tehran, Iran, were reported. Necropsy and histopathologic examination revealed necrotic hepatitis and overall congestive septicaemia in carcasses. *Salmonella enterica* was isolated from 34 examined samples, two samples from each flock, including visceral organs of carcasses and droppings of live diseased birds. All isolates were typed as *Salmonella enterica* samovar Typhimurium by conventional serotyping. Antibiotic resistance profiling using 33 antibiotics and random amplification of polymorphic DNA differentiation by three primers were performed and showed an identical clonal relationship between these isolates and *S*. Typhimurium isolated from a sample of feedstuffs. Changing the feed ingredients along with antibiotic therapy via the drinking water by enrofloxacin solution controlled the outbreaks, and mortalities ceased. The zoonotic nature of S. Typhimurium and close contact of bird owners with pet birds in the home environment made the case significant in relation to public health.

INTRODUCTION

Infections with serovars of *Salmonella enterica* are among the most frequent causes of bacterial infections in animals and humans. Avian species without caeca, like the canary, appear to be more susceptible to *salmonella* infections than birds with fully functioning caeca [4]. *Salmonella* infection has been found in many captive aviary or pet birds and PT infection is one of the important bacterial diseases of canaries and other pet birds. High mortalities were reported in numerous flocks of multi-age canaries in different regions of Tehran. This study was performed in order to confirm an epornitic by a single causative agent. Furthermore, to our knowledge, this is the first study to include molecular typing of *Salmonella enterica* serovar Typhimurium isolated from canaries.

MATERIAL AND METHODS

Samples

Diseased and dead canaries were referred to the Faculty of Veterinary Medicine, University of Tehran. The birds belonged to 17 different collections located in Tehran (Figure 1). The number of canaries in each collection and mortalities are presented in Table 1.

Pathology

All carcasses were examined macroscopically. Tissue samples were obtained in 10% buffered formalin for histopathologic examination after haematoxylin and eosin staining.

Isolation of bacteria

Samples from viscera of dead birds and/or droppings of diseased birds were obtained aseptically in each flock for bacteriologic tests. As almost all canary fanciers in Tehran purchase canary seed from the same downtown market, feedstuffs and water supply were also cultured by conventional microbiological methods and biotyping was performed by conventional biochemical tests. Two isolates from each flock in addition to a feed isolate were selected for further characterization. Serotyping of the isolated salmonellas was performed by commercial antisera (Difco).

Antibiotic susceptibility test

The antibiotic susceptibility test was performed by the standard disk diffusion method in Mueller_Hinton agar (Difco) and the results were interpreted in accordance with the criteria of the National Committee for Clinical Laboratory Standards [8]. The isolates were screened for resistance to the following antibiotics: Tiamulin, Tylosin, Lincospectin, Flumequine, Difloxacin, Neomycin, Sulphamethoxazole, Florfenicol, Trimethoprim, Enrofloxacin, Cloxacillin, Oxytetracycline, Furazolidone, Chloramphenicol, Ampicillin, Ceftriaxone, Co-amoxiclav, Imipenem, Clarithromycin, Ceftazidime, Cefotaxime, Kanamycin, Cephazolin, Ciprofloxacin, Cefixime, Piperacillin, Norfloxacin, Carbenicillin, Ofloxacin, Cefuroxime, Vancomycin and Ticarcillin.

Randomly amplified polymorphic DNA analysis

A single colony of each isolate from an agar plate was picked and suspended in 200 ml distilled water. After vortexing, the suspension was boiled for 5 min, and 50 ml supernatant was collected after centrifuging for 10 min at 25,000 g. The DNA concentration of boiled extracts was determined with a spectrophotometer. The polymerase chain reaction (PCR) was performed as previously described [7]. The selected primers for this study were P1254 (5´-CCGCAGCCAA- 3´), 23L (5´-CCGAAGCTGC-3´) and OPA-4 (5´- AATCGGGCTG-3´).

Treatment and follow-up

The suspect feed materials were exchanged for new fresh sources. Isolation of diseased birds from the rest of the flock, cleaning and disinfection of cages, water and feed containers, and perches with 10% (vol/vol) solution of commercial bleach (containing 5% sodium hypochlorite) and/or commercial disinfectants such as Virkon® S (Antec™ International, Sudbury, UK) were recommended to the bird owners. Treatment of the remaining live birds in flocks was conducted by 10% (w/v) enrofloxacin solution supplied as 200 mg/l drinking water for 5 to 7 days. Three weeks to 6 weeks after treatment, bacteriological examination of a pooled faecal sample was performed to evaluate therapy success.

Table 1: Mortality in each flock

Flock number	Number of canaries in flock	Number of dead birds (%)
1	16	15 (94%)
2	40	9 (23%)
3	20	10 (50%)
4	15	8 (53%)
5	26	16 (62%)
6	50	10 (20%)
7	25	10 (40%)
8	4	2 (50%)
9	100	50 (50%)
10	40	20 (50%)
11	15	8 (54%)
12	100	25 (25%)
13	32	18 (56%)
14	60	25 (42%)
15	44	20 (45%)
16	80	60 (75%)
17	25	20 (80%)
Mean		(51%)

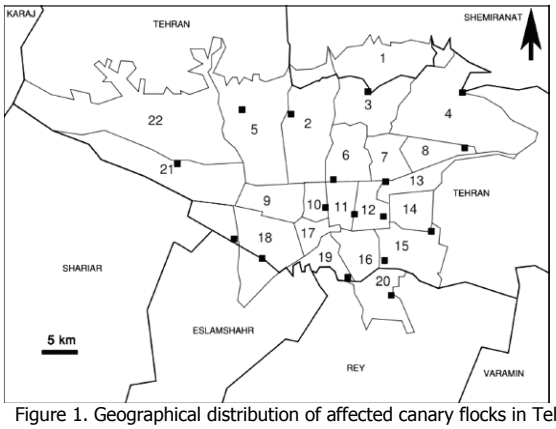

Figure 1. Geographical distribution of affected canary flocks in Tehran (black squares). Numbers refer to regions of municipality of Tehran.

RESULTS

Clinical presentation and pathology

The mean mortality rate was 51% in the referred collections (Table 1). The flocks had no geographical relationship with each other and were distributed in a vast area of Tehran (approximately 700 km2) (Figure 1). All fanciers purchased feed ingredients from the same live bird market located in downtown Tehran. The affected birds were lethargic, and had watery or mucoid diarrhoea with excess urate deposits. Almost all bird owners reported a sudden increase in mortality a week before the mortalities reported in Table 1. Overall congestion of visceral organs and necrotic foci on the liver with a nodular and military appearance were the most significant and consistent macroscopic lesions in dead birds. Dark bloody intestinal contents were also obvious in many carcasses and were probably the result of haemorrhagic diathesis. Microscopic inspection of histopathologic sections revealed disseminated and nodular necrosis of parenchymatous organs, particularly the liver with variable degrees of granulocyte and mononuclear leukocyte infiltration and fibrin deposition

Bacteriology and serology

Salmonellas were isolated from all carcasses and almost all faecal samples. The same bacteria were isolated from fresh feedstuffs that had not been in contact with bird collections or droppings, but not from the water supply of the flocks mentioned. All canary isolates and a tested feed isolate proved to be *S*. Typhimurium.

Antibiotic resistance profiling

All isolated *S*. Typhimurium revealed the same resistance pattern and were susceptible to all antibiotics with the exception of Sulphamethoxazole, Tylosin, Cloxacillin, Tiamulin and Vancomycin.

Random amplification of polymorphic DNA

Random amplification of polymorphic DNA (RAPD) by P1254, 23L and OPA-4 primers revealed identical patterns in all 36 isolates of *S*. Typhimurium. A high similarity between *S*. Typhimurium ATCC 14028 and the canary isolates was observed.

Treatment

No new cases of diseased birds were reported in the affected collections after initiation of antibacterial therapy, but some bird fanciers had faced heavy losses before treatment was initiated. Three weeks to 6 weeks after treatment, all faecal samples were negative for salmonella infection.

DISCUSSION

Diseases caused by non-host-adapted *Salmonella* infection are uncommon in poultry, and are usually seen in chicks, poults or ducklings younger than 2 weeks of age and rarely in birds over 4 weeks of age. The morbidity and mortality varies considerably, and deaths are usually less than 20% of the affected group. In very susceptible young chicks and poults, PT infection can sometimes lead to illness and death at high frequencies. Older birds are considerably less susceptible to the lethal effects of PT salmonellas and may experience intestinal colonization and even systemic dissemination without significant morbidity or mortality [3]. Infection with *S*. Typhimurium in small passerines appears identical with pseudotuberculosis, both clinically and at necropsy [2]. The outbreaks we observed occurred in multi-age canaries with mortality rates that varied from 20% to 94%. These results indicate high virulence of the *S*. Typhimurium isolates or high susceptibility of the canary. Keyvanfar (1968) reported a lethal systemic outbreak of *S*. Typhimurium in a canary flock in Tehran with more than 50% mortality in 100 birds [6]. Raidal (1998) described mortalities in two non-related flocks of canary in Australia due to *S*. Typhimurium infection with almost the same pathological findings as ours [9]. Some authors have reported more chronic courses of PT in canary [2]. This study revealed identical profiles of isolates in the outbreak investigated. Feed contamination with bacteria originating from bird droppings was the suspected cause of this outbreak as all bird fanciers had purchased canary seeds from the same live bird market and the feed isolate was identical to the bird isolates with respect to molecular typing and drug resistance patterns. It seems likely that some predisposing factors and environmental situations, such as breeding or shipping, could affect the severity of PT outbreaks in the canary [9]. As canaries are kept indoors, feed contamination or recently bought birds are the most probable sources of infection to collections. Death caused by non-typhoid salmonellas occurs primarily in humans with serious underlying disease such as HIV infection, cancer or leukaemia and cirrhosis [5]. The human risk for salmonellosis in close association with passerine birds infection was previously reported [1]. Although there is no official report of PT transmission from canaries to humans, considering the zoonotic multihost nature of PT salmonellas, increasing numbers of bird fanciers in Iran and indoor rearing of these kinds of birds, the risk of human infection could be significant. It is obvious that identification and characterization of isolates lethal to birds could be very important for human health surveillance systems. Feed contamination possibly played

an important role in PT spread in both the current case and that reported by Keyvanfar (1968) [6]. This appears to be the first molecular epidemiological study of *S.* Typhimurium in canaries. It is suggested that further studies on canary salmonellosis with more isolates from different areas are performed.

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MICROBIAL COMMUNITY VARIETY LINKED WITH THE INTESTINAL MUCOSA OF FARMED BROWN TROUT (Abstract)

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INTRODUCTION

The intestinal microbiota of fish is sensitive to variety of environmental factors including water chemistry, temperature, stress, conditions salinity, age and diet. The gastrointestinal tract is thought to be a latent way of entry for many fish pathogens. The gut microbiota is played a role as a defensive obstacle against pathogenic species in addition to contributing towards digestive function via the production of a variety of vitamins and enzymes.

MATERIALS AND METHODS

Six fish were euthanized by overdose of tricaine methane sulphonate followed by destruction of the brain, and placed on ice for transportation to the laboratory. After dissecting aseptically, the intestine of each fish was divided into four sampling points: the anterior digesta (AD), posterior digesta (PD), anterior mucosa (AM) and the posterior mucosa (PM). Digesta from the anterior and posterior sections were removed by gentle squeezing separately. Pyloric ceaca was taken to be the fifth sample and the resulting material from two fish was pooled into one sample. Thus, yielding a total of three samples. 100 µL of sample material was then separately homogenized in 900 µL PBS (10^{-1}). Then samples serially diluted to 10^{-7} and 10^{-5} with PBS for pyloric ceaca, anterior digesta and other samples respectively. Plate counts for heterotrophic aerobic viable populations were achieved on TSA at 20 ºC for 7 days and Lactic acid bacteria were enumerated with MRS agar at 20ºC for 7 days. 16S rRNA as analysis of denaturing gradient gel electrophoresis banding patterns, PCR and scanning electron microscope have been used to give accurate assessment about microbial communities.

RESULTS

The number of viable bacteria attached to the epithelial mucosa is less than that of the digesta by approximately 1- 2 log units. The mean viable counts were log 4.31 $CFUg^{-1}$ in the AM; log 4.98 CFUg $-^{1}$ in the PM; log 6.12 CFUg $^{-1}$ in the AD; log 6.23 $CFUg^{-1}$ in the PD; and 4.39 CFUg $^{-1}$ in the PC. The autochthonous level was significantly lower in the anterior region ((P= 50.014)

CONCLUSIONS

Bacterial communities from the posterior and interior of intestinal tract of Brown trout as well as samples from pyloric ceaca will be investigated to assess transient and resident microbial communities using both culture-based and culture-independent techniques. Genera of *Pseudomonas* spp. and *Enterobacteriaceae*, Staphylococcus and Acinetobacter spp. were observed as minor allochthonous populations but were completely absent on the mucosa.

CHARACTERIZATION OF VIRULENCE FACTORS IN ESCHERICHIA COLI ISOLATED FROM DIARRHEIC AND HEALTHY CALVES IN AUSTRIA SHEDDING VARIOUS ENTEROPATHOGENIC AGENTS (Abstract)

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INTRODUCTION

Infectious diseases, especially diarrhoea, are one of the most important disorders in calves. Diarrhoea in neonatal calves is a syndrome of great aetiological complexity that causes economic loss directly through mortality and need for treatment, and indirectly from poor growth. In addition to the influence of varied environmental, managemental, nutritional and physiological factors, the infectious agents capable of causing diarrhoea in the neonatal calf are numerous. In addition to economic losses, diarrhoea in livestock is important because of the public health implications. Numerous infectious agents causing diarrhoea in animals are zoonotic and have been associated with food-borne diseases. *Escherichia coli* and their subtypes (O26, O111, O118 and O157) are firmly associated with emergent food-borne diseases, especially Shiga toxin-producing *Escherichia coli* (STEC). The pathogenicity of STEC O157:H7 is associated with a number of virulence factors, including Shiga toxin 1 (encoded by the *stx1* gene), Shiga toxin 2 (encoded by the *stx2* gene), intimin (encoded by the *eaeA* gene) and enterohaemolysin (encoded by the *Ehly* gene).

ANIMALS, MATERIALS AND METHODS

Faeces samples from 230 diarrhoeic and healthy calves aged 0 to 6 weeks, from 100 farms in Austria were examined, between October 2004 and February 2005 for the presence of bacteria, especially shiga toxin-producing *Escherichia coli* (STEC), viruses and parasites.

RESULTS

Escherichia coli was detected in 17 % of all the faecal samples and was more prevalent in healthy calves. However, *E. coli* F5 was only identified in one calf without diarrhoea. Overall, 35 of the 230 (15.2 %) samples analyzed carried the Shiga toxin gene: *stx1, stx2* or both *stx1* and *stx2* in their faeces, STEC. Nevertheless, from 39 pathogenic *E. coli* positive samples observed, only 2 carried the Shiga toxin genes: *stx1,* in a diarrhoeic calf and both *stx1* and *stx2* in a healthy calf. *eaeA* and *Ehly* genes were more frequently detected in the strains from diarrhoeic calves 57.1 % and 50.0 % respectively. *Clostridium perfringens* was detected in twenty-one samples, the most prevalent toxin-type of *Cl. perfringens* was found to be type A (76.2 %). Other bacteria such as *Klebsiella spp*. and *Proteus spp*. were present in 1.3 % and 0.4 % of all samples. *Salmonella spp* was not detected. The detection rates of other enteropathogens were 25.7 % Bovine Coronavirus, 11.7 % *Cryptosporidium spp*., 10.4 % *Eimeria spp*, 9.1 % Rotavirus group A and *Giardia spp*. 6.1 %.

CONCLUSIONS

We demonstrated the presence of the STEC virulence genes in healthy and diarrhoeic Austrian calves but the importance of the virulence factors of STEC (*stx1, stx2, eae* and *Ehly*) in calf diarrhoea and systemic disease is not well defined. Therefore, further studies are necessary to identify reservoirs or potential sources of virulent STEC strains in order to establish control and prevention strategies for STEC associated diseases in animals and humans.

STUDIES ON AN OUTBREAK OF EQUINE INFLUENZA AT THE SOUTHERN DISTRICT OF EGYPT

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SUMMARY

The present study submitted to record a natural outbreak of EIV in summer 2008 at Edfo district, Aswan governorate, Egypt. Animals of the work were 74 diseased horses and donkeys of both sexes and 3-6 years old. Two blood samples were collected from each animal as a whole blood and other for obtaining serum. Clinical signs of disease were recorded. Serological diagnosis of EIV by ELISA test showed 89.04 % positive while 10.95 % negative. HI tests showed 79.45 % positive and 20.56 % negative. The titre of positive sera ranged between 4 to 64. H3N8 was discovered to be the circulating virus of this outbreak.

Marked drop was reported in values of RBCs. Hb, PCV, WBCs and lymphocytes at late stages of disease. Also, leucocytosis, monocytosis and neutrophilia were recorded in advanced stages. Moderate elevation of AST, CPK and LDH enzymes was recorded. Also, values of G.GT. Bilirubin, BUN and Creatinine were elevated at late stages of disease.

INTRODUCTION

Equine Influenza virus is a highly contagious agent that is capable of causing explosive outbreaks of respiratory disease among susceptible equines. The virus infects the mucosal cells of respiratory tract and induce severe clinical signs. Infection is generally selflimiting and the majority of horses recover uneventfully , however the recovery period may take several weeks to months [5].

Aim of work was to diagnose EIV in a suspected outbreak among equines in Egypt. Also to show effect of virus on general health condition of animals.

MATERIAL AND METHODS

A suspected outbreak of EIV was recorded in summer 2008 at Edfo district, Aswan governorate, Egypt. Animals of work were 34 horses and 40 donkeys of both sexes and 3-6 years old. Control healthy groups of 5 horses and 8 donkeys were used for comparison.

All animals were living under stress factors as high climatic temperature (44 ^{0}C), bnd stabling condition. Low quality feeds given, exhaustion and no vaccination. . The clinical symptoms were observed and recorded.

Blood samples were collected from animals at early and late stages of disease. At each stage two blood samples were collected from each animal , One as a whole blood and other for obtaining serum. Values of RBCs, Hb., PCV, WBCs and differential leucocytes count were estimated [1] . Blood serum samples were used for serological diagnosis of EIV using ELISA [7] and HI [2] tests. Also, blood serum, CPK, LDH, G-GT, Bilirubin, BUN and creatinine determined by using Kits. The obtained date were statistically analyzed [6].

RESULTS

Clinical signs of diseased animals were fever, harsh dry explosive non – productive cough nasal discharge depression, anorexia, hypraemia of nasal and conjunctival mucosa , hurried respiration, tachycardia , limb oedema and stiffness in gait, Serological diagnosis of EIV was arranged in tables 1 – 5.

Table 1: ELISA tests for detection of antibodies against EIV

Rovernorate	Results		Percentage	
Aswan, Edfo	(+)	(-)	(+)	(-)
	65	8	89.04	10.95
Total	73			

Table 2: Competition percentage of positive sera by ELISA

% of competition	(+ ve)				(- ve)
	45-65	65-75	75-85	85-100	% less 40
Serum samples No.	5	30	20	10	
Total	65				

Table 3: Screening of sera for detection of EIV antibodies by HI tests

Governorate Aswan Edfo District	Results		Percentage	
	(+)	(-)	(+)	(-)
	58	15	79.45 %	20.54 %
Total	73			

Table 4: Titration and Identification of sera for detection of EIV antibodies titer and typing by (HI) using H7 N7 and H3 N8 viruses

Titer	4	8	16	32	64
No. of samples	28	10	8	8	4
H3 N 8	+	+	+	+	+
H7 N7	-	-	-	-	-

Table 5: Matching between the results of ELISA and HI for detection of EIV antibodies

Governorate Aswan , Edfo	ELISA		HI	
	+	-	+	-
	65	8	58	15
Total	73		73	

Results of blood Picture and some serum biochemical contents were recorded in tables 6.7 and 8.

Table 6: Some blood Parameters of healthy control and Influenza infected equines

Blood Parameters	Horses		
	Control	Early stages	Late stages
R.B.Cs (T / L)	8.3 ± 1.18	7.5 ± 0.42	6.7 ± 0.5^{+}
HB (gm %)	12.8 ± 1.18	10.2 ± 0.31	9.4 ± 0.2 ++
PVC (%)	36.2 ± 1.2	29.1 ± 12	24.5 ± 11 +++
	Donkeys		
R.B.Cs (T / L)	8.4 ± 0.21	6.9 ± 0.8	6.5 ± 0.4$^{\cdot}$
HB (gm %)	12.3 ± 0.31	10.2 ± 0.5 +	8.7 ± 0.6 ++
PVC (%)	36.7 ± 1.7	30.1 ± 1.2 +	24.3 ± 1.4 ++

+ (P < 0.05),++ ¨ (P < o.o1)

Table 7: Leucogram of healthy control and Influenza infected equines

Blood Parameters	Horses		
	Control	Early stages	Late stages
W.B.CS (G/L)	8.6 ± o.91^{+}	5.2 ± 0.2$^{\cdot}$	9.7 ± 0.3
Band (%)	2.1 ± 0.01	3.2 ± 0.02 +	7.1 ± 0.03
Seg. Neu (%)	51.2 ± 1.4	52.2 ± 1.6 +	55.7 ± 1.3
EOS. (%)	1.7 ± 0.2	1.9 ± 0.1	1.6 ± 0.01
Lymp. (%)	41.8 ± 1.5 ++	32.2 ± 1.3 +++	25.0 ± 1.02
Mon. (%)	2.08 ± 0.03 +	4.23 ± 0.02 ++	9.1 ± 0.2
	Donkeys		
W.B.CS (G/L)	11.6 ± 0.4	8.1 ± 0.3$^{\cdot}$	14.1 ± 0.4$^{\cdot\cdot}$
Band (%)	1.33 ± 0.01	2.2 ± 0.01	6.1 ± 0.06
Seg. Neu (%)	59.31 ± 1.3	58.8 ± 2.01	61.98 ± 1.05
EOS. (%)	3.0 ± 0.01	3-69 ± 0.02 +	3.2 ±0.02 +
Lymp. (%)	35.0 ± 1.2	2.71 ± 1.02 +	21.3 ± 1.11 ++
Mon. (%)	0.69 ± 0.01	2.71 ± 0.4	6.31 ± 0.0 ++1

(P < 0.05) , .. (P < 0.01) , … (P < 0.001)

Table 8 : Levels of some blood constituents of Healthy Control and Influenza infected equines

Blood Paramekss	Horses		
	Control	Early stages	Late stages
AST(mu/ml)	34.01 ± 1.2	38.2 ± 1.01	72.0 ± 1.30 +++
CPK (U/L)	211.9 ± 5.11	7500.0 ± 90.5.+	9013 ± 73.2.++
LDH(U/L)	98.2 ± 1.81	450.6 ± 20.7.++	450.6 ± 20.7.++
G.GT(mu/ml)	11.14 ± 0.9	18.2 ± 1.01.+	18.2 ± 1.01.+
Bilirubin(umol/l)	16.2 ± 1.01	28.1 ± 1.04.+	30.1 ± 1.09.++
BUN(m.mol/l)	7.5 ± 0.4	20.5 ± 2.03.	38.9 ± 2.11.++
Creatinine	109 ± 3.6	109 ± 3.6	1151 ± 2.2
	Donkeys		
AST(mu/ml)	37.5 ± 1.05	65.7 ± 1.07+	1075 ± 2204.04 +++
CPK (U/L)	265.3 ± 7.5	4371.0 ± 20.1+.	6000 ± 372
LDH(U/L)	110.5 ±3.05	607.5 ± 17.4	6524 ± 45.8.++
G.GT(mu/ml)	18.5 ± 1.02	22.1 ± 1.05	27.5 ± 1.12
Bilirubin(umol/l)	12.4 ± 0.09	28.1 ± 1.22+	37.5 ± 1.01+
BUN(m.mol/l)	6.5 ± 0.07	18.5 ± 0.12++.	24.1 ± 1.02+
Creatinine	102.1 ± 3.51	115.9 ± 3.11	119.4 ± 3.4

(P < 0.001),.. (P < 0.011 , (P < 0.05)
Creatin (u mol/l)

DISCUSSION

EIV is an acute contagious respiratory disease caused by two distinct subtypes H7 N7 and H3 N8 Viruses [2] . The reported clinical signs were similar to [5] . Severity of clinical signs is related to exposure dose, virus virulence and the immune status of host. Results of ELISA and H1 diagnostic teses were in agreement with [3 and 7]. The reported anaemia may explain the effect of virus on haemopoietic tissues. Leucocytosis accompanied by neutrophilia may denote bacterial complication . Elevation in the values of AST, CPK and LDH enzymes denotes muscle destruction and myocitis. Changes in G-.Gt, Bilirubin, BUN and Creatinine should not be abnormal unless animals has suffered complications.

CONCLUSIONS

EIV is a severe respiratory disease causes about 90 % morbidities. The virus has a great deleterious effects on health condition of equines. Strict hygienic measurements are required to overcome such outbreaks.

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EXPERIMENTAL EVALUATION OF THE SPREADING ROUTES OF AVIAN INFLUENZA VIRUS, STRAIN H9N2

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SUMMARY

The low pathogenic avian influenza virus, strain H9N2 is extensively spread in commercial flocks in Israel and worldwide, and causes a significant economical burden due to synergism with various pathogens, flock density and other management conditions. As the AIV spreading efficacy is a multi-factorial complex, insight into spreading routes of recent strains of H9N2 were assessed experimentally. Main findings indicated that airborne spread was not effective under the experimental conditions employed. In contrast, contact between infected and uninfected chicks was effective in transmitting the infection for 3-4 days. The present study emphasizes the importance of physical contacts in the spread of H9N2 infection among chickens.

IINTRODUCTION

Low pathogenic avian influenza virus (AIV) (Swayne and Halvorson, 2008), strain H9N2 are extensively prevalent worldwide, causing economical losses. AIV H9N2 spread by the oral-fecal and aerosol inhalation routes in domestic poultry and wild birds, and act synergistically with other pathogens, flock density and management. The spreading efficacy depends on AIV virus strains, load, host and physiology. However, studies were limited, thus characterization of spreading routes of the recent H9N2 strains are required. Surprisingly, Capua and Alexander (2009) described that highly pathogenic AIV possess a decreased spreading ability than low pathogenic AIV.

Although two spreading routes are known for AIV, the airborne and the oral-fecal, disagreements were raised regarding their relative importance, not only for AIV but also regarding influenza infections in humans. Spreading patterns of human avian influenza is also controversial and has been in focus lately in the light of air transportation. While Hanley and Burop (2010) emphasize the critical role of contact transmission by hand contact with the airplane surfaces and less the circulating air, Mubareka et al (2009) brought evidences that the circulating air is the most important role in the virus spread. The studies of Chen et al (2010) provide support for the critical role of the airborne spread to great distances, even in warm and dusty storms.

In the present study we evaluated experimentally two spreading routes of AIV, H9N2 (Banet-Noach et al., 2007) by infecting specific pathogen free (SPF) chicks grown in isolators.

MATERIALS AND METHODS

In the first experiment AIV H9N2-infected chicks were exposed to uninfected chicks by airborne contact in one room, by opening the isolator doors, while operating the ventilation, and closing hermetically the windows and doors.

In the second experiment AIV H9N2-infected chicks were brought into physical contact with uninfected chicks into the same isolators. The infected chicks were moved sequentially to uninfected chicks during 5 days post infection, and left in contact for 24 hr with each group. After each time section of 24 hr, the infected birds were moved to a new group of uninfected birds. The ability of the infected birds to spread infective virus was examined during 5 days post-infection. Each in-contact infected group was monitored and sampled for 7 days. Infection was monitored by real-time PCR of RNA of pooled tracheal swabs from the 5 birds of each group (Ben Shabat et al., 2010), and by inoculation of 3 embryonated eggs per bird and haemagglutination activity of each alantoic fluids separately (Swayne et al., 2008).

RESULTS

Airborne spread of AIV H9N2

The scheme visualizes the experimental setup that was used to assess the airborne spread of AIV H9N2, and Table 1 shows the real-time PCR findings on RNA purified from pooled tracheal swabs at 2, 4 and 7 days post infection of Isolator no. 1.

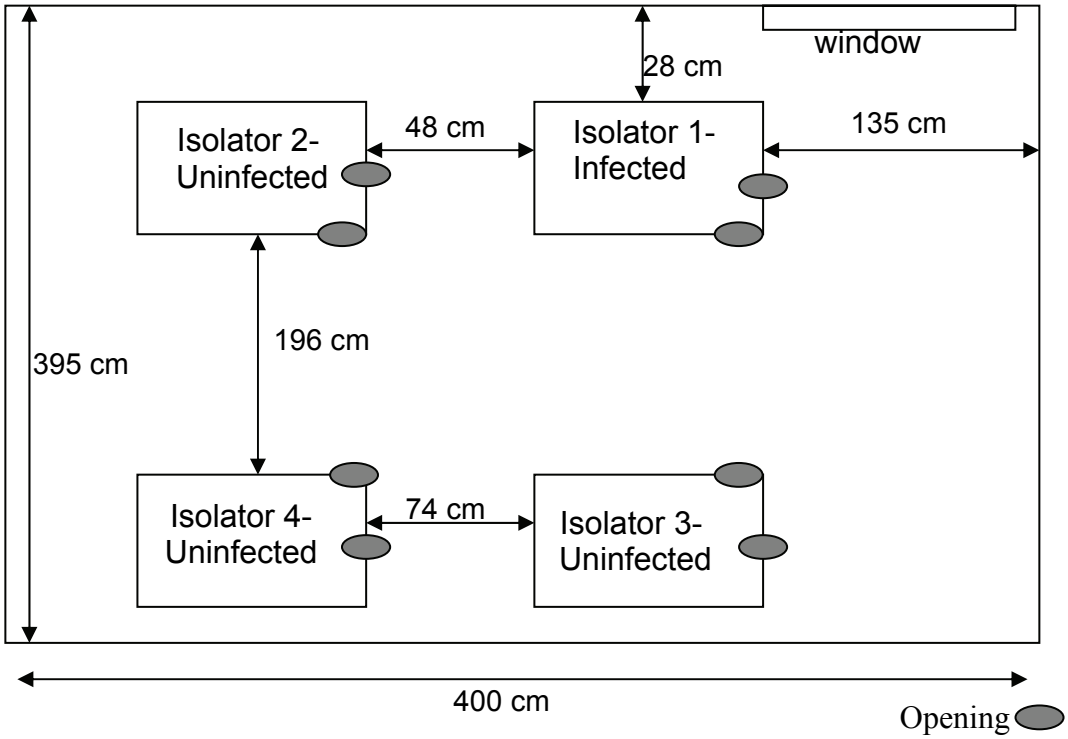

Table 1: Real time PCR for H9N2 in tracheal swabs *
No. birds positive cT ≥ 35/Total no. of birds

	Isolator 1-Infected	Isolator 2-Uninfected	Isolator 3-Uninfected	Isolator 4-Uninfected
2 dpi	*5/6	0/6	0/6	1/6
4 dpi	5/6	0/6	0/6	0/6
7 dpi	0/6	0/6	0/6	0/6

Under the experimental conditions, the airborne virus spread was minimal, indicating that the in-contact infection route contribute to virus dissemination. To evaluate the time length that in-contact infection occurs, experimental trial was performed.

In-contact spread of AIV H9N2

The following experimental groups were monitored for 7 days post AIV infection:

A- Infected at day 0 by dripping of 10^6 EID_{50} to the eye, nose and trachea.
B- 24 hr afterwards, group A was housed for 24 hr with SPF uninfected chicks.
C- 48 hr afterwards, Group A were housed for 24 hr with SPF chicks.
D- 72 hr afterwards, Group A were housed for 24 hr with SPF chicks.
E- 96 hr afterwards, Group A were housed for 24 hr with SPF chicks.
F- 110 hr afterwards, Group A were housed for 24 hr with SPF chicks.

All groups were monitored for embryo mortality after egg inoculation with tracheal swabs (Tables 2, 3) HA (Tables 4, 5), and real-time PCR (Tables 6, 7).

Table 2: Egg mortality after inoculation with tracheal swabs -H9N2 isolate 1567/04

Day 7 % No.	Day 6 % No.	Day 5 % No.	Day 4 % No.	Day 3 % No.	Day 2 % No.	Day 1 % No.	Group
28 4/14	100 6/6	100 13/13	100 2/12	100 14/14	100 8/8	0 0/12	A
77 7/9	100 15/15	70 7/10	100 13/13	35 5/14	66 8/12	0 0/10	B
42 6/14	92 12/13	50 6/12	50 6/12	84 11/13	46 7/15	0 0/11	C
100 9/9	72 8/11	61 8/13	75 9/12	35 5/14	69 9/13	0 0/14	D
54 6/11	9 1/11	36 4/11	0 0/13	12 1/8	18 2/11	0 0/13	E
50 6/12	11 1/9	54 6/11	14 2/14	10 1/10	15 2/13	0 0/14	F

Table 3: Egg mortality after inoculation with tracheal swabs - H9N2 isolate 1525/06

Day 7 % No.	Day 6 % No.	Day 5 % No.	Day 4 % No.	Day 3 % No.	Day 2 % No.	Day 1 % No.	Group
7 1/13	23 3/13	50 7/14	93 14/15	100 14/14	100 13/13	0 /120	A
0 0/13	93 14/15	100 15/15	83 10/12	100 14/14	100 13/13	0 0/12	B
0 0/15	50 7/14	91 11/12	78 11/14	90 10/11	76 10/13	0 0/15	C
0 0/15	0 0/15	57 8/14	86 13/15	100 15/15	64 9/14	0 0/14	D
72 8/11	100 14/14	92 13/14	100 15/15	100 15/15	28 4/14	0 0/14	E
25 3/12	36 4/11	14 2/14	20 3/15	16 2/12	0 0/13	0 0/15	F

Table 4: HA in alantoic fluids of eggs inoculated with swabs - H9N2 isolate 1567/04

Day 7 % No.	Day 6 % No.	Day 5 % No.	Day 4 % No.	Day 3 % No.	Day 2 % No.	Day 1 % No.	Group
20 1/5	100 2/2	100 5/5	100 5/5	100 5/5	100 5/5	100 5/5	A
60 3/5	100 5/5	100 4/4	100 5/5	60 3/5	60 3/5	0 0/5	B
40 2/5	80 4/5	80 4/5	60 3/5	80 4/5	60 3/5	40 2/5	C
60 3/5	40 2/5	40 2/5	80 4/5	40 2/5	60 3/5	20 1/5	D
0 0/5	0 0/5	0 0/5	0 0/5	0 0/1	0 0/5	0 0/5	E
0 0/5	0 0/5	0 0/5	0 0/5	0 0/1	0 0/5	0 0/5	F

Table 5: HA in alantoic fluids of eggs inoculated with swabs - H9N2 isolate 1525/06

Day 7 % No.	Day 6 % No.	Day 5 % No.	Day 4 % No.	Day 3 % No.	Day 2 % No.	Day 1 % No.	Group
0 0/5	20 1/5	80 4/5	100 5/5	100 5/5	100 5/5	100 5/5	A
0 0/5	100 5/5	100 5/5	100 5/5	100 5/5	100 5/5	100 5/5	B
0 0/5	0 0/5	100 5/5	100 5/5	100 5/5	100 5/5	100 5/5	C
0 0/5	0 0/5	80 4/5	100 5/5	100 5/5	100 5/5	100 5/5	D
80 4/5	80 4/5	100 5/5	100 5/5	20 1/5	20 1/5	20 1/5	E
0 0/5	0 0/5	0 0/5	0 0/5	0 0/1	0 0/5	0 0/5	F

Table 6: Real-time PCR of RNA purified from alantoic fluids of embryonated eggs after their inoculation with swabs of chicks infected with H9N2 isolate 1567/04

Day 7 % No.	Day 6 % No.	Day 5 % No.	Day 4 % No.	Day 3 % No.	Day 2 % No.	Day 1 % No.	Group
27.7	25.7	30	26	22.5	22.6	27.7	A
26.7	27.7	25.4	27.5	29.2	28.9	0	B
29.5	27.4	27.9	25.7	27.6	28.2	27.8	C
23.4	23.5	26	26.3	27.2	32	0	D
0	0	0	0	0	0	0	E
0	0	0	0	0	0	0	F

Table 7: Real-time PCR of RNA purified from alantoic fluids of embryonated eggs after their inoculation with swabs of chicks infected with H9N2 isolate 1525/06

Day 7 % No.	Day 6 % No.	Day 5 % No.	Day 4 % No.	Day 3 % No.	Day 2 % No.	Day 1 % No.	Group
27.8	30.5	27.8	23.9	22.6	22.3	21.7	A
30.9	26.8	23.7	22	22.8	21.9	24	B
28	29.4	23.1	22.3	21.9	23	25	C
30	28.7	25.4	22.9	22.8	23.6	27.5	D
25.7	22.9	24.2	23.8	23.9	24.5	29.9	E
0	0	0	0	0	0	0	F

DISCUSSION

The main findings indicated that the airborne spread of the AIV H9N2 strain was not effective under the experimental conditions employed. In contrast, the contact between the AIV H9N2-infected and uninfected chicks was very effective in transmitting the infection for 3-4 days. As the isolates are of different phylogenetic sub-groups, III and IVa, differ genetically by 5-10% in all genes and isolate 1525/06 appeared about 2 years after isolate1567/04, it seems that genetics expresses in biological differences, reflected in-contact infectivity. We conclude and emphasize the importance of physical contacts in the spread of H9N2 infection among chickens.

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EPIDEMIOLOGICAL STATUS OF IRAQ: AREA OF CONCERN

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SUMMARY

A control and prevention the spread of infectious diseases are of major concern in a country like Iraq, which consists of large census and animal complex diversity with reactions of deep elements of the environment. Veterinary services in Iraq were exposed to the severe damage in the wake of years of neglect, unrest, wars and conflicts. These events has turned such authorities from one of the most important existing services in the region to the less effective institutions. of empty, currently Iraq is trying to restore veterinary services to protect animals and humans from possible epidemic diseases, However, Iraq faces a deficit in the field of surveillance and preparedness for animal diseases, as well as the environmental transition that occurs in an expeditious manner which affects the stability of life natural there, The local authority seeks to legislate and document a strategic development which can support Iraq dramatically, The main objectives of this strategy is to reduce risks as well as coordination the mechanisms, strengthening the surveillance system, preparedness and rapid response to the occurrence of diseases. Also such step may encourage multidisciplinary and complementary accession to the scientific research in the coordination and modernization of veterinary services to meet all of these needs

INTRODUCTION

Animal Health in the Middle East has many to epidemic diseases. Such diseases become endemic for several decades, due to and the fact that the countries of the Middle East do not possess mechanisms to provide reports on animal and food borne diseases. These diagnosis were not well diagnosed due to lack of Adequate laboratory diagnostic tools with lack of sufficient number of professionals and public awareness. Notifying serious diseases is not mandatory. Recently Middle East countries are subjected to some diseases for the first time, which become endemic, such as Valley Fever (RVF) [1] and lumpy skin disease (LSD) [2],[3].

The control efforts of countries against these diseases may continuously due to failure to take equivalent action taken by neighboring countries. Hence, these measures should be addressed on the basis of regional cooperation with other countries to unify the programs of control and prevention. There must be an international outlook taken from the natural geographical borders and epidemiological barriers for the beneficial use in the formulation of programs of diseases control [4].

Animal Health in Iraq

Iraq faced an increasingly critical condition of unrest as a result of wars and the siege. These condition hampered the construction and development of all human activities at all levels, including the institutions of veterinary and livestock. In 1989, the number of livestock (sheep, goats, cows, buffaloes and camels) was 15.7 million and the number of large and medium-competent poultry fields were 600 all over Iraq, due to animal smuggling, and poor veterinary health care, malnutrition, ineffective control programs against emergent transbundary diseases which were lessened the number to 9.4 million head and 23 farm in 1998, It was noted that about half of that animals was infected with deferent diseases, such as Foot and Mouth Disease (FMD) and other insect-borne diseases., The occurrence of these diseases is a result of neglect and lack of veterinary care due to the siege, where vaccination was not available to the Iraqi laboratories. Iraq had informed international organizations on this issue several times, However this situation has contributed to the decline in livestock production of red and white meat and other animal products such as milk, leather with subsequent rise in their prices. The ease transportation of such animal products has its impact on the economy of neighboring countries.

Risk factors that contributed to the emergence of epidemic diseases in Iraq

Risk factor for many infectious diseases comes of being in a state of constant evolution and transformation, This development is moving along side the social and cultural changes in population patterns over all the country. Generally we find that the data indicate that the Iraqi population census in 1960, was 6.8, million, 11 million in 1975, 18.7 million in 1991 21.8 in 1998, 22.9 million people in 2000, then rose to 24.5 million people in 2003. It is expected to be up to 34.1 million in 2015 on the basis of United Nations Development Program. The increase ranged between approximately 2.5% and 3.5%, which can be regarded as a very important factor to increase the disease occurrence. Such a dramatic change affects both rural and urban settings, and the evolution of development is also working to influence the spread of disease patterns globally, from the standpoint of

epidemiology, Iraq is characterized by political and economic situation, and environmental characteristics where contagious animal diseases easily scattered within the region causing large economic losses, health authorities confronted, many challenges due to unstable political situation. This condition reflects the need regional cooperation for the exchange of epidemiological information that link three continents Africa, Asia, Europe. Also changes in climatic conditions and diversity of animal production systems as well as uncontrolled movement of nomads, made Iraq one of the more countries in the region that imports livestock and livestock products. Notably, Iraq is located on the main roads of migrating birds between Europe and Africa. Also the tendency of the region's markets is to trade with animals and their products leading to bring exotic diseases. Other related condition include the difficulty of decision-making and population changes as a result of population growth, migration and displacement and the disparities in economic wealth between countries that affect the decision-making and the need to re- structure and support the veterinary services and educators. This need may be due to lack in the supply of field, laboratory and diagnostic tool [5],[6].

The emergence of new generations of pathogens as a result of the diversity of animals, the response of causal factors to environmental changes and the changing of animal feeding over a period of time [7].

In addition to the problem of pollution and environmental characteristics that cause the deteriorative quality of the aquatic environment are due to lack of conformity of vast and major sites of industries e.g industrial parks and power plants with correct qualification of these sites. From environmental point of view much studies are needed to assess the environmental impact and there risks resulting from these industries to the environment and public health, However such procedures are not applied to a large extent now a day. Additionally industrial processes are old having no treatment units, such lack led to high concentration and magnitude and there impacts of pollutants.

Global climate change

There is a great indecation of increase of both temperature of the earth and sea-level since the mid-nineteenth century. Most of this change occurred in 1976, when temperature rose 0.6°c Another increase was recorded in 1980. the Commission of the United Nations was informed that the temperature will increase 1.4 - 5.8°c by 2100, The reason of the latter high temperatures is due to increase human activity and the excessive and inefficient combustion of for the fuel engines in the last fifty years. This change has led to imbalance in the ozone layer around the earth and increase the proportion of green house gases that retains heat [8],[9].

Changing in the patterns of rainfall in arid and semi-arid region led to drought in part of them, with contrast increase the level and heavy rains in other areas [10]. High temperature and humidity affect the life of vectors Which are the intermediate host of serious infectious diseases. With subsequent transmission. and Alamadaiv middle of the causes of disease and therefore the seriousness of transition [10]. Understanding this process requires to highlight the links between environmental change and microbial adaptation [11], as well as the emergence of new generations of pathogens. These change result from diversity of animals and response factors caused by of the environmental changes of animal and change of nutrition over a period of time. [7].

Natural ecosystems and biodiversity in Iraq

Natural ecosystems of Iraq suffered from indefinite neglect and the environmental authority does not take care of the issue, However, much attention was focused on the urban environment in the aspects of engineering, service and construction. So Iraq did not seeks to the establishment of national large-scale protected areas and strict laws protect the wild animal such practice eliminates much animal and plant fauna with subsequent conversion into industrial parks, the absence of legislations that regulates hunting of birds and fish leading to worsen environmental conditions of these livestock wealth. The subsequent impact causes the extinction of many animal and plant species from the environment without assuming serious steps to preserve these species. These changes occurred after drying marshes which is a key site for the proliferation of many types of birds and biological resources. Marshes are regarded as a suitable site for migratory birds migrating from the Arabian Gulf, as well as a niche of certain and many types of migratory species.

Animal Information System in Iraq

The information systems provide animal service in a slightly different manner, However, such differences in view point remain unchanged. The information System include:

1- The case of the disease: The main problem in Iraq in the AGP often is of epidemic diseases due to infection and mortality rate, This problem is due to bad apparition of quarantine, Uncontrollable movement of animals and continuous spreed new diseases [12].

2- The dependence on the agriculture: Most developing countries including Iraq depends upon agricultural and livestock production [13].

3- The Veterinary Infrastructure: generally veterinary services in Iraq is limited. small veterinary services are provided by the governmental authorities, Such authorities often lack financial resources to operate trained and skilled personnel or promotion of employees to a higher level. The majority of veterinarians are assigned in administrative work with lack of technical training curses.

Also a lack is seen in volunteers in this area relating with this field [12], [14],[4].

4- Natural infrastructure resources: Transport and communications fields in developing countries may be difficult in sometimes, especially in remote rural areas in which some animals suffer immense medical problems. In such cases, It is difficult for the breeder to contact with veterinarian which later affects the collection of data and information. [12],[7].

5- Sources of finance: Money that is set for the veterinary sector is often low because this sector has a precedence of a second or third after health and education. veterinary sector in most field cases is supported by foreign aid which is frequently noted of vaccination fields [15],[7],[4], and one of the roles of animal information system is to provide information to support applications that determine the size and material assistance offered by donors or international organizations.

6- Sources of personnels: Since education systems are less advanced there is a lack of essential expertise not only of veterinarians but also from experts in the field of programming computer and statistical experts, this lack includes training courses, the technical infrastructure lack of skills. The result due to lack of experience of veterinarians [14],[7].

7- The integration of technology: as a consequence of limitation of financial resources and staff, these measures make it unable to keep up with changes in information technology. Information systems structure depend on the computer such as geographic information systems which is common in the developed world. However many developing countries, including Iraq uses system based paper, Intrusive modern techniques require new skills with solution of new problems such as the need for different types of data [7].

Animal health cases recorded by the OIE and WHO in Iraq

There were 21 cases recorded by the Organization of Animal Health and World Health Organization concerning the health of the animals viz., Anthrax (1995–2004), Camel Pox (1995), screw fly (1998), PPR (1998), FMD (1999), RVF (2001) which is not confirmed, Rabies (2004), Leshmania (2001-2003-2004 to 2005), avian influenza (2006), and swine flu (2009) [16],[17].

Factors that contributing dynamic nature of the epidemic diseases

1- Increased international transportation which regarded as the most important method in the spread of disease due to the increased movement of animals and there products, There was large increases in transportation by land, sea and air for human, animal and goods as a result of trade growing, increased demand for livestock products, movement of nomads and refugees with their animals away from wars and unrest condition, These factors contributed the spread of diseases through the large costs for the maintenance of effective quarantine barriers at airports, seaports and along the land border.

2- Change in livestock production systems in many countries. This can be seen as an increasing tendency for many countries to focus their trade in animal products especially in the cities surrounding areas. Animal population in such location are large that provide great opportunities for the movement of animal diseases quickly and economic losses be the largest.

3- The decline in the level of governmental veterinary services, and other animal infrastructure activity of animal. This led to uncontrollable animals and their movement, However, financial compensation system of losses due to animal diseases is not followed such decline can be seen by poor diagnostic services and an inability to rapid and practical respond to diseases and outbreaks. Hence, they are forced to sell their apparently healthy animals to alleviate the financial losses. some farmers tend to sell some animals that are in their early subclinical stages of the disease which contributes to the significant spread of the disease.

4- Increased animal raising in areas in the new environment. Such rainy forests and other wildlife that are converted into animal rearing area. Gathering of human and animals in one area make them in direct contact with a wide range of new infectious diseases as a result of environmental pollution occurred by remains of diseased wild animals and contaminated pools of unknown source of water. Some of these diseases have a vulnerability to spread to humans and animals, having high-impact and fast-spreading character in the new hosts.

5- High temperatures led to a change in the level of rainfall and weather patterns in a number of areas which have affected the distribution of generation of insects e.g. mosquitoes, fleas and lice that transmit viral diseases and serious parasitic protozoan. Many of these diseases and infection are transmissible.

6- Evolution of certain types of pathogens due to development of microbial resistance. This resistance resulted from the brood use of antibiotics having more impact on public health [11],[18],[19],[20],[21], [22],[23],[24],[25],[26].

Application of appropriate programs in the resistance to infectious diseases

New views in scientific knowledge occurred in the last ten years, due to the development of genetic engineering and computer fields. Such new techniques and tools helped in the resistance of cross-boundary emergent diseases, this resistance is clear in technically progressed regions through :

1. Investigation of the disease and the legislation of information systems for animal health
2. Other methods to study of the pathology and epidemiology.
3. Diagnosis and characterization of the causative agent of the diseases.
4. Development or of better programs to control and eliminate diseases [27],[28],[29].

Area of interest and the formulation of management strategies

The main area of concern which includes the rapid proliferation of epidemic diseases and their control and prevention can be summarized as follows:

1. Control and prevent the spread of infectious diseases of major concern should be kept in a country like Iraq. Iraq is composed of people and animal having profound interactions with expeditious environmental transition, therefore a policy document must be developed within a strategic framework that could dramatically help countries in the Middle East. The main objectives of this strategy is to reduce the risk, coordinate mechanisms, strengthen the surveillance system, preparedness and response to disease and encourage the accession of the multi-disciplinary complementary scientific research.

2. It is necessary to identify the major areas of foci, work on legislation and public education analysis. This process includes economic and social impacts of infectious diseases and their control and prevention. The prevention involves control of the vector and the definition of priority of communicable diseases and assessing the early warning system. The latter includes the development of the infrastructure animal health services, cooperation between the veterinary and public health authorities and the development of an appropriate strategy to control epidemic diseases in the areas of international borders.

3. Development of a modern surveillance systems should be serious. These system include GPS, GIS, remote sensing and molecular epidemiology, bioinformatics and information technology, and economic sociology. Due to the fluctuating occurrence of many infectious diseases, it is necessary to develop a forecasting system or warning system to develop in vector-borne diseases.

4. It is necessary to develop a link between networks of veterinary laboratories and medical professionals in different levels.

5. Demonstration of geographically designated areas with a support of the important diagnostic laboratories in disease outbreaks. These information must be addressed and sent to the medical authorities in the shortest possible time to start interventions. It is necessary to link laboratories to facilitate the movement of information. Smaller units should be equipped with modern diagnostic tools for rapid diagnosis alongside with different molecular diagnostic tools.

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DETECTION OF HI-ANTIBODIES TO A CIRCULATING HUMAN INFLUENZA B VIRUS AMONG LIVE PIGS IN IBADAN, NIGERIA

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SUMMARY

Influenza B viruses cause natural disease only in humans. However, considering the role of pigs in reassortment of influenza viruses, we investigated the possibility of previous influenza Bvirus infection of pigs in Ibadan, Nigeria. Serum specimens obtained from apparently healthy, unvaccinated pigs were tested for antibodies to two circulating strains of human influenza B virus. Results obtained revealed that 3.3% (n=91) of these pigs were exposed to influenza B/Shanghai/361/2002-like either prior to or during this study. These findings show the need for determination of the susceptibility of pigs to influenza B viruses and investigate their roles in influenza B virus evolution.

INTRODUCTION

Unlike Influenza A viruses which cause natural infection in many species including aquatic birds, Influenza B viruses cause influenza almost exclusively in humans [12]. Two antigenically and genetically distinct lineages, represented by B/Victoria/2/87 and B/Yamagata/16/88 have co-circulated in humans since at least 1983 [10]. Pigs can be naturally or experimentally infected with avian and human influenza viruses [7, 8]. In recent years in south-western Nigeria, increasing agricultural activities, coupled with poor biosecurity have increased the possibility of interspecies transmission of influenza viruses between swine and pig handlers [1, 6]. Using serological survey, this study therefore investigated the possibility of human-to-swine transmission of two influenza B virus strains, one belonging to B/Victoria/2/87 lineage and the other belonging to B/Yamagata/16/88 lineage, which circulated among humans in Ibadan, south-western Nigeria, in 2008 and 2009.

MATERIAL AND METHODS

Serum specimens were collected from ninety-one out of one hundred and ninety-nine (91/199) apparently healthy Landrace pigs at three locations within Ibadan from April to June, 2008 and tested at the Department of Virology, College of Medicine, University of Ibadan (U.I). These animals were sampled as follows: 24/76 boars, 44/64 sows and 23/59 growers. The locations were: Commercial Pig Farm Unit, U.I (11/31 pigs); University Research Farm, U.I (10/35 pigs); and Municipal Abattoir, Bodija (70/133 pigs). These pigs were obtained from different sources and they had never received influenza vaccine. Twenty-four out of 57 (24/57), 42/63, and 25/79 pigs were bled in April, May and June, 2008 respectively. Pigs sampled on farms were bled through the cranial venacava, while blood was collected from the jugular vein immediately after slaughter at the abattoir. About 5ml of blood was collected from each pig into labeled sample bottles (without anticoagulants) and allowed to clot. These were centrifuged in the laboratory at 3000rpm for 10 minutes. Sera were then removed using Pasteur pipettes and stored in labeled eppendorf tubes at -20^{o}C till they were tested.

Virus strains used for Haemagglutination Inhibition (HI) Assay were influenza B/Shanghai/361/2002-like (B/Yamagata/16/88 lineage) and B/Malaysia/2506/2004-like (B/Victoria/2/87 lineage) CDC reference antigens. Sheep Influenza B/Shanghai/361/2002-like (Homologous HI titer 128 / 4HA) and B/Malaysia/2506/2004-like (Homologous HI titer 128 /4HA) CDC reference antisera were used as positive serum controls. Non-specific inhibitors of haemagglutination were removed from all test sera and positive serum controls by receptor destroying enzyme (RDE) treatment, to obtain a 1:10 dilution which had one volume serum, three volumes RDE, and six volumes physiological saline.

HI Assay was performed according to W.H.O Protocol for Animal Influenza Diagnoses and Surveillance Manual [11]. The HI procedure was as follows: Dilutions which contained 4HA units/25µl of reference antigens were obtained before each test and a back-titration of the 4HA was performed to verify its correctness. Dilution (1:10) of each test serum and control serum was then prepared through RDE treatment. Two rows of wells in a V-bottom microtitre plate were labeled for each test and control serum, and 25µl of PBS was added to wells 2 through 12 of each row. Fifty microlitres of each treated serum was then added to the first well labeled for it, from where 25µl was two-fold serially diluted across the row and discarded after well 10 to give a dilution of 1:20 through 1:5120. The last two columns were used as red blood cell (RBC) control wells. Twenty-five microlitres of standardized reference antigens were then added to the appropriate

wells. Plates were agitated manually and incubated at room temperature for 15 minutes, after which 25µl of 0.5% chicken RBC suspension was added to all wells. Plates were manually agitated and incubated at room temperature (25^{o}C) for 30 minutes. Reciprocal of the endpoint of serum dilutions which showed complete inhibition of haemagglutination of 4HA units of the virus with a 0.5% solution of chicken red blood cells were recorded as the HI titre. Results obtained were analyzed by two-way ANOVA and Student's t-test, using GraphPad Prism (GraphPad Software Inc., San Diego, USA). Values of $P<0.05$ were considered significant.

RESULTS

Prevalence of antibodies to influenza B/Shanghai/361/2002-like virus among pigs during this study was 3.3% (n=91). None of the pigs tested had HI antibodies against influenza B/Malaysia/2506/2004-like virus. These results are shown in Table 1. Titres of antibodies to influenza B/Shanghai/361/2002-like virus in each of the three pigs that were seropositive were 160, 160, and 320 HIU/25µl respectively (mean = 213.3 HIU/25µl). Seropositive pigs included 2 boars (n=24) and 1 sow (n=44).

Table1: Prevalence of HI antibodies to influenza B viruses in pigs during the study

Location	B/Shanghai/361/2002-like	B/Malaysia/2506/2004-like
Commercial Pig Farm Unit, U.I (n=11)	3 /27.3*	-
University Research Farm, U.I. (n=10)	-	-
Municipal Abattoir, Bodija, Ibadan. (n=70)	-	-
Total	**3 (3.3)**	**0 (0.0)**
Month		
April	-	-
May	3 (7.1)	-
June	-	-
Total	**3 (3.3)**	**0 (0.0)**

*Prevalence is significantly higher ($p<0.05$) than in the other two locations

DISCUSSION

While Osterhaus and others [9] reported the isolation of an influenza B virus from a seal in the Netherlands, isolation of influenza B viruses from animals has not been reported in Nigeria. In a parallel study conducted by the authors, only influenza A viruses were isolated from these pigs [3]. The only study which reported the presence of HI-antibodies to influenza B viruses in pigs in Nigeria (with very low prevalence) was that conducted by one of the authors of the current paper [5]. However, results from the current study indicate a higher prevalence of 3.3% among pigs in Ibadan for influenza virus B/Shanghai/361/2002-like. Moreover, analysis of results obtained in the present study in the light of a related study by the authors shows that the risk of influenza B virus infection of these pigs tend to increase as the level of exposure of their handlers to these viruses increased. For instance, all pigs which had antibodies to influenza B/Shanghai/361/2002-like were sampled at the two locations, within the University of Ibadan, where people had higher prevalence of antibodies to this virus (100.0% and 90.0%) as compared with results of HI Assay on sera obtained from the same people for influenza B/Malaysia/2506/2004-like virus (50.0% and 40.0% respectively) [2]. Figure 1 clearly shows this comparison. Human influenza B/Shanghai/361/2002-like was also isolated from a pig handler in one of these two locations [4].

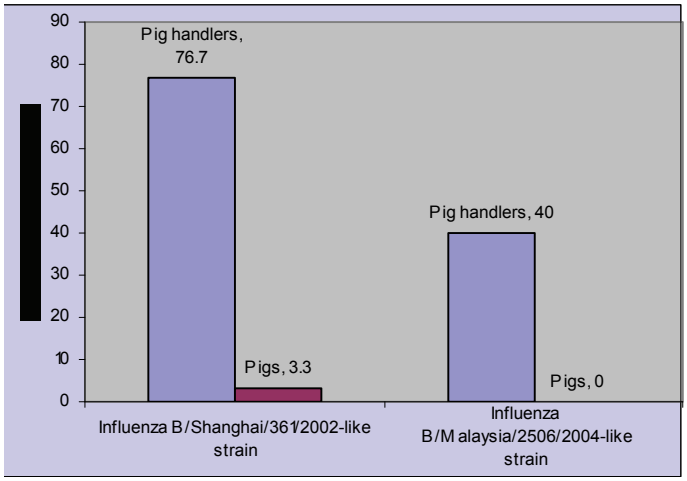

Figure 1: Percentages of pig handlers and pigs with HI-antibodies to influenza B virus

CONCLUSIONS

Although our data do not allow conclusions about susceptibility of pigs to influenza B viruses, considering the role of the pig as an important 'mixing vessel' for influenza A viruses, detection of antibodies to a human strain of influenza B virus among pigs in Ibadan, by HI assay which is considered as the gold standard for detection of influenza antibodies, should prompt more detailed studies to establish the susceptibility of pigs to influenza B viruses and determine the roles of pigs in the evolution of influenza B viruses. It should also serve as an urgent call for effective surveillance among swine herds in south-western Nigeria, not only for influenza A viruses, but also for influenza B viruses.

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SEROLOGİCAL ANALYSİS AND DETECTİON OF BOVİNE HERPESVİRUS -1 İN TİSSUES AND NASAL SWABS BY PCR İN TURKİSH CATTLE

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SUMMARY

Infectious bovine rhinotracheitis (IBR) has been reported in many countries and has economic impact on livestock production by causing respiratory and reproductive disorders. Therefore, this study was conducted to investigate the frequency of IBR infections by using PCR and ELISA. For this 246 sera from non-vaccinated animals, 32 nasal swabs, 7 lungs from dead cattle and 11 aborted fetuses originated from different regions (Aegean, Marmara, Black Sea and South East Anatolia) of Turkey were collected. Sera were analysed by a commercial blocking ELISA (gE) and, nasal swabs and tissue samples from lungs and aborted fetuses were analysed by PCR using the primers spesific for bovine herpes virus -1 (BoHV-1) targeting the gC gene. Antibodies to BoHV-1 were detected in 63 (25.6 %) of 246 cattle sera by ELISA with the smallest rates 1.6 % in Aegean and 7.8 % in Marmara region which borders European Union. A 173 bp product was amplified by PCR in positive control, 6 of 32 nasal swabs and 2 of 11 lung samples. No product was seen in negative control and samples taken from aborted fetuses. Sequence and phylogenetic analysis is going on for the positive samples. In conclusion, this study and previous studies have shown that BoHV-1 has been circulating in Turkish cattle for a long time. Therefore, strict eradication programme is necassary to control BoHV-1 infections in Turkish cattle. Particular care must be taken for the verified risk factors associated with the spread of the infection.

INTRODUCTION

Infectious bovine rhinotracheitis and infectious pustular vulvo-vaginitis (IBR/IPV) are economically important infections of dairy cattle caused by bovine herpes virus-1 (BHV-1). The BHV-1 is an enveloped DNA virus and classified in the family *Herpesviridae* within the subfamily of *Alfaherpesvirinae* (1, 12). BHV-1.1 is generally responsible for the respiratory disorders and abortions in cattle while BHV-1.2 causes mainly balanopostitis and pustular vulvovaginitis (1, 12).

Infected cattle are the major source of infection those cattle spreads the virus via all secrets and excrets including, milk, semen and vaginal fluid. Transmission occurs via respiratory and veneral route by direct and indirect contact. Therefore, in the closed breeding systems the closest animals the fastest is the spread of the virus amoungst the animals. Iatrogeneic transmission also occurs. The genom of the BHV-1 in latency was found in trigeminal and/or sacral ganglions and in tonsils. The virus reactivates when the animals exposed to stres and cortizol given parenterally. As a result of reactivation, viral spread occurs and transmission may realize (1, 12).

In some countries especially in the EU, preventive measurements have been applied to eradicate disease. It has been eradicated in Finland, Switzerland, Denmark, Austria, Sweeden and Norwey using testing, culling and vaccination strategies (1, 9, 12,). The aim of this study was to investigate the frequency of IBR infections by using PCR and ELISA.

MATERIALS AND METHODS

Sera from 246 non-vaccinated animals, 32 nasal swabs, 7 lungs from dead cattle and 11 aborted fetuses originated from different regions (Aegean, Marmara, Black Sea and South East Anatolia) of Turkey were collected (Table 1). Sera were analysed by a commercial blocking ELISA (gE) (IDEXX) and, nasal swabs and tissue samples from lungs and aborted fetuses were analysed by PCR. DNA was extracted using a commercial kit (High Pure PCR Template Preparation Kit-Roche).

The medhod of PCR and primers targeting BoHV-1 "gC gene" used in this study was same as reported previously (14). Test samples and controls were tested for the presence of BHV-1 DNA by PCR after standardization. The nucleotid sequences and predicted product size of the primers are as follows: F-5'-CTG CTG TTC GTA GCC CAC AAC G-3' and R-5'-TGT GAC TTG GTG CCC ATG TCGC-3' with expected size of 173 bp. Each 25 µl PCR reaction mixture consisted of 12.5 µl of Hotstar Taq Master Mix (Qiagen), 0.5 µl of 25 mM $MgCl_2$ (Qiagen), 1,5 µl F primer (10 pmol/ µl), 1,5 µl R primer (10 pmol/µl), 6 µl nuclease free water and 3 µl DNA. The mixture was placed in a thermal cycler (Biorad, Chromo 4) and the polymerase activated by incubation at 95°C for 15 min. The mixture was then cycled at 95°C for 1 min, 60°C for 1min and 72°C for 1 min for 30 cycles and 72°C for 5 min, 4°C 5 min.

RESULTS

Antibodies to BoHV-1 were detected in 63 (25.6 %) of 246 cattle sera by ELISA (Table 1) with the smallest rates 1.6 % in Aegean and 7.8 % in Marmara region which borders European Union. A 173 bp product was amplified by PCR in positive control, 6 of 32 nasal swabs and 2 of 11 lung samples (Figure 1). No product was seen in negative control and samples taken from aborted fetuses. PCR positive samples were also found to be positive by ELISA.

Table 1. Samples and the results of ELISA.

Region	Number of samples	Number of positives
Black sea region	31	1
Aegion Region	62	1
Marmara Region	38	3
South East Anatolia	115	58
Total	246	63

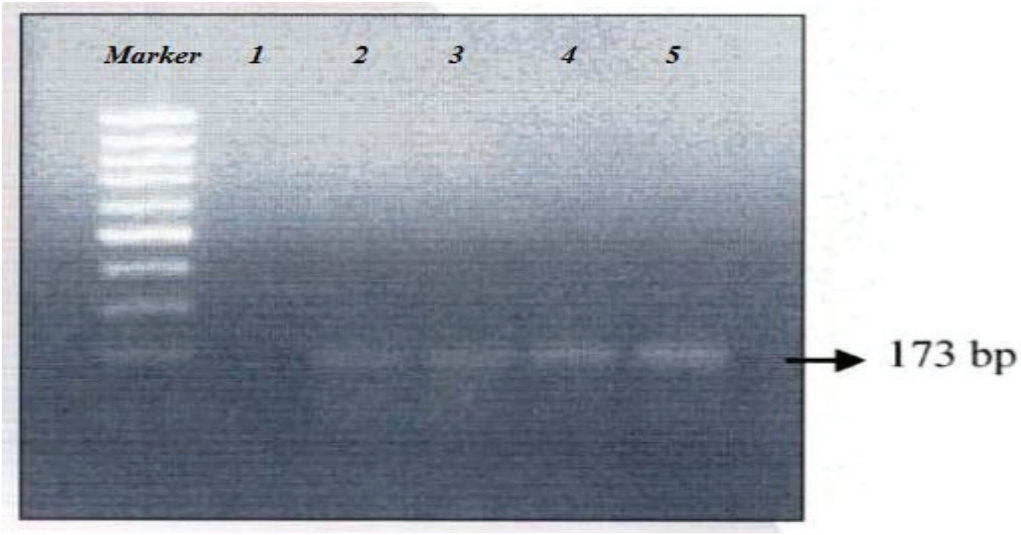

Figure 1. 1. Negative control; 2, 3 and 4. Positive samples; 5. Positive control (173 bp).

DISCUSSION

BHV-1 infections causes economical loses in cattle production and therefore needs to be controlled by applying preventive measurements (1, 9, 12). In order to control the disease spread and transmission of virus and the prevalence in herd level needs to be investigated. ELISA and PCR have been used by many investigators. Recently, marker ELISA to differentiate vaccinated and infected have been applied. However, this is not compulsory in Turkey at present.

In the present study, antibodies to BoHV-1 were detected in 63 (25.6 %) of 246 cattle sera by ELISA (Table 1) with the smallest rates 1.6 % in Aegean and 7.8 % in Marmara region which borders European Union. These findings are similar to those obtained in other countries. In Hungary, Tekes and his colleques (13) investigated small and big farms for the seroprevalence of BHV-1 and 3.4-50% seropositivity was reported. In Italy, 34.9-38.65 % seropositivity was reported (6). In Turkey overall prevalence varies between 0-100 %. Alkan and her colleques (2) performed a study in 10 government farms located in different regions of Turkey and 4-98 % seropositivity was found. The overall prevalence was 59.7 in 480 cattle (2). Çabalar and his colleques (7) have investigated the prevalence of BHV-1 in 20 farms and the seropositivity was between 6.66-100 %. Similar studies were made by other investigators in Turkey, Burgu and Akça (5) 43.3-100%, Bilge-Dağalp and her colleques (4) 55-1-95.8 %, Özkul and his colleques (11) 0-60 % (overall prevalence 21 %) seropositivity. In the Trace district 35% seropositivity was found in 428 cattle (3).

In the present study, nasal swabs and lungs were also analysed by PCR in order to determine the virus spread. PCR was choosen since it is easy to apply and high in sensitiviy and spesificity (8, 10). Mwene and others (10) could detect BHV-1 DNA in nasal and eye swabs after 19 days experimental infection. They performed study in acutely infected animals. In the field like in the present study, it is difficult to know the type of infection of animals (acute, chronic or latent). In this study, 6 of 32 nasal swabs and 2 of 11 lung samples. These samples were also positive in ELISA.

IN CONCLUSION, IBR has been reported in big and small farms in Turkey. The owner of big farms applying preventive measurements such as seperation, hygiene, testing, culling and vaccination but, the small farms are not doing these regularly. The preventive measurments as mentioned above needs to be applied in Tukey in a dedicated governmental control and eradication programs.

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THE INVESTIGATION OF AVIAN MALARIA IN MOSQUITO SPECIES COLLECTED FROM CENTRAL TURKEY (Abstract)

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INTRODUCTION

Vector-transmitted parasites such as *Plasmodium* and *Haemoproteus* rely on the presence of both an appropriate host and a component vector. Species of avian *Plasmodium* are most commonly transmitted by mosquitoes. In avian host, *Plasmodium* spp. And *Haemoproteus* spp. are globally distributed and have been detected in a wide range of species. With the application of molecular diagnostic techniques, both parasite genera have been shown to exhibit varying degrees of host specificity and modes of transmission. This study was carried out to investigate the potential vectors and relative mosquito infection rates of avian *Plasmodium* species throughout two mosquito seasons (2008-2009) in Kayseri province where is located in Central Anatolian part of Turkey. The phylogenetic analyses of determined avian haemosporidian isolates were also performed.

ANIMALS, MATERIALS AND METHODS

During two seasons between June and August of 2008 and 2009, totally 6153 female mosquito samples collected from 46 foci in various regions of Kayseri were included to the research. Each mosquito's head-thorax and abdomen were dissected, categorized with respect to species and collection area and totally 1198 mosquito pools (599 head + thorax, 599 abdomen) were constituted following species identification. After the genomic DNA extraction from the pools, Nested PCR analyses were carried out with two avian haemosporidian protozoa's (Plasmodium, Haemoproteus) specific primer pairs which were amplified to different size regions of partial mitochondrial (mt) cytb gene. Samples, which were determined as positive by Nested PCR's, were gel purified and sequenced in terms of avian haemosporidian mitochondrial (mt) DNA's partial cyt-b gene region. Pairwise analyses of the obtained DNA sequences and multible alignments with some other avian haemosporidias' registered in GenBank were done and phylogenies' were investigated.

RESULTS

One hundred twenty eight (59 head + thorax, 69 abdomen) (%10.7) of totally 1198 Genomic DNA pools were found to be positive with both Nested-PCR analysis. The minimum (MIRs) and expected (P) infection rates in totally 6153 female mosquitoes examined during two seasons were calculated as 1.12 and 1.21, respectively. MIRs and P were determined as 0.60 and 0.63, 2.09 and 2.50, 1.04 and 1.18, 1.06 and 0.69 for *Ae. vexans, Cx. pipiens, Cx. theileri* and *Cs. annulata,* respectively. GenBank records of sequence analyzed 13 isolates (HQ677623-24, JF411401-8, JF430404-6) were performed. DNA sequences obtained from 11 head/thorax and 2 abdomen positive pools were determined as *Plasmodium* sp. and *Haemoproteus* sp., respectively according to the results of Blastn analyses. 11 Plasmodium sp. isolates showed %93-100 identity with each other and shared %89.5-100 identity with some other avian *Plasmodium* isolates from the world. Two *Haemoproteus* sp. isolates showed %98,6 identity with each other and shared %98.6-100 identity with the other avian *Haemoproteus* isolates from the world. Result of phylogenic analysis were revealed that 11 isolates belonging to *Plasmodium* genera were located in 4 main phylogenic groups whereas 2 isolates belonging to *Haemosporidia* genera were located in 1 phylogenic group.

CONCLUSIONS

This study was the first report of molecular detection and characterization of avian *Plasmodium* and *Haemoproteus* from mosquitoes in Turkey and was financially supported by The Scientific and Technological Research Council of Turkey with the project no 110 O 049. *Cx. pipens* was designated as the main vector of avian malaria in the study area. Future research could involve large scale arthropod sampling to identify patterns of parasite endemism, diversity and vector competence in Turkey.

IS AUSTRIAN RED DEER (*CERVUS ELAPHUS ELAPHUS*) A RESERVOIR FOR BVDV-INFECTION IN CATTLE?

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SUMMARY

During the time period 2007-2009 ear notch samples from free-living (n = 527) and farmed (n = 237) Austrian red deer *(Cervus elaphus elaphus)* were tested for bovine viral diarrhea virus type 1 (BVDV-1) and type 2 (BVDV-2) by enzyme-linked immunosorbent assay (ELISA) and single-tube real time reverse transcription polymerase chain reaction (real-time RT-PCR). Ear notch samples were collected from randomly selected hunted-harvested red deer and from individuals originating from deer holdings. All samples were tested negative for BVDV-1 and BVDV-2. Results of this study show no evidence of persistently or acute infected animals. They indicate further that BVDV is playing a minor role in free-living and farmed red deer in Austria and that red deer is not a reservoir for BVDV-infection in cattle.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) causes a complex disease primarily in cattle including respiratory and reproductive symptoms, abortions, mummification, congenital anomalies, stillbirths, birth of persistently infected carrier animals (PI animals) and can lead to fatal mucosal disease [2]. In Austria bovine virus diarrhoea in cattle is a notifiable disease. Most pestivirus isolates from cattle are classified as BVDV-1 showing a high genetic diversity [3]. In the federal state Tyrol, BVDV control is based on antigen detection in newborn calves. Whenever a newborn calf is ear tagged according to EU-regulation, the inserted ear tag is equipped with a tissue sample collection device. Tissue samples from newborn calves are submitted throughout the year to the laboratory for antigen detection as a continuous monitoring process. The same sampling technique was applied to the ear specimens of individual cervids included in this survey.
In free-living and captive cervids, BVDV has been detected in a wide range of European and North American species. In wild ruminants living in the alpine regions the epidemiology of pestivirus infection is not well known and difficult to assess. It is assumed that persistently infected cattle play a key role in epidemiology of BVDV infections in red and roe deer, in the same manner as has been found in white-tailed deer [7]. Common contact with cattle on pastures or licking the same salt stones on mountain pastures might introduce the virus to susceptible animals [4]. In several countries bordering Austria serological investigations to estimate the seroprevalence of antibodies in red deer have been performed. In Italy antibody prevalence was found to be 5.8 %, in Germany 5.4 % and 2 %, respectively [4, 6, 8]. In a study of red deer from the Austrian province of Carinthia antibodies were found in only one out of 59 examined animals [5]. The purpose of our study was to investigate the prevalence of BVDV in free-living and captive red deer originating from all geographic regions from Austria. Further this data should provide evidence as to whether red deer do act as a reservoir for the infection of cattle.

MATERIAL AND METHODS

During the time period 2007-2009, ear tissue specimens from free-living (n = 527) and farmed (n = 237) red deer, all over the age of 18 months, were included in this investigation. Ear notch samples where derived from ears originating from red deer which where included in the national surveillance program on transmissible spongiform encephalopathy (TSE) in cervids according to EU-legislation. Sample size and geographical distribution were randomly selected according to the directives of the Austrian Federal Ministry of Health. In addition to brain samples for the detection of TSE, one ear from each animal was submitted to the laboratory. The majority of samples were delivered during autumn and winter. Tissue sample were obtained by using commercially available ear tagging system. When applying the ear tag, a tissue sample (2-3 mm in diameter) is punched out of the ear of the animal. Two samples were collected of each individual ear, one for testing in the ACE (Antigen Capture ELISA) and one for testing in real-time RT-PCR.

In the laboratory, the barcode of the sample container was electronically identified. The sample container was cut open manually, tissue samples were placed in individual tubes containing dilution puffer, eluted over night and examined for antigen the next day by ACE. In addition, a single-tube real time RT-PCR on the Rotor Gene was performed. The samples were diluted individually and after incubation over night, pools of 10 samples were investigated according to the manufacturer's instructions. The validation of both tests was performed with BVDV-positive ear notch samples of infected white-tailed deer originating from North America.

RESULTS

All tissue samples tested for both BVDV-1 and BVDV-2 by real-time RT-PCR and ACE were found to be virus negative.

DISCUSSION

Experimental infections in non pregnant American elk have shown that infected animals become viremic, seroconverted and were shedding the virus but did not produce acute disease with clinical signs. Virus shedding does not appear to be longer than one week [9]. In the current study most of the animals were investigated during autumn and early winter time. We expected a higher probability of finding an infected animal as the possibility of intraspecies transmission in red deer should be high during mating season and especially in winter when crowding at feeding places can be observed.

Cattle are suggested as a possible source of infection for BVDV in free-living deer [4]. In Austria a compulsory BVDV-control program in cattle was established in 2004. Due to this eradication program the prevalence rate of infected cattle herds is constantly dropping. With ongoing progress of this program the probability of cattle acting as a reservoir for cervids is negligible. Currently in Austria approximately less 0.3 % of all cattle herds under investigation are BVDV infected [1]. In 2003 a serological survey in red deer in the federal Austrian province of Carinthia determined a prevalence rate lower than 2 % in deer [5]. In addition to these findings our results on a national level confirmed the hypothesis that this wildlife species does not act as a reservoir for BVDV-infection in Austrian cattle.

CONCLUSIONS

The results of our study indicate that acute or persistent BVDV infections are uncommon in free-living and farm-raised red deer in Austria. There is no significant evidence that this wildlife species serves as a source of infection for cattle.

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DEVELOPMENT OF SEROLOGIC DIAGNOSTIC SYSTEMS FOR FOOT-AND-MOUTH DISEASE

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SUMMARY

The aim of this paper is to (1) Develop a higher sensitive and specific foot and mouth disease virus (FMDV) non-structural protein (NSP) antibody detection kit in comparison to the current commercial NSP antibody detection kit, and (2) Develop an ELISA detection kit for FMDV structural protein antibody as an alternative to the SN test. The technology platform based on ELISA was developed and evaluated for the detection of anti-SP and anti-NSP antibodies in this study. The results revealed that the methodology of ELISA was effective in detecting antibodies against NSPs of FMDV O/Taiwan/97 in sera of infected pigs and there was also a positive correlation between OD values and SN antibody titers.

INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious disease in cloven-hoofed animals [1-3] that has had severe socio-economical effects in many countries. In 1997, Taiwan experienced a devastating FMD outbreak in swine, which resulted in serious economic loss [4,5]. The outbreak has since been successfully controlled by an eradication program, which included the vaccination of swine. Although many other countries endemically infected with FMDV have also used vaccination as a control method, distinguishing vaccinated animals from infected animals remains a problem. Infected animals produce antibodies to both the structural and non-structural protein (NSPs). Antibodies mainly to the structural proteins (SPs) of FMDV were induced by vaccination. Therefore, assays demonstrating antibodies against NSPs have potential to differentiate infected animals from those that have been vaccinated [6-11]. Serum neutralizing (SN) test is used for the routine detection of antibody in pig serum to evaluate the efficacy of the vaccination. However, it can also be very time consuming, laborious, costly and inefficient, especially for large-scale serological monitoring systems.

MATERIALS AND METHODS

Serum samples

To evaluate the sensitivity and specificity of the tests, total 150 sera from infected pigs (30 field positive and 20 experimental positive) and naïve pigs (20 field negative, 30 field vaccinated, 30 experimentally vaccinated and 20 specific pathogen-free (SPF)) were used.

Serum neutralizing (SN) test

The sera collected from pigs were tested using the SN as previously described [12]. Antibody titers were expressed as the reciprocal of the final dilution of serum in the serum/virus mixture which neutralized an estimated 100 TCID50 of virus at the 50% end-point.

Expression, purification of FMDV polypeptide

In this study, *E. coli* protein expression system was used to express the NSPs and SPs of FMDV O/Taiwan/97.

ELISA

Indirect ELISA assays based on the *E. coli*-expressed FMDV NSP 3ABC polypeptide or VP1 were developed and evaluated for the detection of anti-SP and anti-NSP antibodies from the pig sera mentioned above.

RESULTS

The NSP 3ABC-based ELISA test was evaluated by using the 150 porcine sera and the results indicated the sensitivity and specificity of the test was 92% and 100%, respectively. The correlation value (r) between SN antibody titers and VP1-based ELISA titers (expressed as mean OD_{450} value) was 0.91 as determined by simple regression test. Results revealed the use of ELISA method was effective in detecting antibodies against NSPs of FMDV O/Taiwan/97 infected swine serum samples. And there was also a good correlation between OD values and SN antibody titers.

DISCUSSION

Vaccination is one of the main methods that can be implemented to control FMD, and it is currently the main control strategy used in Taiwan. At present, SN test is used for the routine detection of FMDV antibody in pig serum to evaluate the efficacy of the vaccination in Taiwan. But it can be very time consuming, laborious, costly and inefficient, especially for large-scale serological monitoring systems. In countries and areas where FMD vaccination is implemented, the serological differential diagnosis of FMD can be difficult because vaccinated animals cannot be distinguished from infected animals simply by the SN test, since SN antibodies are elicited by vaccination and infection. It was reported that NSP 3ABC was most reliable indicator of

infection in cattle and sheep and that there was similar antibody response to NSP 3A. However, some animals failed to react against 3B, whilst 3C alone was very weak immunogenic [9]. In the other study, it was shown that FMDV capsid protein VP1 greatly contributates to the antigenicity of FMDV(G-H loop) [1]. For ELISA is suitable for rapidly examining large numbers of sera, therefore in this study, we decided to develope a NSP 3ABC-based and a SP VP1-based indirect ELISA to test for the serum antibody against 3ABC and VP1 respectively. In addition, the 3ABC-based ELISA were compared with two commercial kits (Bommeli Diagnostics/Intervet, and United Biomedical, Inc (UBI)) on our pig sera collections. The results indicated that our the 3ABC-based ELISA was more sensitive than two commercial kits (data not shown). Also a good correlation between OD values and SN antibody titers was obtained from our VP1-based ELISA. Hopefully, from this study, a more efficient technology platform for detecting FMD NSP and SP antibody can be establihed, and further improving FMD control in Taiwan.

CONCLUSIONS

In conclusion these ELISA has high potential as a useful tool for diagnosis of FMD and for the routine detection of FMD antibody in pig serum to evaluate the efficacy of the vaccination in areas where vaccination has been carried out to control the disease.

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OCCURRENCE AND CHARACTERISATION OF ENTEROHAEMORRHAGIC ISOLATES *ESCHERICHIA COLI* FROM DIARRHOEIC CALVES

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SUMMARY

The presence of major virulence factors of enterohaemorrhagic *Escherichia coli* (EHEC; *stx1*, *stx2*, *eae*, *Ehly*) were determined among isolates from 158 diarrhoeic calves by multiplex polymerase chain reaction (PCR). Strains positive for virulence factors were subjected to serotype specific PCR assays for O157:H7 and O111 antigens. Additionally, serogroups were determined by three monovalent antisera for O26, O111 and O157 somatic antigens and enterohaemolysin production were also shown phenotypically. Thirteen (8.2%) calves carried strains positive for one or more of the virulence factors tested, and eleven (6.9%) calves harboured the shiga toxin producing strains (*stx1* or *stx2*). *stx1* was detected in eight (5%) and *stx2* in three (1.9%) calves. *eae* and *Ehly* were observed in the same frequency (6.3%) and were detected in parallel. Of the 13 virulence-positive strains, the predominant genotype was (*stx1*/*eae*/*Ehly*) at 53.8%. None of the EHEC in this study belonged to O157:H7 or O111 serotypes, but four strains (30.7%) belonged to the O26 serogroup. The results show the possible role of *stx1*/*eae* in calf diarrhoea and the particular importance of O26 EHEC. Calves can also act as a reservoir for EHEC and in the transmission of the disease to humans.

INTRODUCTION

Enterohaemorrhagic *Escherichia coli* (EHEC) is a major cause of bloody diarrhoea, haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) in humans. Domestic ruminants and especially cattle have been implicated as a principal reservoir of EHEC that causes human infection [8]. Pathogenic capacity of EHEC resides in a number of virulence factors including shiga toxins (*stx1*, *stx2*), intimin (*eae*) and enterohaemolysin. EHEC may produce two types of shiga toxins (*stx1* and *stx2*), which are functionally and structurally related to shiga toxin of *shigella dysenteriae* type 1. Intimin encoded by the *eae* gene, located on the locus of enterocyte effacement, mediates the intimate attachment of bacteria to intestinal villi and induces attaching and effacing lesions [9]. It has been suggested that enterohaemolysin may complement or enhance the effects of shiga toxins [5]. Strains producing *Ehly* are not haemolytic on standard blood agar but produce a haemolytic zone on washed sheep blood agar supplemented with calcium. EHEC infections have been described in a wide range of both domestic and wild animal species, but their natural pathogenic role has been demonstrated only in young calves, weaning pigs and dogs [2]. Different serotypes of EHEC have been associated with diarrhoea in calves (mainly O5, O26 and O118). Recently, the possible roles of O26 and O111 EHEC have been described in association with calf diarrhoea [5]. Although *E. coli* O157 as an archetype of EHEC is associated with most of the outbreaks in humans worldwide, other serotypes particularly O26 and O111 have emerged as significant causes of human disease. The objectives of this study were to determine the distribution of major virulence factors of EHEC and the presence of important EHEC serotypes associated with human and calf disease, including O26, O111 and O157, in isolates from diarrhoeic calves in Iran.

MATERIAL AND METHODS

Specimen collection and *E. coli* strains

Faecal samples were obtained from 158 diarrhoeic calves from 10 geographically separate farms. Samples were collected using sterile swabs from 7- to 90-day-old calves with symptoms of diarrhoea or dysentery at the time of sampling. Specimens were sent to the laboratory in Amies transport medium and plated within 24 h of collection on MacConkey agar, Sorbitol MacConkey agar (SMAC) and O157chromagar (CHROMagar). Three to four suspected colonies including lactose fermenting colonies on MacConkey agar, sorbitol negative colonies on SMAC agar and mauve colonies on chromagar were randomly picked and sub-cultured.

Detection of virulence genes by multiplex PCR

All *E. coli* isolates were screened by multiplex PCR using four pairs of specific primers for *stx1*, *stx2*, *eae* and *Ehly* as described by Paton and Paton (Table 1) [7]. Total genomic DNA was extracted from overnight LB agar culture by the boiling method, as described by Zahraei Salehi et al. (2007) [10]. Positive controls and negative control (sterile water) were included in all PCRs.

PCR for *rfb*O157, *rfb*O111 and *fliC*H7

Two other PCR assays for the detection of O157, O111 and H7 antigens were carried out on virulence trait-positive strains (according to multiplex PCR results). O157 and O111 somatic antigen *rfb* genes were screened by specific primers in duplex PCR assay. PCR condition and thermal cycles were similar to virulence genes multiplex PCR [10]. PCR for *fliC*H7 was performed according to Gannon et al. (1997) as described previously [3]. Controls (positive and negative) were included in all PCR reactions.

Serogroup determination

Strains which were positive for virulence markers in the PCR assay were subjected to serogroup determination using commercially available O26, O111 and O157 monovalent antisera according to the manufacturer's recommendations.

Enterohaemolysin activity of *Ehly*-positive strains

Production of enterohaemolysin was assessed in *Ehly*-positive strains phenotypically using washed sheep blood agar supplemented with 10 mM $CaCl_2$ (WSBA-Ca) as described previously [1]. Negative controls (*Ehly* negative strain) were used.

RESULTS

A total number of 375 isolates were confirmed as *E. coli* in biochemical tests and were subjected to multiplex PCR for virulence markers. Similar patterns of virulence markers were observed among isolates associated with each PCR positive sample; therefore, one strain per animal was applied for data analysis (n=13). Among 158 diarrhoeic calves, strains from 13 calves (8.2%) were positive in virulence markers multiplex PCR and 11 calves (6.9%) harboured shiga toxin producing strains. The most frequent shiga toxin was type 1 (*stx1*) and was present in 5% of samples (n=8); *stx2* was detected only in 1.9% of specimens (n=3). *eae* and *Ehly* were found in the same frequency (6.3%) and were detected in parallel (n=10). Among the 13 virulence marker-positive strains, the predominant genotype was (*stx1/eae/Ehly*) with a frequency of 53.8% (n=7; Table 1) and individual virulence genes *stx1*, *stx2*, *eae* and *Ehly* were detected at frequencies of 61.5%, 23%, 76.9% and 76.9%, respectively (Table 2). In the serotype specific PCR assays, none of the isolates were found to be positive for O157:H7 and O111. In phenotypic serogroup determination using three monovalent antisera, four virulence marker positive strains belonged to O26 serogroup (30.7%) and were in agreement with the serotype specific PCR. None of these isolates reacted with O111 and O157 antisera. Seventy-five percent of O26 EHEC (n=3) showed prevalent genotype (*stx1/eae/ Ehly*) and 25% (n=1) only contained *stx2*. All *Ehly*-positive strains phenotypically produced enterohaemolysin on WSBA-Ca.

Table 2: Frequency of virulence gene genotypes of EHEC

Genotype	Number	Frequency (%)
stx1/eae/Ehly	7/13	53.8
eae/Ehly	2/13	15.4
stx2	2/13	15.4
stx1	1/13	7.7
stx2/eae/Ehly	1/13	7.7

Table 3: Frequency of individual virulence genes of EHEC

Virulence marker	*stx1*	*stx2*	*eae*	*Ehly*
Number	8/13	3/13	10/13	10/13
Frequency (%)	61.5%	23%	76.9%	76.9%

DISCUSSION

This study investigated the presence of major virulence factors of EHEC among 375 isolates from 158 diarrhoeic calves by efficient multiplex PCR, detecting genes of major virulence factors of EHEC (*stx1*, *stx2*, *eae*, *Ehly*) simultaneously. The findings showed that 6.9% of samples were positive for shiga toxins (*stx1* or *stx2*). *stx1* was present in 5% and *stx2* in 1.9% of samples. *stx*+/*eae*+ strains were identified in 5% and *stx*−/*eae*+ strains in 1.3% of animals tested. In this study, primers for *eae* gene were able to target a conserved region of the intimin gene (*eae*) between EHEC and EPEC (enteropathogenic *E. coli*) [7], Therefore, *stx*−/*eae*+ strains can be considered as EPEC, but coexistence of *Ehly* gene in *eae*+ strains suggests that these might be the former EHEC which lost the genes for shiga toxins during infection or sub-culture. Inability of primers to target *stx* variants could be another possible explanation for this observation [8]. Our results support other findings which reported higher frequency of *stx1* in calves. In contrast, some studies have detected *stx2* as a dominant shiga toxin among EHEC from calves [1, 10] Zahraei Salehi et al. (2007) examined 29 isolates from diarrhoeic calves in Iran and identified 55% of strains as *stx2*+ and 13.7% *stx1*+ [10]. In the present study, 6.3% of samples contained *eae*+ strains; interestingly, all of the *eae*+ strains also carried *Ehly*, and most of them included *stx1*. Another noteworthy observation in the present study was the fact that all *E. coli* strains associated with each EHEC positive sample were positive for similar patterns of virulence genes. In other words, if only one colony from each specimen was tested, similar results would be obtained. It has been documented that in the early stages of infection, EHEC in faeces constitutes more than 90% of aerobic faecal flora, but as the disease progresses, the number may drop dramatically [8]. Hence, it could be concluded that only the initial stages of infection were detected in this study, and many of the infrequent or low number shedders of EHEC remained undetected. On the other hand, this finding indicates that testing of one colony for diagnosis of acute EHEC infection in calves may produce acceptable results, but whenever the prevalence estimation or shedding status is under question, more sensitive method such as PCR on primary faecal culture is recommended. None of the isolates in this survey were positive for O157:H7 and O111 antigens. In comparison, O26 serogroup was frequent among diarrhoeic calves, and among 13 PCR-positive strains, four (30.7%) belonged to O26 serogroup; all of the O26 *E. coli* were positive for *stx*, and most of them belonged to (*stx1/eae/Ehly*) genotype. The results from this study confirm other findings which reported high frequency of *E. coli* O26 in diarrhoeic calves [5,6] and is also in agreement with several studies suggesting that stx1+/*eae*+ strains may be involved in calf diarrhoea [4]. Our results similarly showed that (*stx1/eae/Ehly*) is the predominant virulence pattern among diarrhoeic calf EHEC strains. Based on the literature, it is obvious that different combinations of virulence markers have been reported in different studies. The important criteria for these types of variations may be the geographic area of the herd and time of sampling. Other factors have also been considered such as age, season and diet [2]. Virtually all O157 EHEC and 60–80% of non-O157 EHEC phenotypically induce haemolysis in WSBA-Ca. Surprisingly, all non O157 EHEC isolates observed in this study induced haemolysis. On the other hand, a strong linkage between *Ehly* and other virulence genes, e.g. *eae* and *stx* was observed. The findings indicate that routine use of WSBACa is highly beneficial as a simple, low-cost screening medium for presumptive detection of EHEC in combination with other diagnostic methods. Evidence of enterohaemolysin production could fill at least a part of the gap in diagnosis of highly virulent EHEC in a serotype nonspecific manner.

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SELECTION CRITERIA FOR ANTIMICROBIAL TREATMENT OF SWINE RESPIRATORY DISEASE (SRD) BASED ON TARGET PATHOGENS TO BE INVOLVED (Abstract)

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INTRODUCTION

Veterinarians are requested to use narrow spectrum antimicrobials to treat infectious bacterial disease wherever possible. Pathogens causing Swine Respiratory Disease (SRD) are *P. multocida (PM)*, *A. pleuropleumoniae (APP)*, *Haemophilus parasuis (HPS)*, *B. bronchiseptica (BB)*, *S. suis (SS)*, and *M. hyopneumoniae (MHP)*, *M. hyorhinis (MHR)* and *M. hyosynoviae (MHS)* and viruses. Pathogens may occur concurrently in the same herds on farms. The authors present results of bacteriological observations, prevalence and relevance of bacterial and mycoplasmal pathogens.

MATERIALS AND METHODS

Microbiology

All samples were collected either via deep nasal swab or via bronchoalveolear lavage. Where *Mycoplasma spp*. identification was conducted, specific media for transport were provided.

Selection of farms

From 1999 to 2010, a mean of 9 animals of 64 SRD outbreaks on farms located in Denmark, France, Germany, Hungary, The Netherlands, Slovenia and UK were tested for the presence of pathogens associated with clinical SRD.

RESULTS

Microbiology

MHR was present most frequently in SRD outbreaks (61 %).PM (50 %), APP (45 %), BB (27 %), SC (25 %), MHP (26%), MHS (15% and HPS (13 %) were also isolated.

Multiple pathogens associated with SRD outbreaks

In 50 % of the SRD outbreaks, two or more pathogens were isolated.

DISCUSSION AND CONCLUSION

The results indicate that SRD is frequently associated with the presence of multiple pathogens. SRD outbreaks caused by just one causative pathogen are seldom. If sufficient samples are taken, it is likely that 3 or more different causative pathogens are identified. *Mycoplasma spp.*, PM and APP are most frequently involved. Selecting antimicrobials for treatment of SRD must take into consideration that the active ingredient can reach the target site of infection based on a formulation and treatment duration as required to successfully treat the target pathogens frequently involved in SRD. It appears mandatory to prescribe products that are indicated for all relevant pathogens including *Mycoplasma spp.*.

DIAGNOSIS OF BORDETELLOZIS (TURKEY CORYZA) USING SEROLOGICAL, CULTURAL and MOLECULAR METHODS (Abstract)

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The aim of this work to compare the sensitivity of molecular (PCR), serologic, and conventional methods in diagnose of *B. avium* the cause of Turkey coryza and to prove the disease using direct and indirect methods.

For this purpose, 400 trachea and 1000 serum samples were obtained from a turkey slaughterhouse within a year. Cultural detection and molecular analyses (polymerase chain reaction, PCR) in terms of direct method was used to identified *Bordetella avium*, and blood samples were examined using ELISA for indirect diagnose method. According to ELISA results, 194 serum out of 1000 (21,6%) were found positive, and 4 positive band were found positive using PCR specific to *B. avium*, although isolation of *B. avium* could not be isolated in the organs using cultural method.

Keywords: Bordetellosis, Turkey, *Bordetella avium*, PCR, ELISA, Cultural method

ESTABLISHMENT OF A REAL-TIME RT-PCR METHOD FOR AIRBORNE H9 AVIAN INFLUENZA VIRUS

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SUMMARY

Avian Influenza Virus (AIV) has caused serious epidemics all over the world. Notably, the low pathogenic Avian Influenza Virus H9N2 has spread widely to different areas, resulting in serious damage to the poultry industry. To promptly monitor the occurrence of H9 AIVs and quantify its aerosol particles in chicken houses, in this study, sampling was conducted by using the AGI-30 Impinger, and the specific primers and probe were selected from the conserved regions of the H9 gene to establish the real-time RT-PCR method. This method possessed specificity for H9 AIV. The sensitivity reached 100 copies/reaction when the quantitative detection was carried out with the 10-fold diluted standard plasmids containing H9 genetic fragments. Moreover, by using this method, the concentration of airborne H9 AIVs detected in the chicken house environment was $1.25\sim1.33\times10^4$ copies/m^3 air. The highlight of this particular method lies in its crucial importance to capture of AIVs prevalence information at the times of H9 AIV outbreaks.

INTRODUCTION

Influenza A viruses are enveloped, eight-segmented, signal negative-stranded RNA virus of the family of orthomyxoviridae. Based on the antigenic differences between the two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), this virus was classified into 16 HA (H1-H16) and 9 NA (H1-H9) subtypes (8), of which the H9N2 AIVs have spread widely since it first surfaced in mainland China [5, 7, 14, 18]. Notably, H9N2 AIVs have attracted extensive attention for their lethal impact on poultry and pathogenicity to humans. From 1994 to 2001, H9N2 AIVs had caused severe respiratory diseases in chickens in numerous countries, resulted in laying decline and a lethality rate around 10%~60% [10, 12, 23]. Cases of humans infected with H9N2 AIV have occurred repeatedly since 1999[9, 16], therefore, the significance of public health concerning the virus transmission and infection has aroused worldwide attention. It had concluded in many experimental models that AIVs led to infection via airborne transmission among animals [24, 29]. Airborne transmission of AIV is of critical importance as the viruses may spread by means of aerosolized particles in large crowds [13]. Therefore, it is crucial to monitor the AIV aerosol in the animal house environment to control and prevent this disease.

Currently, the influenza virus isolation is to initially utilize the cells or chicken embryoes for viruses cultivation and type by serological methods, which requires complicated procedures and large time -consuming. More importantly, that is difficult to quantify the influenza viruses in the air, which is disadvantage to develop the rapid disease detection and early warning systems. With the application and improvement of PCR detection methods, the real-time RT-PCR method has gradually become an important technique for influenza virus detection due to its simple operation, strong specificity, high sensitivity, good reproducibility and quick speed [17, 25 28]. In this study, we describe the development of a real-time RT-PCR method with Taq-Man probe for the rapid screening of the low pathogenic airborne H9 AIVs in chicken houses environment in combination with the AGI-30 liquid impinger [4] applied to collect air samples.

MATERIALS AND METHODS

Virus strains

H9N2 AIVs (HQ326722) used for establishing the method were propagated using the 9-day-old SPF (Specific Pathogen Free) chicken embryos according to the method described by Office International des Epizooties (OIE). New Castle Disease Virus (NDV) F48E9 was purchased from China Institute of Veterinary Drug Control. H1N1, H3N2, H5N1, H7N2, and H9N2 AI viral nucleic acids were provided by the Center for Disease Control of Tai'an and the Engineering Center for Animal Disease Control of Shandong Province. Infectious Bronchitis Virus (IBV), Infectious Bursal Disease Virus (IBDV), Avian Leukosis Virus (ALV), Avian Recticuloendotheliosis Virus (ARV), Chicken Anemia Virus (CAV) and Marek's Disease Virus (MDV) were provided by the Engineering Center for Animal Disease Control in Shandong Province. All the virus stocks and viral nucleic acids were stored at -70°C.

Primers and probe design

The hemagglutinin genes of avian-sourced H9N2 AIV were obtained from the GenBank database; homology analysis was conducted through the application of DNASTAR software; primer premier 5.0 software (Applied Biosystems,USA) was applied to design the primers and the Taq-Man probe in the conserved domain of 50 sequences corresponding to the H9N2; comparison analysis was carried out for the nucleotide sequence specificity with the sequences submitted to the GenBank nucleotide database using nucleotide BLAST (http://www.ncbi.nlm.nih.gov/). The fluorescent reporter dye at the 5′ end of the probe was FAM, and the 3′ end was labeled with TAMAR (Available for request).

RNA extraction and cDNA synthesis

Viral RNA extraction was carried out using the TIANamp Virus RNA Kit (Tiangen Biotech Co.Ltd., Bingjing) following the manufacturer's operation instructions. The cDNA synthesis was performed by utilizing the TaKaRa RNA PCR Kit (AMV) Ver.3.0 (TaKaRa DRR019A). The reverse transcription primer of AIV was Uni12 (5′-AGCAAAAGCAGG-3′), while for the other viruses, Random 9 mers were utilized.

Real-time RT-PCR

The Premix Ex Taq^{TM} (TaKaRa DRR039A) kit was applied to conduct real-time RT-PCR amplification. The reaction system included 2×Premix Ex Taq^{TM}10 μL, 50×Rox Reference Dye 0.4 μL, each primer (10 μM) and probe (5 μM) 0.8 μL respectively, and DNA sample 2.0 μL (supplemented to 20 μL with sterile distilled water). The real-time RT-PCR was performed on 7500 Real-Time PCR System (Applied Biosystems, USA). Cycling conditions included an initial denaturation step at 95°C for 30 s, amplification was performed during 40 cycles including denaturation (95°C for 5 s), annealing and extension (60°C for 34 s), and the fluorescent signals were obtained at the end of the extension step. The data was analyzed by means of 7500 System SDS Software Version 1.2 (Applied Biosystems, USA).

Standard plasmid preparation and specificity test

Based on the nucleotide sequence provided on GenBank, the conserved region of the HA gene was selected to design specific primers (Available for request) for subtype H9 AIV. The purified H9 gene fragment was inserted into pMD18-T vector (TaKaRa). Plasmid was transformed into DH5α Escherichia coli.The positive plasmid was determined through nucleotide-sequenced analysis (Shanghai Sangon, China). The mass concentration (C) of plasmid was measured by an ultraviolet spectrophotometer at absorbance 260 nm.

To verify the specificity of the real-time RT-PCR method, the subtypes H1N1, H3N2, H5N1, H7N2, H9N2 influenza virus, NDV, IBV, IBDV, ALV, ARV, CAV and MDV were selected for cross-reactivity.

Sensitivity and reproducibility test

Series diluted positive plasmid (5×10^6~5×10^0 copies/μL) were used to determine the sensitivity of the reaction system, as well as for the standard curve synthesis so as to quantify the samples. Four diluted-series were tested in triplicate in the same PCR reaction for the intra-assay, and the reaction was duplicated with distinct operators on different days, therefore, all the results were used for the inter-assay. The mean value, standard deviation (S.D.) and coefficient of variation (C.V.) were calculated separately for each DNA dilution to evaluate the PCR system reproducibility.

Sampling design

The AGI-30 liquid sampler [4] was applied to collect the air samples from the chicken houses in Qingdao and Tai'an City, Shandong Province. The AGI-30 sampler, containing 20 ml phosphate buffer solution (PBS, 0.1M, pH 7.2), was placed stably 1m above the ground of the sampling point and was continuously operated for 30min at an airflow rate of 12.5 L/min [1,15]. Three samples were obtained at different points of every house and transported in a cold box to the

laboratory, then stored at 4°C and treated within 24 h. After centrifugation at 10 000×g for 45min at 4°C to remove the bacteria and dust particles, the supernatant sampling fluid was ultracentrifuged at 100 000×g for 2 h to collect the viral pellet, and then diluted with 1ml PBS (0.1M, pH 7.2) for RNA extraction and quantitative detection.

Oropharyngeal and cloacal cotton swab samples of 8~10 chickens were collected respectively at all chicken houses, and stored in a centrifuge tube which contained 1ml PBS (0.1M, pH 7.2).

The air and cotton swab samples collected from the four chicken houses were inoculated with 9-day old SPF (Specific Pathogen Free) chicken embryos. RNAs were extracted from the chicken embryo's allantoic fluid for PCR amplification and nucleotide sequencing of subtype H9N2 AIV haemagglutinin [20].

RESULTS

Specificity of real-time RT-PCR

To assess the potential cross-reactivity with other microorganisms, the primers were analyzed by nucleotide BLAST (http://www.ncbi.nlm.nih.gov/) and had no significant homologies to other sequences. Compare to other viruses, only H9 AIV yielded a positive signal.

Standard curve and sensitivity of real-time RT-PCR

The 10-fold diluted (5×10^6~5×10 copies/μL) plasmid containing the H9 gene formed the standard curve using the 7500 System SDS Software Version 1.2 (Applied Biosystems, USA) (Fig. 1). Ct (threshold cycle) values served as the cross point at which the amplification curve exceeded the threshold line, and increased in proportion to the dilutions. With the slope of 3.117 and the correlation coefficient R^2 of 0.9988 exhibiting on the standard curve, the linear equation for the quantitive RT-PCR concentration detection was y = 3.117x + 38.4971, and the detection sensitivity of this method was 100copies/reaction.

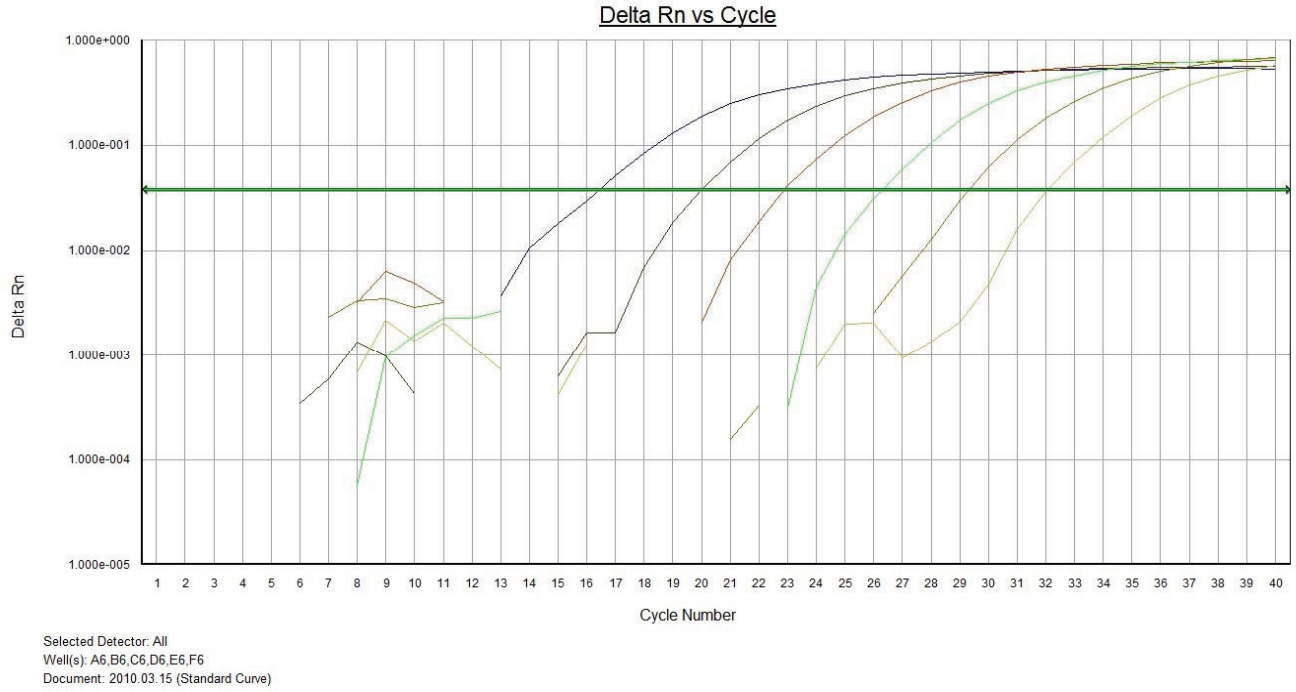

Fig.1 Amplification curve of the AIV H9 standard plasmid using real-time RT-PCR
From left to right, the plasmid DNA ranges from 10^7 to10^2 copies per reaction respectively

Reproducibility of real-time RT-PCR

The standard plasmids with four diluted-series were selected and respectively analyzed for identification of reproducibility through the use of intra- and inter-assay methods. The Mean±S.D. of the coefficient variation (C.V.) for intra-assay was 0.822±0.64% (variation range: 0.36~1.76%), and that for inter-assay was 1.35±0.40% (variation range: 0.95~1.36%). All showed good reproducibility (Table 1).

Table 1. Intra- and inter-assay variability recorded for the real-time RT-PCR assay

Sample	Intra-assay			Inter-assay		
	Mean	S.D.	C.V. (%)	Mean	S.D.	C.V. (%)
1	16.48	0.29	1.76	16.43	0.20	1.20
2	19.63	0.07	0.36	19.41	0.26	1.36
3	22.70	0.11	0.50	22.53	0.21	0.95
4	25.62	0.17	0.67	25.43	0.28	1.09

Field samples detected by real-time RT-PCR

Four chicken houses (A, B, C, D) of Shandong province were detected using this real-time RT-PCR assay, the average concentrations of airborne H9 AIV of three chicken houses (A, B, C) were 312.5, 165.4 and 348.8 copies/reaction respectively. After calculated through the formula, the average concentrations of the airborne H9 AIVs of chicken houses A, B, and C were 2.38, 1.25 and 2.66×10^4 copies/m^3 air respectively. H9 AIVs were also detected in the oropharyngeal and cloacal cotton swab samples from chicken houses A and C, while none was detected in samples from B and D (Table 2). Haemagglutinin nucleotide sequence of air isolated strains (A1, C1) and cotton swab isolated strains (A2, C2) in chicken houses A and C were analyzed through DNAStar software, and the homology of A1 and A2, C1 and C2 was 100% (GenBank accession no. HQ225839 and HQ378727).

Table 2. The detection results of 4 chicken houses

Chicken houses	Real-time RT-PCR		AIVs aerosol concentration ($\times10^4$copies/m^3 air)
	AIVs aerosol (copies/reaction)	Swab samples	
A	312.5	+ [a]	2.38
B	165.4	- [b]	1.25
C	348.8	+	2.66
D	-	-	-

[a] Positive signal in the real-time RT-PCR; [b]Negative signal in the real-time RT-PCR.

DISCUSSION

Real-time RT-PCR is mainly used to detect AIV from clinical samples[2, 17, 21, 27], but little was used for airborne AIV. By collecting the virus aerosol particles from poultry markets with a filter, Chen [6] indicated that the concentration of airborne avian influenza A viruses was lower than 3.7×10^4 copies/m^3 air. However, the quantitative detection of airborne H9 AIVs in breeding environments has not yet been reported. In this study, a real-time RT-PCR detection method for H9 AIV was established. This method had high sensitivity of detection, and could detect 100 plasmid DNAs in one reaction, which is conducive to the identification of AIV aerosol particles in the environment. It took less than 3h from the collection of air samples to the delivery of results.

The detection results of four chicken houses showed the content of H9 AIVs ranged between 1.25 ~ 2.66×10^4 copies/m^3 air. The AIV aerosol in chicken house environments not only causes an infectious hazard to the chicken flocks, but also forms a threat to the neighboring breeding farms. Therefore, the monitoring of environment could acquire the disease information rapidly for diagnosis and early warning, as well as verify the disinfection of chicken house environments.

The H9 AIV has spread widely in China, contributing to great economic losses to poultry industry. When mixed with other pathogens such as *infectious bronchitis virus*, *Mycoplasma gallisepticum and Escherichia Coli*, high disease incidences and fatality rates may be caused [3, 11, 22]. The reason for the rapid spread of this disease is correlated with its airborne transmission and infection. Virus aerosol was formed when the chicken was infected by H9N2 and could infect other chicken flocks at a certain distance [26, 30]. Therefore, the detection of AIV aerosol in breeding environments could further confirm its airborne infectivity and enrich the epidemiological information.

The homology of the air environment and cotton swab isolated strains was 100%, which suggested that airborne H9 originated from the chicken flock at the viruses excreting phase. On the contrary, no H9 was detected from the oropharyngeal and cloacal cotton swabs in chicken houses B and D. The possible reason for this might lie in the fact that the chicken group had passed the virus excreting

phase. The study conducted by Yao [30] pointed out that it could take 7-10 days for chicken flocks infected with H9N2 to excrete the virus. In this study, the results showed that H9 aerosol particles still existed in the air of chicken house B, and the reason might possibly be correlated with the survivability of this virus in the chicken house environment or the failure of the breeding hygiene management. Lowen [19] indicated that the aerosol transmission of AIV was related to the relative humidity and temperature of the surrounding environment; specifically, low temperature and dry environment were more conducive to the transmission of this virus. In order to further investigate the survival time of H9 aerosol, future studies are planned.

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ISOLATION AND PATHOLOGICAL INVESTIGATIONS OF EHV-1 AND EHV-4 INFECTIONS IN ABORTED FETUSES IN TURKEY

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SUMMARY

This study was aimed at isolation and pathological investigations of equine herpesvirus-1 (EHV-1) and equine herpesvirus-4 (EHV-4) in aborted fetuses in Turkey. Two aborted fetuses were necropsied and the results of isolation and pathological studies reported herein. EHV-1 was isolated from the lung, liver and brain of 1 aborted fetuses. EHV-4 was not isolated from the fetuses. EHV-1 DNA was detected by PCR in the lungs, livers, and spleens of 2 necropsied fetuses and brain of one fetus while no EHV-4 DNA was detected. At necropsy, sub-milier focal necrosis in the liver and spleen were observed. Microscopically, focal coagulation necrosis and marked eosinophilic intranuclear and intracytoplasmic inclusion bodies in the hepatocytes localised around the necrotic areas in the liver. Severe coagulation necrosis in white pulp of the spleen was also observed.

INTRODUCTION

Horses are the natural hosts for 5 recognized herpesviruses: equine herpesviruses 1, 3, and 4 (EHV-1, 3, and 4) that belong to the *Alphaherpesvirinae* subfamily and EHV-2 and EHV-5 that are classified within the *Gammaherpesvirinae* subfamily (11). The role of EHV-1 in equine abortion is well known while the involvement of EHV-4 is not frequent and the presence of EHV-2, EHV-3 and EHV- 5 in tissues from aborted foetuses remains poorly documented (6,7,14 ,) Both EHV-1 and EHV-4 are endemic in horse populations worldwide including Turkey (1, 4 8, 10, 12, 14,16, 15).

In the present study, molecular studies of EHV-1 and EHV-4 including PCR detection in tissues and pathological investigations in aborted fetuses in Turkey were carried out and the results here in presented.

MATERIALS AND METHODS

Two aborted fetuses were necropsied and isolation, detection by PCR and pathological investigations were carried out. For this, lung, liver, brain, kidney and spleen of the aborted fetuses were taken. Conventional methods were used for tissue preparation (9) and isolation of infectious EHV-1 and EHV-4. The cells (MDBK) were examined daily for the presence of cytopathic effects (CPE) for 7 days. Detection of EHV-1 and EHV-4 in cell culture and fetus tissues were performed by PCR after the isolation of DNA using commercial kits (Qiagen). The method of PCR and primers were the same as reported previously (16). For histo-pathology, tissue samples were fixed in 10% formalin solution and further processed and stained by haematoxylin & eosin. The lesions were examined under a light microscope.

RESULTS

EHV-1 was isolated from the lung, liver and brain of 1 aborted fetuses. EHV-4 was not isolated from the fetuses. CPE consisting of rounding and clustering of cells and lysis were seen by the third day after inoculation and CPE increased by the 5th day. The EHV-1 DNA was detected in cell cultures but not EHV-4 DNA. No CPE and EHV-1 and 4 DNA were detected in control cells.

EHV-1 DNA was detected by PCR (Figure 1) in the lungs, livers and spleens of 2 fetuses and a brain of 1 fetus while no EHV-4 DNA was detected. At necropsy, sub-milier focal necrosis in the liver and spleen were observed. Microscopically, focal coagulation necrosis and marked eosinophilic intranuclear and intracytoplasmic inclusion bodies in the hepatocytes localised around the necrotic areas in the liver (Figure 2B) and severe coagulation necrosis in white pulp in the spleen (Figure 2A) were seen.

DISCUSSION

Infectious diseases due to equine herpesvirus (EHV-1 and EHV-4) affect equines all over the world and decrease the performance and production of racing horses by causing reproductive, respiratory and neurologic disorders.

Rapidness of the diagnostic test is also important, since aborting mares need to be separated from the healthy ones immediately until a negative EHV1 result is obtained (11,12). Therefore, molecular detection is a good tool for the identification characterisation of circulating EHV-1 and EHV4 strains in Turkey.

Successful detection and monitoring of EHV-1 and EHV-4 in horses depends on the test and samples used and the time of sampling. PCR for EHV1 and EHV4 showed a greater superiority to virus isolation in terms of rapidness, sensitivity and specificity (4, 10,13,17). In the present study, PCR was used for the detection of these closely related viruses because of its superiority compared to other dignostic tests. Tissues and placenta can be used for the diagnosis of abortions (2,3). In this study, tissues of aborted fetuses were used for PCR.

EHV-1 and EHV-4 have been detected in variety of samples including nasal swabs, blood, tissues, lymph nodes, trigeminal ganglia and palcenta (2,3,5,8). In this study, EHV-1 was detected by PCR in the lung, liver, spleen and brain. Other researchers (6,8) have reported more EHV-1 than equine herpesvirus-4 in nasal swabs and tissues of aborted aborted fetuses. Similarly, EHV-4 was not detected in the present study. This is the first study to present molecular detection of EHV-1 and EHV-4 in aborted fetuses in Turkey. EHV-1 was detected in two aborted foetuses and isolated from 1 fetus. Previous studies reported that 5 out of 36 neonatal dead foals were positive for EHV-1 (16). On the other hand, 19% (4/21) buffy coat samples and 22.5% (9/40) nasal swab samples were found to be positive for EHV-4 DNA in Turkey (1). No aborted fetuses were analysed in those studies.The gross pathological and histo-pathological findings reported in this study were similar to those reported previously (18).

IN CONCLUSION, the results of this study show that PCR is a rapid, sensitive and specific technique for the detection of EHV DNA in tissues of aborted foals. Using this technique 2 EHV-1 positives were detected and ı EHV-1 isolated from a aborted fetus. In the future more samples will be analysed to have a better understand of epidemiology, pathogenesis, genetic and phylogeny of equine herpesviruses in Turkey.

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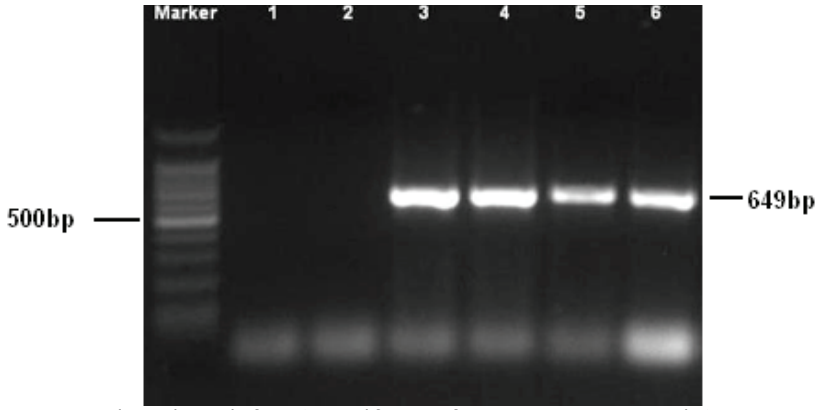

Figure 1. Products obtained after PCR amplification of EHV-1. 1. Negative control; 2. Negative sample previously analysed; 3, 4 and 5. Positive samples from aborted fetuses; 6. Positive control (649bp).

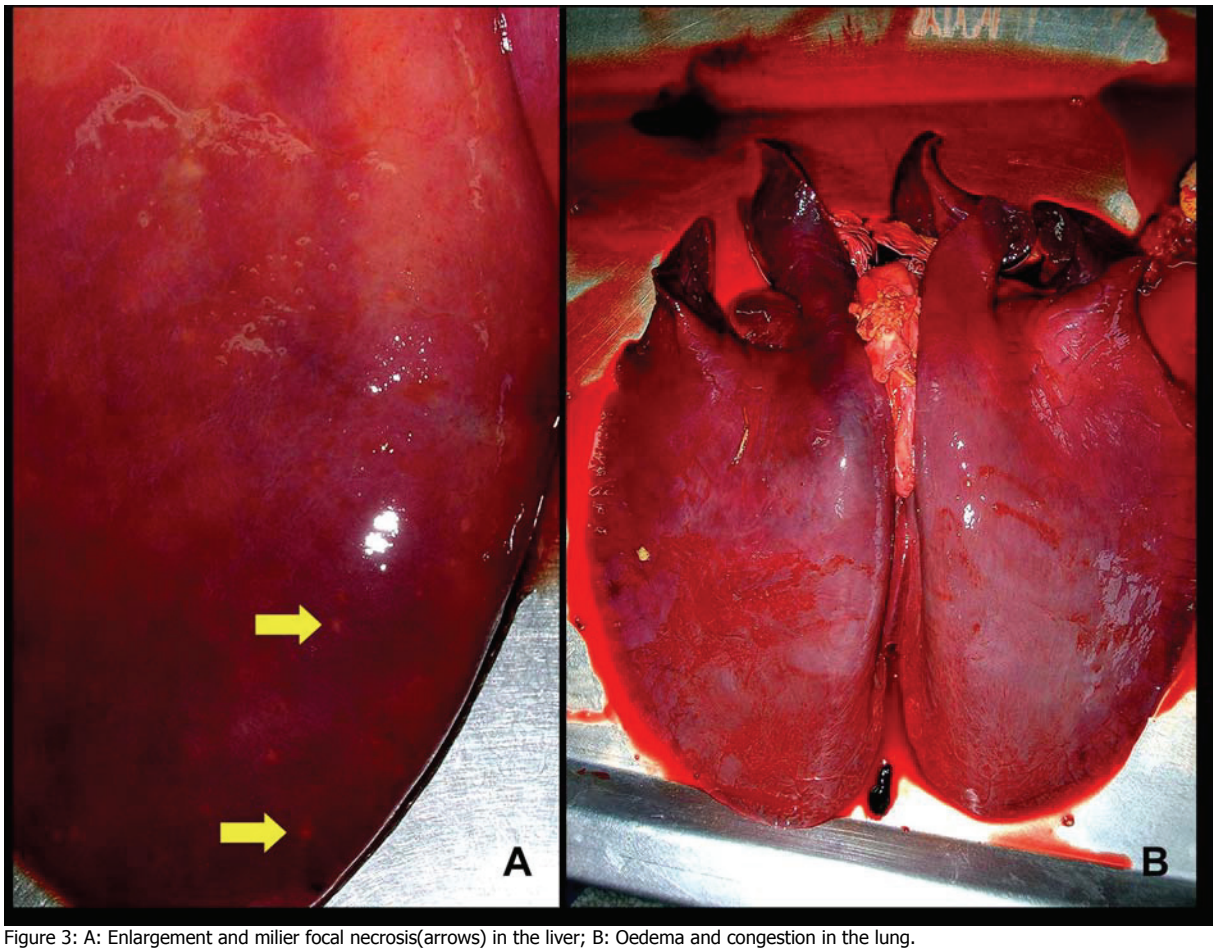

Figure 3: A: Enlargement and milier focal necrosis(arrows) in the liver; B: Oedema and congestion in the lung.

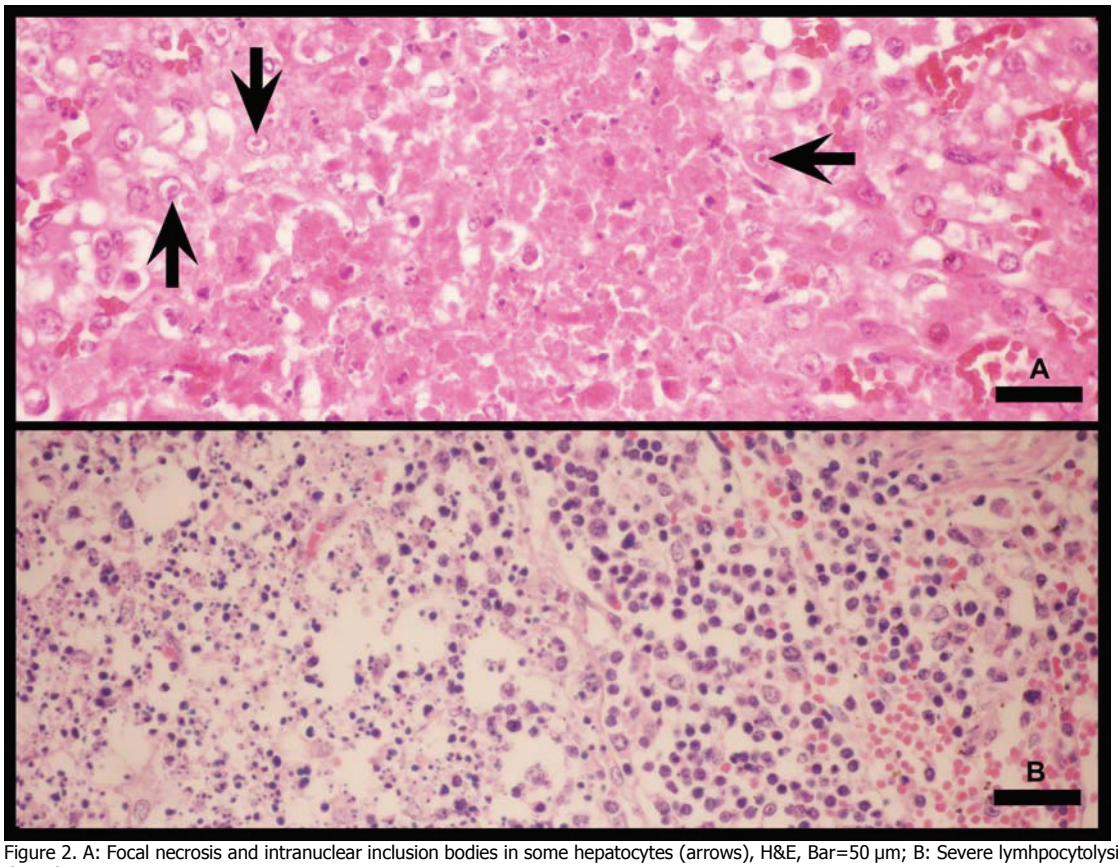

Figure 2. A: Focal necrosis and intranuclear inclusion bodies in some hepatocytes (arrows), H&E, Bar=50 µm; B: Severe lymhpocytolysis in white pulps of the spleen, H&E, Bar=50 µm.

OCCURRENCE AND SEASONALITY OF DOMESTIC SHEEP PARASITES

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SUMMARY

The aim of this study was to determine the occurrence and seasonality of parasites in domestic sheep (*Ovis aries*). From March 2009 to February 2010, a survey of the parasitological state a flock of Romanov sheep (15 rams, 15 ewes and 15 lambs) in a village in northern Bohermia was researched. Of all the parasites in this study, the genus *Trichostrongylus* spp. was the most common representative. The ram group had the highest values of the genus *Trichostrongylus* spp. in May and October. For ewes, the highest prevalence was in April. The lambs had the highest prevalence of the genus *Eimeria* spp..

INTRODUCTION

With rising economic development and world population the global demand for meat, milk and other animal products is increasing dramatically. Controlling parasitic diseases in livestock, in particular helminth infections, could rapidly improve productivity and resource utilization. (Piedrafiat et al., 2010) Sheep can be parasitized trough a diverse range of parasites, with well over 150 species of internal and external parasites reported worldwide. (Taylor, 2010) The most widespread endoparasitic disease seen in sheep is parasitic gastroenteritis (PGE), which is caused by a range of gastrointestinal (GI) nematodes. The reason for its relevance is the cost it imposes on sheep farming (West et al., 2009). Therefore we studied the situation in a flock of sheep to determine strategy: how to fight parasites of sheep.

MATERIALS AND METHODS

In our research we investigated the occurrence of parasites in a flock of Romanov sheep that are bred in an upland village in the north of the Czech Republic. We chose 45 subjects. Sheep were divided into 3 groups: 15 rams, 15 ewes, and 15 lambs. Faeces from sheep were collected every month, from March 2009 to February 2010. The samples were examined through flotation techniques for the presence of Coccidia and nematodes eggs. Results were processed in Microsoft Excel, where we calculated the EPG, prevalence, average, minimum, and maximum of each parasite in the groups.

RESULTS

We found the following genera: *Eimera* spp. (Coccidia), *Trichostrongylus* spp., *Strongyloides* spp., *Trichuris* spp. (Nematoda). Of all the parasites in this study, the genus *Trichostrongylus* spp. was the most common representative. The highest average prevalence of the genus *Trichostrongylus* spp. was found in ewes (58 %) and rams (69%). The lambs had the highest average prevalence of the genus *Eimeria* spp. (91%). Lower values occurred in the genus *Strongyloides* spp. (average prevalence was 25% rams). The lowest prevalence was evident with respect to *Trichuris* spp., where it average 12% in lambs.

The number of *Eimeria* spp. was decreased in August and in the winter months, and it reached a maximum prevalence for the lamb group in April, May, September and November 2009 (100%). The ram group had the highest values of the genus *Trichostrongylus* spp. in May and October, however, these values decreased in the following month. For ewes, the highest prevalence was in April, later the values fell to below 60 % by January 2010. The genus *Strongyloides* spp. had values that reached a maximum in the autumn months for ram and lamb groups, in ewes Strongyloides spp. occurred sporadically reaching peak in March 2009. The genus Trichuris spp. occurred irregularly with maximum values appearing in late summer and autumn in all groups.

DISCUSSION

The most commonly occuring parasites for ewes and rams in the spring appeared to be the genus *Trichostrongylus* spp.. For lambs, a higher number of the genus *Eimeria* spp. occurred in spring and autumn. Similar results can be found in studies in Canada and in the Slovak Republic. In the Canada, fecal cultures demonstrated that the most predominant nematode genera were *Teladorsagia* spp., *Haemonchus* spp. and *Trichostrongylus* spp.. Although the overall mean EPGs were not remarkably high, there were months with higher EPGs such as May–June for ewes and July–August for lambs in both provinces (Mederosa et al., 2010). In the Slovak Republic strongyle eggs were identified in 1255 samples (82.6%), *Nematodirus* spp. in 481 samples (31.7%), *Strongyloides papillosus* in 431 samples (28.4%) *Moniezia* spp. in 291 samples (19.2%) and *Trichuris* spp. in 148 samples (9.7%) (Cernanska et al., 2005). However, these works did not include Coccidia, which we found markedly in lambs. In Iran, the intensity of infection by *Eimeria* spp. was significantly higher in young sheep than in older animals. ($P<0.05$) (Yakhchali et al., 2008). In the Turkey, according to statistical analysis, Eimeria spp. oocysts were significantly higher ($P < 0.01$) in lambs aged between 31 and 60 days (76.81%, 53 of 69) than in those aged 16-30 days (50%, 21 of 42) as well in those aged 1-15 days (28.57%, 6 of 2 1) (Ozdal et al., 2009). We observed prevalence of Coccidia of up to 100% during our study.

CONCLUSION

It is clear that *Eimeria* spp. is a significant parasite for lambs. For adult ewes and rams, however, Nematoda parasites are serious specifically the order Strongylida, genus *Trichostrongylus* spp.. Treatment of lambs should focus on Coccidia while treatment of adult sheep should concentrate on Nematoda. The biggest development of these parasites occurred in spring. Treatment of sheep in early spring is essential.

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EFFECT OF STRONGYLOSIS ON SOME BLOOD CONSTITUENTS IN DONKEYS

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SUMMARY

The goal of the present study was to investigate the effect of different degrees of strongyle infection on haematological and some serum biochemical constituents in donkeys. Based on fecal egg count; animals were classified into mild, moderate and severe infected groups. Results revealed anaemia in a moderate ($p<0.05$) and severe infected group, leucocytosis in a moderate and severe infected groups. There were significant increases in total proteins and globulins in moderate and severe infected groups. Significant increases in serum triglycerides and very low density lipoprotein (VLDL) levels only in severe infected group, significant decrease in high density lipoprotein (HDL-C) in severe infected group. In conclusion, moderate and heavy strongyle infection in donkeys resulted in anaemia, leucocytosis, hyperproteinaemia, hyperglobulinaemia. On the other hand, lipoprotein profile is disrupted only in severe infected donkeys and result in increases serum triglycerides and VLDL and decrease serum HDL-C levels.

Keywords: lipoproteinsAnaemia, strongylosis, donkeys.

INTRODUCTION

The donkey (trlle-ass) is an important draft animal in many parts of the world [11]. In underdeveloped countries, equine and particularly donkeys play an essential role in the agricultural economies, but these animals have not yet been given sufficient care, and they are subjected to many diseases, which affect their viability and lower their ability to work [5]. Despite' their importance, several constraints are hindering improvement including nutrition, farmer attitudes, wondering diseases and poor health [1]. Their ability to thrive in a harsh environment is derived from their immunity to certain disease [2].

Internal parasitism is one of the major causes of ill-thriftiness in animals and cause losses through morbidity and hidden effects on feed intake and efficiency of nutrient utilization. The red worms (strongyles) are nematodes commonly found in the large intestine of horses and other Equidae [12]. Strongylosis is a common disease of horses throughout the world and causes deaths when control measures are neglected [6]. The present study aimed to investigate the effect of different degrees of strongyle infection on haematological and some serum biochemical constituents in donkeys.

MATERIAL AND METHODS

Animals: A total number of 17 adult donkeys were subjected to study. Animals were divided into four groups based on precise fecal examinations; control group (No.=3) and strongyle infected group (No.=14). Animals belonged to the control group were clinically healthy and free from any parasitic infections.

Samples: Fecal samples were collected in clean plastic bags from the rectum of all animals under investigation. Fecal samples were examined directly after collection by direct smear, sedimentation and floatation techniques [3]. Faecal egg counts were performed and eggs per gram of faeces (epg) were determined using McMaster technique [8].

Blood samples:
Whole blood samples were drawn from the jugular vein in vacutainer tubes containing EDTA. Haematological analysis was carried out using automatic blood cells counter (Medonic CA 620, Sweden). The following parameters were measured; total red blood cells count (T.RBCs), haemoglobin concentration (HGB), mean corpuscular volume (MCV), red blood cell distribution width (RDW), haematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets count (PLT), mean platelet volume (MPV), platelets distribution width (PDW), plateletcrit (PCT), large platelets concentration ratio (LPCR) and total white blood cells count (T.WBCs)

Blood samples were collected from the jugular vein in a plain vacutainer tube and processed for separation of serum [3]. Serum samples were used for measuring serum total proteins, albumin, globulins, triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoproteins

(VLDL) using commercial test kits supplied by Spectrum Diagnostics (Cairo, Egypt) and by means of Digital VIS/Ultraviolet Spectrophotometer (Cecil instruments, Cambridge, England, Series No. 52.232).

Statistical analysis was conducted using SPSS 16.0 for windows (SPSS, Chicago, USA) and was carried out using one way analysis of variance. Data were expressed as mean ± SD.

RESULTS

Fecal egg counts

Fourteen donkeys were found infected with *Strongyle spp.,* Animals were classified into three groups based on fecal egg count; samples contain less than 500 EPG, samples contain 500-1000 EPG and samples contain more than 1000 EPG were considered mild, moderate and severe infected groups respectively [13].

Haematological findings

There were significant decreases in T.RBCs count in moderate ($p<0.05$) and severe ($p<0.01$) infected groups when compared with the control group, Significant decrease in HCT (%) only in severe infected group, significant decreases in HGB concentration in moderate ($p<0.01$) and severe ($p<0.05$) infected groups, significant increase in MPV ($p<0.05$) only in the moderate infected group. Significant increases in T.WBCs count in moderate ($p<0.05$) and severe ($p<0.05$) infected groups (Table 1).

Table 1: Haematological findings in different degrees of parasitic infection in donkeys

	Control (No.=3)	Mild infection (No.=3)	Moderate infection (No.=4)	Severe infection (No.=7)
T.RBCs count (10^6)	5.65 ± 0.12	5.11 ± 1.31	5.02 ± 0.37*	4.79 ± 0.18**
MCV (fl)	53.20 ± 1.70	50.66 ± 5.95	55.16 ± 1.14	53.15 ± 4.45
RDW (%)	23.53 ± 1.35	22.43 ± 1.12	23.10 ± 1.27	23.42 ± 1.58
HCT (%)	30.03 ± 0.35	25.43 ± 4.44	27.90 ± 1.91	25.47 ± 2.30*
HGB (g/dl)	10.80 ± 0.00	8.90 ± 2.17	9.56 ± 0.40**	8.77 ± 0.99*
MCH (pg)	19.17 ± 0.45	17.53 ± 1.59	19.10 ± 0.65	18.27 ± 2.02
MCHC	35.90 ± 0.30	34.80 ± 2.69	34.60 ± 1.24	34.35 ± 1.20
PLT (103)	175.0 ± 10.0	158.67 ± 93.18	194.5 ± 41.73	169.43 ± 16.94
MPV (fl)	5.26 ± 0.05	5.46 ± 0.55	5.75 ± 0.28*	5.51 ± 0.40
PDW (%)	8.00 ± 0.10	8.53 ± 1.19	8.85 ± 0.65	8.51 ± 0.72
PCT (%)	0.08 ± 0.01	0.08 ± 0.06	0.11 ± 0.02	0.09 ± 0.012
LPCR	5.06 ± 0.45	7.43 ± 5.00	6.72 ± 3.29	6.54 ± 2.52
T. WBCs count (10^3)	12.26 ± 0.15	10.60 ± 4.68	16.75 ± 2.96*	20.1 ± 7.43*

Data Expressed as mean ± SD, *:p<0.05, **:p<0.01

Biochemical findings

There were significant increases ($p<0.05$) in total proteins and globulins in moderate and severe infected groups. Significant increases in serum triglycerides and VLDL ($p<0.05$) levels only in the severe infected group, Significant decrease in serum HDL-C level in severe infected group (Table 2).

Table 2: Serum biochemical constituents in different degrees of parasitic infection in donkeys

	Control (No.=3)	Mild infection (No.=3)	Moderate infection (No.=4)	Severe infection (No.=7)
Total proteins (g/dl)	6.86 ± 0.08	7.20 ± 0.29	8.48 ± 0.81*	8.00 ± 0.84*
Albumin (g/dl)	2.77± 0.06	3.05 ± 1.10	2.62 ± 0.26	2.33 ± 0.37
Globulins (g/dl)	4.09 ± 0.02	4.15 ± 1.02	5.86 ± 0.75*	5.67 ± 0.90*
Total cholesterol (mg/dl)	76.01 ± 13.85	66.44 ± 9.31	71.30 ± 12.88	65.54 ± 8.05
Triglycerides (mg/dl)	20.0 ± 4.14	27.12 ± 3.91	14.48 ± 14.931	38.44 ± 11.29*
HDL-C (mg/dl)	48.73 ± 0.28	39.86 ± 5.89	50.09 ± 3.75	39.37 ± 8.00*
LDL-C (mg/dl)	23.37 ± 14.96	21.14 ± 15.88	18.74 ± 14.82	20.31 ± 8.01
VLDL-C (mg/dl)	4.00 ± 0.83	5.43 ± 0.79	2.89 ± 2.98	7.68 ± 2.26*

Data Expressed as mean ± SD, *:p<0.05

DISCUSSION

This study described the effect of *strongyle sp.* infection on haematological and biochemical constituents of donkey's blood. The study revealed that majority of the donkeys had high faecal egg counts, which is consistent with the results from previous studies [4]. According to Soulsby [13], an epg of 500 suggested mild strongyle

infection, an epg of 500-1000 suggested moderate infection and an epg above 1000 suggested severe infection in horses. On this basis, the majority of working donkeys in the current study were severely infected with nematodes,

In the current study, moderate and severe infected groups were anaemic as indicated by the significant decreases in T.RBCs count in moderate ($p<0.05$) and severe ($p<0.01$) infected groups, significant decrease in HCT (%) only in the severe infected group, and significant decreases in HGB concentration in moderate ($p<0.01$) and severe ($p<0.05$) infected groups. The presence of anaemia in moderate and severely infected groups indicated that when anaemia associates *Strongyles spp.* infection in horse fecal egg counts above 500 EPG is expected. Leucocytosis was observed in moderate ($p<0.05$) and severe ($p<0.05$) infected groups [10].

Total proteins concentration in serum is one of the common measurements in clinical laboratory diagnosis. Changes in the levels of plasma proteins may result from alteration in synthesis, catabolism or from protein losses. Hyperproteinaemia in moderate and severe infected groups may be attributed to the increased globulin's levels ($p<0.05$) in response to parasitic infection [12].

Large species' differences in lipoproteins profiles and the percentage of total cholesterol and triglycerides carried by each lipoprotein class were recorded in different animals [9]. Whereas in human and pigs, the majority of cholesterol is transported as LDL, in cattle, cholesterol is equally divided between LDL and HDL, while in sheep and horses, the majority of cholesterol circulates as HDL [7]. According to the present study, the majority of cholesterol circulates in the blood as HDL in donkeys (Table 2).

The significant increase in serum triglycerides levels indicated increase lipolysis in donkeys severely infected with strongyle spp., the increased lipolysis results in liberation of triglyceride and non-esterified fatty acids, which is processed by the liver into acetylCoA in the tricarboxylic acid (TCA) cycle for energy production [14]. The significant increase in serum VLDL in the severe infected group may be attributed to increased serum triglycerides. Very low density lipoproteins export hepatic triglycerides and cholesterol and distribute triglycerides to adipose tissue and striated muscles [7]. The significant decreases in serum HDL-C in the severe infected group may be attributed to the non-significant decrease in serum cholesterol level in heavy infected group, as the majority of cholesterol circulates as HDL [7].

In conclusion, the current study revealed that moderate and heavy strongyle infection in donkeys resulted in anaemia, leucocytosis, hyperproteinaemia, hyperglobulinaemia. On the other hand, lipoprotein profile is disrupted only in severe infected donkeys and results in increases serum triglycerides and VLDL and decrease serum HDL-C levels.

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DETECTION OF THEILERIA ANNULATA BY PCR AND ITS COMPARISON WITH CONVENTIONAL METHOD (Abstract)

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INTRODUCTION

Bovine theileriosis in Egypt is a tick-borne disease known as Egyptian fever since 1947, caused by protozoan parasite known as *Theileria annulata*. The disease is one of the most destructive obstacles to the livestock production in Egypt **(Al- Gaabary, 1995).** Early and accurate diagnosis plays a crucial role for thileriosis control. The present study aimed to compare the conventional and PCR methods for diagnosis of *Theileria annulata* in tick infested cattle.

ANIMALS, MATERIALS AND METHODS

In the current study, a total number of sixty-eight of cattle infested with ticks and clinically suspected cases with *Theileria annulata* infection were examined for confirmation of the infection using both conventional method compared to genetic assay as golden standard assay. These animals belonged to farms and villages of EL-wady EL-geded, Assiut, ELfayoum, EL- minia and Sohage Governorates in Upper Egypt.

RESULTS

The clinical examination of the animals during this study concluded that there were different degrees of tick infestation and some common clinical signs recorded on the infected animals. The rate of conventionally confirmed infection among clinically suspected animal were recorded based on examination of thin blood film the results revealed that according to Giemsa stained thin blood film examination, the percentages of infection were 27.94 %. The genotypic finding using Tams-1 target based PCR assay was used for molecular confirmation of *T. annulata* infection among selected cases revealed that. The infection confirmed in 64.71 % among clinically suspected cattle using blood. The finding of the evaluation study of (68) examined cattle recorded a true positive, true negative, false positive and false negative results of the conventional test as 19, 24, zero and 25, respectively. Accordingly the estimated sensitivity, specificity, PPV, NPP and CPV of the conventional diagnostic method were 43.18 %, 100 %, 100 %, 48.98 %, 63.24% respectively.

CONCLUSION

- The disease was greatly affected by hygienic and management systems specially tick eradication program which influence the presence of the ticks (The only vector for transmission of the protozoan parasite of the disease.
- Conventional method of diagnosis (thin blood film) is an important method for diagnosis of clinical cases.
- Tams-1 target–based PCR is the most sensitive and specific test used for diagnosis of the disease in either acute or chronic cases and also in carrier animals of tropical theileriosis.

STUDIES ON CONTAGIOUS SKIN NECROSIS AND TRYPANOSOMOSIS IN CAMELS

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SUMMARY

Camels showed clinical signs of contagious skin necrosis (CSN), with or without trypanosomosis, were subjected to study. The following samples were collected; sterile bacteriological swabs from skin necrosis area, whole blood samples for hematological analysis and for diagnosis of trypanosomosis, and serum for measuring lipid peroxidation product (Malondialdehyde, MDA). The bacteriological examination of collected swabs from dermal lesion of CSN revealed that *Staphylococcus aureus* was the predominant bacterial isolate alone in 6 cases and coupled with other bacteria in the remained 4 cases, coupled with coagulase negative staphylococci 3 cases and coupled with *Streptococcus agalactiae* in one case. *Trypanosoma evansi* infection was identified using polymerase chain reaction in 5 camels that had CSN. Malondialdehyde showed significant increase in camels affected with CSN that associated with trypanosomosis. The current study revealed that *Staphylococcus aureus* was the predominant bacterial isolate, camels may be infected with both trypanosomosis and CSN, lipid peroxidation products increased in the blood of camels with CSN that associated with trypanosomosis, it is recommended to supply camels with antioxidants to overcome the deterioration of blood antioxidants.

Keywords: Camels, CSN, Trypanosoma, MDA

INTRODUCTION

Contagious skin necrosis (CSN) is a chronic inflammation of the skin primarily caused by *Staphylococcus aureus*, occurred mainly in young dromedaries and localized mostly in the shoulder and neck regions [8]. The disease began with signs of folliculitis, which frequently progressed to a furunculosis with individual or grouped small abscesses. These abscesses could become large and when lanced yield whitish-green pus, and the disease could be chronic and difficult to treat medically depending on the pathogenicity of the staphylococcal strain present [7, 12, 13, 20]. Arthropods were incriminated as transmitting vectors of the various types of the isolated bacteria [17].

Trypanosomosis in camel caused by Trypanosoma evansi is still a serious problem in camel husbandry, causes considerable economic losses in many camel-rearing regions of the world [6, 15]. The course of the infection is often chronic and the parasitological diagnosis is usually difficult, because the parasitaemia is low or no trypanosomes are found in the blood [21]. With the introduction of molecular diagnostic techniques, several diagnostic assays based on the detection of trypanosomal DNA by PCR have been developed [1]. The goals of the present study are to identify the causative microorganism of CSN in the dromedary camels and to evaluate the effect on the health status of camels.

MATERIAL AND METHODS

The investigated camels were clinically inspected [14] for the presence of skin necrosis or abscesses, the suspected cases were subjected to detailed clinical examination and samples collection. The following samples were collected; sterile bacteriological swabs from skin necrosis area, whole blood samples for hematological analysis [5], and for diagnosis of trypanosomosis, and serum for measuring lipid peroxidation product (Malondialdehyde, MDA).

Sterile bacteriological swabs were taken from the opened cutaneous abscesses of the infected camels after disinfection also the necrosed skin was detached and the underlaying tissue was swabbed. These swabs were taken for identification of the bacterial pathogens following standard bacteriological techniques [4, 16].

Serum malondialdehyde (MDA) was measured by using commercial kits (Bio-diagnostic, Egypt) and by means of Digital VIS/Ultraviolet Spectrophotometer (Cecil instruments, Cambridge, England, Series No. 52.232).

For detection of trypanosoma infection 2-5 thin blood films from each camel were prepared [5]. Negative cases were subjected to molecular diagnosis using PCR technique, the PCR technique was performed according to established method [2]. Primers of *Trypanosoma evansi* were as follow: Primer 1: 5'-CGAATGAATATTAAACAATGCGCAGT-3', and Primer 2: 5'-AGAACCATTTATTAGCTTTGTTGC- 3'

Statistical analysis

Data were expressed as mean ± SD, Statistical analysis was conducted using SPSS 16.0 for windows (SPSS, Chicago, USA).The difference in the blood constituents among the investigated groups were compared using one way analysis of variance followed by least significant difference (LSD) post-hoc analysis, significant difference was considered at $p<0.05$.

RESULTS

Contagious skin necrosis

Ten camels showed characteristic dermal lesions of contagious skin necrosis. These lesions were demonstrated on different parts of the animal body, as an area of skin necrosis, in which the skin looked black in color and not covered with hair. These areas sharply separated from the surrounding healthy skin, and they were cold and very hard in consistency. When an area of necrosed skin was detached, circular ulcer of varying diameter, usually 2-10 cm, remained and clearly demarcated from surrounding healthy tissue (Fig. 1). This ulcer was filled with large amounts of whitish pussy material tinged with blood and may reach up to 10cm in depth. The bacteriological examination of collected swabs from dermal lesion of CSN revealed that *Staphylococcus aureus* was the predominant bacterial isolate alone in 6 cases and coupled with other bacteria in the remained 4 cases, coupled with coagulase negative staphylococci 3 cases and coupled with *Streptococcus agalactiae* in one case.

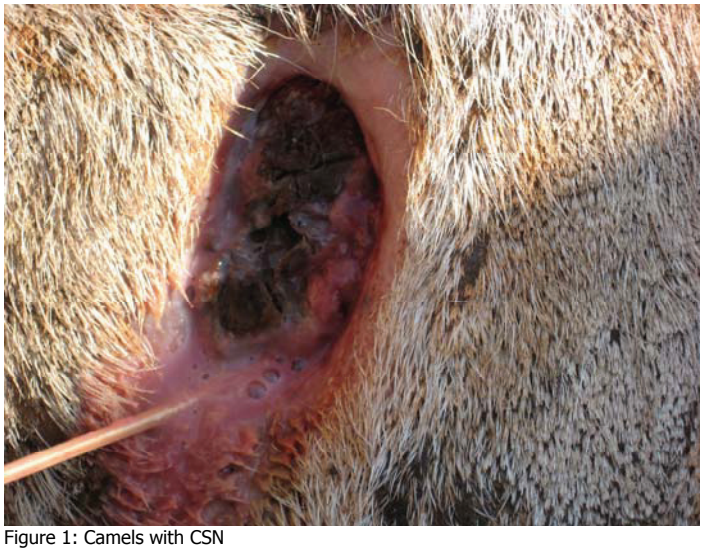

Figure 1: Camels with CSN

Trypanosoma evansi infection was identified using polymerase chain reaction in 5 camels that had CSN.

Comparing data from camels with CSN groups with the control group revealed significant decrease in total RBCs count ($p<0.05$) in camels suffering from CSN alone. Serum malondialdehyde showed significant increase in camels affected with CSN with trypanosomosis ($p<0.01$), when compared with the control healthy camels.

Table 1. Measured blood constituents and MDA levels in investigated camels

	RBCs ($x10^6$/ul)	Hb (g/dl)	PCV (%)	MCV (pg)	MCH (fl)	MCHC (g/dl)	MDA (nmol/ml)
Control	8.35±1.21a	12.2±1.73a	28.5±2.3a	34.7±5.6a	14.7±1.6a	43.3±7.5a	1.0±0.6a
CSN	6.2±1.8b	10.7±2.2a	25.4±3.8a	40.0±6.5a	15.7±3.3a	39.4±5.3a	3.2±1.7ab
Tryp.+ CSN	7.2±1.3ab	11.2±2.2a	26.8±3.3a	37.9±7.5a	15.8±4.1a	41.6±5.3a	5.0±2.6b

In each column, different letter means significant, Tryp.: Trypanosomosis,

DISCUSSION

In the current study, lesions of CSN were found on the back of the animal, sides, shoulder region, gluteal region and in the ventral aspect of the neck. Similar field observation was previously recorded [7, 11], who noted that lesions of staphylococcal dermatitis were usually situated in the gluteal, perineal and lower cervical regions. The main clinical findings in camels suffering from CSN are agreed with previous studies [7, 11, 14, 20].

The obtained results revealed that the main isolated bacterial species from CSN affected camels was *S. aureus* (60.61%), while coagulase negative staphylococci

represented 27.27% and *Streptococcus agalactiae* was 12.12%, these results greed with previous studies [20], which stated that staphylococcal dermatitis primarily caused by *S. aureus*. The obtained results are in harmony with previous studies [7, 8, 14], they reported that CSN in camels caused by a number of bacteria including *S. aureus*. In contrast to other studies [9], which concluded that the main isolated bacteria from CSN was *Streptococcus agalactiae* followed by *S. aureus.*

Lipid peroxidation in biological samples [19] and the metabolic fate of malondialdehyde has been extensively studied [3, 18, Hjelle and Petersen 1983). Unlike reactive free radicals, aldehydes are rather long lived and, therefore, can diffuse from their site of origin (i.e., membranes) to reach and attack other targets intracellularly or extracellularly [10].The increased MDA in the current study indicated increased oxidative stress in blood of camels with CSN coupled with trypanosomosis, and this may attributed to decreased antioxidant levels or may be due to excessive release of free radicals.

CONCLUSIONS

The current study revealed that *Staphylococcus aureus* was the predominant bacterial isolate, camels may be infected with both trypanosomosis and CSN, lipid peroxidation products increased in the blood of camels with CSN and it is recommended to supply camels with antioxidants to overcome the deterioration in blood antioxidants status.

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COCCIDIAN INFECTIONS IN HOUSED LAMBS IN KOSOVO

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ABSTRACT

In this study were investigated 10 sheep farms in Kosovo with a number 22 up to 100 lambs per farm, to detect coccidian infection in housed lambs. In the study were monitored zoo - hygienic conditions, temperature and humidity of floor in the farms and floor was treated with the disinfection material Neopredisan® (chlor chresol 3%) to see, if the disinfection material will reduce or eliminate coccidian oocyst in the farm. After collecting samples of feces from floor and directly from rectum, they were analyzed with floating method. These analyses were repeated every 2 weeks, parallel with measuring temperature and humidity in the farm and in the floor. In every farm were found coccidian infection in lambs with *Eimeria spp.*. The highest intensity of infection it was in the end of month February and in the beginning of month April, respectively in the period when lambs were in ages between 4 and 9 weeks. During this infection period temperature in the farm were between 20 to 30 ºC, humidity in the floor was between 80 up to 90%. In all farms was a massive detection of *Eimeria spp.* in lambs. In some cases it was shown a clinical signs of coccidiosis and death of lambs, respectively in the farm 1 with 6 deaths (6/100), farm 2 with 3 deaths (3/40), farm 3 with 4 deaths (4/70), farm 4 with 1 death (1/22), farm 5 with 3 deaths (3/45), farm 6 with 4 deaths (4/51), farm 7 with 5 deaths (5/55), farm 8 with 6 deaths (6/90), farm 9 with 2 deaths (2/90) farm 10 with 8 deaths (8/60). After treating the floor with disinfection material Neopredisan® (chlor chresol 3%) it was shown after some hours, reduction and elimination of coccidian oocyst in the farm.

Keywords: Coccidiosis, lamb, oocistes, temperature, humidity

EFFECT OF DIETARY VITAMIN E ON PLASMA OXIDATIVE STRESS IN BROILER CHICKS INFECTED WITH *EIMERIA TENELLA*

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SUMMARY

The aim of the present experiment was to study the alterations in plasma oxidative stress parameters in broiler chicks fed with graded dietary vitamin E whilst infected with *Eimeria tenella*. Ninety six new-born chicks were assigned into 3 treatment groups by adding 0, 316 or 562 ppm of vitamin E premix to their regular diet. On day 21, half of the experimental birds were inoculated with 4×10^4 sporulated oocysts of *Eimeria tenella* per bird; whereas the remaining chicks served as non-infected controls. Blood samples were taken and assayed for total antioxidant activity (TAA), lipid peroxidation level and vitamin E content. Oocyst shedding was also examined in all treatments. Results showed that TAA and vitamin E levels in plasma were not affected by dietary treatment ($p>0.05$). The lowest level of plasma lipid peroxidation ($p<0.001$) was noticed in the chicks treated with 562 ppm of dietary vitamin E, but the difference between the chicks fed a regular diet or 316 ppm dietary vitamin E was not significant ($p>0.05$). The oocyst shedding was the lowest in the chicks treated with 316 ppm dietary vitamin E ($p<0.001$), but there was no significant difference between the other two dietary treatments ($p>0.05$). In conclusion, the addition of vitamin E at a rate of 316 ppm to broiler basal diet can improve cellular defence system against *E. tenella* infection without any effect on the plasma antioxidant status, but at higher values it may have an adverse effect.

Keywords: *Escherichia coli*, Antibiotic resistance, Broiler chicks

INTRODUCTION

Lack of efficient commercially-available vaccines to eimerian parasites and the expense of developing novel drugs have forced poultry industry to rely upon prophylactic chemotherapy by present drugs to control the disease. One of the major drawbacks of extensive use of anticoccidial drugs is the development of resistance, which has been described for all anticoccidial drugs introduced so far. In addition, there is growing concern regarding the chemical residues of anticoccidial drugs in meat and eggs, encouraging researchers to investigate alternative prevention and treatment strategies such as in-feed supplements.

Vitamin E is a well-known classical lipid soluble antioxidant scavenging free radicals in a hydrophobic milieu and plays an important role in growth, reproduction, prevention of various diseases and protection of tissue integrity. Among the four saturated forms, α-tocopherol is considered as the most biologically active and plentiful form of vitamin E in plasma and most tissues. To afford increased stability, vitamin E is generally added to poultry feeds as the fully racemic form, all-rac-α-tocopheryl acetate, at levels from 17mg/kg to 48mg/kg [9]. This ester, which is not an antioxidant, is stable to oxidizing conditions, hydrolyzed after ingestion to be absorbed through the small intestinal epithelium in its un-esterified form and then readily incorporated into cellular membranes where it promotes integrity by functioning as an antioxidant and protecting cells against free radical oxidative processes.

In spite of the wide safety marginal of vitamin E, Bowry and Stocker [2] documented that in high doses it can have pro-oxidative effect on human lipoprotein. *E. tenella*, causing caecal coccidiosis, is highly pathogenic and one of the most widespread *Eimeria* species throughout the world. Therefore, the aim of the present experiment was to study the effect of higher levels of dietary vitamin E supplements on the oocyst shedding of *E. tenella* and also to follow alterations in plasma antioxidant and malondialdehyde (MDA) levels in broiler chicks.

MATERIAL AND METHODS

Chicks and diets

This experiment was undertaken on a total of 96 unsexed one-day-old broiler chicks (Ross 308). Immediately after arrival, the chicks were randomly allotted into 3 dietary treatment groups with 32 birds in each. The chicks in treatment group 1 received mash corn-soybean-based diet during the experiment and served as controls, but those in

treatments 2 and 3 were fed similar diet supplemented with 316 or 562 ppm of vitamin E premix, respectively. On day 21, half of the experimental birds were randomly separated into treatments 4, 5 and 6, respectively, transferred to another house and inoculated with 4×10^4 sporulated oocysts of *E. tenella* per bird; whereas the remaining chicks were kept on their corresponding diets and served as non-infected controls. To exclude unwanted infections, salinomycin Na was also added at 60 ppm to the diet of all treatments up to 17 days of age. The diet was formulated to meet the nutrient requirements of broiler chicks according to National Research Council, 1994 [11]. Throughout the experiment, the birds were kept in wire-floored cages with constant lighting and free access to feed and water.

Experimental infection

At first, the parent Houghton strain of *E. tenella* kindly provided by Dr. Damer Blake (Department of Pathology and Infectious Diseases, The Royal Veterinary College, University of London) was refreshed by two continuous propagations on 3-week-old chicks in cage condition. At each propagation the oocysts were purified by salt flotation technique, sporulated in 2% potassium dichromate solution and then stored at 4°C until used. To produce challenge infection, the dichromate was washed out using PBS through repeated centrifugations, and concentration of oocysts was determined by means of a bright-line Improved Neubauer haemocytometer (Germany). On day 21, each bird in treatment groups 4, 5 and 6 was inoculated via crop intubation with 4×10^4 sporulated oocysts of *E. tenella* in 1 ml of PBS, whereas the chicks in non-infected treatment groups were sham-inoculated with the same volume of PBS. All birds were off-fed overnight before the day of inoculation.

Oocyst shedding

Faecal samples were collected separately from all treatments every 12 hours during 5 to 8 days pi. Then, the mean daily oocyst shedding was determined by conventional McMaster technique, and expressed as the number of OPG per bird. Faecal samples were also taken on the day before inoculation to confirm the lack of any *Eimeria* contamination.

Blood collection

Blood samples were taken via the brachial vein from 8 birds in each treatment on day 8 pi, transferred into 5-ml sterile EDTA-K_3 containing tubes and immediately centrifuged at 2,500 rpm for 10 min at 4ºC. Then, the obtained plasma was aliquoted into microtubes and stored at -80ºC until required.

Biochemical analysis

Total antioxidant activity (TAA) of plasma was determined by ferric reducing/antioxidant power (FRAP) assay as per the method described by Koracevic *et al*. [7]. In brief, the assay measures the capacity of plasma to inhibit the production of thiobarbituric acid-reactive substances (TBARS) from sodium benzoate under the influence of hydroxyl radicals ($OH^{\cdot}$) derived from Fenton's reaction. Antioxidants from the added sample cause suppression of TBARS production, and the inhibition of colour development is defined as TAA. Finally, absorbance values were measured spectrophotometrically at 532nm (Shimadzu, UV-120-12; Kyoto, Japan). The total amount of plasma lipid peroxidation as TBARS was estimated according to the method of Placer *et al*. [13] and the values of TBARS material were determined in terms of MDA (nmol/ml) at 532 nm. Plasma vitamin E values were measured spectrophotometrically on each specimen according to Martinek method [8]. In this oxidimetric colour reaction, ferrous ion is produced by reduction of ferric iron by vitamin E contents of plasma and its concentration is expressed as mg/dl. All measurements were done in duplicate.

Statistical analysis

The obtained data were submitted to one-way ANOVA using the Sigma State software (version 2.03, Systat software Inc., Point Richmond, CA, USA). Values were compared using Tukey's post hoc test when they passed the normality test and Dun's post hoc test in case of failure to pass normality. The significance of differences between mean values was set at $p<0.05$.

RESULTS

There were no signs of infection in the non-infected treatment groups. The infected birds showed clinical signs such as depression, weakness and ruffled feathers without any mortality. However, recovery was observed in all infected chicks.

There was no oocyst observation on the day before inoculation. Regardless of the diet composition, the oocyst shedding started in all infected treatments from day 6 pi and reached a peak on day 7 pi. Furthermore, the chicks in treatment group 5 had the least significant amount of shedding during 6 to 8 days pi ($p<0.001$); whereas those in treatment groups 4 and 6 had comparable values ($p>0.05$).

The plasma levels of vitamin E, TAA and MDA in all experimental treatments are presented in Table 1. As shown, a noticeable difference in the plasma level of

vitamin E was only found between treatment groups 3 and 6 fed on 562 ppm vitamin E diet ($p<0.001$). The plasma level of TAA was not affected by dietary treatment ($p>0.05$), but infection in the chicks in treatment groups 4 and 6 caused a significant drop ($p<0.05$). The plasma level of MDA was not affected by infection, but it was significantly decreased by supplementation of diet with 562 ppm vitamin E both in the infected and non-infected treatments ($p<0.001$).

DISCUSSION

In the present study, the oocyst shedding started in all infected treatments from day 6 pi and reached a peak on day 7 pi (Figure 1). This finding is consistent with Chapman and Shirley [3], who suggested day 7 pi as the best time for the collection of the oocysts from the caeca of broilers infected with *E. tenella*. Furthermore, the shedding value was significantly reduced by dietary supplementation with 316 ppm but not 562 ppm vitamin E, suggesting that dietary vitamin E at 316 ppm could have a suppressive impact on the replication and consequently the severity of infection with *E. tenella*; whereas at higher doses it may have some adverse effects on the cellular antioxidant defence system. This claim is supported by a previous report by Yamamoto and Niki [13], where they demonstrated that high amounts of vitamin E could destroy intestinal cells through the increase of free radicals.

It was observed in the current study that almost all experimental treatments had comparable plasma levels of vitamin E. It seems that the level of vitamin E in the control diet is sufficient to make the maximum plasma levels. Besides, there is apparently a homeostatic system controlling the plasma levels of vitamin E. The observation that non-infected chicks treated with 562 ppm vitamin E had the least significant plasma value is in agreement with that reported by Meydani *et al*. [10]. They discussed that the absorption of vitamin E correlates negatively with increasing doses of vitamin E. Allen and Fetterer [1] fed broilers from 1 day of age on diets supplemented with 25 or 225 ppm dietary vitamin E, but found no consistent effect of dietary treatment on oocyst shedding of *E. maxima*. They proposed that malabsorption of vitamin E from damaged cells in the mid-small intestine and consequently less biologically available vitamin E to infected tissues during the acute phase of infection, as the cause of this ineffectiveness.

It was found in the current study that the plasma level of TAA was only affected by infection in the chicks of treatment groups 4 and 6. It seems that the enterocyte antioxidant status against the parasite is independent on its plasma level. In this respect, there is some controversy about the changes in the plasma antioxidant status during infection with *E. tenella*. For example, Georgieva *et al*. [5] observed an increase in plasma catalase activity during infection with *E. tenella*, whereas Ersalan *et al*. [4] reported just the contrary. The evaluation of plasma MDA level showed that there was no significant difference between infected treatments and their corresponding controls ($p>0.05$), but dietary supplementation with 562 ppm of vitamin E caused a significant decrease in the plasma MDA levels. In this respect, Paşaoğlu *et al*. [12] discussed that changes of plasma MDA levels may be independent of their cellular levels. Furthermore, research in patients with type 2 diabetes mellitus have been established that changes in the cellular and plasma TAA values can be in counter-direction manner [6].

CONCLUSION

With due attention to the results presented here, it may be concluded that addition of vitamin E at a rate of 316 ppm to broiler basal diet can improve cellular defence system against *E. tenella* infection without any effect on the plasma antioxidant status, but at higher values it may have adverse effect. Nevertheless, further investigations should be undertaken on the entrocyte level of vitamin E in chickens to reveal the possible cellular target component in response to high vitamin E supplementation.

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Table 1 Effect of graded levels of dietary vitamin E on the plasma levels[1] of tocopherol, total antioxidant activity (TAA) and malondialdehyde (MDA)

		Parameters		
Treatment	Description	Tocopherol (mg/dl)	TAA (mmol/l)	MDA (nmol/ml)
1	(Non-Infected +0 ppm)	0.53±0.13	6.57±0.04^{AE}	1.56±0.65^{AB}
2	(Non-Infected +316 ppm)	0.45±0.03	6.65±0.03^{BCD}	1.25±0.36
3	(Non-Infected +562 ppm)	0.35±0.03^{A}	6.16±0.10	0.72±0.46^{B}
4	(Infected+0 ppm)	0.43±0.07	4.46±0.32^{AB}	1.25±0.14
5	(Infected+316 ppm)	0.43±0.11	4.67±0.19^{C}	1.23±0.63
6	(Infected+562 ppm)	0.58±0.15^{A}	4.45±0.19^{DE}	0.75±0.30^{A}

[1]Values represent means±SE for 8 samples per treatment.
$^{A-E}$Values in column with common upper-case superscript letters differ significantly ($p<0.05$).

PREVALENCE OF *NEOSPORA CANINUM* IN DOMESTIC CATS FROM AHVAZ, IRAN

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SUMMARY

Antibodies to *Neospora caninum* were determined in serum samples of 100 feral cats from Ahvaz, Khouzestan province, Iran. IgG antibodies were assayed by the modified agglutination test using whole tachyzoites of *T. gondii* and *N. caninum*, incorporating 2-mercaptoethanol, NAT. Anti –*N. caninum* antibodies were detected in 14(14%) of 100 cats with titers of 1:80 in 6, 1:160 in 5, 1:320 in 3.. There was no difference between presence of antibodies for both parasites in male and female cats, but occurrence of antibodies was increased with age.

Keywords*:* Prevalence; *Neospora caninum*; Domestic cat; Iran.

INTRODUCTION

N. caninum is an intracellular apicomplexan protozoan parasite belonging to family sarcocystidae [5]. This parasite is distributed worldwide, have a two stage life cycle including asexual stages in intermediate hosts and sexual stages producing oocysts, and can induce serious fetal mortality in cattle [2,10,11]. Canids are definitive hosts for *N. caninum* and oocysts of this parasite has not been demonstrated or isolated from cats [7].

N. caninum has similar morphologic and biologic characteristics to *T. gondii* and was first described in the 1980s [4], and now researchers showed that many warm-blooded animals including cat have antibodies against this parasite [1,10,8,9].

Little is known about *N. caninum* infection in Iranian cats. So, the objective of the present study was to determine the seroprevalence of *N. caninum* in naturally infected domestic cats in ahvaz, Iran.

MATERIAL AND METHODS

Blood samples were collected from jugular vein of 100 trapped domestic Persian cats (52 male and 48 female) of the ages ranging between 6 months to 7 years. Animals had not any clear symptom of diseases at sampling. The samples were centrifuged at 1000 ×g and the supernatants were frozen at −20°C until the examinations were performed.

Sera were tested for the presence of *N. caninum* antibodies using the agglutination tests based on the direct agglutination of fixed parasites with sera pre-treated with 2-mercaptoethanol to prevent non-specific IgM agglutination, as described by Romand et al. [13], NAT (*Neospora* agglutination test). Sera were started at 1:40 serum dilution for *N. caninum*. A titer of 1:80 and greater was indicative for *N. caninum* infection [6,8]. Sera with doubtful results were re-examined.

A complete carpet of agglutination was considered as positive result. Clear-and cut button-shaped deposit at the bottom of the well was interpreted as a negative reaction.

The results obtained for serum evaluation, were analyzed statistically by logistic regression and chi-square tests using SPSS software, version 16. Alpha was 0.05 for all the tests for the associations.

RESULTS

Antibodies to *N. caninum* in 100 examined cats were detected in 14 (14%) with titers of 1:80 in 6, 1:160 in 5 and 1:320 in 3. The seroprevalence of *N. caninum* was 3.61% in males (7 of 52) and 3.36% in females (7 of 48). There was no statistical difference between the prevalence of infection in males and females ($P>0.05$). Logistic regression showed that the prevalence rate of seroreactivity increased significantly with age *for N. caninum* ($P<0.05$).

DISCUSSION

This study aimed to estimate the seroprevalence of *N. caninum* in domestic cats distributed all over Ahvaz city, Iran.

In the current study, we used NAT for detecting antibodies against *N. caninum*. NAT revealed a good sensitivity and specificity compare with Indirect Fluorescent Antibody Test (IFAT) [12,13]. Furthermore, reported by Dubey et al. 2002 [6], a good correlation found between NAT and Western blotting at dilutions of 1:80 or higher for screening *N. caninum* positive cats.

To our knowledge, this is the first study to investigate the occurrence of *N. caninum* antibodies among cats in Iran. Supporting the studies of Dubey et al. 2002, (11.9%) [6] and Ferroglio et al. 2005 (24.8%) [8]], our results confirmed the serological presence of specific antibodies to *N. caninum* in cats (14%) and indicate that populations of feral cats are exposed to *N. caninum* infection. Different prevalences in such studies may be based on the different methods, geographical distribution of the parasites, sampling criteria and cut-off values.

Current data suggest that occurrence of anti- *N. caninum* antibodies in domestic cats of Ahvaz city is relatively considerable, but similar to most of the studies, lower than that to *T. gondii* [14]. This suggests that *N. caninum* is less widespread in the environment. Infected dog excreate few oocysts when infected with *N. caninum* in comparison with cats that shed large amount of oocysts in environment.

In present study, there was no significant difference between genders for *N.caninum*. These finding has also reported by other authors [3].

Clinical neosporosis has not yet reported in naturally infected cats, but experimental infection in immunocompetent cats and cats given corticosteroids showed lesions those observed in dogs [8].

CONCLUSIONS

This study revealed that cats in Ahvaz are the suitable intermediate hosts for *N. caninum* and may have a role on epidemiology of this parasite in other hosts.

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PREVALENCE OF BOVINE CYSTICERCOSIS FROM 2005 TO 2009 IN FEDERAL SLAUGHTERHOUSE IN BAHIA STATE - BRAZIL

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SUMMARY

The Taeniasis-cysticercosis complex is a parasitic disease found in various parts of the world, especially in underdeveloped countries with sanitary problems. In Brazil this zoonosis is endemic, with epidemic character in some regions, especially among the diseases of importance on the economic aspects and public health. The bovine cysticercosis is a zoonotic disease found in the most of slaughterhouses, also the main cause of condemnations of organs and carcasses of cattle. The aim of the present work was to study the prevalence of cysticercosis in cattle slaughtered under federal inspection in Bahia state, Brazil. During the data collection period, 312,336 cattle (males and females), were subjected to standard postmortem procedures, including incision of the masseter and heart muscles, according the rules of the Ministry of Agriculture, Livestock and Supplies of Brazil. The results showed that the prevalence of cysticercosis found alive was 0.08%. The living and calcified cysts diagnosed during slaughter were more found in heart muscle of cattle. The veterinarian inspector and his team play an important role in controlling this zoonosis, preventing contaminated meat to reach the table of the consumer. The data obtained by the professional inspection service can be used for programs of health education and control this disease. The Taeniasis-cysticercosis complex is a public health problem and should not be disregarded by public agencies and the community either.

Keywords: 1. Cysticercosis; 2. Taeniasis; 3. Cattle

INTRODUCTION

The agribusiness system in Brazil's beef supplies the domestic and international market share through exports but faces several problems, among which stands out illegal slaughter, which accounts for approximately 50% market share. There are many costs resulting from illegal logging, particularly in relation to public health due to contaminated meat trade for several zoonoses (2). Bovine cysticercosis is a zoonotic disease most commonly found in slaughter plants and also the main cause of condemnation of organs and carcasses from slaughtered cattle in Brazil (6).

The Taeniasis-cysticercosis complex, determined by *Taenia saginata*, has a worldwide distribution and is widespread in most countries where there is bovine farming. However, despite worldwide distribution, this zoonosis is mostly found in countries with lower development index, due to poor sanitation and low socio-economic and cultural aspects. The infection is often underestimated the difficulty of clinical diagnosis, but both the Pan American Health Organization (PAHO) and the World Health Organization (WHO) consider the contents of 1% for human taeniasis, 0.1% to human cysticercosis and 5% to animal cysticercosis as endemic problem (7). In Brazil, it is estimated that the prevalence of cysticercosis is between 0.7 and 5.3% (9). Besides its importance to public health, this disease is also responsible for a large amount of losses and economic losses for farmers and slaughterhouses, resulting in condemnations and conditional use of carcasses, depreciating its market value (3).

The post-mortem examination carried out on meat inspection in slaughterhouse for slaughtered cattle is the only practical way of detecting the *Cysticercus bovis*. The veterinary inspector and his assistants play a key role in the slaughterhouse, as with the inspection of meat during slaughter, interrupts the life cycle of the parasite, preventing meat unfit for consumption from reaching the consumer (6). Furthermore, also play an important role of epidemiologists, and holds important information about this zoonosis, which could be used by authorities to be developed and control programs to eradicate this disease (9).

Bovine cysticercosis is a zoonotic disease of significant occurrence, one of the major causes of condemnation of carcasses in slaughterhouses in Brazil, causing losses for farmers, industries and public health, it is important to study and monitoring the epidemiological data of this disease. Thus, in order to generate epidemiological data on this important zoonosis and enable greater safety of meat products for the maintenance of public health, this study aimed to determine the prevalence of the *Cysticecus bovis* in slaughtered cattle from 2005 to 2009 in a slaughterhouse under federal inspection system, located in the state of Bahia, Brazil.

MATERIAL AND METHODS

This study was conducted from 2005 to 2009, and the data were obtained from the federal inspection service of the cattle slaughterhouse located in Bahia state, Brazil. Data contained the monthly number of cattle slaughtered, their origin and number of animals positive for cysticercosis with living and/or calcified lesions in various organs and carcasses of animals affected. The methods used in-line inspection in slaughter plants for the presence of cysticercosis are recommended by the Ministry of Agriculture, Livestock and Supply of Brazil, through the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin (RIISPOA) (4).

RESULTS

During the study period, were subjected to sanitary inspection of 312,336 cattle (males and females), obeying the rules of ante-mortem inspection and post-mortem recommended by the Federal Inspection Service, by the standards of RIISPOA (4). The animals were culled from various municipalities in four different states: Bahia, Tocantins, Mato Grosso and Pará.

It can be seen through the data provided by the federal inspection of the establishment where he developed the present study, the prevalence of cysticercosis with viable cysts in slaughtered animals in the slaughter plant in Bahia, during this period, was 0.08%, representing 221 positive animals, as shown in Table 1.

Table 1: Total of slaughtered animals in the slaughterhouse under federal inspection in Bahia state, Brazil, during the period 2005 to 2009, and prevalence of cows positive for cysticercosis alive.

Year	Slaughtered animals	Positive animals	Percentage
2005	46,101	54	0.12
2006	72,882	42	0.06
2007	69,546	39	0.06
2008	94,283	56	0.06
2009	29,554	30	0.10
Total	312,366	221	0.08

Regarding the localization, were found living cysts in the heart, tongue and head, while the calcified cysts were diagnosed in the heart, head, tongue and liver. Table 2 presents a summary of the percentages of occurrence of calcified and alive cysts were diagnosed during the study period in slaughtered cattle in that slaughter plant. There is a higher prevalence of heart and head, both living and calcified, showing that post-mortem examination of these organs is crucial for correct diagnosis, avoiding contamination of the consumer for this zoonosis

Table 2: Percentages of viable and calcified cysts from 2005 to 2009.

Year	Viable cysts (%)			Calcified cysts (%)			
	Heart	Tongue	Head	Heart	Head	Tongue	Liver
2005	48	27	25	69	15	11	5
2006	67	0	33	61	29	8	2
2007	56	16	28	63	21	8	8
2008	25	23	52	34	33	10	23
2009	80	0	20	67	15	13	5

DISCUSSION

In other studies conducted in the state of Bahia, Almeida (1) observed much higher percentage (4.20%) in the city of Teixeira de Freitas, located in the south of Bahia, while in the study conducted by Paiva (8), in the municipality of Barreiras, west region of Bahia, the results were lower (0.52%). The indices reported in this paper demonstrate how Brazil in its huge territory, can simultaneously display characteristics of developed countries, when the next show indices of absence for bovine cysticercosis in some regions, and reach much higher rates of up to 10% as seen in the study realized by Costa (5) in the municipality of Nova Friburgo, state of Rio de Janeiro. In the present study, the mean percentage of live cysticercus found in slaughtered cattle in Bahia was 0.08%, below the majority of works found on the subject in the first decade of this century

The results demonstrate the importance of consuming beef from slaughter plants under official inspection service, for the second RIISPOA (4), carcasses with severe infestation by *Cysticercus bovis*, which corresponds to the identification of one or more cysts in various parts of muscles are convicted. These inspection procedures carried out in ensuring a better quality of life of the consumer, preventing serious health problems.

CONCLUSIONS

The present study demonstrated a significant percentage of bovine cysticercosis in slaughtered animals, with higher prevalence of cysts (alive and calcified) in the myocardium and head muscles. Finally, the presence of this zoonosis in the study area shows that it is important and necessary to perform a work of public awareness of the taeniasis/cysticercosis complex, with the creation of educational campaigns involving all segments related to public health.

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COMPARISON BETWEEN CONVENTIONAL AND RECENT METHODS FOR DIAGNOSIS OF BOVINE THEILERIOSIS

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SUMMARY

The present study was conducted on the period from April 2008 to July 2009 and included a total number of 150 cattle and 35 buffalo. The age of these animals ranged from one day to above five years old. The animals belonged to farms and villages of EL-Wady EL-geded, Assiut, ELFayoum, EL- Minia and Sohage Governorates.

The positive cases from blood film examination was 25.3% and 8.6% of cattle and buffaloes respectively .While examination of the lymph smear was recorded 55.8% and 15.4% in cattle and buffaloes respectively, So, the using of lymph smears methods is more accurate than using stained blood films.The (TaSP) ELISA test were sensitive and specific for detection of *T. annulata* infection in both cattle and buffaloes through detection of its specific antibodies the infection rate in clinically suspected cattle was 64.7% while in clinically suspected buffaloes was 74.3%. So, it will be recommended for using it during the epidemiological survey to evaluate the incidence of the infection. Serological method, as (TaSP) ELISA test, revealed that the sensitivity of the test was 72.9% and 75% in cattle and buffaloes, respectively. While the specificity were 87.5% and 37.5% in cattle and buffaloes, respectively. So, the serological assay, (TaSP) ELISA is considered the suitable test that is recommended for epidemiological survey of the disease especially in cattle. It can be also used for diagnosis of chronic and carrier stages of the disease.

INTRODUCTION

Theileria annulata, a protozoan parasite of cattle and water buffalo (*Bubalus bubalis*). It transmitted by ticks of genus Hyalomma. The parasite first invade lymphatic system cells of vertebrate hosts , where they develop into macroschizonts. Eight days later, they appear in the peripheral blood as intra–erythrocyte forms which are mostly rounded in shape and therefore were formally named Piroplasma annulatum. In Egypt, clinical cases of the disease in imported and crossbreed cattle have been reported. However, Theileriosis, caused by *Theileria annulata* and transmitted by the prevalent tick vector *Hyalommaanatolicum anatolicum*, represents one of the main obstacles of cattle herd industry in this area **[9,2].** Accurate diagnosing of carrier animals is necessary for determining the immune status of these animals that is very important for implementing suitable control programs. Serological tests are suitable for the diagnosis of the disease during its late phases as well as in carrier cases where antibody titers are usually high and piroplasmparasitemia drops to microscopically undetectable levels **[7,4].** Serological methods are usually employed for determining sub clinical infection. Enzyme linked immunosorbent assay (ELISA), due to its ease of standardization and application, would fulfill the requirements for epidemiological surveys.ELISA, being a highly sensitive, specific and objective assay, is recommended for the detection of antibodies against *T . annulata* for sero- epidemiological studies **[6,8,12].** *T. annulata* surface protein (TaSP) is present as a single copy within the parasite genome and transcribed in the sporozoite and schizont stage. The potentiality of the recombinant surface protein of *Theileria annulata* (TaSP) to produce a humeral response would make it a suitable candidate for diagnostic systems as well as immunization trial.

Recently, *T. annulata* surface protein (TaSP) has been characterized and its application in indirect ELISA has been documented and validated **[10].**

MATERIALS AND METHODS

Animals: A total number of 150 cattle and 35 buffalo belong to different localities in EL- Fayoum, EL-Mania, Assiut, Sohage and EL-Wady EL-Gaded governorates were subjected to this study. All animals showed acute or chronic forms of tropical theileriosis with different degrees of tick infestation (Fig 8).

Sampling: 1.Blood sample was collected directly from the ear vein and used for preparation of thin blood films. which fixed by using methyl alcohol(methanol)for about 3-5 min.,then stained with Giemsa stain for about 30-45 min. Dried and examined by using Oil immersion lens at x1000 magnification.

2.Lymph node aspiration were collected from enlarged lymph nodes these samples were marked with numbered labels in the field and used for preparation of lymph smears immediately after collection.

3.Blood sample were collected without anticoagulant for serum separation by centrifugation of the samples at 12000 g for 20 min. using (Hettich, Zentrifugen EBA8SD–78532 Tutilingen, Germany); that used in serological test as (ELISA).

Clinical examination: All animals were subjected to clinical examination ,those animals showed various degrees of the characteristic clinical signs for the tropical theileriosis like fever (>40c), enlargement of the superficial lymph nodes(acute form), in appetence, pale or congestion of visible mucous membranes, conjunctivitis, severe congestion of the eyes, excessive lacrimation, corneal opacity, various degrees of respiratory signs from serous nasal discharge to bloody ,purulent discharge, cough and dyspnoea (chronic form). In addition to various degrees of ticks infestation.

Serological diagnosis Enzyme linked immunosorban assay (ELISA)

RESULTS

The study revealed that out of the clinically suspected cases (13%) were conventionally confirmed positive at EL-wady EL-gaded governorate as compared to 12.4%, 8.6%, 3.8%, 2.7% were recorded at Assiut, EL- fayoum, EL-mania and Sohage governorates, respectively. The results of the diagnostic assay by conventional tests for detection of *T. annulata* infection among randomly selected samples of clinically–suspected cases were recorded as in blood films the infection rate was (25.3%,8.6%) and (55.8%,15.4%) in case of lymph smears, among clinically suspected cattle and buffaloes ,respectively (Table 1). Serological finding using TaSP-based ELISA test which was used for detection of *T. annulata* specific antibodies revealed that the infection rate of 64.7% and 74.3% among clinically suspected cattle and buffaloes, respectively (Table 2). The over all results revealed an infection rate of 48.4% and 57.8% among selected cattle, respectively. On the other hand an infection rate of 16.7% and 66.7% were recorded among selected buffaloes using conventional and serological methods, respectively.

DISCUSSION

In the present study it found that using of conventional methods for diagnosis of tropical theileriosis, although they are cheap and easy to use, was limited for the detection of acute cases especially with high level of parasitemia. The test was less sensitive during the chronic form or in case of carrier animals. The obtained results for the conventional method of diagnosis were (48.4% and 16.7%) in cattle and buffaloes, respectively. If compared with (TaSP) ELISA test (57.8% and 66.7%) in cattle and buffaloes, respectively, This finding was supported by previous finding of **[13]** in Sudan (Khartoum) who reported that blood smears is quick and cheaper for diagnosis but less sensitive (20%) than ELISA test (64%) in cattle. The current study revealed that examination of Giemsa stained lymph smears can provided more diagnostic value (48.2%) if compared with the Giemsa stained thin blood films (36.1%). This was manifested by the high percentage rate of positive cases which recorded from examination of lymph smears in comparison with others obtained from blood smears**.** This could be attributed to the easier detection of Koch's blue bodies inside the lymphocytes of the stained lymph smear than detection ofintraerythrocytic trophozoites of the *T. annulata* in stained thin blood film. This result was in agreement with **[5]** in Germany and **[1]** in Upper Egypt they reported that it was the method of choice for diagnosis of tropical theileriasis in suspected cases specially those with enlarged lymph nodes.The evaluation study of the Tasp-based ELISA test revealed that a relatively low sensitivity in both cattle and buffaloes (72.9% and 75%), respectively. This could be attributed to the inability of the test to detect the acute infection in cattle due to low titer of specific-antibodies **[3,10].** On the other hand, the evaluation study revealed that low specificity in buffaloes (37.5%) as compared with cattle (87.5%). This could be attributed to the fact that buffaloes can resist the infection with tropical theileriosis through innate factors and cell mediated immune response.. Also, there is low affinity of the protozoan parasite to the buffaloes' cells so the animal may be have high titer of the specific antibodies but have no infected cells**).** This could explain the higher sensitivity and low specificity of the test in case of buffaloes if compared with cattle. The Tasp-based ELISA test revealed that there was a relative high PPV (94.6%) in cattle if compared with buffaloes (37.5%). It means that, the ability of TaSP-based ELISA test to correctly identify true positive cases as being positive is higher in cattle as compared with buffaloes. So, TaSP-based ELISA test could be recommended for epidemiological survey of the disease in cattle **.** The study also revealed that there was a lower NPV in cattle (51.9%) as compared with buffaloes (75%). It means that, the TaSP-based ELISA test have a lower ability to detect the negative cases as being negative in cattle as compared with buffaloes. It could be contributed to the inability of the test to detect the acute infection in cattle due to low titer of specific antibodies, cross reaction with antibodies for other closely related blood parasites **[3,10].**

CONCLUSION

It seems that the use of TaSP- based ELISA test for diagnosis of tropical theileriosis is more sensitive and accurate especially in cases of chronic and carrier cattle if compared with the use of different conventional method.

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Table 1: Rate of conventionally confirmed infection among clinically suspected cases.

Animal species	Blood film			Lymph smears		
	No. of tested animals	positive	%	No. of tested animals	positive	%
Cattle	150	38	25.3	95	53	55.8
Buffaloes	35	3	8.6	13	2	15.4

Table 2: Rate of serologically diagnosed infection among clinically suspected cases by using (TaSP) protein - based ELISA test

Animal species	(TaSP) ELISA test			
	Range Mean +/-SD	Cutoff*	positive	%
Cattle (150)	0.045 - 0.873 0.334 ± 0.148	0.285	97	64.7
Buffaloes (35)	0.190 - 1.14 0.462 ± 0.207	0.285	26	74.3

*****The cutoff value set at 44 percentage positively (Salih et al., 2005)

CATTLE THEILERIOSIS: EFFECT ON SERUM CONSTITUENTS, ERYTHROCYTES AND PLATELETS PICTURES

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SUMMARY

The goal of the present study was undertaken to investigate the influence of the tropical theileriosis on some serum constituents, erythrocytes and platelets pictures. A total number of 26 cows were subjected to study. Out of them 16 cows was suffered from Theileria infection. Comparing Theileria infected group with the control group revealed significant decreases in total RBCS counts ($p<0.01$), haemoglobin concentration ($p<0.01$), packed cell volume ($p<0.01$), platelets count ($p<0.01$), plateletcrit ($p<0.01$), significant decreases in serum total proteins ($p<0.01$), albumin ($p<0.01$), calcium ($p<0.01$), and phosphorus ($p<0.01$) levels, and significant increases in serum blood urea nitrogen (BUN) ($p<0.05$) and creatinine ($p<0.05$) levels, and in serum aspartate aminotransferase ($p<0.01$) and gamma glutamyl transferase (GGT) ($p<0.01$) activities. Correlations between the studied parameters revealed significant positive correlations between total proteins and albumin ($r= 0.598^*$), Albumin/Globulin (A/G) ratio and albumin ($r=0.860^{**}$), calcium and albumin ($r=0.729^*$), calcium and A/G ratio ($r=0.752^*$), GGT and BUN ($r=0.539$), and significant negative correlations between A/G ratio and globulins ($r=0.809^{**}$) and between glucose and albumin ($r=0.614$). It could be concluded that Theileria infection in cattle resulted in anaemia, thrombocytopenia, hypoalbuminaeia, hypoproteinaemia and hypophosphataemia.

Keywords*: Theileria annulata*, cow, Buparvaquone, serum

INTRODUCTION

Tropical theileriosis is one of the most prevalent and economically important diseases of cattle. The principal causative agent of bovine theileriosis is the protozoan parasite *Theileria annulata* and transmitted by the ticks of the genus Hyalomma. The disease is observed in South Europe, North Africa, middle and South Asia and the Middle East and Threatens approximately 250 million cattle [11]. Theileriosis causes serious economic losses through mortality and loss of productivity [1,5]. *T. annulata* sporozoites infects the host mononuclear cells (macrophages/monocytes and B lymphocytes) in the lymph nodes draining the site of inoculation of the sporozoites by ticks. The sporozoites transform into schizonts in the mononuclear cells. Host cells become transformed and proliferate in synchrony with the parasite during this process, named the macroschizont stage [6]. The schizonts undergo further differentiation to merozoites, which are released by the lyses of the infected cells. Later, the merozoites invade red blood cells. It is followed by the development of piroplasms in erythrocytes, and these intra-erythrocyte piroplasms become available to the vector [2, 4]. The severity of infection with theileriosis is indicated by the change in blood serum biochemical parameters. Based on the above considerations, the present study was designed to investigate the influence of the tropical theileriosis on some serum components, erythrocytes and platlets pictures.

MATERIAL AND METHODS

A total number of 26 cows were subjected to study. Out of them 16 cows were presented to the Veterinary Teaching Hospital, Assiut University, showed high fever 40-41.5°C, enlargement of superficial lymph nodes, and infestation with ticks. Some cases accompanied with corneal opacity and lacrimation and represent the Theileria infected group. The remained animals (No. =10) were selected from healthy cows and kept as the control group. Theileria infection was confirmed by blood and lymph smears.

Drop of blood from the ear vein was used for making blood film [3]. Lymph node aspirate was used for making lymph smear immediately after collection. Blood and lymph smears were fixed in absolute methyl alcohol, stained with Giemsa stain and examined under the oil immersion lens (x100). Two blood samples were collected from the jugular vein; one sample was collected on EDTA vacutainer tube for haematological analysis. The other one was collected in a plain vacutainer tube and processed for separation of serum [3]. Haematological analysis was carried out by automatic blood cells counter (Medonic CA 620, Sweden). Serum samples were used to measure serum total proteins, albumin, blood urea nitrogen (BUN), creatinine, calcium, phosphorus and glucose levels, and serum aspartate aminotransferase (AST, U/l), gamma glutamyltransferase (GGT, U/l), lactate dehydrogenase (LDH, U/l) and creatine phosphokinase (CK, U/l) activities by using commercial kits (Spectrum-diagnostics, Cairo,

Egypt) and by Digital VIS/Ultraviolet Spectrophotometer (Cecil instruments, Cambridge, England, Series No. 52.232).

Statistical Analysis Data are presented as means and standard deviation. Statistical significance was determined by the analysis of variance using Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA). Statistically significant differences were determined at $p \leq 0.05$.

RESULTS

Besides clinical signs of theileriosis, diagnosis of Theileria infection was confirmed by findings intracellular signet ring of Theileria trophozoites in blood smear or intralymphocytic schizonts in lymph smear. As shown in table 1, there were significant decreases in total RBCS counts ($p<0.01$), haemoglobin (Hb) concentration ($p<0.01$), packed cell volume (PCV) ($p<0.01$), platelets (PLT) count ($p<0.01$), plateletcrit (PCT) ($p<0.01$) in Theileria infected group when compared with the control group.

Table 1: Haematological findings in cattle theileriosis

	Control group	Theileria infected group
Total RBCs count $(x10^6)/mm^3$	7.59 ± 0.78	4.47 ± 0.35**
Haemoglobin (g/dl)	11.43 ± 0.51	6.38 ± 0.10**
PCV%	36.66 ± 1.52	17.98 ± 0.46**
MCV (fl)	48.53 ± 3.54	40.44 ± 3.88
RDW (%)	26.96 ± 1.22	31.78 ± 6.29
MCH (pg)	15.90 ± 0.70	14.34 ± 1.21
MCHC (%)	32.93 ± 1.85	35.58 ± 0.42
Total WBCs $(x10^3)/mm^3$	11.10 ± 0.40	12.24 ± 2.73
PLT $(x10^3)/mm^3$	461.0 ± 107.61	178.80 ± 106.32**
MPV(fl)	6.30 ± 0.17	6.16 ± 0.59
PDW %	10.70 ± 0.30	10.28 ± 1.43
PCT%	0.29 ± 0.06	0.10 ± 0.04**

There were significant decreases in serum total proteins ($p<0.01$), albumin ($p<0.01$), calcium ($p<0.01$), phosphorus ($p<0.01$) levels and LDH ($p<0.01$) activity. In addition, there were significant increases in serum BUN ($p<0.05$) and creatinine ($p<0.05$) levels, and in serum AST ($p<0.01$) and GGT ($p<0.01$) activities (Table 2).

Table 2: Serum biochemical constituents of Theileria infected cattle

	Control group	Theileria infected group
Total proteins (g/dl)	6.79 ± 0.60	5.42 ± 0.45**
Albumin (g/dl)	3.56 ± 0.33	2.39 ± 0.45**
Globulins (g/dl)	3.23 ± 0.32	3.03± 0.40
A/G ratio	1.12 ± 0.09	0.81 ± 0.21**
BUN (mg/dl)	10.48 ± 1.57	17.57 ± 9.77*
Creatinine (mg/dl)	0.75 ± 0.26	1.52 ± 0.74*
Phosphorus (mg/dl)	6.58 ± 0.75	3.63 ± 1.06**
Calcium (mg/dl)	9.39 ± 0.95	6.56 ± 2.06**
Glucose (mg/dl)	76.47 ± 12.18	74.28 ± 30.62**
GGT (U/l)	8.08 ± 2.06	20.84 ± 7.61**
AST (U/l)	30.01± 6.47	290.52 ± 36.79**
CK (U/l)	72.54 ± 34.22	91.65 ± 54.38**

Correlations between serum biochemical parameters revealed significant positive correlations between total proteins and albumin (r= 0.598*), A/G ratio and albumin (r=0.860**), calcium and albumin (r=0.729*), calcium and A/G ratio (r=0.752*), GGT and BUN (r=0.539*), and significant negative correlations between A/G ratio and globulins (r=0.809**) and between glucose and albumin (r=0.614*).

Table 3: Correlation between serum biochemical constituents in theileria infected group

	Albumin	Globulin	A/G ratio	BUN	Creat.	Phosph.	Calcium	Glucose	GGT	AST	CK
Total protein	.598*	.455	.131	.359	.377	-.006	.350	-.290-	.016	.287	-.164
Albumin	1	-.442-	.860**	.052	.164	-.078	.729*	-.614*	-.416	.072	.070
Globulin		1	-.809**	.340	.235	.083	-.453	.357	.478	.240	-.260
A/G ratio			1	-.183	-.036-	-.118	.752*	-.484	-.493	-.107	.134
BUN				1	.294	-.011	-.262	-.021	.539*	.337	.045
Creatinine					1	.247	-.051	-.294	-.108	.085	-.194
Phosphorus						1	-.566	.068	-.073	-.272	.141
Calcium							1	-.356	-.216	.190	-.258
Glucose								1	.287	-.245	.008
GGT									1	.155	-.154
AST										1	.329

DISCUSSION

Haematological findings of the current study revealed significant decreases in total RBCs count, Hb concentration, PCV, PLT and PCT, which indicated severe anaemia [8]. Normocytic normochromic anaemia in cross-bred calves experimentally infected with *T. annulata* was reported previously [12]. The significant decrease in PCT ($p<0.01$) is attributed to the significant thrombocytopenia ($p<0.01$), the latter together with the normocytic normochromic anaemia may indicate that the anaemia may be partially attributed to depression of the bone marrow. In the present study, theileria infected group showed hypoproteinaemia ($p<0.01$) and hypoalbuminaemia ($p<0.01$), which may be attributed to liver damage [9]. The elevation in serum AST ($p<0.01$) and GGT ($p<0.01$) activities (Table 1) in the theileria infected group support the occurrence of hepatic dysfunction and agree with previous studies [10]. *Theileria annulata* infection causes hepatic tissue damage that includes coagulative necrosis, distortion of hepatic cords and heavy infiltration of lymphocytes in the periportal areas, including severe damage to the hepatobiliary system (Sandhu et al., 1998). Not only the liver was affected by theileria infection, but the kidney function also disturbed as confirmed by the rise in serum blood urea nitrogen and creatinine levels. Sandhu et al. (1998) reported an increase in BUN and uric acid in calves infested with theileria, with focal to diffuse coagulative necrosis, severe damage to collecting tubules, haemorrhages and lymphoid aggregations in interstitial spaces. It has also been stated that parasitized lymphoid cells can infect non-lymphoid organs such as liver and kidney inducing tissues damage [4].

In the current study, theileria infected cattle showed hypophosphataemia and hypocalcaemia, the decrease in serum calcium level may be attributed to hypoalbuminaemia, It is important to appreciate that a high proportion of calcium is bound to albumin. However, it is the unbound calcium that is the most important physiologically. For this reason when albumin is low, the total calcium level may be misleading [7]. The reduction in the serum phosphorus concentration may be due to the renal tubular defects. The same was reported by Sandhu et al. (1998). The non significant changes in CK activity indicate absence of muscle damage in both groups. Correlations between the studied parameters in Theileria infected group revealed significant positive correlations between total protein and albumin (r= 0.598*), A/G ratio and albumin (r=0.860**), calcium and albumin (r=0.729*), calcium and A/G ratio (r=0.752*), GGT and BUN (r=0.539), and significant negative correlations between A/G ratio and globulins (r=0.809**) and between glucose and albumin (r=0.614). The above correlations confirm the findings of the present study, for example the positive correlations between calcium and albumin may be attributed to the bound part of calcium which decreases with hypoalbuminaemia. The negative correlations between albumin and glucose may be attributed to the destruction of liver cells, which results in the release of glucose to the circulation and decrease albumin synthesis.

CONCLUSIONS

It could be concluded that Theileria infection in cattle resulted in anaemia, thrombocytopenia, hypoproteinaemia and hypophosphataemia. It is recommended to supply cows suffered from theileriosis with supportive treatment to help the animal resume normal productivity early.

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COMPARISON BETWEEN CONVENTIONAL AND ELISA METHODS FOR DIAGNOSIS OF SARCOCYSTOSIS IN BUFFALOES

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SUMMARY

The present study was undertaken to compare the conventional and ELISA methods for diagnosis of sarcocystosis in Buffaloes. A total number of 100 female buffaloes were subjected to study. Macroscopic sarcocystis was collected from the esophagus of buffaloes slaughtered in Mosha slaughterhouse (Mosha, Assiut Governorate, Egypt) during the period from February to June 2010. Part from the esophagus containing the sarcocystis was fixed in 10% formol saline and was processed for histopathological exam. Serum samples from all animals were subjected to ELISA for detection of antibody to sarcocystis. The prevalence of macroscopic sarcocystis was 23%. On the other hand, ELISA technique revealed that 94.44% of examined animals were infected with sarcocystis. The sensitivity of the macroscopic method was 27%. Specificity was 100%. Positive predictive value was 100%, and negative predictive value was 7.46%. Histopathological sections of infected muscles showed cross and longitudinal sections of sarcocystis with different shape and size. The current study revealed that macroscopic examination for detection of sarcocystis is not sufficient. Animals must be subjected to ELISA to ensure that the animals are free from the parasite. It is recommended to apply control measures for the source of infection at the area of study.

Keywords: arcocystis, ELISA, Buffaloes.

INTRODUCTION

Sarcocystis is a protozoan parasite. It has an obligatory prey-predator two host life cycle, that has carnivorous predator hosts (dogs, cats and man) and a wide variety of prey hosts (sheep, cattle, buffaloes, pigs, camels, birds, fish and man), species of sarcocystis are more specific for their prey hosts than for their predator hosts. There are three species of Sarcocystis, which naturally infect buffaloes namely; *Sarcocystis fusiformis*, *Sarcocystis levinei sp. no.*, and *S. buffalonis* [2, 9, 12]. The diagnosis of sarcocystosis has long been based on the identification of the encysted parasites in the muscles by direct microscopic examination. Such technique, although is simple and valuable in screening is not adaptable for diagnostic purposes. ELISA is generally preferred as being efficient, sensitive, objective and less time consuming [14]. The present study was carried out to compare the macroscopic diagnosis of sarcocystis in buffaloes with the ELISA method in Assiut governorate, Assiut, Egypt.

MATERIALS AND METHODS

A total number of 100 female buffaloes were subjected to study. Macroscopic sarcocystis was collected from the esophagus of buffaloes slaughtered in Mosha slaughterhouse (Mosha, Assiut Governorate, Egypt) during the period from February to June 2010. Part from the esophagus containing the sarcocystis was fixed in 10% formol-saline and was processed for histopathological examination [15]. Serum samples from all animals were subjected to ELISA for detection of antibody to sarcocystis [17]. Antigen was prepared according to procedures in a previous study [14].

RESULTS

The prevalence of macroscopic *Sarcocystis sp.* (Fig.1) was 23%. Examination of the sarcocystis by using the light microscope depending on gross and histopathological sections revealed that all sarcocystis in the present study are *Sarcocystis fusiformis*, both small and large cyst was detected. On the other hand, ELISA technique revealed that 94.44% of examined animals were infected with sarcocystis. The sensitivity of the macroscopic method was 27%. Specificity was 100%. Positive predictive value was 100%, and negative predictive value was 7.46%. Histopathological sections of infected muscles showed cross and longitudinal sections of sarcocystis with different shape and size (Fig. 2).

Fig. 1. *Sarcocytis sp.* in esophageal muscles

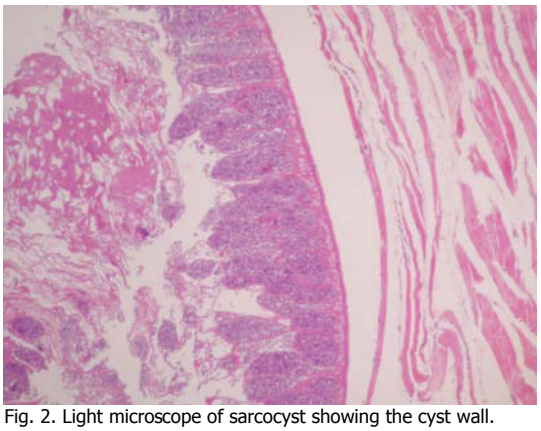

Fig. 2. Light microscope of sarcocyst showing the cyst wall.

DISCUSSION

Sarcocystosis is distributed worldwide. Many investigations have concerned the prevalence of Sarcocystis spp. infection in muscles of slaughtered cattle and buffaloes. In the present study, Sarcocystis sp. was detected in the esophagus and tongue muscles of slaughtered buffaloes. Esophagus is the most frequently infected organ with either macroscopic or microscopic sarcocyst [5, 7]. Distribution of sarcocyst does not follow a specific pattern in most of the infected organs in buffaloes, except for macroscopic cysts, which tend to be located in the esophagus. In addition, hearts of these animals do not usually contain any macroscopic forms [10].

The prevalence of macroscopic Sarcocystis sp. was 23%, ELISA technique revealed that 94.44% of examined animals were infected with sarcocystis. Similar results were reported by previous studies, a prevalence rate of 76.8% was reported in Assiut Governorate [16], 72.6% in examined buffaloes in Qena Governorate, Egypt [4] and 94% among cattle in Egypt [3]. Higher infection rates have also been recorded in other countries that have similar climatic conditions, such as 87% in India [13], 79% in Vietnam [8] and 82.9% in Iraq [11]. However, there have also been reports indicating lower prevalences, among them 65% in the Philippines [1] and 57% in Iran [6].

The current study demonstrated that ELISA is efficient in diagnosis of sarcocystosis in buffaloes. Because of the low sensitivity of the macroscopic method (27%), it can't be used as the only method for diagnosis. It is recommended to diagnose sarcocystosis by using ELISA method, which will benefit both diagnosis and control of sarcocystosis.

CONCLUSIONS

The current study revealed that macroscopic examination for detection of sarcocystis is not sufficient. Animals must be subjected to ELISA to ensure that the animals are free from the parasite. It is recommended to apply control measures for the source of infection at the area of study.

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CRYPTOSPORIDIOSIS AND ITS RISK FACTOR IN CALVES OF HUSBANDRIES AROUND OF TEHRAN, IRAN

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SUMMARY

Cryptosporidium is an intracellular protozoan, whose affect human and animals as a major cause of diarrhea. In present study, incidence rate of cryptosporidiosis in diarrheic calves in husbandries around of Tehran was studied. Moreover, the role of risk factors in the infection was evaluated. Two hundred fecal samples from diarrheic calves in husbandries around of Tehran, Iran including: Varamin, Gharchak, and Shahriar districts were collected as cluster sampling. After transfer to laboratory and concentration of oocyst with Formol-ether method, all of slides were stained with modified Ziehl-Neelsen's acid fast method and were studied for presence of oocyst with photomicroscope. Results confirmed that cryptosporidiosis exists in 9% of studied calves. Statistical analyses showed that incidence rate of the infection did not have any relationship with sex of animals and diarrhea history in the past. Moreover, some of risk factors including; age, type of animal bed, and animal lactating process can effect on the infection to *Cryptosporidium*. Therefore, with regard to presence of cryptosporidiosis in the different regions of Iran, study and diagnosis of major risk factors can be important in the control of the infection.

INTRODUCTION

Cryptosporidiosis is a zoonotic protozoan disease of worldwide distribution, affecting a wide range of vertebrate hosts (6). Its life cycle undergo two asexual generations in the epithelial cells of the intestine. Finally, gametogony phase ends with the formation of the oocyst, which is excreted with the feces to the external environment (5). This phase is relevant for the detection and identification of the parasite (6). Infection to this protozoan may cause cryptosporidiosis. This disease is considered as one of the most important enteric pathogen and is endemic in developing countries (20). The major clinical signs are diarrhea, malnourishment, weight loss, growth retardation, and decrease of milk production (9). Clinical signs in infected animals are relate to some factors like parasite type, age and immunity system of host, completely (20). Infected calves can eliminate a large number of oocyst in their feces and contaminates fresh food, drinking and recreational water causing infection affecting humans and other animals (5 & 23). The presence of *Cryptosporidium* in feces of calves and children has been reported in other countries in the world (1, 3, 5, 13, 17 & 18). Also protozoan was isolated from different regions of Iran in animals and human (12, 16 & 21). With regard to extension of the infection in Iran and different areas of the world, the best method for the control of this parasite is management strategy and it is necessary for diagnosis of its potential risk factors. Therefore, the objective of this study was to evaluate of the incidence rate and potential risk factors of cryptosporidiosis in calves of husbandries around of Tehran, Iran for the suggestion of its better control strategies.

MATERIAL AND METHODS

The study was performed between May and October 2009. Two hundred fecal samples from diarrheic calves in husbandries around of Tehran, Iran including: Varamin, Gharchak, and Shahriar districts were collected as cluster sampling. After transfer to laboratory and concentration of oocyst with Formol-ether method, all of slides were stained with modified Ziehl-Neelsen's acid fast method and were studied for presence of oocyst with photomicroscope. Moreover, for study on the role of potential risk factors of cryptosporidiosis in calves, some factors including: sex, age, diarrheic history, bed type, and lactating process were recorded. Finally, Chi square (χ^2) was used to detect significant differences between the various groups at 5% level of significance. SPSS 10.0 for Windows was used to do the statistical analyses.

RESULTS

The prevalence of infection to *Cryptosporidium* in calves of husbandries around of Tehran was 9% (Table 1). Statistical analysis did not show significant relationship between infection to protozoan and sex of studied animals ($p=0.928$).

Table 1: Frequency of infection to Cryptosporidium in diarrheic calves based on their sex

Sex	Infection to *Cryptosporidium* No. (%)	No Infection to *Cryptosporidium* No. (%)	Total No. (%)
Male	8 (4)	78 (39)	86 (43)
Female	10 (5)	104 (52)	114 (57)
Total	18 (9)	182 (91)	200 (100)

The prevalence of the infection according to age groups is shown in Table 2. Results showed that the maximum rate of infection is in 0-2 month old. Moreover, Results showed that prevalence of *Cryptosporidium* infection in calves according to age groups was statistically significant (p=0.000).

Table 2. Frequency of infection to Cryptosporidium in diarrheic calves based on their age

Age (months)	Infection to *Cryptosporidium* No. (%)	No Infection to *Cryptosporidium* No. (%)	Total No. (%)
0-1	6 (3)	54 (27)	60 (30)
1-2	6 (3)	26 (13)	32 (16)
2-3	2 (1)	32 (16)	34 (17)
3-4	2 (1)	24 (12)	26 (13)
4-6	2 (1)	46 (23)	48 (24)
Total	18 (9)	182 (91)	200 (100)

Furthermore, role of some potential risk factors including: bed type, Lactating process, and diarrheic history were studied (Table 3). Results showed that infection to *Cryptosporidium* in calves with dry feces cluster is more than wheat stubble type (p=0.000). Moreover, our findings showed that 7% of calves were infected to parasite and the infection in infant calves was more than non infant calves, significantly (p=0.000).On the other hand, study on the diarrheic history of calves showed that this factor can not be cause for infection to *Cryptosporidium* and there is no significant relationship between this factor and infection to protozoan (p=0.640).

Table 3: Frequency of infection to Cryptosporidium in diarrheic calves based on some potential risk factors

Some potential risk factors		Infection to *Cryptosporidium* No. (%)	No Infection to *Cryptosporidium* No. (%)	Total No. (%)
Diarrheic history	Diarrheic	2 (1)	20 (10)	22 (11)
	No diarrheic	16 (8)	162 (81)	178 (89)
Bed type	Wheat stubble	12 (6)	146 (73)	158 (79)
	Dry faeces	6 (3)	36 (18)	42 (21)
Lactating process	Non infant	4 (2)	104 (52)	108 (54)
	Infant	14 (7)	78 (39)	92 (46)
Total		18 (9)	182 (91)	200 (100)

DISCUSSION

In the present study, infection to *Cryptosporidium* was reported in 9% of calves in husbandries around of Tehran. Infection to this protozoan was reported from different areas of the world [3, 4, 8, 11, 13, 14 & 19]. The first report of parasite in Iran was in 1985 and was isolated from a native roster by histopathologic method [10] and then reported in many cases [7, 12, 15 & 22]. Evaluation of infection rate showed that 24% of healthy calves fewer than three months, 4% of calves above three months and 20% of diarrheic calves were infected [12]. Vahedi et al. (2009) in Amol region showed that 4.09% of calves fewer than three months and 3.92% of calves above 6 months were infected to *Cryptosporidium* [21]. The infection rate was reported 4.1% in Sanandaj (Kordestan province) by Yakhchali and Gholami (2008) that the maximum rate was in 1-4 months calves [22] and this finding is with agreement of our findings that the maximum rate of infection was reported in calves with fewer than 2 months old. In another study, infection was observed in 18% of calves less than one month in Shahre-kord [2]. Fotouhi Ardakani et al (2008) were reported cryptosporidiosis in 18.9% of studied cattle. Also in this study, age of studied animals was one of important factors in incidence of the infection and the maximum of the infection rate was observed in infant calves with less than 2 months age [7]. Therefore, it appears that age of calves is a potential risk factor for the infection and cryptosporidiosis was seen in animal with lower age. Of course there are other influential factors in this regard can be to manage livestock, particularly density of animals, malnutrition, poor sanity conditions and cluster type noted [22]. Importance of cluster type is for its role in direct transmission of the infection and contact to excreted feces by infected calves can be cause cryptosporidiosis in healthy animal, easily.

Our findings showed that the rate of infection in animal with dried feces bed is double over in bed animals that they had been using wheat stubble. One of the potential risk factors in rate of the infection was Lactating process of studied calves. Our findings showed that the infection to *Cryptosporidium* in the infant calves is more than non infant them, significantly and its cause is for direct contact between infant calves and their infected cattle. Although,

cryptosporidiosis in cattle feces is not a symptom for cryptosporidiosis, but in diarrheic calves, infection to protozoan makes the disease worse [21]. In another study, the rate of infection was determined 34% in studied lambs [15]. Therefore, relationship between presence of parasite and diarrhea in animals was determined. Azizi et al (2007) showed that the rate of diarrhea in infected calves is 2.3 times more than non infected calves [2]. Also in Kerman, the rate of infection to *Cryptosporidium* in diarrheic calves was 35.7% [7]. Of course, the most reported cases of cryptosporidiosis mainly focused on cattle, sheep and goats and is less about the other animals. But other animals like camel can be act as a reservoir and may transfer protozoan to healthy animals [16]. So according to the life circle of parasite and its direct transmission, infection can spread among animals in the outbreak area easily. In another study, results showed that infected calves and lambs excreted protozoan wit their feces for 14 days after infection [21].

CONCLUSIONS

So it is necessitate that presence of diarrhea resistant to antibiotic therapy can be existence of infection to *Cryptosporidium*. On the other hand, this protozoan can transfer to human and it is a zoonotic disease [15]. Thus, the importance of the above-mentioned studies about parasite is necessary and it is advised for better control of *Cryptosporidium*.

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A STUDY OF INFECTION RATE WITH STRONGYLES IN HORSES OF TEHRAN PROVINCE REGARDING TO AGE, SEX AND SEASON

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SUMMARY

For a 12 consecutive months (April, 2009 to April 2010) fecal samples were collected seasonally from 169 horses residing within 60 km of Tehran, Iran. Samples were taken directly from rectum and transferred to the laboratory in lidded plastic containers. Fecal samples were examined by floatation method. Diagnosis was based on the observation of strongyle eggs in microscopic examination of fecal samples. In this study 13 of 169 horses (7.69%) were infected with at least one of the strongyles. Infection rate was not significantly different among age categories and seasons ($p>0.05$). Data also showed no significant difference between the infection rate in mares and stallions ($p>0.05$).

INTRODUCTION

Horses are susceptible to infection with a variety of gastrointestinal nematode parasites [14]. Strongylosis is a common disease of horses and other equidae throughout the world and causes deaths when control measures are neglected. The redworms (strongyles) are nematodes generally found in the large intestine of equidae. They belong to two subfamilies: the Strongylinae (large stronglyes) and Cyathostominae (small strongyles) [12]. A schematic description of the life cycle of strongyles is presented in *Figure 1.*

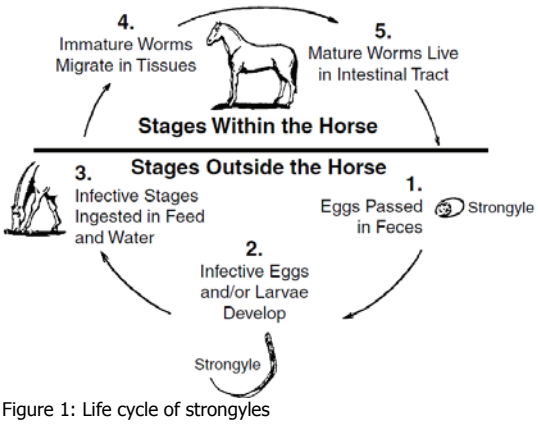

Figure 1: Life cycle of strongyles

Strongylosis in horses causes a wide spectrum of effects from in-apparent infection to sudden death [14]. Infected animals show anorexia, diarrhea and loss of weight, moderate anemia [13], poor performance [12] and lethargy [4]. Control is best achieved by a treatment and management regimen based on environmental conditions and by limiting the number of horses on the pasture [9]. Preventive anthelmintic strategies are designed to limit exposure to infective parasite stages [14].

Although numerous surveys on the prevalence and abundance of strongyles in equines were conducted in various areas of the world, including United States of America and several European countries [2], only a few detailed quantitative studies has been carried out in Iran.

This survey was carried out to investigate the infection rate with strongyles in horse-clubs located in Kordan area in Tehran province, during April, 2009 to April 2010. More than 20 horse clubs exist in this area. Kordan is located in west of Tehran province. It is mountainous and has moderate to cold climate. *Table 1* reveals data obtained from IRI Metrological Organization concerning this area.

Table 1. Data obtained from IRI Metrological Organization from April 2009 to April 2010.

	Spring	Summer	Autumn	Winter
Mean Minimum Temperature (˚c)	10	17	6	3
Mean Maximum Temperature (˚c)	22	31	16	12
Relative Humidity (%)	48	37	56	60
Amount of Precipitation (mm/day)	1.2	0.3	1.1	1.1

MATERIAL AND METHODS

Infection usually is diagnosed by microscopic examination of slides prepared by concentration and floatation of parasite eggs from feces [3]. Fresh fecal samples were collected from 169 horses, 72 females and 97 males, residing on seven horse-clubs, directly from rectum, put in lidded plastic containers and transferred to the laboratory. Fecal samples were examined by floatation method. Diagnosis was based on the observation of strongyle eggs in microscopic examination of fecal samples using the magnification of 40X *(Figure 2).* All equine strongyles produce similar thin-walled eggs, with 4-16 brownish cells [9].

Figure 2. Strongyle egg from fresh equine feces

Infection rates were calculated regarding to age and sex of animals, as well different seasons. Based on age, horses were assigned to three categories: up to 3, 3-10 and over 10 years old.
Results were analyzed using SPSS software.

RESULTS

In this study 13 of 169 horses (7.69%) were infected with at least one of the strongyles. 11.34% of stallions and 2.77% of mares were infected *(Figure 3)*.

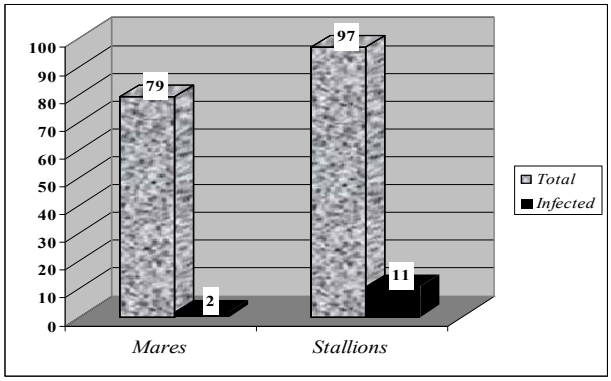

Figure 3. Infection rate with strongyles in mares and stallions in Kordan

Infection rate was 6.89%, 7.14% and 10.71%, respectively in up to 3 year-old, 3-10 year-old and over 10 year-old horses *(Figure 4).*

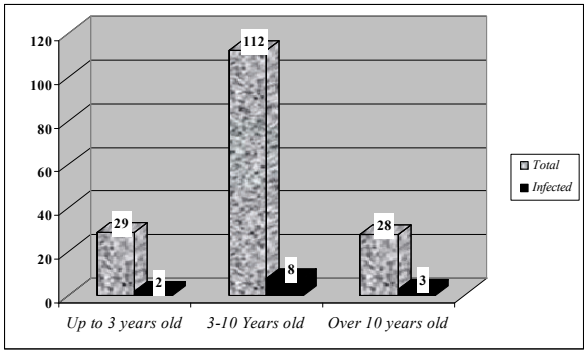

Figure 4. Infection rate with strongyles in different ages in Kordan

Prevalence of strongylosis was the highest in spring (4.24%) and the lowest in winter (0.84%) *(Figure 5)*.

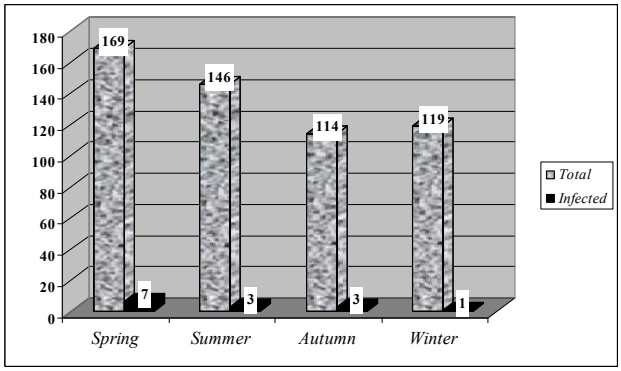

Figure 5. Infection rate with strongyles in different seasons in Kordan

DISCUSSION

The group of equine strongyle parasites is very diverse and consists of about 60 described species [6].Climatic variations, pasture and stable management [14], anthelmintic treatment [4] and nutritional status of horses are the major epidemiological and management features which have been recognized.

Regarding the sex of the host, parasitological differences are seldom reported in horses and seem to be associated to specific management (e.g. stable vs pasture) rather than to sex itself [10]. In the present study 7.69% of horses were infected with at least one of the strongyles. Data showed no significant difference between the infection rate in mares and stallions ($p>0.05$). Our results support the irrelevance of horse gender on strongyle infections.

Although disease may occur also in horses of any age [8, 15], foals are especially susceptible [7]. Adult animals are less susceptible to parasitic disease, suggesting some degree of acquired immunity to infection [14]. First year grazing foals are the age group at risk [15] and mares are the main source of infection for them as many adults carry appreciable strongyle burdens and pass large number of eggs [14]. In this survey difference of infection rate between different age groups was not statistically significant ($p>0.05$). Chapman et al. reported a reduction in large strongyles prevalence and intensity in older ponies [1], and it is not consistent with our results. However, as suggested by the same authors, differences may be due to their pre-selection of highly parasitized animals, while our horses were all sampled.

Most animals had a mixed (small and large) strongyle infection. Eggs which have passed onto pasture develop in 1 to 3 weeks into infective third-stage larvae [14]. As in many other parasitic conditions, the survival of eggs and larvae is favored by shade, moisture and moderate temperature [12]. Climate has a major influence on development and survival of free-living stages of strongyles, and so the size of the parasite refugium varies during different seasons of the year [11]. It is known that strongyle larvae can develop under the temperature from +8 to +38 ^{0}C and with a soil humidity of more than 30% [5]. In areas with cold winters and mild summers, egg deposition peaks in spring and remains high over summer [12]. Cold temperature may temporarily halt maturation of eggs and larvae, but development resumes when the temperature rises. Under optimum conditions larvae survive 2 years on pasture [14]. Stable-infection is also possible with contaminated hay or bedding [15]. There was no significantly difference among seasons ($p>0.05$) in this study. Analysis of the meteorological data *(Table1)*, demonstrated that climatic conditions of Kordan were favorable for the development and survival of strongyle infective larvae from April to January.

CONCLUSIONS

According to the results of this study, it seems that strongylosis may occur in different sex and age of horses, as well different seasons; so care must be taken to minimize the risk of infection. The control of internal parasites of the horses is based on sanitation, management and drug treatment. Stable manure should be composted or spread on cropland or other ungrazed lands. Feed should be placed in mangers. The water supply should be clean and fresh. Treatment for worms should be based on recommendation of veterinarian and periodical fecal examination must be done.

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THE PREVALENCE OF *DIROFILARIA IMMITIS* IN STRAY DOGS IN BURDUR REGION

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SUMMARY

This study was performed to investigate the prevalence of *Dirofilaria immitis* in stray dogs in Burdur region. A total of 142 at different ages, sexes and breeds were used as material in this study. Blood samples were examined with nativ, modified Knott's and antigen ELISA techniques. Of the total of 142 dogs, 31 were positive for *D. immitis* with a prevalance value of 22%.In addition 35.5% of positive dogs determine to have occult *D. immitis* infections.The highest prevalance of *D. immitis* infections were observed in ≥7 age group (%53,3), and this prevalance was followed by 4-6 (% 20,5) and 0.6-3 age group (% 17).The difference between ≥7 age group and other age groups (0.6-3 and 4-6 age groups) were found significant ($P<0.05$), whereas no statistically difference was observed between 0.6-3 and 4-6 age groups ($P>0.05$).The infection was more prevalant in males.Among the controlled regions, the maximum infection rate was found in Burdur centrum (% 37), followed by Tefenni (% 23,5) and Gölhisar (% 18,8). Our results suggest that heartworm treatment and prophylaxis should be considered in Burdur region.

Key Words: *Dirofilaria immitis*, Dog, Prevalance, Burdur,

INTRODUCTİON

Dirofilaria immitis is commonly found in the pulmonary arteries and right ventricle of dogs and other canids where they cause canine heartworm disease. It also occurs in cats and human beings (13, 25). The geographical distribution of heartworm infection is associated with availability of mosquitoes, the intermediate host. Mosquito population dynamics are influenced by environmental factors such as suitable components of still water and warm temperatures (21). Hypertrophy of heart, liver congestion, cirrhosis and ascites are commonly symptoms of heartworm infection in dogs (11, 14, 26).

In recent years, several epidemiological studies have been performed in different countries. The parasite is widely distributed in Africa, Asia, Australia, Latin America and Mediterranean countries (3, 6, 10, 12, 17, 24). Turkey is suitable country for development of this parasite due to climatic conditions and abundant intermediate hosts. Heartworm infections were reported different region of Turkey in previous studies (4, 5, 18, 19, 22, 27, 29-32). This study was performed to investigate the status of *D. immitis* infection by native, antigen detecting Elisa and Knott technique among stray dogs in Burdur region.

MATERİALS AND METHODS

Study area: The study was conducted in seven districts (Burdur city center, Bucak, Yesilova, Aglasun, Golhisar, Karamanli, Tefenni) of Burdur Province (Turkey), including Burdur city.

Experimental animals: Study was performed on total of 142 dogs (85 female and 57 male) from various villages in Kırıkkale from December 2008 to September 2009. The dogs examined were randomly selected.

Blood samples: A 10 ml of whole blood was drawn from the cephalic vein of each dog, half of the sample was stored with heparin and the other half was allowed to clot. Serum was harvested following centrifugation of clotted blood and was stored at -20 C^o until analysis. All samples were obtained during the day.

Microfilaria Examination: Blood samples were examined with native and modified Knott technique for determine microfilarias.

Antigen testing: DiroCHEK- Lab Pack Heartworm Antigen kits (Synbiotics Corp., 96-0230) for detection of sexually-mature female worms were used to examine serum samples. The test is based on an Elisa and was evaluated spectrophotometrically (Bio-Tek Instruments, ELX800), using a 630 nm filter (Reference wave length: 450 nm) according to manufacturer's instructions. Cutoff value was calculated by adding 0.020 to negative control's optic density.

Statistical analysis: Pearson's chi-square (x^2) test was performed to compare prevalence among sex, age and breed categories.

RESULTS

Thirtyone (22 %) of the 142 samples tested with antigen detecting ELISA kits showed a positive reaction for D. immitis in this study. Microfilariae were detect of 64.5 % (20/31), 54.8 % (17/31) dogs respectively with Knott technique, native. The regional distribution and prevalence values are presented in Table 1. Occult infection rate was seemed as 35.5 % of dogs examined (11/31).

Table 1: The Prevalence of *Dirofilaria immitis* in dogs in Burdur.

Region	No. of examined dogs	Infected dogs	
		No.	%
Burdur City Center	54	20	37
Bucak	12	1	8,3
Yeşilova	12	1	8,3
Ağlasun	16	-	-
Gölhisar	16	3	18,8
Karamanlı	15	2	13,3
Tefenni	17	4	23,5
Toplam	142	31	22

The seroprevalence rates in males and females in Burdur were 22.8 % and 21.2 %, respectively. There was no significant difference between these groups (P > 0.05).

Seroprevalence was the highest (53.3 %) in the ≥7 year-old age group in Burdur, followed by 20.5 % in the 4-6 year-old age group and 17 % in the 0.6-3 year-old age group. The difference between ≥7 year-old age group and the other groups is significant (p < 0.05) but the difference between 4-6 year-old group and 0.6-3 year-old group is not significant (p > 0.05).

Seroprevalence was similar between clean-bred and cross-bred groups. There was no significant difference between these groups (P > 0.05).

Sex, age and breed distributions of dogs with D. İmmitis infection are shown in Table 2.

Table 2: The Prevalence of *D. İmmitis* correlated with age, sex, breed

	Examined dogs	İnfected dogs	
Sex	No.	No.	%
Feamale	85	18	21,2
Male	57	13	22,8
Age			
0,6-3	88	15	17
4-6	39	8	20,5
≥ 7	15	8	53,3
Breed			
Clean-bred	47	9	19,1
Cross-bred	95	22	23,1

DİSCUSSİON

Thirtyone (22 %) of the 142 samples tested with antigen detecting ELISA kits showed a positive reaction for *D. immitis* in this study.

D. immitis has been reported by many researchers in dogs in Turkey. Different prevalence rates (0.2–46.2 %) reported in previous studies in Turkey should be related to these factors (1, 8, 15, 18, 20, 28).

Our studies' prevalance is higher then the other studies in Turkey. Because climate is critical factor in the prevalence of heartworm infection. Especially, the environmental temperature is important factor for *D.immitis* maturation to infective third-stage larvae (L3) in the mosquito (15). The population of mosquito species was increased from July to September in Turkey (2). Burdur which the climate allows the development of a large population of mosquitoes is localised in temperate region of Turkey. All dogs are stray dogs, for this reason, these dogs could be more contact with the intermediate mosquitoes.

ELISA test is commonly used for diagnosis of *D. İmmitis*, especially for occult infections (9, 18, 32), because of this reason in our study we preferred DiroCHEK- Lab Pack Heartworm Antigen kits.

The circulating microfilariae were not found in peripheral blood in some dogs with adult heartworm. This type of infection is known occult infection (7). Serological techniques are used to detect of occult infection in dog (15). In previous study, the occult infection was reported in dog in Turkey as 1.52 – 29.6 % (5, 19, 32). In present study, the occult infection rate was detected in dog as 35.5 % in Burdur.

Selbey et al. (23) found that male dogs had the highest relative risk for heartworm infection. They are more likely to be bitten by mosquitoes. In the present study, infection rate in male dogs is higher than female dogs altought statistically no significant differences in seroprevalence were observed between male and female dogs (P > 0.05).

Montoya *et al.* (17) were suggested that age of dog was important risk factor of heartworm infection. The infection was more prevalent in old dog than that of younger one because of long exposure period in endemic areas, (16, 18). In present study, the highest (53.3 %) seroprevalence was in the ≥7 year-old age group in Burdur, followed by 20.5 % in the 4-6 year-old age group and 17 % in the 0.6-3 year-old age group. The difference between ≥7 year-old age group and the other groups is significant ($p < 0.05$) but the difference between 4-6 year-old group and 0.6-3 year-old group is not significant ($p > 0.05$).

The breed of dog may be important for dirofilariosis. The prevalence of heartworm infection is usually higher in larger dog species than that of small ones (17, 31). In present study, clasification was not like this study, we only clasificate as clean-bred and cross-bred and there was no significant difference between breed groups (P > 0.05).

CONCLUSİON

D. immitis infection in dogs was determined in Burdur province. Patent and occult infection rate were detected as 22 % and 35.5 %, respectively. According to these results, heartworm treatment and/or prophylaxis are needed in this area.

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SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH NEOSPOROSIS IN SHEEP AND DOGS FROM FARMS

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SUMMARY

This study aimed to establish the seroprevalence and risk factors associated with neosporosis in sheep and dogs from rural properties. 1497 blood samples were collected from sheep and 42 from dogs that cohabited with sheep from 16 farms located in the central region of São Paulo State, Brazil. For the detection of N. caninum antibodies it was performed the indirect fluorescent antibody test (IFAT ≥ 25). For the epidemiological study it was applied a questionnaire for the owners or responsible from the sheep and dogs regarding informations related to neosporosis. The seroprevalence obtained out of the 1497 sheep sera tested was 8.0% (LB95%=6.7%; UB95%=9.2%) and out of the 42 dogs 4.8% (LB95%=0%; UB95%=7.2%). Variables statistically related to seropositivity for N. caninum in sheep were: artesian well as water supply (P=0.0004; OR=2. 15), presence of other domestic canids (P=0.0013; OR=2.38) and absence of reproductive problems (P=0.0031; OR=1.75).

INTRODUCTION

Neospora caninum is an obligate intracellular protozoan that causes neosporosis, a worldwide distribution disease, which affects a considerable range of species of domestic and wild animals [5]. It is considered an emerging disease associated with reproductive problems and neurological disorders, with a progressive character, manifesting itself more severely in young animals, and recognized as an important cause of abortion in cattle [2].

The sheep industry is in growing expansion in Brazil, especially in regions with no traditional culture in this type of economic activity. Thus, the production chain must be alert to general health problems, including the reproductive problems that has a direct effect on production and productivity of herds. Among the parasitic diseases that affects reproduction, neosporosis stands out for being responsible for abortions, embryonic death, fetal malformation and birth of weak or persistently infected lambs [1].

The indirect fluorescent antibody test (IFAT) was the first method described and is now widely used for diagnosis of infection in sheep and dogs, considered the gold standard test when compared to others [3].

The aim of this study was to evaluate risk factors for the occurrence of antibodies against N. caninum in naturally infected dogs and sheep from farms in the central region of São Paulo state, in the counties of Ibitinga, Itápolis, Borborema and Tabatinga.

MATERIALS AND METHODS

Out of a total of 4228 sheep from 16 farms, blood samples were taken from 1497 sheep and all the dogs that inhabit these properties, located in São Paulo state and belonging to the counties and number of properties: Ibitinga (5), Itápolis (5), Borborema

(3) and Tabatinga (3). The criteria for inclusion of the farms were: belonging to the central region of São Paulo state and having dogs cohabiting with sheep.

Serum samples were assayed for N. caninum antibodies by IFAT, considering 1:25 as the cut-off value, and titrated doubling dilutions from 1:25 to 1:800.

During the experiment an epidemiological questionnaire was applied in each property by interviewing the owner or person responsible for animal management.

The variables were analyzed by Chi-square and G-test with a significance level of 5%. The Bioestat 5.0 was used to calculate the associations were significant $P < 0.05$.

RESULTS

A total of 120/1497 (8.0%) sheep samples reacted to N. caninum, especially at titles 25 (19; 15.8%), 50 (35; 27.5%), 100 (29; 29.2%) and 200 (6; 24.5%), and a total of 2/42 (4.8%) dog samples were soropositives at titles 50 (2; 100%).

In this study, we found 8 (50%) farms with seropositive sheep. The sizes of 16 (100%) farms were quite heterogeneous, ranging from 15 to 200 alqueires. In 13 (81.25%) farms the main activity was mixed (agriculture and livestock), followed by 2 farms which had as main activity agriculture (12.5%) and 1 which had livestock (6.25%). All of them were dedicated mainly to sheep meat production and an extensive production system. The association between farm size and main activity with the results obtained in sheep, showed no statistically significant difference (P=0.4164 and P=0. 1614).
Two (12.5%) of 16 studied properties had seropositive dogs. Out of the eight farms with positive sheep, only two (25%) dogs were positive.

The water supply of the animals was an artesian well in 14 (87.5%) properties and dam in 2 (12.5%). Significant differences were observed regarding the results and the association between water supply (P=0.0004, OR=2.15).
All properties have reported the presence of other dogs, 2 (12.5%) reported the presence of wild canids and 14 (87.5%) of dogs from neighboring properties. The association between the presence of other domestic or wild dogs with the results showed statistical difference (P=0.0013, OR=2.38).

In all of the 16 farms studied the farming of domestic poultry was reported (Gallus domesticus), and in 15 (93.75%) they were in free-ranging system and in one (6.25%) in intensive system. The association between the type farming system of the poultry and seropositivity of the sheep showed no statistical difference (P=0.8665).

In 14 (87.5%) properties rodents were found (Rattus norvegicus, Rattus rattus or Mus musculus), however, there was no association between the presence of these and the serology results (P=0.88 19) (Table 4).

All the properties reported slaughtering sheep, poultry and pigs. Dogs have access to the raw meat and offal of slaughtered animals in 10 (62.5%) properties. The association between the slaughter of animals on the property and access of dogs to raw meat and offal from these animals showed no significant difference (P=0.3989 and P=0.8984).

Reproductive problems occurred in 14 (87.5%) properties, more frequently reported: abortion (47.7%), repeat breeding (40.7%) and stillbirths (11.6%). The abortions occurred on the first third of gestation in 30.4% of cases, 26.0% on the final third and 2 1.7% on the second third of gestation. Not all properties had a complete diagnosis of the reproductive failures. Moreover, there was an association of seropositivity with the presence or absence of reproductive problems in sheep (P=0.0031, OR=0.25).

DISCUSSION

In Brazil, serologic studies of infection by N. caninum in sheep and dogs have shown variable rates of seropositivity. It is noteworthy that the comparison between studies using different serological tests with different cutoffs should be used with caution, since there are differences in sensitivity and specificity between the tests used [5].

Figliuolo et al. [8], in 30 properties in the state of São Paulo, found that 86.6% sheep were positive by IFAT and Munhóz [13] in Paraná state, found in 9 (8 1.82%) and 11 farms sheep seropositive. The comparison with results from other studies is limited because the properties differ in number of animals, size, management and technical standards.

All properties were adopting extensive production system, however, it is believed that the occurrence of disease is lower in properties with semi-intensive or intensive system due to better sanitation, less crowding of animals, separation from flock by age, sex and purpose of production, and more qualified manpower [4].

This study suggests vertical transmission of the parasite to sheep [6], although stray dogs of nearby properties and even from urban area were found among the flocks. The dogs presented antibody but no cinical signs. Other problem is to more exposure of the dams to dog feces, definitive hosts in neosporosis [10].

In Brazil, few serological studies were performed in wild canids. Among the Brazilian species tested for N. caninum, positive results were found in Chrysocyon brachyurus, Lycalopex gymnocercus and Cerdocyon thous [9]. The presence of wild dogs on farms has increased significantly, making it difficult to control and enhances the transmission of the disease in flock, especially when the access to food and water sources of the animals is possible.

Until 2007, only mammals had been identified as natural hosts of N. caninum, but the parasite was also reported in chickens, which gives an even broader distribution of the agent, a fact of great epidemiological importance [7].
Natural infection by N. caninum in mice and rats, cosmopolitan considered synanthropic animals, was reported [11,12]. It is unknown the efficiency of transmission of the parasite from infected rodents to other natural species, but the carnivorism does contributes to the spread of the parasite.

The risk factors could be determined because not all herds were positive for the presence of N. caninum. The questionnaire revealed occurrence of reproductive

wastage in some herds. Thus, further investigations are needed in order to know the causative agents and the actual role played by neosporosis on reproductive and economic losses of ovine breeding in that region.

CONCLUSION

The results indicate seroprevalence of antibodies against N. caninum in 50% (8/16) of the flocks, 8% (120/1497) of sheep, and 4.8% (2/42) of dogs of the farms. The intake of water from dams, the presence of wild or domestic canids, and the presence of reproductive problems demonstrated positive association with the occurrence of infection by N. caninum in sheep.

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PREVALENCE OF *BABESIA* INFECTION ON RURAL AND URBAN DOG IN SOUHGTWEST IRAN (AHVAZ)

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SUMMARY

Canine Babesiosis is an important worldwide, tick-born disease caused by apicomplexan hemoparasitic from genus *Babesia*. The aim of the present survey was to identify the current state of *Babesia* infection in rural and urban dogs from soughtwest Iran (Ahvaz). For this reason within 2 years, 200 rural dogs from 5 village around Ahvaz and 200 urban dogs referred to the veterinary hospital of Shahid Chamran Uneversity were examined for presence of *Babesia* spp. After recording the sex and the age of dogs, blood samples were taken from cephalic vein. Then peripheral thin blood smears prepared and stained with gimsa for parasitological examination. The result revealed that among 400 dogs, 15 dogs (3.75%) were infected with *Babesia canis.* This study was showed from 200 rural dogs, 11 dogs (5.5%) and from 200 urban dogs, 4 dogs (2%) were infected with *B. canis.* In this survey there was no significant relationship between sex, age and season in urban dogs regard to infection with *Babesia canis*. Significant relationship between season and infected rural dogs in Ahvaz was revealed (P<0/05). The infectivity rate to this parasite was low, but transmission of the protozoan to dogs should be intentioned.

INTRODUCTION

Babesiosis is a tick borne blood protozoon disease of domestic and wild animals, occurs in the southern USA, Central and South America, Africa, Asia and Southern Europe. Out of 100 species, three *Babesia* species are known to cause natural infection in dogs, i.e, *Babesia canis, Babesia gibsoni and Babesia vogeli* [2]*. Babesia canis* are considered to be the most important species affecting the dogs. [6]. Few epidemiological studies addressing the prevalence of babesiosis have been conducted in large urban centres in Iran. *Babesia canis* found as a trophosoites in erythrocytes, multiply by binary fission to produce pairs of piriform bodies. This parasite cannot survive outside the dog or tick vector. Dogs become infected during parasitemia, which lasts few days after infection. Clinically recovered dogs may have periodic parasitemia. A tick vector *Rhipicephalus sanguineous* (Brown Tick). *Haemaphysallus leach, Hyalomma plumbeum, Demacentor andersoni* and *D marginata* can also transmit these diseases [6].

The objective of the present study was to find out the percentage of Babesiosis in Ahvaz, Iran. This may help to present true picture of this disease in Iran.

MATERIAL AND METHODS

Study area

The study was conducted in the Ahvaz (Sought west of Iran; 31/24 N, 48/49W; altitude 18 m), which is located in an area known as the Khouzestan plain.

Collection of blood samples

In order to determine the prevalence of canine babesiosis within the dog population of the area, blood samples were collected in 2009 from 400 dogs (200 rural and 200 urban) of both sexes and of various breeds. The number of samples collected was based on the expected prevalence of infection (5%). The animals were selected through a systematic sampling procedure based on a list of the existing dogs in the municipality.

About 1ml of blood was collected from cephalic or jugular vein of each dog with the help of sterile disposable syringe and needle.

Additional data including the name, sex, age, breed and phenotypic description of the dog,
and the residential address, were recorded .

Diagnosis

The blood smear were stained with Diff. Quik and Giemsa stain.The stained smears were examined under the microscope for the demonstration of *Babesia*. [3].

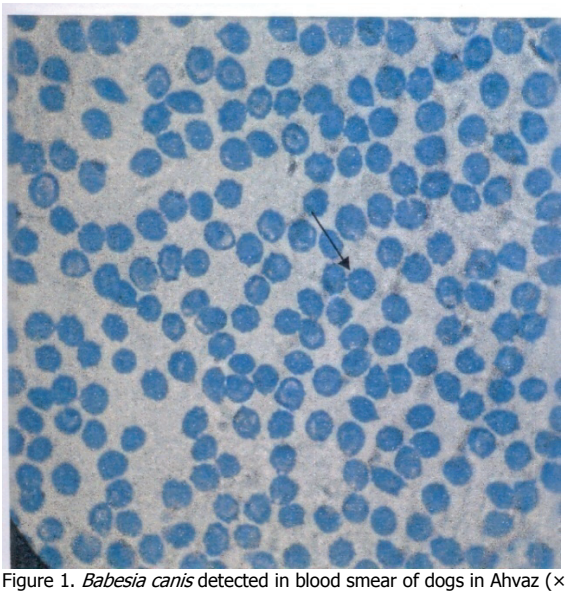

Figure 1. *Babesia canis* detected in blood smear of dogs in Ahvaz (×1000).

RESULTS

As shown in Table 1, the overall prevalence of *Babesia* spp. in dogs was 3.75% (15 dogs) among 400 dogs by Giemsa staining.

Table 1: The number of dogs infectet in Babesia spp. In Ahvaz

origin	Numbers examined	Numbers infected	Infection Rate (%)
Rural	200	11	5.5
Urban	200	4	2
Total	400	15	3.75

Sexual distribution of canine babesiosis in Ahvaz presented in the Table 2 revealed that 10 of 250 male dogs and 5 of 150 female dogs were positive.

Table 2: The number of dogs infected with *Babesia spp*. by sex.

Sex	No. of positive	Total
Male	10	250
Female	5	150
Total	15	400

DISCUSSION

Babesia spp. are protozoa; organism that parasitize erythrocytes, causing anemia in the host. Many different species exist with varying host specificity [4]. *B. canis* and *B. gibsoni* are two organisms commonly known to infect dogs. Both organisms have Ixodid tick vectors and are found throughout Asia, Africa, Europe, Middle East and North America, with *B. canis* being more prevalent [6]. However, *Rhipicephalus sanguines* and *Dermacentor variabilis* are believed to be potential vectors of disease [2]. The data collected showed that Babesiosis is a common disease with low prevalence of dog in the Ahvaz region.

Brown dog ticks, when submitted to unfavorable conditions (low temperature, low humidity and absence of hosts) reduce their metabolic rate in a phenomenon known as diapauses [1]. Such changes reduce oviposition, increase incubation time of the eggs and can even inhibit the questing behavior typical of the host-seeking process [5]

CONCLUSIONS

This study suggests that the epidemiology of infection by this protozoon in the Ahvaz differs from that found in the other cities of Iran. It may be that transmission of babesiosis within different regions of Iran is related to the variants of the ixodid tick found in those areas The infectivity rate to this parasite was low, but transmission of the protozoan to dogs should be intentioned..

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DETECTION OF CRYPTOSPORIDIUM-SPECIFIC ANTIBODY IN COLOSTRUM OF CATTLE

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SUMMARY

Cryptosporidium parvum is a coccidian parasite that causes enteric disease in humans and animals. Determination of specific antibodies in colostrum and sera of dam could be helpful for the management of cryptosporidiosis. The aim of this study was to detect antibodies against *C. parvum* in colostrum of dam.The results showed that 17 samples out of 63 colostrum samples were positive in dot blot. In 7 cases, the antibody against p23 could be detected in serum from cow, its colostrum and serum from corresponding calf. P23 can be used as a suitable target for detection of antibody against Cryptosporidium in sera and colostrum.

INTRODUCTION

Cryptosporidium parvum is a widely distributed coccidian parasite and causes enteric disease in humans and animals[1]. It is known that new born and young calves are more susceptible than adults[10]. The animals are mostly infected with *C. parvum* between day 4 and the third week of age[3]. Since the immunoglobulins cannot be transmitted through placenta, transmission of *C. parvum*-specific antibodies through the colostrum is very important to protect new borne[8,11]. Determination of specific antibodies in colostrum and sera of dam could be helpful for the management of cryptosporidiosis.

For the identification of the *C. parvum*-specific antibodies in sera and colostrum, many suitable antigens such as CP15/P60 and P23 were applied [5, 2, 9,7]. Perryman et al. (1999) introduced the recombinant P23 as a suitable antigen for vaccination in dam resulting desirable protective immunity through colostrum in calves.

P23 was previously used successfully for detection of *C. parvum* specific antibody in sera of dam in our laboratory[7]. In the present study the application of the recombinant protein was tested for detection of presence of specific antibody in colostrum.

MATERIAL AND METHODS

Samples of colostrum were collected from 63 cattle holding in 2 farms near Tehran. After collection, the samples were transported on ice to the laboratory. Subsequently samples centrifuged at 1800 X g for 10 min at 4ºC in a refrigerated centrifuge. After centrifugation three layers was observed. The superior layer contained the lipid, the intermediate layer was liquid and the bottom layer was a pellet. The lipid layer was removed with Pasteur's pipette and the liquid phase was transferred into 1.5 mL microtubetubes and stored at –20ºC until the use.

The P23 protein gene as a suitable target for antibody against *cryptosporidium parvum* was cloned in pGEX-5X-2 and subsequently the recombinant P23 was expressed in *E. coli BL21* and extracted using Microspin GST purification Module kit and FactorXa (Amersham, USA) according to the manufacturer's instruction.

In our previous works recombinant P23 was separated in 12% SDS-PAGE, then protein bands were either stained with Coomassie brilliant blue solution or transferred to the nitrocellulose membrane and determined the immunogenic bands. To determine the immunogenic bands, free binding sites on the membrane were first blocked with 3% bovine serum albumin in TBS buffer (20 mM Tris base and 0.15 M NaCl in H2O) containing 0.05% Tween 20 for 1 h at 37°C. Subsequently, the membrane was incubated in a corresponding diluted positive serum (dilution of positive serum in TBS containing 0.05% Tween 20:1, 1:10, 1:100, 1:200, 1:500, 1:1,000, and 1:10,000) for 1 h at room temperature (RT). The membrane was then washed three times with TBS containing 0.05% Tween 20 for 5 min at RT. Horseradish-conjugated rabbit anti-bovine Ig (Dako, Denmark) (1:1,000) were added to the washed membrane and incubated for 1 h at RT. After incubation, the membrane was washed three times as described above. The positive reaction was developed using DAB (Sigma, USA) as substrate under visual observation within 5 min. In all experiments, serum from newborn calf
before first feeding with colostrum was used as negative serum control. Optimized serum dilution (1:200) was then selected for the test sera.

Purified P23 was used for detection of antibody against Cryptosporidium in colostrum collected from cows by dot blot analysis. For Dot-Blot analysis, one microliter of recombinant P23 (0.15 µg/1 µl PBS) was dotted on one corner of a 1×1 cm square nitrocellulose membrane. As negative control, purified Newcastle virus antigen (provided by the Department of Virology, Faculty of

Veterinary Medicine, University of Tehran) was used. Dot blot membrane was first blocked and subsequently incubated with diluted colostrum (1:200).The analysis was continued as described above. As positive control, positive serum supplemented colostrum and as negative control serum from new born calf before the first feeding was used.

RESULTS

We have previously shown that P23 can be used for detection of antibody against Cryptosporidium in serum of infected cattle. P23 was also used for comparative detection of antibody against Cryptosporidium in serum of infected cows and their new born calves. In the present study we used P23 for detection of antibody against Cryptosporidium in colostrum. The results showed that 17 samples out of 63 colostrum samples (27%) were positive in dot blot (Fig1). In 10 cases, sera from cows, their colostrum and sera from corresponding calves were available. In 7 cases, the antibody against p23 could be detected in serum from cow, its colostrum and serum from corresponding calf. In 3 cases no antibody could be detected in serum from cow, its colostrum and in serum from corresponding calf.

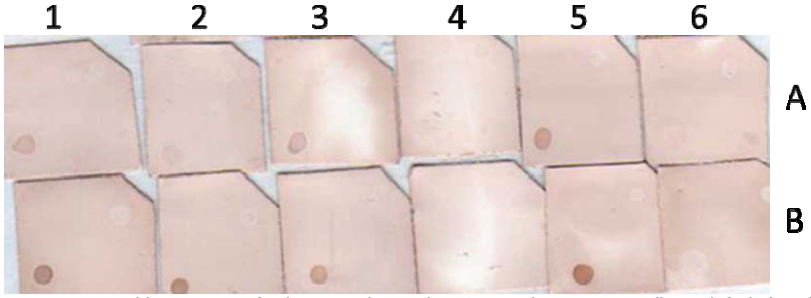

Figure 1: Dot blot reaction of colostrum whey with p23 recombinant protein (lower left dot) and antigen from Newcastle virus (upper right dot) as negative control. Column 5/rows B, and column 6/rows B showed the positive and negative respectively.

DISCUSSION

C. parvum is a coccidian parasite that infects entrocytes young calves. It is known that immunized cows can protect calves through colostrum[1,2].

Western blot analysis showed that p23 could be detected in lysate prepared from P23 recombinant pGEX-5X-2 transfected *E. coli BL2* as fusion protein using *C. parvum* positive serum. The purified p23 could also be detected with the same serum.

It can be concluded that this protein is an important antigen which stimulates host immune system resulting in the specific antibody production under natural infection[7]. Therefore, P23 was applied for the antibody screening of colostrum whey in dot blot.

Our results showed that the antibody against p23 could not be detected in 73% colostrum samples. Interestingley, in accordance with the positive colostrum results, antibody against p23 could also be detected in corresponding serum.

The use of milk for surveillance of different diseases in cattle has become routine in recent years and milk antibody testing now plays a significant role in cattle disease control [4,6], but milk sampling is easier, cheaper and non-invasive compared to blood sampling.

Since the majority of the tested colostrum was p23 negative, a high risk of cryptosporidial infection could be estimated in new born calves. The successful animal health management requires knowledge of epidemiology and suitable equipment employed for rapid and simple diagnosis.

In the present study, we confirmed easy and rapid diagnostic tool for the analysis of cryptosporidial infection, which may help to investigate the epidemiological status of herds. Since it is not clear that the seroreactivity of naturally infected cows against P23 and protection are in fact correlated, it remains to be investigated whether seropositivity, clinical, and parasitological status of animals are interrelated.

CONCLUSIONS

P23 can be used as a suitable target for detection of antibody against Cryptosporidium in sera and colostrum.

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IN VITRO EFFECTS OF PENTOXIFYLLINE ON KINEMATIC PARAMETERS OF RAM EPIDIDYMAL SPERM

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SUMMARY

To evaluate effects of different concentrations of pentoxifylline, as phosphodiesterase inhibitor, on kinematic parameters, spermatozoa were separated from ram caudal epididymis. Epididymal sperm were incubated at concentrations of 0.01 mM, 0.1 mM, 1 mM, and 10 mM pentoxifylline for 60 min. Motion parameters were assessed using the CASA system.

Pentoxifylline at 0.01 mM appeared significant ($P<0.05$) in increasing progressive motility, straight line velocity (VSL), VCL, average path velocity (VAP), average amplitude of lateral head displacement in micrometers (ALH), and mean angular displacement (MAD) and significant ($P<0.05$) in decreasing BCF in spermatozoa compared with controls. Pentoxifylline at 10 mM caused a significant ($P<0.05$) decrease in progressive motility, VSL, VAP, ALH, LIN, and WOB compared with controls. In conclusion, a low concentration of pentoxifylline was able to increase most kinematic parameters while high concentration had the opposite effect.

INTRODUCTION

In vitro fertilization (IVF) of oocytes and pregnancy rates are critically dependent on sperm motility which can be enhanced by the addition of sperm motility stimulants such as pentoxifylline (PF) and progesterone. There is increasing interest in the use of such stimulants in assisted reproductive technology for the treatment of malerelated infertility [17]. PF, a methylxanthine derivative, has been shown to enhance motility of human spermatozoa in vivo [14] and in vitro [10]. Improved IVF rates have been reported using PF-treated spermatozoa from infertile males [12]. Additionally, this drug successfully been used to increase fertilization rates in bovine in vitro fertilization [13] and as pretreatment to stimulate epididymal and testicular sperm motility for ICSI [16]. PF inhibits cyclic adenosine monophosphate (cAMP) phosphodiesterase, thus increasing intracellular cAMP concentration and tyrosine phosphorylation at the tail level [3]. cAMP has a role in sperm kinematics and in the acrosome reaction second-messenger system. Treatments that increase intracellular cAMP concentrations often cause an increase in sperm motility and kinematics as well as in the agonist-induced acrosome reaction and fertilization rates. In assisted reproductive technology, the advantageous effect of PF in improving sperm motility and fertilization capacity in asthenozoospermia has been confirmed [8]. However, the effect of pentoxifylline on the motility of ram epididymal spermatozoa has not been reported previously. Therefore, the specific aim of this study was to evaluate the ability of different concentrations of pentoxifylline to influence motion parameters of ram epididymal spermatozoa.

MATERIAL AND METHODS

Preparation of cauda epididymal spermatozoa from ram

Ten cauda epididymas from healthy adult ram were retrieved in the abattoir and transported to the laboratory on ice in less than 2 h. The cauda epididiymal sperm was obtained as previously described by Blash et al. [2] and transferred to 35 mm petri dishes containing 2 ml of equilibrated HEPES-TLP-PVA (114 mM NaCl, 3.1 mM KCl, 0.3 mM NaH2PO4, 2.1 mM CaCl2, 0.4 mM MgCl2, 2 mM NaHCO3, 0.2 mM sodium pyruvate, 10 mM sodium lactate, 10 mM HEPES, 1 mg/ml polyvinyl alcohol, and 0.7 mg/l Pen/Strep). All dishes containing samples were incubated at 38°C with a humidified atmosphere of 5% CO2 in air. All samples were analyzed by computer-assisted sperm analysis (CASA). Only specimens with progressive motility >60% were used in the experiments. Preparation of sperm suspensions. Semen samples were obtained from cauda epididymidas, and aliquots of sperm suspension in HEPES-TLP-PVA medium at 100-µl each, containing 5× 107 cells were incubated for 60 min at concentrations of 0.01 mM, 0.1 mM, 1 mM, and 10 mM pentoxifylline (Sigma, UK, L-6638) and an equal volume of HEPES-TLP-PVA medium (control).

Sperm motility analysis

Sperm motility parameters were analyzed by CASA (Computer Assisted Sperm Analysis, WLJY-900, China) with the following settings: image collection speed: 20 frames per second; analysis time per frame, less than 15 s; sperm velocity that can be analyzed, 0–180 μm/s; number of vision fields that were selected, 6 per sample; magnifying power of microscope (object lens), ×10; measurements were performed in Makler chambers 20-μm depth. Sperm motility parameters were analyzed at a time of 60 min following incubation with different concentrations of PF. The studied motion parameters can be defined as follows: progressive motility which is rapid and slow movements of sperm ahead in percentage; straight line velocity (VSL) which represents the average velocity measured in a straight line from the beginning to the end of one track in micrometers per second; the curvilinear velocity (VCL), which is the average velocity measured over the actual point-to-point track followed by the cell in a micrometers per second; the average path velocity (VAP), which corresponds to the average velocity of smoothed cell's pathway in micrometers per second; the average amplitude of lateral head displacement in micrometers (ALH); the beat cross frequency (BCF), the frequency at which the sperm cell's head crosses the sperm cell's average pathway in Hertz; the linearity (LIN) which estimates linearity of a curvilinear path in percentage; the straightness (STR) which estimates the proximity of the cell's pathway to a straight line with 100% corresponding to the optimal straightness in percentage; the wobble (WOB), which is the measure of oscillation of the actual path about the average path; and the MAD which is the time average of absolute values of the instantaneous turning angle of the sperm head along its curvilinear trajectory in degree [1].

RESULTS

Exposure of spermatozoa to concentration of 0.01 mM PF appeared significant ($P<0.05$) and showed an increase in progressive motility, VSL, VCL, VAP, ALH and MAD and significant ($P<0.05$) decrease in BCF after 60 min incubation compared with controls, whereas concentrations of 0.1, 1 mM PF had an insignificant effect on motion parameters. Concentration of 10 mM PF resulted in decreased progressive motility, VSL, VAP, ALH, LIN, and WOB significantly ($P<0.05$) after 1 h compared with controls (Table 1).

DISCUSSION

An alternative and/or complementary approach to sperm preparation for assisted reproductive technology is to treat the spermatozoa in vitro in order to improve their functionality, i.e., motility, or to supply a protective environment to maintain or improve their functional capacity for successful fertilization. Many substances including serum, peritoneal fluid, and follicular fluid or other chemically defined pharmacological substances like progesterone, adenosine analogs, or methylxanthines have been proposed to stimulate human sperm functions [8]. The beneficial effect of pentoxifylline on sperm motility and motion characteristics like sperm velocity or hyperactivity has been described for both fresh [9] and cryopreserved spermatozoa [7; 15]. The results on the stimulation of sperm motility are conflicting. Many studies have found no effect in normozoospermic patients while others observed a significant increase in motility and the number of progressively motile spermatozoa in patients with asthenozoospermia [8]. The present study has shown that pentoxifylline at a low concentration (i.e., 0.01 mM) had an increasing effect on progressive motility and other kinematic parameters while, at higher concentrations, either no change (0.1 and 1 mM PF) or a decrease was observed (10 mM PF) in the motion parameters. It is probable that a high dose of PF has an adverse effect on sperm motility. Centola et al. [4] also confirmed these negative effects on human sperm motility. Maxwell et al. [11] showed that pentoxifylline can increase ram sperm motility after thawing. Considering that different families of this enzyme have been described, of which six PDE's are present in spermatozoa and stimulate different sperm functions, i.e., acrosome reaction and motility, respectively [6; 5], an unspecific inhibition of PDE's will obviously result in both stimulation of motility and acrosome reaction. Taken together, the function of pentoxifylline apparently is dependant upon the conditions, the time of stimulation, and, most importantly, on the concentration of pentoxifylline in the medium.

CONCLUSIONS

In conclusion, a low concentration of pentoxifylline is able to increase most kinematic parameters while a high concentration caused a decrease.

Table 1: Effects of pentoxifylline on sperm kinematic parameters after 1-h incubation

Parameters	Control	Pentoxifylline (mM)			
		0.01	0.1	1	10
Progressive motility (%)	51.1±3.21	62.5±3.19[a]	53.4±4.69	46.8±5.30	37.8±3.77[a]
Curvilinear velocity (VCL; µm/s)	66.2±2.06	77.3±2.29[a]	69.2±3.67	61.9±4.02	55.4±3.93
Straight line velocity(VSL; µm/s)	41.7±1.42	49.3±1.31[a]	41.6±2.94	34.8±3.59	26.0±2.33[a]
Average path velocity (VAP; µm/s)	48.5±1.28	56.1±1.32[a]	48.72±2.81	42.3±3.36	34.0±2.18[a]
Lateral head displacement (ALH; µm)	1.58±0.06	1.77±0.07[a]	1.65±0.07	1.47±0.11	1.29±0.09[a]
Linearity (LIN; %)	55.6±1.16	58.2±0.97	53.9±1.95	49.9±2.80	43.7±1.95[a]
Straightness (STR; %)	75.5±1.31	79.5±0.65	75.4±2.16	71.1±2.98	67.4±2.90
Mean angular displacement (MAD; º)	40.8±2.87	48.8±2.47[a]	45.4±2.42	42.6±2.10	46.1±3.61
Beat cross frequency (BCF; Hz)	4.79±0.07	4.51±0.10[a]	4.76±0.16	4.93±0.14	5.05±0.10
Wobble (WOB; %)	72.1±1.20	70.3±0.92	69.4±1.07	68.3±0.85	64.8±1.80[a]
Number of cases	10	10	10	10	10

[a] Significantly different vs. corresponding control (P<0.05)

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INVESTIGATION OF SOIL CONTAMINATION WITH ASCARIDOID NEMATODES IN PUBLIC PARKS OF TURKEY (Abstract)

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INTRODUCTION

Although most gastrointestinal parasites are found worldwide, they are more prevalent in tropical and subtropical regions. Some of these parasites live in the soil during their development and for protection until they infect their next host. The main source of soil contamination with helminthes is infected dog and cat feces. These animals can be reservoirs for gastrointestinal parasites that occasionally cause infection in humans. *Toxocara canis*, *Toxocara cati* and *Toxascaris leonina* are ascaridoid nematodes of dogs and cats, causing significant health problems in these animals. While the infection of dogs with *T. canis* is common worldwide, the larvae of *T. canis* are also capable of infecting humans, causing ocular larva migrans, visceral larva migrans, eosinophilic meningoencephalitis and/or covert toxocariasis. The infection of cats with *T. cati* is also relatively common and *T. cati* is also zoonotic. Larvae of *Ta. leonina* can invade the tissues of laboratory animals and has the potential to cause human disease. Analyses of fecal samples found in public places can predict levels of soil contamination. This study was designed to determine the contamination status of children playgrounds with ascaridoid nematodes of dogs and cats and was carried out on totally 248 sand samples collected from 20 picnic areas and public parks which are all in the Kayseri province located in Central Anatolian part of Turkey.

MATERIALS AND METHODS

250-300 gr sand samples were technically collected from each park between August-October 2009 and each sampling area was inspected for the presence of dog and/or cat faeces. Sand samples were examined by modified Kazacos technique. Zinc sulphate flotation technique was used to investigate helminth eggs in faecal samples. Genomic DNA's were extracted from ascarid eggs recovered from slides after determination by modified Kazacos technique. PCR was performed on extracted genomic DNA's with primer pairs specific to *Toxocara canis* (YY1, 5'-CGGTGAGCTATGCTGGTGTG-3'; NC2, 5'-TTAGTTTCTTTTCCTCCGCT-3'; amplification of partial ITS2 gen region), *Toxocara cati* (JW4, 5'-ACTGTCGAGGATGAGCGTGA-3'; NC2; amplification of partial ITS1 gen region) and *Toxascaris leonina* (YY2, 5'-ATATCGGAAAAGGACGCACA-3'; NC2; amplification of partial ITS2 gen region). The amplification products were analyzed by electrophoresis in 1.5% agarose gel, stained with ethidium bromide and visualized in CLP Gel Documentation System (UVP INC Uplant, CA).

RESULTS

According to parasitological results, 10 (50.0%) of 20 parks and 33 (13.3%) of 248 sand samples were contaminated with ascaridoid and some other helmint eggs. *Toxocara* sp. was found as the most prevalent species with the ratio of 7.3% and this was followed by *T. leonina* (4.0%), *Spirocerca lupi* and *Taenia* sp. (0.8%) and *Ancylostoma caninum* (%0.4). Molecular analysis results revealed that *T. canis* was the most prevalent species with the ratio of 12.0% in the ascarid contaminated sand samples, and this was followed by *T. leonina* (7.5%) and *T. cati* (3.0%). The differences among the prevalence rates of species were found statistically significant ($p<0.001$).

CONCLUSIONS

The prevalence of zoonotic ascaridoid nematodes was designated in the playgrounds of public parks in Central Anatolian Part of Turkey and obtained data present strong need for control programs which should be implemented for prevention of zoonotic ascarid infections.

A SURVEY OF SHEEP LIVER FLUKES IN SARI INDUSTRIAL SLAUGHTER HOUSE, MAZANDARAN PROVINCE, IRAN

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SUMMARY

In period of September 2008 to September 2009, 33394 sheep were slaughtered in Sari industrial abattoir in the north region of Iran. In the present survey we determined the prevalence of sheep liver flukes (*Fasciola spp.* & *Dicrocoelium spp.*). Information was reported seasonal and the survey conducted from September 2008 to September 2009. Data analyze showedthat 12.2% and 6.95% of sheep livers were infected with *Fasciola spp.* and *Dicrocoelium spp.*, respectively. Total condemnation of liver about *Fasciola spp.* infection was 5% and for *Dicrocoelium spp* was 4.8%. The highest level of infection for fasciolosis was shown in spring (13.8%) and for dicrocoeliosis in summer (9.2%). Two reason of increasing rate of infection during season are humidity rate of seasons and snail abundance for *Dicrocoelium spp.* and *Fasciola spp.* infection respectively. Choosing the best anthelmintic drug strategy and sever inspection in abattoirs can prevent sheep fluke infection and consequently guarantees human health and prevents economic losses.

Key words: *sheep liver flukes, fasciola spp., dicrocoelium spp.*, *sari industrial slaughter house, Iran.*

INTRODUCTION

Liver flukes are parasitic trematodes that cause fasciolosis and dicrocoeliosis in sheep and other species of ruminants. These diseases are important in many commercial aspects and leading to economic losses because of liver condemnations. Fasciolosis caused by trematodes *Fasciola spp.* And Dicrocoeliosis caused by *Dicrocoelium spp*.[7]There are many surveys about the prevalence of these diseases in sheep , in the world, but the there are a few surveys about sheep liver flukes prevalence in processing plants of Asia, as well as in Iran. Some of these limited surveys in Asia conducted in Pakistan[5], Saudi Arabia[1,2],Turkey[4]and in Iran[1,8]. In the present survey we determined the prevalence of sheep liver flukes in Sari industrial slaughter house in the north region of Iran.

MATERIAL AND METHODS

Each liver from slaughtered sheep was examined in the course of process and liver condemnations due to flukes infection recorded daily on data sheet. Diagnosis of liver flukes was based on macroscopic observations by inspectors.Information was reported seasonal and the survey conducted from September 2008 to September 2009. The collected information included total number of sheep slaughtered, season of slaughter, number of infected and condemnated livers. All collected data analyzed using SPSS ver.12.

RESULTS

In period of September 2008 to September 2009, 33394 sheep were slaughtered in Sari industrial abattoir. Data analyze showed(table1&2) that 12.2% and 6.95% of sheep livers were infected with *Fasciola spp.* and *Dicrocoelium spp.*, respectively. Total condemnation of liver about *Fasciola spp.* infection was 5% and for *Dicrocoelium spp* was 4.8%. The highest level of infection for fasciolosis was shown in spring (13.8%) and for dicrocoeliosis in summer (9.2%).

Table 1: The liver infection and condemnation for Fasciol spp. from September 2008 to September 2009 in sari industrial slaughter house , Mazandaran province, IRAN.

Season	Number of liver examined	Number of liver infection	percentage of liver infection (Mean:12.2)	Total of liver condemnation	percentage of liver condemnation (Mean:5)
Autumn	7542	990	13.1	398	5.3
winter	7390	680	9.2	330	4.5
spring	10780	1487	13.8	602	5.6
summer	7682	980	12.7	357	4.6

Table 2: The liver infection and condemnation for Dicrocoelium spp. from September 2008 to September 2009 in sari industrial slaughter house , Mazandaran province, IRAN.

Season	Number of liver examined	Number of liver infection	percentage of liver infection (mean:6.95)	Total of liver condemnation	percentage of liver condemnation (Mean:4.8)
Autumn	7542	528	7.0	354	4.7
winter	7390	680	5.0	310	4.2
spring	10780	712	6.6	528	4.9
summer	7682	835	9.2	414	5.4

DISCUSSION

Data has showed a floating trend for *fasciola spp.* infection percentage during different seasons (chart1), but for *dicrocoelium spp.* showed a relative order (chart2). Unlike the percentage of infection, condemnation percentage has not any large variation and has relative equal values for both of parasites. The number of infected livers has been more than the number of condemnated, for the both parasite. The interval between The number of infected and condemnated livers due to *Fasciola spp.*, has more than *dicrocoelium spp.*. The interval between The number of infected and condemnated livers has been close, in the winter about both parasites.

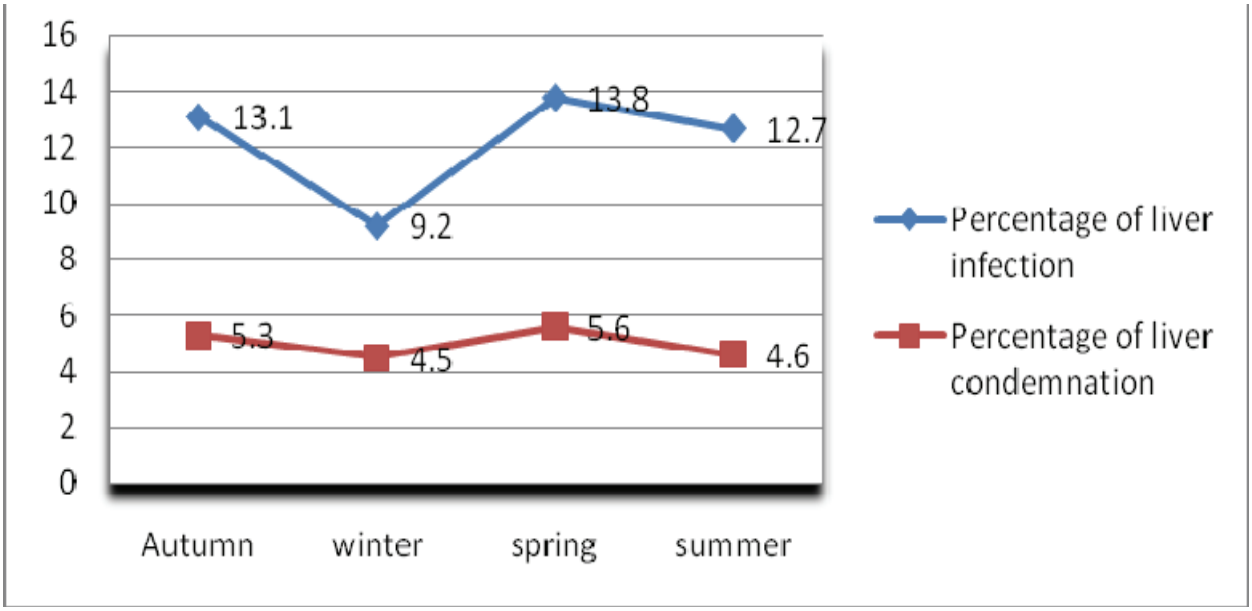

Chart 1: Percentage of liver infection and condemnation for Fasciola spp. From September 2008 to September 2009 in Sari industrial slaughter hause

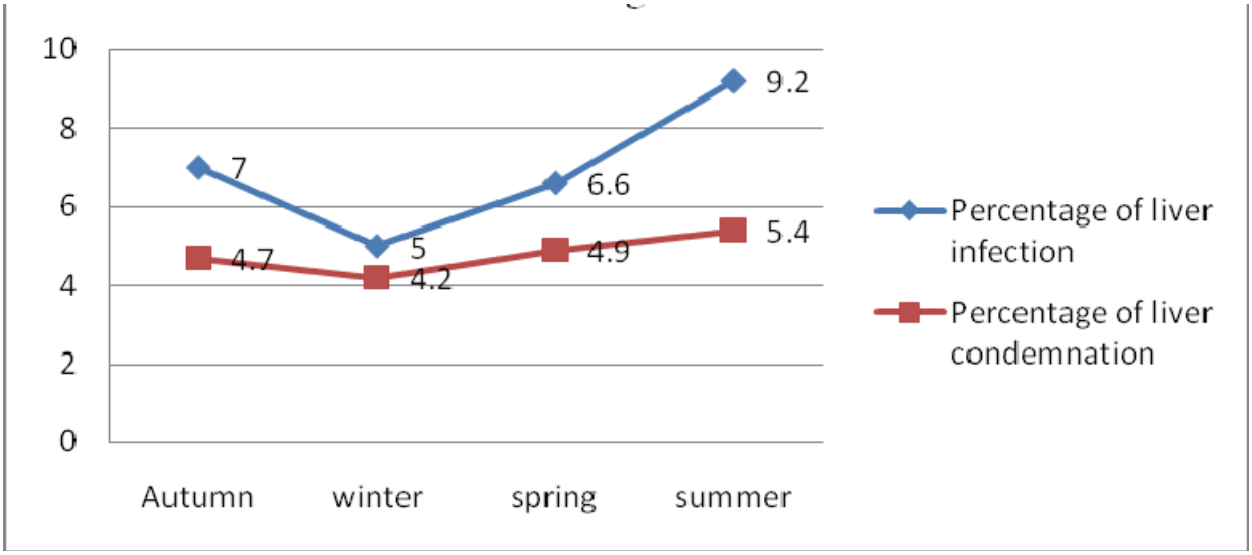

Chart 2: Percentage of liver infection and comnation for Dicrocoelium spp. From September 2008 to September 2009 in Sari industrial slaughter hause

CONCLUSIONS

Results for *dicrocoelium spp.* infection had a significant relationship with climatic conditions specially, humidity rate of seasons. The prevalence of infection which reported in this survey is higher than other surveys in Iran[1,8]. It seems that this increase is because of higher humidity in Mazandaran province from the other province of Iran.[8] Data analysis about *Fasciola spp.* has showed that there was no significant relationship with humidity rate of seasons and infection rate but it seems that related to snail activity and abundance seasons. The different interval between condemnation percentages in almost all values is because of that the inspectors cut the spoiled part of livers and the rest of liver was utilizable. Also the higher level of the number of infected and condemnated livers due to *Fasciola spp.* than *dicrocoelium spp.*[2,3] is because of fewer number and larger size of *Fasciola spp.* than *dicrocoelium spp.* Overall infection and condemnation percentage of livers due to *Fasciola spp.* was higher than dicrocoelium spp. Reason of this difference has no establishment in literatures. Choosing the best anthelmintic drug strategy and sever inspection in abattoirs can prevent sheep fluke infection and consequently guarantees human health and prevents economic losses. [7,9]

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IS THE SHEEP TAPEWORM (*MONIEZIA EXPANSA*) ABLE TO ABSORB LEAD AND CADMIUM FROM SHEEP TISSUES?

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SUMMARY

This paper describes investigations into heavy metal accumulation by intestinal helminths. The sheeptapeworm *Moniezia expansa* and naturally infected sheep were investigated with respect to their lead and cadmium accumulation. 2g $Pb(CH_3COO)_2$ or 0.2g $CdCl_2$ was added to 10 ml of distilled water and administered orally to the sheep every day for a period of 1 week. After the exposure period the sheep were killed and the metal levels were determined in the muscle, liver, kidney and blood of the sheep as well as in the cestode parasites (*Moniezia expansa*). The impact of an infection with the cestode *Moniezia expansa,* and simultaneous Pb or Cds exposure, on the concentrations of heavy metals in the host kidney, liver, muscle, and cestodes was studied. The concentration of lead in the cestodes (85.2 $mg.kg^{-1}$ dry weight) averaged 458, 5 and 4-fold higher in the cestodes than in the muscle, liver and kidney of the host, respectively. Parasitized sheep accumulated significantly less lead in their tissues than their uninfected conspecifics. Also, the differences between host tissues and tapeworms were found to be significant. The cadmium content of *Moniezia expansa* was lower than that of the liver tissues of sheep, although this difference was not significant. The highest mean cadmium concentrations were found in the liver of sheep infected with *M. expansa* (24.5 $mg.kg^{-1}$ dry weight). The mean cadmium concentration measured in *Moniezia expansa* was 21.5 $mg.kg^{-1}$ dry weight, which was 31 and 1.5 times higher than levels determined in the muscle and kidney of the host respectively, however, 0.9 times lower than levels determined in the liver of host. Sheeps with *Moniezia expansa* infection always had higher cadmium concentrations in the tissues than their uninfected conspecifics.

INTRODUCTION

Several helminths are able to accumulate considerable concentrations of heavy metals. It remained unclear if conspicuous metal accumulation of the parasitic worms affects the metal levels in the tissues of the definitive host, as very few comparative studies on heavy metal concentrations in tissues of infected and uninfected hosts are available. A very common farm animal (*Ovis aries*) and their common tapeworm (*Moniezia expansa*) were selected for the present study. Sheep tapeworm and naturally infected sheep were investigated with respect to their lead and cadmium accumulation.

MATERIAL AND METHODS

The sheeptapeworm *Moniezia expansa* and naturally infected sheep were investigated with respect to their lead and cadmium accumulation. 2g $Pb(CH_3COO)_2$ or 0.2g $CdCl_2$ was added to 10 ml of distilled water and administered orally to the sheep every day for a period of 1 week. After the exposure period the sheep were killed, and the metal levels were determined in the muscle, liver, kidney and blood of the sheep, as well as in the cestode parasites (*Moniezia expansa*). The impact of an infection with the cestode *Moniezia expansa* and a simultaneous Pb or Cd exposure on the concentrations of heavy metals in the host kidney, liver, muscle, and cestodes was studied. Concentrations of heavy metals were compared among individual tissues and treatments using one-way analysis of variace (ANOVA, $P \leq 0.05$). Additionally, the bioconcentration factor as a ratio of the metal concentration in the parasites and the host tissues ($C_{parasite}/C_{host\ tissue}$) was determined according to Sures et al. (1999).

RESULTS

The concentration of lead in the cestodes (85.2 $mg.kg^{-1}$ dry weight) averaged 458, 5 and 4-fold higher in the cestodes than in the muscle, liver and kidney of the host, respectively. Parasitized sheep accumulated significantly less lead in their tissues than their uninfected conspecifics. Also the differences between host tissues and tapeworms were found to be significant. The cadmium content of *Moniezia expansa* was lower than that of the liver tissues of sheep, although this difference was not significant. The highest mean cadmium concentrations were found in the

liver of sheep infected with *M. expansa* (24.5 mg.kg^{-1} dry weight). The mean cadmium concentration measured in *Moniezia expansa* was 21.5 mg.kg^{-1} dry weight, which was 31 and 1.5 times higher than levels determined in the muscle and kidney of the host respectively, however, 0.9 times lower than levels determined in the liver of host. Sheeps with *Moniezia expansa* infection always had higher cadmium concentrations in the tissues than their uninfected conspecifics. Parasitized sheep accumulated less lead in their tissues than their uninfected conspecifics. Lead concentrations in infected sheep livers, kidneys and muscles were as follows: 16.584±0696; 19.599±2.496 and 0.183±0.008 mg Pb/kg in dry weight contrary 22.046±0.751; 39.173±1.595 and 0.600±0.057 mg/kg in non infected sheep. Although the cadmium content of *Moniezia expansa* was lower than that of the liver and kidney tissues of sheep, the concentrations of lead in the cestodes were averaged 431.15, 4.76 and 4.03 times higher in the cestodes than in muscle, liver and kidney of the host. Cadmium concentrations were found to be the highest in the sheep liver (23.785±11.482 mg/kg). It was 34.52, 4.86 and 1.62 higher in the liver than in the muscle, cestodes and kidney of the host, respectively.

DISCUSSION

Tapeworms can absorb bile-bound lead through their tegument. The cestode tegumentary surface is functionally equivalent to the brush border of the vertebrate intestinal mucosa [5]. Cestode tegument serves as a highly efficient digestive-absorptive layer that competes with the vertebrate mucosa for nutrients including heavy metals [1].

The main object of the present study was to monitor the relationship of farm animals (sheep) to their lead and cadmium burden, and to their cestode parasites. Heavy metal bioaccumulation by intestinal parasites of farm animals from the host tissues is a new method of natural detoxication for organisms. Due to the fact that muscle tissue makes up the bulk of a carnivorous diet, and biologically incorporated heavy metals (lead above all) transfers upwards in the food chain only in scarce amounts, herbivorous species may be more susceptible to lead and other heavy metal poisoning than predatory species [4].

Another purpose of this study was to test the potential suitability of the cestode/sheep model *Moniezia expansa/Ovis aries* as another promising bioindicator system for Pb and Cd pollution under farm and natural conditions. Sheep are also used as a low-cost means of maintaining the landscape (through grass-feeding). These species were chosen in view of the fact that there are very few models for farming conditions: *Fasciola hepatica*/cattle for the digenean [10] and *Macracanthorhynchus hirudinaceus*/pig for the archiacanthocephalan [8]. A cestode/rodent model such as *Hymenolepis diminuta* and *Rattus rattus* was successfully evaluated for urban areas [9,11]; for non urban areas the cestode/rodent model was comprised of *Gallegoides arfaai/Apodemus sylvaticus* [12] and *Skrjabinotaenia lobata/Apodemus sylvaticus* [13] and *Paranoplocephala* spp./*Microtus agrestris*) [2]. Also the cestode/carnivore model (*Mesocestoides* spp./*Vulpes vulpes*) is a suitable bioindicator system [3].

In terrestrial mammals the kidney is known as one of the main accumulation organs for metals. Accordingly, in our study, the kidney of sheep both with or without *Moniezia expansa* infection exhibited the highest lead content among all the investigated tissues. The kidney lead content was 19.9 mg.kg^{-1} dry weight compared with the liver and muscle, which contained lower dry weight lead levels of16.3 and 0.186 mg.kg^{-1}, respectively. Lead concentrations in the sheep muscles were approximately 108 times lower than in the sheep kidneys. Although when taking into account the parasites, the highest concentration of lead within the host-parasite system was recorded for *Moniezia expansa* (85.2 mg.kg^{-1} dry weight) from exposed sheep. A significantly higher accumulation of lead was recorded for the cestode parasite *Moniezia expansa* when compared to the organs of its host. Bioconcentration factors for lead in *Moniezia expansa* ($Pb_{parasite}/Pb_{host}$) were 432, 5, 4 times higher than in the host muscle, liver, kidney, respectively.

The mean cadmium concentration measured in *Moniezia expansa* was 21.455 mg.kg^{-1} dry weight. This was 31 and 1.5 times higher than levels determined in the muscle and kidney of the host, respectively, however, only 0.9 times the amount determined in the liver of the host. Also, Torres et al. found that Cd concentrations were higher in the host tissues than in the tapeworms [12,13]. Furthermore, Cd concentrations in sheep tissues were higher in sheep with *Moniezia* infection in comparison to uninfected sheep. This is an indication that anoplocephalid tapeworms do not absorb Cd from the tissues of the host (contrary to Pb).

The results presented here demonstrate that sheep tapeworms (*Moniezia expansa*) accumulate much higher lead levels than do the host tissues. Thus, *M. expansa* might be used as an accumulation indicator for heavy metals in terrestrial biotopes, especially as it is a very abundant parasite of sheep and cattle. Further investigations are necessary to decide whether or not intestinal parasites of mammals are able to affect metal levels in host tissues.

CONCLUSIONS

This study reveals that lead accumulation also occurs in mammal parasitizing cestodes.

The host-parasite system sheep-*Moniezia expansa* appears to be a useful and promising bioindication system for lead, especially in farming (rural, agricultural) and in the natural ecosystem. It may also be used, to a certain extent, to decontaminate sheep tissues of lead.

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THE ROLE OF MHC CLASS II GENES IN RESISTANCE TO NEMATODE INFECTIONS OF SHEEP (Abstract)

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INTRODUCTION

Nematodes adversely affect animal health and cause serious economic losses; one option for control is the use of genetically resistant stock. There is an association between the MHC class II loci and resistance to nematode infections of sheep such as DRB1 gene. Our long-term aim is to fine-map the causative mutations but in order to design efficient and powerful experiments, we need to determine the extent of diversity at the different loci. We have therefore sequenced the second exons of all alleles at the DQA1, DQA2, DQB1, and DQB2 loci in 600 naturally infected lambs from the Scottish Blackface breed and Texel breeds.

ANIMALS, MATERIALS AND METHODS

400 Scottish Blackface and 235 Texel rams were used in this work. Blood was collected by jugular venepuncture into evacuated glass tubes containing the anticoagulant disodium EDTA Afterward the plasma layer was collected and then the leucocytes (buffy coat) was collected and stored at -20 °C. DNA was extracted from the blood buffy coat and then we have genotyped 4 genes at the MHC Forward and reverse primers of ovine MHC class II genes (DQA1, DQA2, DQB1 and DQB2 (100pmol/ul, eurofins MWG Operon) were diluted in sterile water (invitrogen™, Carlsbad, CA) to make a final concentration 20pmol/ul for each primer. The PCR products of the novel alleles were TOPO® cloned into the One Shot® Mach1™ -T1^R Competant Cells (chemically competent E. coli cells) were transformed with the recombinant vector according to the manufacturer's instruction. The assays for IgE and IgA specific antibodies to L3 and L4 stage were performed as described previously (Pettit et al., 2005), employing the muse monoclonal anti-ovine IgE, clone 2F1, (Bendixen et al., 2004), and anti-ovine IgA (Serotec, Oxford, UK). Detection was with the goat anti-mouse – HRP (Dako).

RESULTS

The MHC of sheep contains highly variable DR and DQ regions. In the Blackface breed, there were at least 10 alleles at DQA1, 19 alleles at DQA2, 24 alleles at DQB1, and 25 alleles at DQB2 gene. In comparison, the GenBank database, which contains sequences from all breeds of sheep, contains 18, 45, 19, and 29 alleles at DQA1, DQA2, DQB1, and DQB2 respectively. In this study, we have identified 16 novel DQB1 alleles; 8 of them have been submitted to the GeneBank and their accession numbers (GU191453, GU191454, GU191455, GU191456, GU191457, GU191458, GU191459, and GU191460), and 11 novel DQB2 alleles, 3 of them have been submitted to the GeneBank.

CONCLUSION

There was a significant association between some of the DQA2, DQB1 and DQB2 alleles and the FECs which is considered one of the genetic markers for resistance to nematode infection and hence, this research reported here is considered an important achievement by providing better understanding of the genetic structure of the Ovar-MHC which improve the methods used in selective breeding to identify resistant and susceptible sheep.

FATTY ACID CONTENT OF EGG YOLKS FROM HERITAGE BREED HENS* (Abstract)

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Compared to commercial hybrids, the populations of heritage breed hens, which are not selected for productive traits, are characterized by lower egg production but are more resistant to varying and often adverse environmental factors that occur under free-range conditions. Due to the ban on battery cages in the European Union from 1 January 2012 and the promotion of alternative housing systems, there is an urgent need to find breeds/lines of hens that are suitable for raising in alternative housing systems, in particular the free-range system.

Eggs from laying hens of three breeds included in the biodiversity conservation programme in Poland: Leghorn (G-99), Rhode Island Red (R-11) and Sussex (S-66), were studied. Within each breed, one group of hens (60 birds) was kept under floor conditions on litter (LF) and the other (60 birds) under free-range conditions (FR). Egg quality was evaluated at 44 weeks of age. The content of higher fatty acids in egg yolks was analysed using gas chromatography by determining acids as methyl esters.

R-11 hens raised with outdoor access (FR) produced smaller eggs characterized by a greater proportion of yolk and a more intensive yolk colour compared to the eggs laid by hens raised without outdoor access (LF). Eggshell weight and thickness were similar in R-11 and S-66 hens raised with and without outdoor access and lower in G-99 hens. The vitamin A content of egg yolks was higher, and the vitamin E of egg yolks lower in FR hens compared to LF hens. Egg yolks from G-99 FR hens contained as much cholesterol as did egg yolks from LF hens of the same breed. The content of *n-3* unsaturated fatty acids and the *n*-6:*n*-3 PUFA ratio in the yolks of eggs from FR hens was more favourable compared to the yolks of eggs from LF hens.

It is concluded that the increased concentration of *n:3* PUFA and the higher vitamin A content of egg yolks from free-range hens compared to confined hens, obtained in our study, support the opinion about the superior dietetic qualities of free-range eggs and justify the use of this housing system for hens included in the genetic resources conservation programme. In our opinion, of the analysed breeds/lines R-11 hens are best suited for free-range rearing because they reduce their productivity to a lower extent than other breeds when exposed to free-range conditions.

*Supported by grant no N R12 0083 10 financed by the National Centre for Research and Development.

A COMPARISON BETWEEN THE ROUTINE TREATMENT OF EQUINE DERMATOPHYTOSIS AND TREATMENT WITH GARLIC-ALOE VERA GEL

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SUMMARY

Ringworm is an infectious disease of animals caused by different species of keratinophilic fungi. Disease in horses can be ranging from mild to severe lesions. In horses, most dermatophyte lesions are found in areas of contact with saddles or other tack. Treatment is generally topical because systemic therapy is expensive. Herbal medicine has been formulated and used as an integral part of primary health care in some countries. These natural plants involve Garlic and Aloe vera. In this study a total of 10 affected horses in two groups, were subjected under the routine treatment and treatment with Garlic and Aloe vera gel, respectively. Periodic observations of two groups showed the comparable results. Results of 5-day intervals suggested a progressive improvement in both groups. A desirable response to treatment was seen by the 25th day in each method.

INTRODUCTION

Skin, hair, nail, and subcutaneous tissues in human and animal are subjected to infection by several organisms, mainly fungi named dermatophytes and cause dermatophytoses [3]. It is a major public and veterinary health problem reported from different parts of the world and causes great economic loss [4]. It has been reported that animals housed in close proximity to each other for long periods and the presence of infected debris in buildings account for both the higher incidence and the greater infection rate in winter. Although *Trichophyton* and *Microsporum* species are the main causes of ringworm [8], in horses, *T. equinum* is most commonly involved. Disease in horses can be quite variable, ranging from mild or subclinical disease to severe lesions mimicking pemphigus foliaceus [10]. In horses, most dermatophyte lesions are found in areas of contact with saddles or other tack. *T. equinum* lesions are usually pruritic, with exudative lesions and areas of hairless, thickened skin. *M. equinum* lesions are usually less severe and consist of small scaly areas with brittle hairs [7]. Systemic treatments recommended for use in farm animals include the injection of sodium iodide as a 10% solution repeated on several occasions, and, if the high cost of the treatment can be overlooked, the oral administration of griseofulvin [8]. Treatment is generally topical because systemic therapy is expensive and of unproven efficacy. Whole-body rinses and individual lesions treated with clotrimazole or miconazole preprations [6]. There are some evidences that garlic and aloe vera can be used as anti-dermatophytic agents [1]. Garlic (*Allium sativum*) is an intriguing herb with a long history of medicinal use for a variety of diseases including ringworm infections [11]. The Aloe vera gel has been found to promote wound healing due to the presence of some components like anthraquinones and hormones, which posses antibacterial, antifungal and antiviral activities. Most of the constituents are found in the gel and not in the leaf; hence the gel is likely to be more active than the leaf. The fact that Aloe vera extracts on microorganisms gave credence to the popular use of both Aloe vera gel and leaf [1]. This study was designed to compare the routine treatment of dermatophytosis with garlic and aloe vera gel treatment in horses.

MATERIAL AND METHODS

Animals

A total of 10 horses in a horse-riding club in Isfahan were used in the present study. The club contained about 100 animals of different ages. There was a history of skin lesions on mentioned horses shortly after introducing a non-quarantined horse with a history of cutaneous lesions. The horses were cross-bred in a good nutritional status and of 2-12 years of age.

Clinical examinations

The skin of all animals was examined and a complete clinical examination of all affected animals was done. Evaluation of the general state of the animals, temperature, pulse, respiratory rate and appetite were recorded. The shape, size, position, distribution and time of the appearance of skin lesions as well as the age of the animals were also recorded.

Sampling

The surface of the affected area was first rubbed with a cotton swab impregnated with 70 % ethyl alcohol to remove surface adhering organisms. Skin scales were collected by scraping of the margin of the lesion using a sterile scalpel blade into sterile petri dish. Hairs were collected by removing dull broken hairs from the margin of the lesion. Each sample collected was divided into two portions: One portion was used for direct microscopic examination with 10% KOH/ DMSO. The second portion was cultured on sabouraud dextrose agar, incubated at 28 °C for up to 3 weeks and checked daily for the colony formation. To identify the pathogenic fungi, macroscopic and microscopic examinations were performed and time of appearance of the growth, colony morphology, and also color, shape, size and colony reverse side morphology was noticed. Microscopic examination for positive fungi cultures was done using the Lactophenol cotton blue wet mount method [5].

Treatment method

All affected horses were kept in the same conditions such as light, ventilation, bedding and stable disinfection. They were divided into two groups and each group was subjected under different treatments. Horses in the first group were administered griseofulvin orally at the daily dose rate of 250mg/kg for seven days as well antifungal lotions were scrubbed topically, two times a day, for three weeks. Horses in the second group were given five pills of garlic daily for 25 days. Aloe vera gel was rubbed on the lesions, as well. Results were recorded in five-day intervals.

RESULTS

Fungal elements could be seen in KOH preparation of affected horses *(Figure 1)*. Fungi *C*ulture was positive in all cases. Fungal species were identified on the basis of cultural characteristics, pigment production and microscopic examination in lactophenol cotton blue preparation. Although some species of dermatophytes were isolated, the predominant pathogen was *T. equinum*.

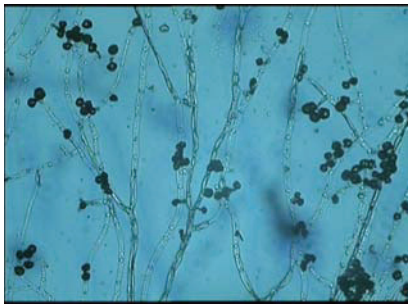

Figure 1: Trichphyton equinum isolated from skin samples of affected horses

Periodic observations of two groups showed the comparable results. Results of five-day intervals suggested a progressive improvement in both groups. In horses with signs of dermatophytosis *(Figures 2,3)*, fungal spots on the fifth, tenth, Fifteenth and twentieth day gradually become smaller and itching went away *(Figures 4,5)*. A desirable response to treatment was seen by the 25th day in each method. Unwillingness to eat, lethargy and depression were improved, so that at the end of 25th day, all of the horses tended to feed, wounds become very small or disappeared, and only there was a mild alopecia in some areas.

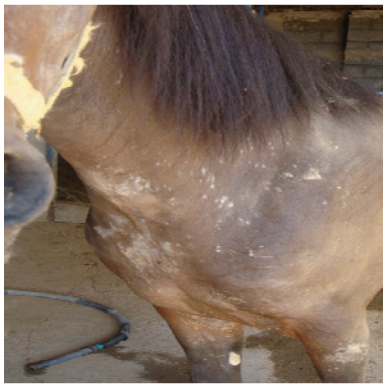

Figure 2: Affected horse before treatment

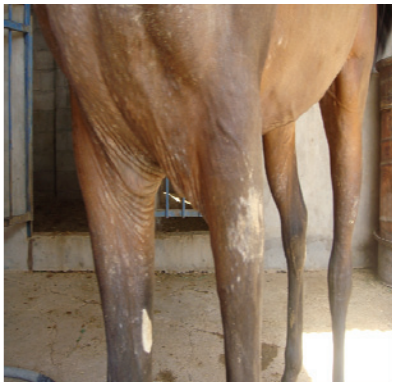

Figure 3: Affected horse before treatment

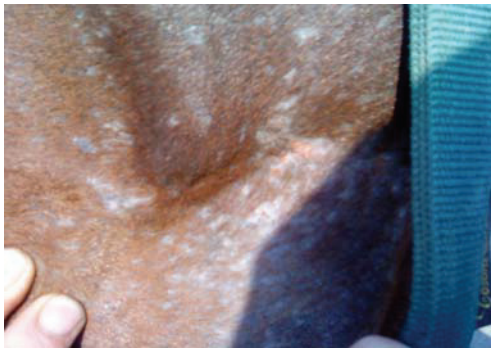

Figure 4: Affected horse on the 25th day of treatment

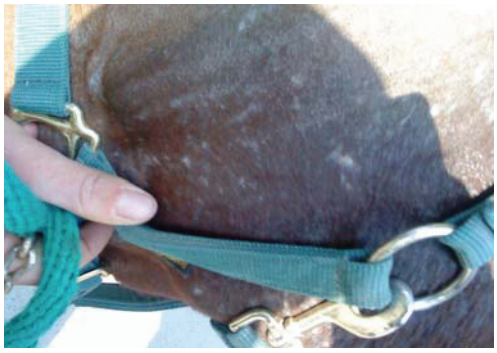

Figure 5: Affected horse on the 25th day of treatment

DISCUSSION

Animals often have self-limiting dermatophytosis that resolve within a few months, but treatment can speed recovery, decrease the spread of lesions on the animal, and decrease the risk of transmission. Treatment may include topical antifungal creams or shampoos, and/or systemic antifungal drugs [7]. The choice of proper treatment for dermatophytosis is determined by the site and extent of the infection and the species involved, as well as by the efficacy, safety profile and pharmacokinetics of the available drugs.

Development of more effective and less toxic antifungal agents is required for the treatment of dermatophytosis. Different treatments have been recommended to control dermatophtes. In general, pharmacological treatment options include antifungal agents, but recently the use of some natural plant products has been emerged to inhibit the causative organisms. A number of reports are available in vitro and in vivo efficacy of plant extract against plant, animal and human pathogens causing fungal infections [3]. Shams et al. (2003) has showed that *Trichophyton mentagrophytes* growth was significantly inhibited by garlic extract in vitro [9]. Adejmo et al. (2009) have reported satisfactory results of applying Aloe vera extract for controlling the dermatophyte-causing agents (*Trichophyton mentagrophytes*) in vitro [1]. Also, Antickchi et al. (2009) reported acceptable results in treating dermatophytosis with Aloe vera in calves [2]. The results of this survey showed that Aloe vera extract and garlic can be used as potential candidates for preparation of anti-dermatophytic drug formulations.

CONCLUSIONS

The use of medicinal plants in the treatment of dermatomycoses will help to reduce the dependence on the use of microbial or chemically synthesized antimicrobials and thus overcome the problem of the emergence of fungi being resistant to antifungal chemicals on various etiological agents of dermatophyte infections [1].

The ultimate conclusion of this study supports the traditional medicine use of different plant extracts in treating different infections caused by pathogenic fungi, either by using a single or combined extracts. It also suggests that a great attention should be paid to medicinal plants which are found to have plenty of pharmacological properties that could be sufficiently better when considering a natural food and feed additives to improve human and animal health

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FARMER VISITS AS A POTENTIAL ROUTE FOR DISEASE TRANSMISSION

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SUMMARY

Foot- and mouth disease may affect several species such as cattle, pigs and sheep. In simulation studies as well as contingency planning, it is important to know whether there are contacts between the different species, enabling the disease to spread. Persons visiting on farms are one possible route for FMD spread.

Two questionnaires to cattle and pig farmers were performed in order to study contacts between different animal sectors. The results revealed that farmers mostly visit farms within the same production sector. This means that the likelihood of spread of disease through visitors is smaller from one animal species to another than within the production sector.

INTRODUCTION

There are some animal diseases, such as foot and mouth disease, which can infect cattle, pigs and sheep. When simulating diseases for risk assessment, it is important to know what events may lead to spread within a production sector and between different production sectors. One potential way for diseases to spread between farms is people visiting farms without using efficient biosecurity precautions. In this study, cattle and pig farmers in Finland were asked how frequently they visit other farms, which type of farms they visit, and whether they use protective clothing and boots during their visits. There were 20 211 cattle farms, 3 225 pig farms and 1 885 sheep farms in Finland in 2006.

MATERIAL AND METHODS

In this study we used the data from two questionnaires directed to Finnish farmers in 2007. The questionnaires were sent to 2699 cattle farmers and 1118 pig farmers in Finland in the spring 2007. Responses were received from 1180 (44%) cattle farmers and 571 (51%) pig farmers. The farmers were asked about the number of visits they did to other farms during the previous year (2006). In addition they were asked about the habits of visitors of using protective boots and clothing. The analyzed questions and answers were a part of a larger questionnaire regarding the production structure and habits on farms in general [1].

In the results the means and the 95% confidence intervals of means (estimated by normal approximation to the binomial distribution) are given in parenthesis (±).

RESULTS

Among the respondents, 562 cattle farmers and 316 pig farmers have answered the questions regarding the number of visits to other farms during year 2006. Cattle farmers visited mostly other cattle farms: 94% of the cattle farmers' visits were to other cattle farms. Only a small fraction (5%) of the visits was to pig and sheep farms. Pig farmers, on the other hand, visited mostly other pig farms (60%); but also quite often (38%) they visited cattle and sheep farms (Figure 1).

In cases where the pig farmers have reported to have entered the animal unit, the farmer has more often used protective boots and clothes when he visited other pig farms. Most of the pig farmers (86%, ± 8) used boots and (76%, ±10) used protective clothing when visiting another pig farm. When the pig farmer visited cattle and sheep farms, their usage of boots and protective clothing were less frequent (47%, ±15 and 36%, ±15, respectively). There were no such statistical significant differences among cattle farmers. Cattle farmers used boots 58% (±5) and protective clothing 40% (±6) when visiting other cattle farms. When the cattle farmers visited pig and sheep farms they used boots in 37% (±19) of the visits and protective clothing in 27% (±20) of the visits.

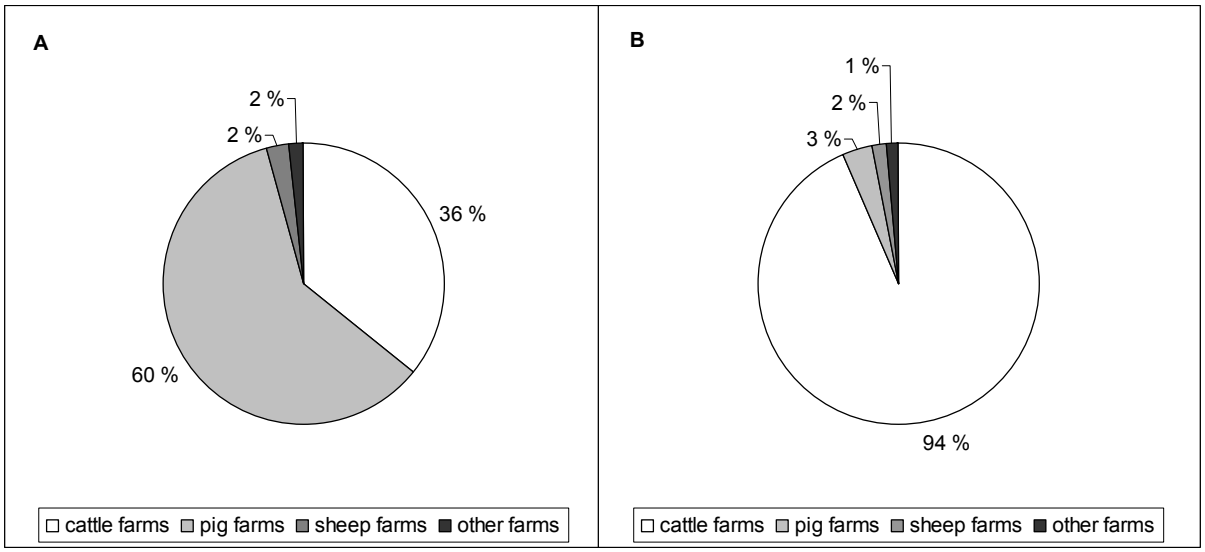

Figure 1. The pie charts represent the number of visits to other farms that A) pig farmers and B) cattle farmers have reported during year 2006 according to two questionnaires [1].

DISCUSSION

Potential contacts between farms through people visiting are more prominent within the same production sector. Thus the possibility of contacts and thereby transmission of diseases by this route in Finland is more likely to happen *within* a production sector than between the sectors.

This result is much clearer in the cattle than in the pig sector – as pig farmers tend to visit also cattle farms. Sheep farms were not visited frequently either by cattle or pig farmers and it seems to be quite a separate sector in the Finnish animal production scene and indicative for the few sheep farms and small size of sheep production in Finland.

The questionnaire also revealed that the use of boots and protective clothing is more common on pig farms than on cattle farms. It seems that pig farmers are more eager to keep a strict biosecurity barrier and require protective boots and clothing at their farms than cattle and sheep farmers do. Notable is also that, cattle farmers do not apply protective measures at the same level when they visited pig farms as pig farmers do when they visited another pig farm.

The results from the survey, that the different production sectors are not in frequent contact with each other, at least concerning the farmer visits, is important information in contingency planning as well as in future simulations of animal diseases in Finland.

CONCLUSIONS

Potential contacts between farms through people visiting are more prominent within the same production sector. Thus the possibility of contacts and thereby transmission of diseases by this route in Finland is more likely to happen *within* the production sector than between the sectors.

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MAIN CAUSES OF CATTLE CONDEMNATIONS IN FEDERAL SLAUGTHERHOUSE IN BAHIA STATE – BRAZIL

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SUMMARY

Brazil is one of the most important cattle producers and the largest exporter of cattle meat in the world. The food production out of the standards of hygiene and sanitary quality can result in serious damage to health consumers. Failure to detect lesions of potential zoonotic diseases at slaughter poses a health risk to consumers especially when meat is eaten undercooked. This study aimed to determine the major causes of condemnation in slaughtered cattle under federal inspection in Bahia state, Brazil, and was conducted through analysis of reporting forms, kindly provided by the Ministry of Agriculture, Livestock and Supply of this country. Data were collected in 2008, and the total number of animals slaughtered during this period was 64,889. Were considered major causes of condemnation those who showed a percentage more than 10% of all cattle slaughtered in the period studied. From official data, were observed the following main causes of condemnation: blood aspiration in the lungs (12.8%), pulmonary congestion (12.9%) and nephritis (30.9%). The present study confirms the importance of veterinarians and others professionals qualified and trained in food industry, resulting in a better quality product, increasing exports and reduce economic losses to industry, and ensuring the maintenance of public health.

Keywords: 1. Condemnations; 2. Cattle; 3. Nephritis

INTRODUCTION

In 2008, estimates of Food and Agriculture Organization (FAO) showed that Brazil holds the world's largest herd of cattle, followed by India, China, USA and Argentina. Brazil is a privileged country in terms of conditions for production of animal protein. Climate, soil, technology and human resources have long ceased to be obstacles and came to constitute comparative advantages, added to the immense territorial extent, enable the country to produce competitively priced animal protein in increasing amounts, with the quality desired by consumers. However, there are still some pending issues of both health and lack of management of some supply chains that need to be resolved. Still, one can say that the country is possessed of an adequate infrastructure to serve the domestic market and increase export volumes to grow as the demand for protein foods. Specifically in the case of beef, the advances of the last thirty years, especially in the 90s, in the areas of pasture, forage production and conservation, mineralization, animal breeding, health, slaughter, processing and marketing of meat, are very significant (3). In contrast, government investment for the recruitment and training of veterinarians and technical aids for Defense and Health Inspection have not followed the same trend (4). However, while such technological advances and production were important for increasing the production and sale of beef in Brazil, encouraging internal and external competitiveness, there are still some problems in handling the animals, as well as the stages of transport and slaughter that can directly affect the quantity and quality of the meat. The contamination of meat during slaughter of animals, occurs primarily by contact with skin, hair, feet, gastrointestinal contents, milk from the udder, hands and clothing of workers, water used for washing carcasses, equipment and air in places where slaughtering and storage (7). Foods of animal origin, even if obtained from healthy animals, can be vehicles for biological physics and chemistry contaminants, which could lead to possible outbreaks of diseases that bring serious damage to health of the population and, consequently, economic losses (6). Therefore, considering the importance of meat in the diet of the population, the sanitary quality, the maintenance of public health and economic issues associated with the production of animal products, the present study was carried out to determine the major causes of condemnation in cattle slaughtered under federal inspection in Bahia state, Brazil.

MATERIAL AND METHODS

This work was carried out using data obtained from post-mortem inspection of cattle slaughtered in a slaughter plant under federal inspection in Bahia state, Brazil, with a daily slaughter capacity of 520 animals. Data were collected from January to December 2008 and the total number of animals slaughtered during this period was 64,889, providing an average of approximately 178 heads per day.

A post-mortem inspection of large ruminants (cattle and buffaloes) is based on so-called "inspection lines". In establishing where were performed the present study, the

steps directly related to the slaughtering room, are quoted below: stunning, bleeding, removal and inspection of the feet, manual skinning followed by mechanical skinning, removal of the head, the head assembly and inspection language, gutting, inspection of the viscera and carcass, toilet, weighing of whole carcasses, and wash in cold storage. The convictions made during the post-mortem inspection were based on the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin - RIISPOA (1) and recorded on the notification forms, which were removed from the data concerning the number and causes the same for this work. Finally, for purposes of calculation and discussion in this work were considered as major cause of condemnation all the alterations that percentage equal to or greater than 10%.

RESULTS

Were initially categorized all data relating to convictions made of carcasses and offal from animals slaughtered in the establishment during the period of this study. Taking into account the percentage equal to or greater than 10% used as a criterion of analysis of this work, although other causes of condemnation have been observed, they were not considered important. Thus, according to the reporting forms, it was found that the main causes of condemnation in cattle slaughtered were blood aspiration into the lungs (12.8%), pulmonary congestion (12.9%) and nephritis (30.9%). In addition, even if the percentage of condemnation have not been equal to or greater than those established in this study as the most important causes, it is possible to observe that some causes of condemnation of that slaughterhouse are associated with technology failures occurred during the slaughter and handling of animals, the example of traumatism in feet (3.24%) and aspiration of food (1.18%).

DISCUSSION

The results of this study regarding pulmonary congestion are very close to those found by Lima et al. (5), where in the study about pathological changes in 1,840 cattle slaughtered in the slaughter plant in Rio Grande do Norte - Brazil found pulmonary congestion present in 16.7% of the organs condemned, this holds the second greatest alteration in the number of condemned organs on that slaughter plant, behind only to the pulmonary emphysema that holds 66.7% of all cases of organ condemnation. Aspiration of blood to the lungs was alteration found in 12.8% of total animals slaughtered in this study. Daguer (2) defines the aspiration of blood as a "technopathy" that is, an operational lesion that is non-pathological, so it has no correlation with clinical status or health of animals.

Thomson (9) confirm that the granulomatous nephritis in domestic animals may be associated with various infectious agents including viruses, bacteria, fungi and parasites, being common to these etiologies the formation of macroscopic granulomas distributed throughout the kidney, and more steeply in renal cortex. Salgado et al. (8), following a period of one month at a slaughter plant under federal inspection in São Paulo state, Brazil with a slaughter of 1,200 animals a day, reported the presence of nephritis in 24.51% of the total 36,000 animals during the study period, relating this percentage to failures in the sanitary herd. In this study, confirming the major causes of condemnation found by Salgado et al. (8), nephritis was present in 30.9% of cases condemnation, and this index is very worrisome, since it suggests a high rate of infectious diseases of livestock in this area, revealing the importance of veterinarian inspector presence in slaughter plant to ensure quality food to the human population as well as the importance in having in the property qualified technical labour ensuring the herb health. Moreover, the condemnations of edible viscera cause significant economic losses in the slaughter plant.

CONCLUSION

In this study we observed that the main causes of condemnation in cattle slaughtered under federal inspection in Bahia state were aspiration of blood into the lungs, pulmonary congestion and nephritis. Finally, we emphasize that the role of the veterinarian in activities related to sanitary inspection of animal products is critical to preserving the health of population and economic growth of the country.

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MAIN CAUSES OF SMALL RUMINANTS CONDEMNATIONS IN FEDERAL SLAUGTHERHOUSE IN BAHIA STATE - BRAZIL

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SUMMARY

Brazil is one of the largest exporters of meat in the world. The exploitation of small ruminants is an activity that is growing every year in Brazil, and the Bahia state holds the largest and the second largest herd of goats and sheep, respectively. Meat is one of the main products obtained with this type of farming. Subjecting it to rigorous processes of sanitary inspection by properly trained professional preserves the benefits of this food as well as reduces the risk that its ingestion can cause, ensuring product quality. The aim of the present was to determine the major causes of carcasses and organ/offal condemnations in slaughtered goats and sheep under federal inspection in Bahia state, Brazil, and was conducted through analysis of reporting forms, kindly provided by the Ministry of Agriculture, Livestock and Supply of this country. Data were collected from August 2005 to February 2006, the total number of animals slaughtered in this study period was 13,125, and where 3,387 were goats and 9,738 were sheep. Were considered major causes of condemnation those who showed a percentage more than 10% of all goats and sheep slaughtered in the period studied. From official data, the main causes of condemnation were blood aspiration, responsible for 56.6% of convictions made in sheep and 38% of goats slaughtered, and intestinal parasitosis, responsible for 57.5% of convictions in sheep and 31.8% of goats slaughtered. The present study confirms the importance of veterinarians and others professionals qualified and trained in food industry, resulting in a better quality product, increasing exports and reduce economic losses to industry, and ensuring the maintenance of public health.

Keywords: 1. Condemnations; 2. Small ruminants; 3. Blood aspiration

INTRODUCTION

The physico-chemical and nutritional products of animal origin such as meat and meat products, contributing to configure these foods among those most concerned about humanity because of the dangers they offer. Thus, to minimize the risks from the ingestion of these products, it must be the need for prior inspection of food in retail establishments. Such control, in its specific nature, makes nondelegable character of this activity exclusively performed by veterinarians, as described in Brazilian laws (3, 4, 7).

According to the Ministry of Agriculture, Livestock and Supply in Brazil there are about 30 abattoirs under federal inspection carrying out the slaughter of small ruminants. In Bahia, the limited number of establishments under the control of Ministry of Agriculture who perform the slaughtering of goats and sheep favors the practice of illegal slaughter. Besides not being subjected to inspections ante and post-mortem, the animals are killed by people who are unprepared, with the use of inadequate equipment and without respect to the hygienic-sanitary standards required, failing in this way the legislation. Due to the scarcity of records available in the literature on convictions made more relevant in small ruminants, the present study was carried out to determine the major causes of condemnation in goats and sheep in a slaughter plant under federal inspection in Bahia state, Brazil.

MATERIAL AND METHODS

This work was carried out using data obtained from post-mortem inspection of caprine and sheep slaughtered in a slaughter plant under federal inspection, located in Bahia state, Brazil, with a daily slaughter capacity of 200 animals. Data were collected from August 2005 to February 2006, the total number of animals slaughtered during this period was 13,125 (3,387 goats and 9,738 sheep), providing an average of approximately 62 heads per day.

A post-mortem inspection of sheep and goats resembles the inspection of large ruminants (cattle and buffaloes) and is based on so-called "inspection lines". In establishing where were performed the present study, the steps directly related to the slaughtering room, are quoted below: stunning electronarcosis; bleeding, removal and inspection of the feet, followed by skinning manual mechanical skinning, removal of the head, the head assembly and inspection language, gutting; inspection of the viscera and carcass, toilet; weighing of whole carcasses, and wash in cold storage. The convictions

made during the post-mortem inspection were based on the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin - RIISPOA (1) and recorded on the notification forms, which were removed from the data concerning the number and causes the same for this work.

RESULTS

Were initially categorized all data relating to convictions made of carcasses and offal from animals slaughtered in the establishment during the period of this study. Taking into account the percentage equal to or greater than 10% used as a criterion of analysis of this work, although other causes of condemnation have been observed, they were not considered important. Thus, according to the reporting forms, it was found that the main causes of condemnation in both goats and sheep slaughtered were blood aspiration into the lungs and intestinal parasites. The condemnation of the lungs due to aspiration of blood was found in 56.6% of sheep and 38% of goats slaughtered. Aiming to verify macroscopic changes resulting from aspiration of blood, cuts were made in lungs using his own blade inspection, and were observed an accumulation of blood in the pulmonary parenchyma (Figure 1)

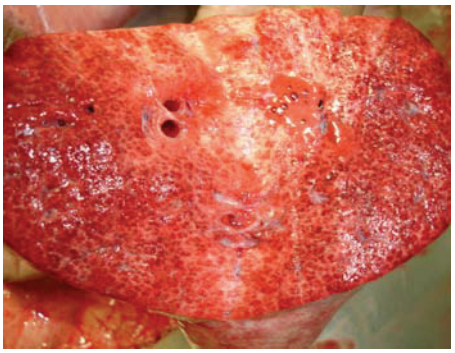
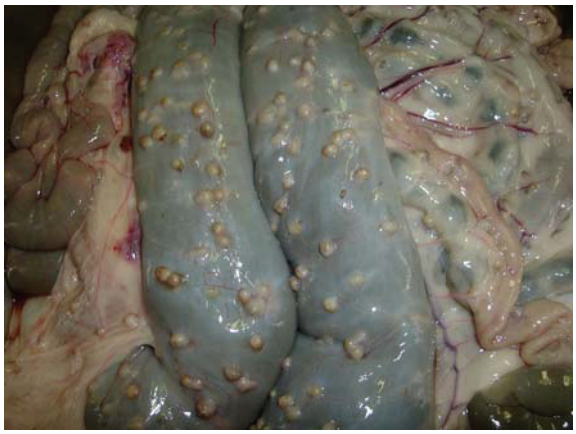


Figure 1: Representative image of the lung showing accumulation of blood in their parenchyma, characteristic of blood aspiration in goats and sheep slaughtered in the slaughterhouse under federal inspection in Bahia state, Brazil.

With regard to intestinal parasites, it was observed that this was the major cause of condemnation (57.5%) in sheep slaughtered during the study period, with percentages higher than those found in goats (31.8%). Figures 2 and 3 shown representative images of intestines condemned due to intense parasitism.



Figures 2 and 3: Representative images of intestines with lesions caused by parasites in goats and sheep slaughtered, from August 2005 to February 2006, in the slaughterhouse under federal inspection in Bahia state, Brazil.

DISCUSSION

In the slaughter plant under study carried out the stunning of animals through electro, which is produced by passing alternating electrical currents through the brain. The electric discharge acts as a cardiac stimulant and peripheral vasoconstrictor, resulting in a rapid elevation of blood pressure (8). The aspiration of blood is common in animals as a result of the section of the trachea in the act of bloodletting and that similar lesions occur in various organs and tissues, especially in sheep, when subjected to high voltages on the electro, is often observed multiple bleeding capillaries (6). Although the electro can cause bleeding in some animals, based on results found in this study, no correlation could be established as the

conviction rates of the lungs by inhalation of blood and this technique of desensitization.

Although this study has not been possible to correlate this type of circulatory disorders in the presence of blood in the lungs of slaughtered animals, it is believed that human failures and / or technological process during the electro and / or bleeding of the animals may be associated with high percentage of conviction. Accordingly, it is suggested that new techniques for stunning and / or bleeding of small ruminants are studied as well as professionals involved in these transactions are properly trained in order to minimize the conviction rates of lung for this particular cause. Besides the economic advantages brought about by reducing the number of convictions, the stunning / bleeding effectively reduces the unnecessary suffering of animals, the essential foundation for humane slaughter, as recommended in Brazilian law (2).

During the adaptive process of sheep in the tropics, animals that survived under these environmental conditions have become less resistant to diseases, particularly those parasitoses (5). This may be due to the decrease in quality and forage availability observed in the dry season of semi-arid northeastern Brazil, which leads to a picture of malnutrition in the animals at this time. At the beginning of the rainy season, these animals usually are weak and therefore more susceptible to infections. During the rainy season is an increase in rainfall in semi-arid region, promoting the development and survival of infective larvae on pasture, resulting in increased infection of animals through the ingestion of these larvae, contributing to a greater number of cases of intestinal worms, as has been observed in this study. However, considering the higher susceptibility of sheep to intestinal parasites, combined with possible failures of management, it is suggested that different schemes are adopted for the species worming goats and sheep, since the high percentage of conviction of sheep slaughtered in slaughter plant where this study was conducted may be associated with this type of problem, giving significant losses to producers and state and national economies

CONCLUSIONS

In this study we observed that the main causes of condemnation both in goats as in sheep slaughtered in a slaughter plant under federal inspection in Bahia state, Brazil, were aspiration of blood into the lungs and intestinal parasites. Finally, we emphasize that the role of the veterinarian in activities related to sanitary inspection of animal products is critical to preserving the health of population and economic growth of the country.

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IDENTIFICATION OF MEAT SPECIES IN SOME RAW MEAT PRODUCTS IN ASSIUT CITY, EGYPT

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SUMMARY

By using Agar gel immunodiffusion (AGID), higher adulteration rate occurred in raw kofta with chicken was detected at 34%, while with pork at 26%. Donkey detected only in beef burger at 2%. Higher adulteration rate with polymerase chain reaction (PCR) was detected in beef burger with chicken at 69%, in raw kofta with pork and donkey at 45.5% and 18%, respectively.

INTRODUCTION

A number of analytical techniques have been developed for the inspection and detection of these illegal meats and feed products. Overall, these methods can be classified into five types: chromatography, electrophoresis, spectroscopy, immunochemical techniques including enzyme-linked immunosorbent assay (ELISA) kits and deoxyribonucleic acid (DNA) based techniques [5].
Recently, due to various constraints in identification of meat species, DNA-based techniques were widely utilized due to stability at high temperature and highly conserved structure of DNA within all tissues of an individual [4].
The objectives of this study were carried out to fulfill the following; estimation of the adulteration in meat products in Assiut retail markets and detection of the banned animal tissues, estimation of the accuracy of meat products labeling in samples collected from Assiut city retail markets and comparison between the results of identification of species by AGID and PCR technique.

MATERIALS AND METHODS

Experimental animals: Female New Zealand white breed rabbits at (10 – 12 weeks old) were used. Rabbits were divided into groups according to the number of antigens used. Three rabbits used for each group and 3 rabbits as control. Rabbits were immunized for production of the target antisera.

Meat antigens: Antigens from beef, chicken, pork and donkey meats were prepared and kept frozen at -20 until be used.

Samples: Two hundreds beef meat products samples of minced meat, raw kofta, sausages and beef burger (50 of each) were collected from Assiut city retail markets and analyzed for detection of meat adulteration with other meat species.

Preparation of antigen or meat extraction: Two hundreds of meat products samples of beef burger, sausages, minced meat and raw kofta were tested for presence of the previously mentioned antigens. The samples were prepared for immunization of rabbits according to [3 & 6].Testing of a blood sample by taking the sample from marginal ear. Bleeding of rabbits occurred by 10-14 days after the last injection. To prevent contamination add sodium azide 0.02 % ,[2].

Methods for species identification: By AGID [6].Fifty samples were chosen from the suspected and negative adulterated samples previously examined by AGID to be reexamined by PCR. QIAamp DNA Mini Kit (Catalog no. 51304, Qiagen Pvt. Ltd) was obtained for extraction of DNA from tissue samples.
Three oligonucleotide primer sets (synthesized by Sigma Genosys and MWG-Biotech AG, USA) for amplification of sequences for detection of chicken, pork and donkey.

Details of primers used for PCR

Species (bp)	Product size	Sequence	Name
Chicken	420	CCTAGCCCTAAATCTAGATACC	Ch-F
		TTTTGAGGGTGACGGGCGGTGT	Ch-R
Donkey	350	AAAATAGCTCACATAACAAAGC	Don-F
		TTAATTTACTACTAAATCCTCC	Don-R
Pork	343	TTCAAACTGGGATTAGATACCC	Po-F
		TGAGGGTGACGGGCGGTGTGTG	Po-R

Steps of PCR:
Initial denaturation at 94°C for 4 minutes, then denaturation at 94°C for 1 minute, Annealing at 55°C for1 minute, extension at 72°C for 30 seconds and final extention at 72°C for 10 minutes. The basic three steps; denaturation , annealing and extension repeated 35 cycles.

RESULTS

Table 1: Incidence of adulteration of minced meat, raw kofta, sausage and beef burger samples examined by AGID).

Species	Minced meat		Raw kofta		Sausage		Beef burger		Total*	
	No	%	No	%	No	%	No	%	No	%
Beef	50	100	50	100	50	100	50	100	200	100
Chicken	3	6	17	34	16	32	16	32	52	26
Pork	3	6	13	26	7	14	1	2	24	12
Donkey	-	-	-	-	-	-	1	2	1	0.5

Table 2: Incidence of adulteration of minced meat, raw kofta, sausages and beef burger samples examined by PCR.

Species	Minced meat		Raw kofta		Sausage		Beef burger		Total	
	No	%	No	%	No	%	No	%	No	%
Chicken	8	57	7	63.6	8	66.7	9	69	34	68
Pork	5	35.7	5	45.5	5	41.7	3	23	18	36
Donkey	1	7	2	18	1	8	1	7.7	5	10

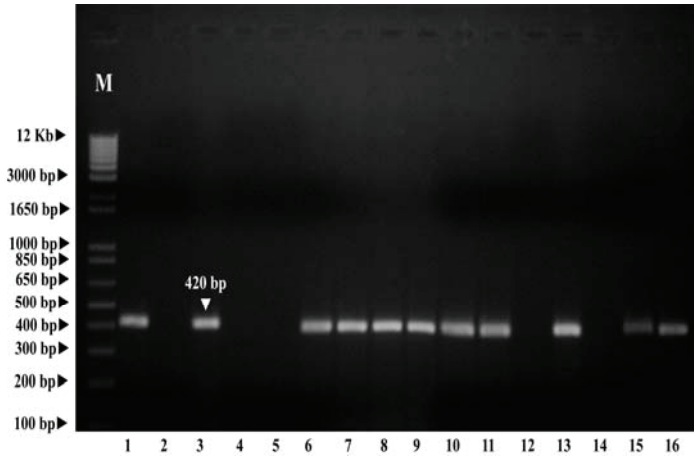

Figure (1): Agarose gel electrophoresis of PCR amplicon (420bp) showing chicken adulteration in samples No. from 1 to 16 at lanes 1, 3, 6, 7, 8, 9, 10, 11, 13, 15and 16. Lane M, 1kb plus DNA ladder.

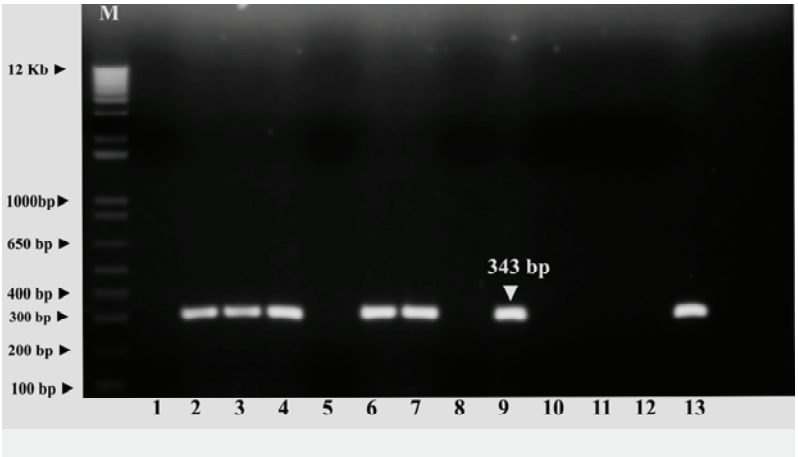

Figure (2): Agarose gel electrophoresis of PCR amplicon (343bp) showing pork adulteration in samples No. from 1 to 13 at lanes 2, 3, 4, 6, 7, 9 and 13. Lane M, 1kb plus DNA ladder.

DISCUSSION

The results of the examined samples with AGID are recorded in (Tables 1). Identification is possible by electrophoresis of PCR products. Beef, chicken, pork and donkey can be qualitatively identified and differentiated by PCR. Samples appeared to be negative or suspected to be adulterated when examined by AGID method. The results of PCR showed that the adulteration rates for minced meat, raw kofta, sausages and beef burger with chicken were 57%, 63.6%, 66.7 %and 69% respectively (Table 2, figure 1). By using PCR, the incidence of adulteration rates for minced meat, raw kofta, sausage and beef burger with pork were 35.7%, 45.5%, 41.7% and 23%, respectively (Table 2, figure2). The obtained results agree with the finding obtained by [1]**,** who found that the most common species were pork and beef mixed. The adulteration rates with donkey were 7% for minced meat, 18% for raw kofta, 8% for sausages and 7.7% for beef burger (Table 2).

CONCLUSION

Beef, chicken, pork and donkey can be qualitatively identified and differentiated by PCR**.** It was noticed that the sensitivity and accuracy of PCR in detection of species of meat and its adulteration greatly overcome potency of AGID test.

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DIVERSITY OF *LISTERIA MONOCYTOGENES* FISH AND SEAFOOD ISOLATES DETERMINED BY MOLECULAR SUBTYPING (Abstract)

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Listeriosis is a rare but severe illness with a mortality rate of 20- 30%. This disease is caused by the environmental widespread bacterium *Listeria monocytogenes*, and many studies have shown that both sporadic and epidemic cases of listeriosis in humans are mainly of food-borne origin. Soft cheese, milk and milk products, meat and meat products, vegetables, and fish and seafood were always counted to the high risk food. Especially, ready-to eat fish and seafood products have been shown to be often contaminated with *L. monocytogenes*. In addition, *L. monocytogenes* can grow to significant levels under storage conditions on such products, even in pre-packaged food. The aim of the present work was to examine the diversity of *L. monocytogenes* strains from fish and seafood products. For this purpose, *L. monocytogenes* isolates recovered from fish and seafood products available in Austria were typed by API Listeria, classical O:H serotyping, serovar-PCR, pulsed-field gel electrophoresis (PFGE) according to CDC protocol and a strain subset by Multilocus Sequence Typing (MLST). A special focus of the study was to analyse the potential persistence of strains in products of single producers. Furthermore, a subset of isolates from fish and seafood products was tested for virulence genes by PCR. An electronic strain database library was created for tracing similar PFGE patterns e.g. in fish and seafood products in the future. Preliminary results of 58 *L. monocytogenes* strains reveal short and long-time persistence in smoked salmon and trout samples of six producers at retail level. Serotyping showed an equal proportion of genetic linage I (1/2b, 4b) and linage II (serovar 1/2a) strains. A low number of cases have been linked to fish associated listeriosis outbreaks when compared to listeriosis outbreaks associated with other foods. Some authors showed that especially persistent strains have a lower virulence potential in cell assays. To gain more insight into virulence of the *L. monocytogenes* fish isolates included in this study a comparison with clinical isolates in a virulence model should be performed.

1ST NATIONAL RING TRIAL ON DETECTION OF ANTIBODIES TO *TRICHINELLA* IN PIGS

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SUMMARY

Regulation (EC) 2075/2005 ensures official inspection of food of animal origin with specific rules on official controls for *Trichinella* in meat. Fattening pigs with a negligible risk need not be examined for *Trichinella* if the farm is subjected for a monitoring by use of digestion or serological methods. The aim of the ring trial was to evaluate a new ELISA regarding test accuracy and practical usage. The participants of the ring trial tested 22 sera prepared by the German National Reference Laboratory for Trichinellosis and additionally 22 field samples from their own sample collection using the commercial ELISA kit. This ELISA demonstrated a very good diagnostic sensitivity and robustness in the ring trial.

INTRODUCTION

Trichinellosis is a rare food-borne disease caused by nematodes of the genus *Trichinella*. Most human infections are related to the consumption of raw or insufficiently heated meat (products) from high risk areas such as Eastern Europe. According to Regulation (EC) 2075/2005 fattening pigs with a negligible risk need not be examined for Trichinella if the farm is subject to a monitoring by use of digestion or serological methods. ELISAs for the diagnosis of infection with *Trichinella* in swine are well established [2, 4]. To evaluate a new commercial ELISA a ring trial was organised by the German National Reference Laboratory for Trichinellosis with 21 participating laboratories in September 2009.

MATERIAL AND METHODS

21 laboratories from 11 states of Germany tested 22 serum samples from experimentally infected pigs, as well as 212 serum, 33 plasma and 169 meat juice samples from pigs and 26 sera from wild boars, respectively, from their routine submission using the PIGTYPE® Trichinella Ab ELISA (Labor Diagnostik GmbH Leipzig). The number of correct, false positive and false negative results per laboratory was compared to the sample status obtained by the German National Reference Laboratory for Trichinellosis. The repeatability of the assay was analysed by calculation of the coefficient of variation for the OD-values. Additionally z-scores were calculated to determine deviation of the laboratory mean from the overall mean and the result variance of the labs was compared to the mean variation by Mandel´s k [1, 3]. Pearson correlation coefficient was determined to evaluate the reproducibility of the test results in different laboratories.

RESULTS/DISCUSSION

14 of 21 participants reported all results for the reference samples as expected. Incorrect result calculation and testing performed by two different lab technicians were identified as one cause of laboratories reporting false positive or false negative results. All tested field samples but four wild boar samples scored negative. Overall the ELISA results for laboratories demonstrate a good sensitivity and specificity of ELISA with 98.93% and 95.39%, respectively. As only 6.8 % of the sera showed a variation coefficient above 30 %, the repeatability of the ELISA was good for the participating laboratories [5]. Z-scores and Mandel´s k were calculated to analyse the variability of the test results in more detail and also demonstrated that the test results of both tests were reproduced by most laboratories.

CONCLUSION

Taken together the PIGTYPE® Trichinella Ab showed a stable performance in both repeatability and reproducibility in this ring trial. The close correlation for S/P ratios between participants and the reference laboratory also demonstrate a good performance of the ELISA.

In conclusion monitoring of pigs determined for human consumption for *Trichinella* by serological examination using ELISA seems to be a suitable tool, since the method is well established and standardized.

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POSSIBILITY OF ELECTROLYZED OXIDIZING WATER DECONTAMINATION OF POULTRY MEAT

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SUMMARY

Introduction: Durability of poultry meat is due to contamination of different microorganisms. Contamination of chicken carcasses with intestinal contents during the slaughter happened frequently. They report about 40 – 85% contamination with *Campylobacter spp.* Neutral electrolyzed oxidizing water (NEOW) is a new generation of biocide which mainly operates on the abstraction of electrons from the environment does not leave residuals on the land and the land it is not necessary to rinse. We investigated whether the NEOW used for washing carcasses of chickens contaminated land and whether this increases the stability and safety of food.

Materials and Methods: The investigation was taken randomly selected parts of a chicken flock, which was selected parts of the skin, without skin. We randomly selected control samples and samples that were previously treated with 5% NEOW. All samples were then sealed with gastro closure procedure (70% N_2 and 30% CO_2) and stored at +4 ° C. The samples were studied from first to tenth day of storage, and observed count and the presence of bacteria *Campylobacter jejuni | coli*.

Results: On average, we found a reduction of the total number of microorganisms on parts of the meat with the skin. We found a reduction of micro-organisms between 77.62 to 86.17%.

Conclusions: The results suggest the possibility of NEOW on chicken carcases and parts of the meat with the skin. We believe that to achieve extended shelf life of foods. Equally, using NEOW can reduce the presence of *Campylobacter jejuni | coli* during the working process. The potential to achieve greater safety and health of the consumer more control in preventing food-borne infections.

INTRODUCTION

C. jejuni and *C. coli* cause the most common gastrointestinal bacterial zoonosis. In 2009 the incidence rate within the EU countries was 43.9 per 100.000 population. (1). Salmonelosis, the second most frequent zoonosis achieved the incidence 23.7 per 100.000 population. Although the disease usually occurs sporadically (2), it can appears also as an outbreak. Main source of the infection with Campylobacter is the poultry meat. Many broiler chicken flocks are infected with the bacteria *C. jejuni* and *C. coli* (1, 2). The researchers are indicating that 10.8 – 90 % of the poultry meat is contaminated with the above mentioned microorganisms. The contamination occurs during the slaughtering of the animal – the critical points are hanging of the animal, stunning, bleeding to death, mating, depluming, evisceration and meat chilling. The most important role has the exenteration, because the intestinal injury occurs frequently, which is followed by the leakage of the intestinal content all over the skin and into the pectoral-abdominal cavity. During the cutting of the poultry carcasses there is also a possibility of the cross-contamination (4). Campyobacters are termolabel; the most common cause of the infection is inappropriate thermic treatment of the meat and the cross-contamination during the foodstuff preparation.

It is well-known that many factors, e.g. the presence of carbon dioxide and oxygen, cooling, freezing, heating, drying, disinfection etc. have a major impact on the survival of the microbes.

In last 15 years many studies have been done on the survival of the *Campylobacter* spp. on the poultry carcasses.

Thus electrooksidyzing water act with a number of mechanisms:

• Redox potential: EOV has a pronounced lack of electrons. Therefore, it has a tendency to electrical neutral environment, which can be achieved by acquiring electrons from the environment. If they find themselves near the EOV microorganisms, they will pick up electrons from the membrane, thus disturbing the micro-organism collapses and such.

• Oxide and superoxide ions: The formation of oxide and superoxide ions in the EOV group classifies biocidal resources that emit oxygen. Notable features of this group are fast-acting, degradation products are ecologically acceptable. Major disadvantages of this group are violent reaction to the presence of organic matter and method of storage (requires special packaging with breathers that emit excessive pressure generated in the package), corrosively and respiratory irritant, and caustic. All these disadvantages of EOV is not found.

• Chlorine, chloride and sodium hypochlorite: These compounds formed by electrolysis and it rank among the biocidal compound. However, the quantities generated in the electrolytic are low.

• Acidity: Acidity is also an important factor in disinfection. pH of 1-2 is highly acidic and has been very low values to some extent biocidal action. Due to the low pH, the areas exposed to corrosive action. Recently in this area progress has been made. EOV is being produced in the physiological pH (6.6-6.7). Thus, in concentrated form has no corrosive action.

The principle of obtaining water elektrooksigenirane (EOV) is known for a long time. Basically, obtained from a solution of table salt without iodine and water alkaline and acidic product, where an alkaline pH range fraction 11-12, while acidic pH reaches 1-2. Alkaline phase is attributed to a treatment effect, while the phase distinctly acidic biocidal effect. They are generally attributed to the performance of only pH changes. However, closer analysis revealed that a key factor in the operation of the biocidal redox potential, formation of oxide and superoxide ions in small quantities, but there are also some chlorides of sodium hypochlorite and chlorine.

In the beginning, EOV produce for direct application for water in plumbing systems. Here he used the so-called "Aqueous two" EOV acquisition system, where they were concentrated 1-2 acidity and redox potential of +1200 mV. Disadvantages are mainly the corrosiveness and the relative instability of EOV, it was necessary EOV used immediately after its manufacture.

The latest acquisition system EOV made at these "Four aqueous" system, where the physiological acidity of EOV is not corrosive, but is acquired resistance, which reaches with the present data at least 10 months. Thus it can be packaged in special plastic packaging.

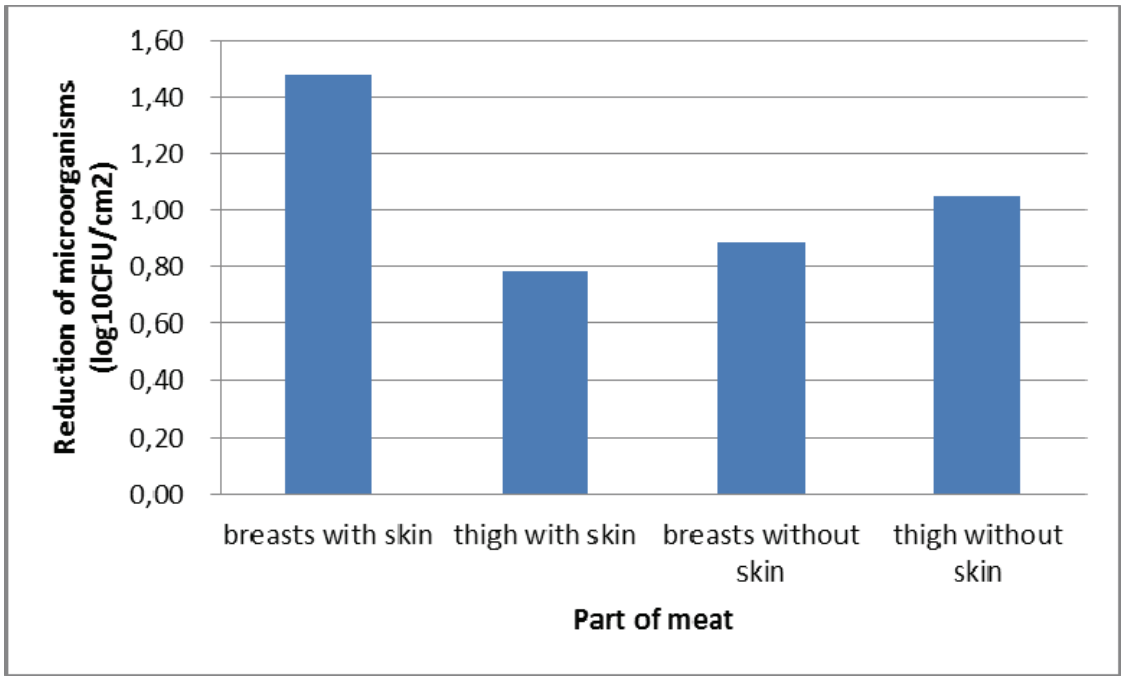
MATERIAL AND METHODS

Experiment was conducted in a poultry slaughterhouse. After cutting up chicken carcasses, the trays are loaded by meat with skin and without skin. Chicken breast and legs with skin and without skin were packed. This was followed by preparation of 5% solution of EOV. Tested samples were spraying with hand sprayer and so effloresced meat surface. Subsequently, the meat pieces were packed in special atmosphere with 70% N_2 and 30% CO_2. Packed meat was chilled at +4 ° C and transport to the laboratory in cooling bags.

In the laboratory, samples were daily opened and examined for total count of microorganisms, presence of *Campylobacter spp.* and controlled the organoleptic quality of meat.

RESULTS

The experiment was concluded in 10 days. Comparing the results of total count we observed a reduction of contamination on meat with the skin, between 0.78 to 1.48 $\log_{10}$ CFU/g, after 10 days. On the meat without skin the contamination with micro-organisms was reduced from 0.89 to 1.05 $\log_{10}$ CFU/g.



The untreated control meat samples were tested for the presence of *Campylobacter* spp. The contamination was low (10-40 CFU/g). After treatment with EOV Sun campylobacters were not isolated.

Organoleptic examination of threated meat samples we did not showed any change in the color or smell.

DISCUSSION AND CONCLUSIONS

First results of the EOV treatment of meat chickens to reduce the presence of microorganisms, showed encouraging results. After using EOV, the contamination of meat surface as concerned total count and *Campylobacter* spp. was lower. The contamination of the packaged chicken meat in controlled atmosphere was stable for at least 8 days. According to the results we can conclude that durability of meat packaged that way could be prolonged. t. After 10 days the total number of microorganisms did not exceed $4x10^5$CFU/g at treated meat, while the values of control group (not treated) rise to $3x10^6$CFU/g. The organoleptic characteristics were not changed. Due to our results we can conclude, that the use of EOV in the process of slaughtering can extended the durability of fresh meat.

Adding the EOV to tap water when washing carcasses after slaughtering can reduce the number of microorganisms on poultry carcasses.

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MICROBIAL CONTAMINATION OF HONEY OF LATVIA (Abstract)

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INTRODUCTION

Honey is the natural sweet substance produced by honeybees from the nectar of floral parts. Honey is a most commonly consumed in its unpreserved state. Due to the natural properties of honey, it is a product with minimal types and levels of microbes. Known that microorganisms in honey may influence safety. The aim of the study was to clarify distribution of microorganisms in honey and bees and to determine changes of microbiological contamination of honey during the storage.

ANIMALS, MATERIALS AND METHODS

A total of 38 honey samples (6 samples of honey in combs and 32 samples of strained honey) were bought from three apiaries of Vidzeme region in Latvia. Six bees samples were aseptically collected directly from bee houses. All samples were collected and transported in sterilised bottles. Samples for microbiological examination were analysed in the Research Institute „Sigra". The standard plate count method was used for culturing and isolating the different microorganisms, but microbiological tests for specific genus were performed in accordance with generally accepted methods. The pH was measured using a pH meter.

RESULTS

The results showed that the total plate counts found in the honey samples vary from zero to 2700 (average 174) CFU/g. The average total plate count in honey in combs (average 420 CFU/g) was lower than in strained honey (average 590 CFU/g). We can't found statistically valid results ($p>0,1$) regarding increasing microbiological contamination of honey during the storage 3 months (average 387 CFU/g) and essential reduction after storage 1 year (average 76 CFU/g). The highest bacteriological contamination were establish in honey harvested in May (average 884 CFU/g), but lowest – in June and July honey (average 55 CFU/g). The bacteriological examination show a considerable contamination with microorganisms in the samples of bees and honey *Aeromonas spp., Corynebacterium spp.*, *Enterococcus spp.*, and *Staphylococcus spp*, but in the honey samples were isolated *Bacillus spp., Escherichia coli, Yersinia spp., Klebsiella spp., Micrococcus spp.* and *Weeksella spp.*. The mean pH values of honey were from 5 to 6,5 (average 6,1).

CONCLUSION

Honey obtained from apiaries in Latvia is contaminated with microorganisms indicating inadequate hygiene condition during harvesting, handling, processing and/or storage.

OCCURRENCE OF LISTERIA MONOCYTOGENES IN POULTRY, FISH & THEIR PRODUCTS AS WELL AS ITS PUBLIC HEALTH HAZARD ON WOMEN

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SUMMARY

The present study was undertaken to determine the incidence and distribution of *Listeria* spp. in poultry and some food samples and to investigate listeriosis in pregnant women and their newborns. Genotyping of *L. monocytogenes* isolates were determined to detect *inlA* gene as a target by using polymerase chain reaction. 400 samples comprising, poultry (100), chicken pâté (50), hen's egg (100), fish (100) and smoked herring (50) were collected from different poultry slaughter houses, shops, supermarkets and fish markets in Assiut province, Egypt. The study also, included 25 women suffered from intrauterine fetal death, 25 premature labored women and their 25 newborns admitted to Special Care Baby Unit (SCBU), Assiut University Hospital. The overall incidence of *Listeria* spp., *L. monocytogenes*, *L. innocua*, *L. ivanonii*, *L. welshimeri*, *L. seeligeri* and *L. grayi* was 81 (17.05%), 15 (3.15%), 40 (8.42%), 4 (0.84%), 11 (2.7%), 10 (2.1%) and 1 (0.21%), of the all examined samples respectively. The study revealed that 10%, 10% and 57% of poultry, hen's egg and fish samples were contaminated with *Listeria* spp., respectively and *Listeria* was not detected in chicken pâté, egg contents and smoked herring samples. *L. monocytogenes* was isolated from 2%, 4% and 7% of the examined poultry, eggs and fish samples, respectively. Incidence of human listeriosis was 5.3%, *L. monocytogenes* was isolated from 2 (2.6%) of both a woman suffered premature labor and her newborn while, *L. innocua* was isolated from 2 (2.6%) of women suffered intrauterine fetal death. Out of 15 *L. monocytogenes* isolates detected, 6 (40%) were found to harbor *inlA* gene. The existence of *Listeria* species and *L. monocytogenes* in the examined food samples warrants the need for appropriate control measures as this would pose a serious threat to human health.

INTRODUCTION

L. monocytogenes is the primary human pathogen although there have been several reports of illnesses caused by *L. seeligeri, L. ivanovii*, *L. innocua, L. welshimeri* and *L. gyayi* in human **[41**& **16].** *Listeria* species are tolerant to extreme conditions such as low pH, low temperature and high salt conditions **[27].** Therefore, they can be found in a variety of environments, including soil, sewage, silage, water, effluents and foods. There has been more attention given to incidences of *Listeria* occurring in raw poultry and other poultry products **[42]** and *L. monocytogenes* was the most common bacterial contaminant on broiler chickens at slaughterhouses **[26].** Eggs may constitute, a public health hazard. So, it was noted that presence of *L. monocytogenes* in eggs is most likely due to contamination from the shells during the breaking process or from the processing environment **[11]**. Since *L. monocytogenes* is commonly found in surface waters of lakes, fish captured or cultivated in these waters may possibly carry this microorganism **[8]**. Several studies have implicated *L. monocytogenes* in fish and smoked fish [**38, 34** & **39].** Although *L. monocytogenes* is infective to all human population groups, it is more likely to cause severe problems among pregnant women, immunocompromised individuals, the elderly and neonates and in these groups, the mortality from listeriosis is high, typically 20-30% **[32** & **22].** The present study was undertaken to determine the incidence and distribution of *Listeria monocytogenes* in poultry, eggs and poultry product, determine the incidence and distribution of *L. monocytogenes* in fish and fish product, investigate listeriosis in pregnant women and their newborns and genotype of the *L. monocytogenes* isolates to detect *inlA* gene.

MATERIALS & METHODS

A sum of 400 random samples of poultry, eggs, fish and some products (chicken pâté and smoked herring) were collected for this study, including, poultry wings (25), its legs (25) and its intestinal contents (50), chicken pâté (50), egg shell (50), egg content (50), *Tilapia nilotica* slime (25) and its intestine (25), *Clarias angiullaris* slime (25) and its intestine (25) and smoked herring (50). Samples were collected from different poultry slaughter houses, shops, supermarkets and fish markets in Assiut province. The human samples included 2 groups; the first group consisted of 25 pregnant mothers suffered intrauterine fetal deaths (IUFD) during 2nd and 3rd trimester from Obstetric and Gynecology Department at Assiut University Hospital. While, the second group consisted of 25 pregnant mothers with preterm labor (more than 28 weeks and less than 37 weeks) and 25

blood samples from their newborns admitted to Special Care Baby Unit (SCBU), Assiut University Hospital. All mothers were questionnaired for residence, maternal age, bad obstetric history like recurrent abortions or still birth and attack of fever or flu-like illness. The vaginal swabs and neonatal blood samples were collected by the obstetrician.

The Isolation of *Listeria species* have been done by using Selective enrichment procedure and Cold enrichment procedure according to [**18 & 21]**. The identification of the listeria species was done by using gram stain, motility test **[30**], Urease test, Catalase test **[10],** Sugar fermentation test **[7],** Beta hemolysis**,** Nitrate reduction tes**t** and The Christie-Atkins-Munch-Peterson (CAMP) test **[6 & 7]**. Polymerase chain reaction (PCR) was applied according to [**1]** using oligonucleotide primers sequence of the inlA gene **[35]** to amplify a 760 bp fragment that corresponds to the region of repeats B of inlA gene. The reaction products were separated by electrophoresis, visualized by UV transilluminator (Biometra) and photographed by Gel Documentation System containing BioDocAnalyze (BDA) Software (Biometra, 035-114) for measuring and analyzing the PCR results.

RESULTS

Table (1): Distribution of *Listeria* species in the examined samples

Sample type	No. of samples	Recovered *Listeria* species											
		L. monocytogenes		*L. innocua*		*L. ivanonii*		*L.welshimeri*		*L. seeligeri*		*L. grayi*	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Poultry	100	2	2	2	2	-	-	3	3	3	3	-	-
Chicken pate	50	-	-	-	-	-	-	-	-	-	-	-	-
Hen's egg	100	4	4	3	3	1	1	-	-	2	2	-	-
Fish	100	7	7	33	33	3	3	8	8	5	5	1	1
Smoked herreing	50	-	-	-	-	-	-	-	-	-	-	-	-
Human	75	2	2.7	2	2.7	-	-	-	-	-	-	-	-
Total	475	15	3.15	40	8.42	4	0.84	11	2.3	10	2.1	1	0.21

Table (2): Incidence of *Listeria* species in the examined poultry samples

Sample type	No. of Samples	Recovered *Listeria* species								Total	
		L. monocytogenes		*L. innocua*		*L.welshimeri*		*L. seeligeri*			
		No.	%	No.	%	No.	%	No.	%	No.	%
Poultry wings	25	-	-	1	4	1	4	1	4	3	12
Poultry legs	25	-	-	1	4	1	4	1	4	3	12
Poultry intestine	50	2	4	-	-	1	2	1	2	4	8
Total	100	2	2	2	2	3	3	3	3	10	10

Table (3): Incidence of *Listeria* species in hen's egg

Egg sample	No. of Samples	Recovered *Listeria* species								Total	
		L. monocytogenes		*L. innocua*		*L. ivanonii*		*L. seeligeri*			
		No.	%	No.	%	No.	%	No.	%	No.	%
Egg shell	50	4	8	3	6	1	2	2	4	10	20
Egg contents	50	-	-	-	-	-	-	-	-	-	-
Total	100	4	4	3	3	1	1	2	2	10	10

Table (4): Distribution of *Listeria* species in the examined fish

Samples	No.	%												Total	
		L. monocytogenes		*L. innocua*		*L. ivanonii*		*L.welshimeri*		*L. seeligeri*		*L. grayi*			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No	%
Tilapia nilotica slime	25	2	8	7	28	1	4	2	8	2	8	1	4	15	60
Clarias angiularis slime	25	4	16	10	40	2	8	2	8	2	8	-	-	20	80
Tilapia nilotica intestine	25	-	-	3	12	-	-	2	8	-	-	-	-	5	20
Clarias angiularis intestine	25	1	4	13	52	-	-	2	8	1	4	-	-	17	68
Total	100	7	7	33	33	3	3	8	8	5	5	1	1	57	57

Table (5): Distribution of *Listeria* species in women and feti

Source of samples	No. of samples	Recovered *Listeria* species				Total	
		L. monocytogenes		*L. innocua*			
		No.	%	No.	%	No.	%
Women with IUEF (vaginal swap)	25	-	-	2	8	2	8
Women with premature labor (vaginal swap)	25	1	4	-	-	1	4
Premature labored feti (blood)	25	1	4	-	-	1	4
Total	75	2	2.7	2	2.7	4	5.3

Table (6): Clinical summary of individual's history

Patient	Age	Pregnancy	History of previous pregnancies	Symptoms	Complain at time of sampling	Examined samples		Recovered *Listeria spp.*
						Vaginal swabs	Neonatal blood	
1st	25	2nd pregnancy	Normal	Flu-like symptoms	Premature labor	+	+	*L.monocytogenes*
2nd	32	3rd pregnancy	Normal	Normal	Intrautrine fetal death	+	-	*L. innocua*
3rd	29	3rd pregnancy	Normal	Normal	Intrautrine fetal death	+	-	*L. innocua*

Table (7): Residence-wise incidence of *Listeria* species in women

Residence	No. of samples	*L.monocytogenes*		*L. innocua*		Total	
		No.	%	No.	%	No.	%
Rural	26	1	3.84	2	7.69	3	11.53
Urban	24	-	-	-	-	-	-
Total	50	1	2	2	4	3	6

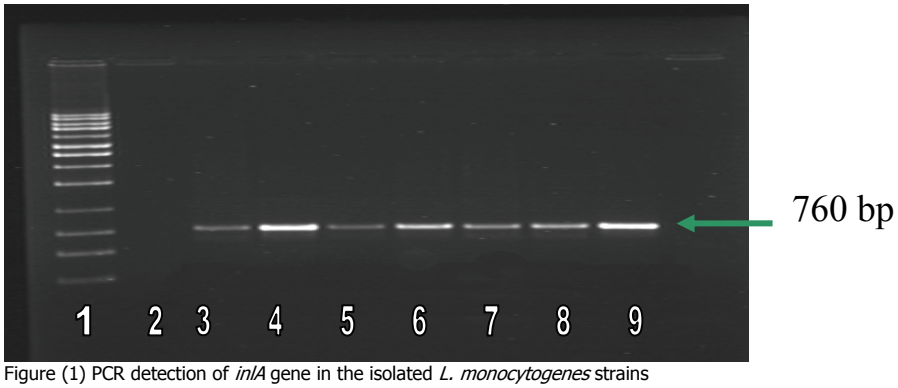

Figure (1) PCR detection of *inlA* gene in the isolated *L. monocytogenes* strains

Lane 1: DNA Ladder (300 bp to 10,000 bp), Lane 2: negative sample, Lanes 3 and 5: *L. monocytogenes* isolated from poultry intestine, Lane 4: *L. monocytogenes* isolated from *Tilapia nilotica* slime, Lane 6: *L. monocytogenes* isolated from *Clarias angiularis* intestine, Lanes 7, 8 and 9: *L. monocytogenes* isolated from *Clarias angiularis* slime.

DISCUSSION

Listeria monocytogenes is an important food-borne pathogen and is widely distributed in food, environmental and clinical samples **[13].** Although *Listeria* have been less frequently identified compared to other food-borne diseases, they account for the majority of death of any food-borne pathogen resulting in a high mortality rate of about 30% **[29]**.

Concerning the distribution of *Listeria* spp. in the examined samples (Table 1); out of the 475 samples examined in this study, 15 (3.15%), 40 (8.42%), 4 (0.8%), 11(2.3%), 10 (2.1%) and 1 (0.21%) of the examined samples were contaminated with *L. monocytogenes*, *L. innocua*, *L. ivanonii*, *L. welshimeri*, *L. seeligeri* and *L. grayi*, respectively. According to [**46]**, the presence of any *Listeria* spp. may predict the presence of *L. monocytogenes.*

The main source of contamination of poultry and poultry products probably originates from improper cleaning and disinfection of the processing line and the environment and to a lesser degree from the broilers **[37].** In this study, *Listeria* was isolated from 10 (10%) out of 100 samples of poultry samples (Table 2). *Listeria* spp. isolated from the examined poultry samples were *L. monocytogenes* (2%), *L. innocua* (2%), *L. welshimeri* (3%) and *L. seeligeri* (3%). Incidence of *Listeria* spp. in poultry wings (meat and skin) was 12% including, *L. innocua* (4%), *L. welshimeri* (4%) and *L. seeligeri* (4%). However, *L. monocytogenes* was not isolated from the examined wings in this study. While in poultry eggs, the incidence of *Listeria* spp. was, 12% including, *L. innocua* (4%), *L. welshimeri* (4%) and *L. seeligeri* (4%). Poultry legs may be responsible for the contamination of chicken carcasses, and the surfaces that come into contact with these pieces of meat which play an important role in spreading *Listeria* spp. [**12].** The incidence of *Listeria* spp. in the examined poultry intestine was 8% including *L. monocytogenes* (4%), *L.welshimeri* (2%) and *L.seeligeri* (2%). The presence of *L. monocytogenes* in the intestinal contents of poultry may be attributed to the ingestion of *Listeria*-contaminated feed and soil **[19].** However, the intestinal tract of birds is a usual habitat of *L. monocytogenes* **[4]**.

Incidence of *Listeria* spp. in the examined egg shell samples was 20% (Table 3). The egg shell contamination observed in this study might be due to contamination with the bacteria present in the laying hen's environment. This is because *L. monocytogenes* is very frequently present in broiler poultry farms and flocks of laying hens **[5].** Also, no *Listeria* was isolated from the examined egg contents. Absence of *Listeria* in egg content may be attributed to the unsuitability of pH of raw egg albumen for growth of *L. monocytogenes*. Furthermore, presence of the antibacterial properties of eggs which hydrolyze the polysaccharide bacterial cell wall causing cell lysis **[47].**

The high incidence of the *Listeria* spp. (57%) obtained in the examined fish samples (Table 4), reflects the high level of contamination of the lakes or streams from which the fishes were caught and this may be due to disposal of sewage effluents in waterways, this suggestion agreed with **[43 & 44].** The incidence of *Listeria* species in *Clarias anguillaris* slime and *Tilapia nilotica* slime was 80% and 60%, respectively and the distribution of *Listeria* spp. in the *Tilapia nilotica* slime was 8%, 28%, 4%, 8%, 8% and 4% were contaminated with *L. monocytogenes*, *L. innocua*, *L. ivanonii*, *L. welshimeri*, *L. seeligeri* and *L. grayi*, respectively. While, 16%, 40%, 8%, 8% and 8% of the examined *Clarias anguillaris* slime samples were contaminated with *L. monocytogenes*, *L. innocua*, *L. ivanonii*, *L. welshimeri* and *L. seeligeri*, respectively. The incidence of *Listeria* species in *Clarias anguillaris* and *Tilapia nilotica* intestine was 68% and 20%, respectively. Only *L. innocua* and *L. welshimeri* were isolated from *Tilapia nilotica* intestine in a rate of 12% and 8%, respectively. While, 4%, 52%, 8% and 4% of the examined *Clarias anguillaris* intestine were contaminated with *L. monocytogenes*, *L. innocua*, *L. welshimeri* and *L. seeligeri*, respectively. Listeriae are ubiquitous organisms whose main reservoirs are soil, silage and water **[45].**

Listeriosis in human can result in premature labor, stillbirth and septicemia in premature infants. Several outbreaks of the disease have been associated with the consumption of foods contaminated with *L.*

monocytogenes **[33** & **36].** Incidence of human listeriosis in this study was 5.3%. Regarding, women examined in this study (Table 5 & 6), *L. monocytogenes* was isolated from 1 (2%) of the examined vaginal swabs, a woman at 2nd pregnancy, aged 25 year, suffered preterm labor for the first time. The obtained result agreed with **[43 & 2]** who mentioned that pregnant women are particularly prone to infection as the placenta provides a protective niche for the growth of *L. monocytogenes***.** Out of 25 examined neonatal blood samples only 1(4%), newborn of a woman at 2nd pregnancy, aged 25 year, suffered preterm labor for the first time proved to be infected with *L. monocytogenes*. This finding was agreed with that of **[15** & **9]** who reported that *L. monocytogenes* infection causes premature labor in pregnant mothers and the fetus suffers more damage than the pregnant mother **[23]** Moreover, transmission of infection to the fetus occurs through infection of the amniotic fluid from infected birth canal **[31].** It was found that all positive cases, in this study, were women coming from rural areas (Table7). *Listeria* organisms are widely disseminated in the rural environment **[14]** and, thus can contaminate food. Consumption of infected raw or improperly cooked food is a common in rural areas.

The PCR represents a rapid procedure with both high sensitivity and specificity for the immediate detection and identification of pathogenic bacteria from different food materials **[25 & 17].** In this study, a PCR detection system based on another gene of the virulence cluster of *L. monocytogenes*; the internalin (*inlA*) gene (Fig.1) **[40 & 1].** The internalin gene play a critical role in the pathogenesis of human listeriosis **[20]** and the risk should be evaluated not only on the basis of bacterial contamination level but also the functionality of internalin. The absence of *inlA* may be attributed to that these strains were avirulent while, its presence indicated that these strains were virulent. Moreover, the presence or absence of some *inlA* family genes are good indicators of the level of virulence of *Listeria* strains [**28].**

In this study we concluded that *L. monocytogenes* should be put in consideration in differential diagnosis of women suffering from abortion, intrauterine fetal death and preterm labor. Certain recommendations should be followed to safe guard consumers from listeriosis and to prevent contamination of food-products with *L. monocytogenes* during the manufacturing processing.

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CORRELATIONS BETWEEN FRESHNESS OF BROILER CHICKEN LIVER AND FOOD SAFETY

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SUMMARY

The liver of broiler chicken is an important foodstuff due to the high amount of nutrient contained. As the liver is a perishable organ, the conservation rules must be strictly observed, in order to follow the food safety principles and to avoid the potential damage to consumer's health. This investigation proposed to link the assessment of freshness degree in liver of broiler chicken submitted to different conservation methods (refrigeration, freezing, defrosting) with the hepatocyte degeneration.

Seventeen livers harvested immediately after slaughter from young broilers, have been submitted to refrigeration (0-4°C). After 72 hours of refrigeration, three of these livers have been put to freezing temperature (-18°C) for 24 hours. Then, these samples have been examined after 24, 48 and 72 hours after defrosting (thawing in 0-4°C). The refrigerated samples have been monitored at different hours (1; 7; 25; 31; 37; 43; 49; 55; 61 and 73 hours). First, gross assessment was performed for the refrigerated and defrosted samples. For all liver samples, the imprints of liver were used for cytological exam (May Grünwald Giemsa stain). The changes of the hepatocyte structure inducted by conservation techniques were considered.

Between the first and fifty-fifth hour of refrigeration, the liver had the following features: elastic consistency, normal brownish gray color and normal, fresh smell. The cytological exam has revealed normal shaped hepatocytes with clearly featured nucleus (central position into the cell, fine arranged chromatin). Between the sixty-first and seventy-third hour of refrigeration, the consistency became friable, with darker nuances of color and characteristic smell of putrefaction process. The cytological exam has shown nude nuclei and a high number of rot bacteria. Generally, defrosted samples presented the hepatocytes with normal morphology, minor alteration of nuclei and bacterial contamination.

Gross assessment has shown that between the first and fifty-fifth hour of refrigeration, the liver is a wholesome product. Cytological investigation has shown that the liver contained a high number of bacteria after 61 hours of refrigeration, which makes it unsafe for consumption. The freezing process had a beneficial effect in order to maintain the safety of broiler liver.

INTRODUCTION

The liver of broiler chicken is an important foodstuff due to the high amount of nutrient contained. As the liver is a perishable organ, the conservation rules must be strictly observed, in order to follow the food safety principles and to avoid the potential damage to consumer's health. The increasing consumer's demands require the slaughterhouses' owners and processors of poultry meat to provide high quality and hygienically safe products. Thus careful monitoring should be imposed on all factors, the process flow, which could in any way affect the finished product, by applying the principle "from farm to table". [1].

Also the liver lesions can cause impairment of quality of the organ and thus unable to be given for human consumption.

MATERIAL AND METHODS

This study was conducted on two types of samples, using the same examination protocol.

The first category of samples was represented by livers harvested immediately after slaughter from young broilers. The samples were collected from a midlle sized slaughterhouse from Vrancea county Romania.

The second category of sample was represented by refrigerated broiler liver coming from a shop from Bucharest. This product was in validity period, respectively 6 days after packaging.

Seventeen livers harvested immediately after slaughter from young broilers, have been submitted to refrigeration (0-4°C). After 72 hours of refrigeration, three of these livers have been put to freezing temperature (-18°C) for 24 hours. Then, these samples have been examined after 24, 48 and 72 hours after defrosting (thawing in 0-4°C). The refrigerated samples have been monitored at different hours (1; 7; 25; 31; 37; 43; 49; 55; 61 and 73 hours). First, gross assessment was performed for the refrigerated and defrosted samples. For all liver samples, the imprints of liver were used for cytological exam (May Grünwald

Giemsa stain). The changes of the hepatocyte structure inducted by conservation techniques were considered.

RESULTS

Between the first and fifty-fifth hour of refrigeration, the liver had the following features: elastic consistency, normal brownish gray color and normal, fresh smell. The cytological exam has revealed normal shaped hepatocytes with clearly featured nucleus (central position into the cell, fine arranged chromatin). Hepatocytes showed relatively polygonal shape with round or slightly oval nucleus, positioned in the center of the cell. Due to the imprinting method, the majority of hepatocytes were arranged in cords keeping the classical arrangement in one or two cell formations. In this cords, sinusoids were observed containing nucleated erythrocytes and leukocytes. The cytoplasm had a homogeneous blue color and small lipid vacuoles were observed, which did not change the central position of the nucleus. At this stage no bacterial or yeast microflora was revealed.

Between the sixty-first and seventy-third hour of refrigeration, the consistency became friable, with darker nuances of color and characteristic smell of putrefaction process. The cytological exam has shown that a large number of nuclei were degraded (nude nuclei). In addition, a high number of rot bacteria was presented.

After ninety-seven hours of refrigeration, the consistency was friable, on the surface of the organ appeared small, green spots and characteristic smell of putrefaction process. Hepatocytes kept the microscopic recognition drawing.

Generally, defrosted samples presented the hepatocytes with normal morphology, minor alteration of nuclei and bacterial contamination.

For the liver coming from the shop the following features were observed: pasty consistency with tendency of drainage on section, gray pink color and strongly repulsive altered organ smell. The hepatic structure was almost disintegrated; rarely, nuclei and erythrocytes were identified. The large amount of bacterial and yeast microflora was revealed

DISCUSSION

Broiler liver is a biological product that easily may be contaminated by infectious, toxic or physical hazardous agents [2].

In general, broiler liver is contaminated, in a more or less proportion, from microorganisms with intestinal origin.

The mechanism of microbial translocation from the intestinal level to liver is relatively simple. Exposure to various stressors (e.g. nutritional, physical, toxic factors) can determinate an increased enteric epithelial permeability, which allow penetration of bacteria into mucosa and colonization of liver via blood flow. [3, 6]

Intestinal origin bacteria and their products are subsequently distributed to Kupffer cells and hepatocytes [5].

Also, the liver can be contaminated during the specific slaughter operations and by usual putrefaction microorganisms.

Yeasts were isolated from the majority of the samples. Probably, these are contamination microorganisms coming from the slaughtering process, manipulation and transport of samples [4].

This study revealed that freezing process has proven to be an effective method of preserving and maintaining the sanitation of broiler liver. This fact has been proven by the extremely small bacterial contamination even after 72 hours after defrosting.

These results and correlations represent the first step in our study concerning freshness of broiler liver and food safety.

CONCLUSIONS

The gross assessment of the broiler liver showed that between the first and fifty-fifth hour of refrigeration time, the liver has kept all the attributes corresponding to a wholesome product.

After sixty-one hours of refrigeration, the cytological exam showed that the broiler liver contained a large amount of bacteria that made the liver unsuitable for consumption.

The freezing process has proven to be an effective method of preserving and maintaining the sanitation of broiler liver. This fact has been proven by the extremely small bacterial contamination even after 72 hours after defrosting.

The liver from the shop showed all the gross, sensory and cytological features of an altered and unwholesome product.

The cytological exam, using May Grünwald Giemsa stain, represented a good method of examination, because it revealed the hepatocytes structure modification and the associated micro flora.

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MEAT QUALITY OF BROILERS CARCASSES AND CONDEMNATION RATE DURING THE VETERINARY CONTROL IN THE BATNA SLAUGHTERHOUSE (ALGERIA)

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SUMMARY

Chicken meat, is more and more evaluated by consumers who search a better quality of the finished product. This quality depends primarily on the breeding conduct, and then the animal collecting, transport and slaughtering conditions. Therefore, the aim of this study focused on a survey conducted at the poultry slaughterhouse of Batna city (Algeria) on 3 flocks of 3000 broiler chickens *ISA 15*, was to assess the frequency and characteristics of external lesions observed on broiler's carcasses before and after slaughter and the condemnation rate. The results showed three types of lesions: lesions superficial, intermediate and deep. The frequency of traumatic injuries before putting on the chain is estimated to average 12.43% of bruises, 3.73% of fractures and 2.71% of wounds. The condemnation rate of carcasses for 3 groups of 8.4%. Major causes of sanitary condemnation in slaughterhouse are diverse (congestion, skin, lesions, cachexia, ascites, arthritis, abnormal coloration).These results show that many carcasses lose their quality during transport and during the slaughter process and can be removed from consumption, which is detrimental to farmer.

INTRODUCTION

The chicken meat quality on the market is increasingly valued by consumers. The achievement of the physical integrity of animals during transport and slaughter is claimed by associations of animal welfare. Many authors have reported that the transport conditions and slaughter can lead to significant economic losses and negatively affect the sanitary quality of products [1, 2, 3]. In poultry production, there has been trauma during the carcasses inspection. These are mostly bruises and fractures. Nevertheless, the effect of the stages prior to slaughter (harvest and transport) has been few studied. Therefore, the main objectives of this study, focused on a survey conducted at the poultry slaughterhouse of Batna (Algeria) on three batches of 3000 broilers *ISA 15*, is to assess the frequency and characteristics of external lesions observed on chicken carcasses before and after slaughter and the condemnation rate.

MATERIAL AND METHODS

The study was conducted at the poultry slaughterhouse in the Batna city (Algeria). The survey was carried out on 3 flocks of 3000 broiler chickens ISA 15 aged between 49 and 56 days to determine the impact of collection, transport and unloading of animals. The characteristics of the animal state description are performed on a batch of 350 chickens. They are based on the description of the color of damaged tissue, the presence or absence of edema and traumatic observations (bruises, fractures and wounds). Each type of injury has been described in terms of its nature, its location and its severity.

Chickens are manually suspended on the slaughter without shock. The time of suspension, before the bleeding, is estimated at 1 minute. The 3 groups of broiler chickens from different farms were slaughtered in the same conditions. For each batch of animals the condemnation rate was calculated. It is based on the results of the ante and post mortem. The causes of sanitary condemnation included in this study were: cachexia, congestion, skin lesions, ascites, abnormal color or odor.

The results were presented by calculating the mean and standard deviation (SD). Statistical analyses were performed by SPSS 15.0. The differences were tested by analysis of variance (ANOVA), they are considered significant at $P < 0.05$.

RESULTS

Characteristics of lesions observed on broiler chickens carcasses: Each type of injury has been described in terms of its nature, its location and the degree of severity (Table 1). There are 3 types of lesions: superficial, intermediate and deep lesions. Unopened fractures, mean bruises located on the wing, thigh, or the filet are the main superficial lesions. Intermediate lesions are mainly represented by open fractures of the wing or elbow. Most lesions are characterized by deep tissues tumefaction in the whole carcass.

Table 1: Characteristics of lesions observed on carcasses of chickens.

Superficial lesions	Intermediate lesions	Deep lesions
- Unopened fracture -Hematoma means located on the wing, thigh or the filet -Hematoma of the vein	-Open fracture of the elbow -Fracture of the wing bones -Extended violet hematoma	-Swollen wing -Extended violet hematoma -Dislocation of the hip and rupture of the femoral artery

The frequency of lesions in the 3 groups before slaughter is 12.43% of bruises, 3.73% of fractures and 2.71% of wounds (Table 2). The bruises prevalence varied significantly between the 3 groups (10.0 VS to 15.0 and 12.3% p< 0.05). Indeed, the transport conditions were different. The birds of groups 2 and 3 were transported in small vehicles. No significant differences were observed between groups with fractures (3.6 VS 4.2 VS 3.4%).

2.71% chickens has presented wounds, a significant difference was observed between groups 2, 3 and 1(4,2 VS 1,3 and 2,6%). This is caused by the conditions of collection and unloading of unskilled workers.

Table 2: Prevalence of lesions before slaughtering (%).

Groups	1	2	3	P	Means ± SD
Hematoma	10.0 ± 1.2^{b}	15.0 ± 2.9^{a}	12.3 ± 2.5^{b}	*	12.43 ± 2.20
Fractures	3.6 ± 0.5^{a}	4.2 ± 0.7^{a}	3.4 ± 0.2^{a}	NS	3.73 ± 0.46
Wounds	4.2 ± 0.2^{a}	1.3 ± 0.09^{b}	2.6 ± 0.3^{b}	*	8.10 ± 0.19

a,b values with different superscripts are significantly different (p< 0.05)
*(p< 0.05)
NS: Not significant

Estimating the condemnation carcasses frequency at slaughterhouse level is based on prospective observation of lots of animals from their receipt to the slaughterhouse to the sanitary inspection. The condemnations prevalence varied significantly between the group 2 and groups 1, 3 (11.2 VS 6.8 VS 7.2% p<0.05) (Figure 1). For each group, the carcasses removed from the food chain have been accrued by reason of regulatory condemnation.

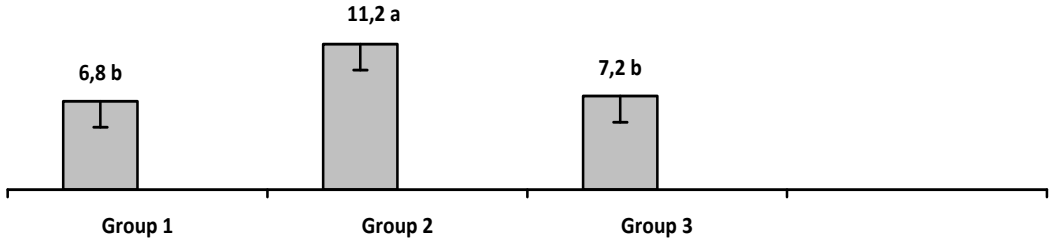

Figure 1: Condemnation of chicken carcasses after veterinary control for each group (%)

The main reasons for condemnation were cachexia, congestion, ascites but some associations between skin lesions, abnormal conformations and the color or odor. The carcasses were seized mainly for the following reasons in increasing order (Figure 2): congestion (30%), skin lesions (28%), cachexia (25%), ascites (8%), abnormal coloration (4%), arthritis (3%) and conformation (2%).

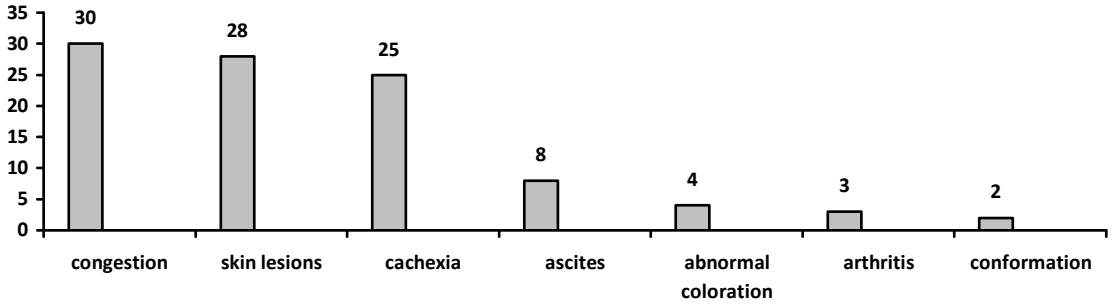

Figure 2. Major causes of sanitary condemnation in slaughterhouse (%)

DISCUSSION

Studies of catching conditions, transport and discharge of poultry are rare in Algeria. Barbuat and his team [4] showed that the transport conditions have led to many lesions in poultry (wing bruises, fractures, dislocation). However, Gregory and Wilkins [5] reported that the beating of wings of birds in cages transport cause the appearance of bruises, fractures and trauma. The condemnation frequency observed in our experiment is lower than that reported by Bremner [6], Herrenda and his coworkers [7] and by Lupo et al. [8]. However, the causes of seizure were heterogeneous in their work. This first study in Algeria, has demonstrated an association of several risk factors leading to a depreciation of the carcass quality and assessment of different condemnation causes.

The losses with contusions, fractures and dislocation can be reduced by improving the management of harvesting and transportation as well as the adjustment of the equipment used in slaughterhouses. The sanitary control of slaughterhouse should be improved, since it is crucial and have direct effects on the quality of the final product.

CONCLUSIONS

These results show that many carcasses lose their quality during transport and the slaughter process and can be removed from consumption. Therefore, drastic measures must be applicated before, during and after the slaughtering process for best performance and to minimize the economic losses and guarantee best meat quality for the consumers.

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MAIN CAUSES OF PIGS CONDEMNATIONS IN FEDERAL SLAUGTHERHOUSE IN BAHIA STATE - BRAZIL

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SUMMARY

Brazil is one of the largest exporters of meat in the world. The food production out of the standards of hygiene and sanitary quality can result in serious damage to health consumers. Failure to detect lesions of potential zoonotic diseases at slaughter poses a health risk to consumers especially when meat is eaten undercooked. This study aimed to determine the major causes of carcasses and organ/offal condemnations in slaughtered pigs under federal inspection in Bahia state, Brazil, and was conducted through analysis of reporting forms, kindly provided by the Ministry of Agriculture, Livestock and Supply of this country. Data were collected in 2006, and the total number of animals slaughtered during this period was 6,758. Were considered major causes of condemnation those who showed a percentage more than 10% of all pigs slaughtered in the period studied. From official data, were observed the following main causes of condemnation: blood aspiration in the lungs (36.22%), pulmonary emphysema (21.85%), food aspiration into the lungs (12.59%) and tongue contamination (11.13%). The present study confirms the importance of veterinarians and others professionals qualified and trained in food industry, resulting in a better quality product, increasing exports and reduce economic losses to industry, and ensuring the maintenance of public health.

Keywords: 1. Condemnations; 2. Pigs; 3. Blood aspiration

INTRODUCTION

The swine is of great importance to the national economy. According to data obtained from the Brazilian Association of Swine Breeders, the export of pork products has been growing in recent years. In 2005 Brazil exported 625 thousand tons, which has this activity become increasingly attractive to investors in the segment. Countries that import more Brazilian pork meat are Russia, Argentina and South Africa.

The physico-chemical and nutritional products of animal origin such as meat and meat products, contributing to configure these foods among those most concerned about humanity because of the dangers they offer. Thus, to minimize the risks from the ingestion of these products, it must be the need for prior inspection of food in retail establishments. Such control, in its specific nature, makes nondelegable character of this activity exclusively performed by veterinarians, as described in Brazilian laws (3, 4, 6).

Thus, in order to provide data to the productive sector, with regard to health status of flocks, and describe the procedures that involve improvements in meat quality, emphasizing the importance of sanitary supervision of the slaughter of animals for meat producers by the clinician, ensuring quality and safety of the final product, the aim of this study was to identify the main causes of condemnation in pigs slaughtered in a slaughter plant under federal inspection in Bahia state, Brazil.

MATERIAL AND METHODS

This work was carried out using data obtained from post-mortem inspection of swine slaughtered in a slaughter plant under federal inspection in Bahia state, Brazil, with a daily slaughter capacity of 150 animals. Data were collected from January to December 2006, the total number of animals slaughtered during this period was 6,758.

A post-mortem inspection of swine is based on so-called "inspection lines". In establishing where were performed the present study, the steps directly related to the slaughtering room, are quoted below: stunning electro narcosis, bleeding, scalding bath, gutting; inspection of the viscera and carcass, toilet; weighing of whole carcasses, and wash in cold storage. The convictions made during the post-mortem inspection were based on the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin - RIISPOA (1) and recorded on the notification forms, which were removed from the data concerning the number and causes the same for this work.

RESULTS

Were initially categorized all data relating to convictions made of carcasses and offal from animals slaughtered in the establishment during the period of this study. Taking into account the percentage equal to or greater than 10% used as a criterion of analysis of this work, although other causes of condemnation have been observed, they were not considered important. From official data, were observed the following main causes of condemnation: blood aspiration in the lungs (36.22%), pulmonary emphysema (21.85%), food aspiration into the lungs (12.59%) and tongue contamination (11.13%). The lungs convictions as blood aspiration showed deep red color and increase in volume at one or both lungs. When were made cuts in the lungs, which had an appearance suggestive of aspiration of blood, it was proved the presence of blood in the trachea and lung parenchyma (Figure 1).

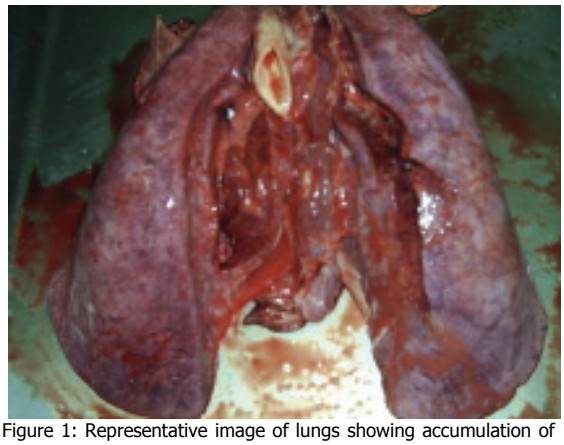

Figure 1: Representative image of lungs showing accumulation of blood in their parenchyma, seen to cut inspection, featuring blood aspiration in pigs slaughtered at the slaughter plant under federal inspection in Bahia state, Brazil.

DISCUSSION

In the slaughter plant under study carried out the stunning of animals through electro narcosis, which is produced by passing alternating electrical currents through the brain. The electric discharge acts as a cardiac stimulant and peripheral vasoconstrictor, resulting in a rapid elevation of blood pressure (7). The aspiration of blood is common in animals as a result of the section of the trachea in the act of bloodletting and that similar lesions occur in various organs and tissues, when subjected to high voltages on the electro is often observed multiple bleeding capillaries (5). Although the electro can cause bleeding in some animals, based on results found in this study, no correlation could be established as the conviction rates of the lungs by inhalation of blood and this technique of desensitization.

Although this study has not been possible to correlate this type of circulatory disorders in the presence of blood in the lungs of slaughtered pigs, it is believed that human failures and /or technological process during the electro and /or bleeding of the animals may be associated with high percentage of conviction. Accordingly, it is suggested that new techniques for stunning and /or bleeding of pigs are studied as well as professionals involved in these transactions are properly trained in order to minimize the conviction rates of lung for this particular cause. Besides the economic advantages brought about by reducing the number of convictions, the stunning / bleeding effectively reduces the unnecessary suffering of animals, the essential foundation for humane slaughter, as recommended in Brazilian law (2).

CONCLUSIONS

In this study we observed that the main causes of condemnation in pigs slaughtered in a slaughter plant under federal inspection in Bahia state were blood aspiration in the lungs, pulmonary emphysema, food aspiration into the lungs and tongue contamination. Finally, we emphasize that the role of the veterinarian in activities related to sanitary inspection of animal products is critical to preserving the health of population and economic growth of the country.

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NEW CRITERION FOR THE ASSESSMENT OF THE FOOD SAFETY (Abstract)

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Studies show that regarding the risk factors regulated by Regulation № 31 should be applied a differentiated approach. In this elaboration we combine the criterias for chemical inhomogeneity: along with the developed and applied by us bioconcentration factor, we offer safety criterion clark (SC).

Analyzing the bioaccumulation of lead and cadmium in the body of kids (Capra hircus), raised in an Ecotopia with a different Clark of the two components was showen that there is no typical xenobiotics dependence biokontsentrirane – increasing concentration of each trophic level. The second criterion – SC is defined as the ratio between the quantity of toxic element in the research sample, the maximum limit in the Regulation № 31.

In displaced samples of meat from kids was established the amount of lead 1,0 mg/kg PDS that according the Regulation could be 0,2 or the SC is 2,0. The hygiene assessment is that in all cases where SC is more than 1 there is a risk for the human health.

The studies reveal differences in the values of BF and SC, but the use of the second criterion is an opportunity to unify the assessment on comparable dimensions, as desaga used mg/kg,%, etc.

Keywords: food safety

MICROBIOLOGICAL AND PHYSICOCHEMICAL ANALYSIS OF HONEY FROM SOUTHERN ROMANIA

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SUMMARY

The present study was carried out in order to characterize 10 types of commercial honeys available in the Bucharest market. First, in order to verify the declared commercial quality, each sample of honey was subjected to color determination, with Pfund colorimeter. Afterwards, their microbiological quality was assessed, by determining the aerobic mesophiles, moulds, yeasts, fecal coliforms and sulphite-reducing clostridia. Each honey sample was subjected to: moisture content, ash content, acidity, pH, electrical conductivity and HMF. Concerning these parameters, all honey samples were found to meet the European legislation requirements, except for HMF. Microbiologically, the commercial quality was considered overall good, showing negative results in respect to safety issues.

Keywords: honey, physicochemical properties, microbiological quality, HMF content.

INTRODUCTION

Honey is considered a very high nutritive food, being composed of a mixture of carbohydrates (with fructose and glucose 85-95%) and other substances in minor quantities, such as organic acids, amino acids, proteins, minerals and vitamins [3, 7, 18].

Honey quality is determined by sensorial, chemical, physical and microbiological analysis, with some criteria of major importance, like: moisture content, electrical conductivity, ash content, free acidity, HMF content. All there specifications are included in EC Directive 2001/110. On the other side, EU legislation does not specify anything about the microbiological safety of honey, although different studies have showed that honey can be contaminated in a serious manner, as to put human being life in danger, especially due to Clostridium botulinum [1, 2, 10, 18, 22]. They consider that honey can be contaminated from different sources, like: pollen, digestive tracts of bees, dust, air, soil. These are very difficult to eliminate. The secondary sources, considered to be the handlers and the processing, are controllable through GMP. Scientists have showed that honey can be also contaminated with moulds, yeasts, spores of Bacillus cereus, all these destroying the commercial quality and safety of honey [5, 9, 14].

The study below aimed to characterize 10 types of honeys available on Bucharest markets, in respect to their physicochemical parameters, microbial safety and commercial quality. The microbial contaminants considered of interest were: aerobic mesophiles, moulds, yeasts, fecal coliforms, sulphite-reducing clostridia and Salmonella.

MATERIALS AND METHODS

Honey samples: was harvested ten commercial honeys of different floral sources and geographical origins were purchased from local markets and left at room temperatures until further analysis. This analysis was carried by refractometry, using an Abbe refractometer. All measurements were performed at 20^{o}C, obtaining the corresponding moisture (g/100 g honey) from the refractive index of each honey sample, and using a standard table to obtain the specific results. The estimation if total ash was estimated by conductimetry using the next equation [12]:

Ash content (%) = 0,083 * conductivity – 0,092.

The pH was measured using a pH-meter, from a solution of 10 g honey in 75 ml distilled water.
After homogenization and filtration, honey samples were subjected to free acidity analysis. Ten grams of honey were dissolved in 75 ml of distilled water and afterwards, a solution of phenolphthalein was added. The solution was titrated with NaOH 1/N. Acidity was determined by using 10 times the volume of NaOH in titration.

Hydroxymethylfurfural (HMF) was determined using the standard analysis technique. Five grams of honey were dissolved in 25 ml of distilled water, treated with a clarifying agent until 50 ml. The solution was filtered and the first 10 ml were discarded. The absorbance was measured at 284 and 336 nm against a solution of $NaHSO_3$, filtered.
The HMF content was determined by using the next equation:

$$HMF/100\ g\ of\ honey = (Abs_{284} - Abs_{336}) \times 14,97 \times (5/g\ of\ sample)\ [12]$$

The microbial contamination from each sample, was made in an aliquot of 10 grams ere homogenized in 90 ml of peptone water solution. Decimal dilutions were made into the same solvent. Aerobic mesophilic bacteria were counted onto standard plate count agar and incubated for 48 h at 30°C [12], while moulds and yeasts counts followed the standard protocol [13]. Sulphite-reducing clostridia were analyzed using SPS agar media, poured into tubes with culture, and incubated at 37°C, for 5 days. Fecal coliforms and Salmonella were enumerated by the Most Probable Number.

RESULTS

All the samples collected from the markets showed specific Pfund values (table 1) for the declared quality, with 8-10 for acacia honey, 1st quality, a maximum of 13 for 2nd quality, linden honey 1st quality with a maximum of 35 and 2nd quality between 35 and 40, polyfloral honey 1st quality with a maximum of 40 and 2nd quality between 40 and 50.

Table 1 Commercial types of honeys purchased for analysis from Bucharest markets, their floral source, origin and Pfund values.

Sample	Floral source and declared quality level	Pfund values	Origin
1	Acacia 1st quality	8	Southern Romania Olt county
2	Acacia 1st quality	9	Southern Romania Dâmboviţa county
3	Acacia 2nd quality	11	Southern Romania Ialomiţa county
4	Acacia 2nd quality	12	Southern Romania Prahova county
5	Linden 1st quality	33	Southern Romania Călăraşi county
6	Linden 1st quality	35	Southern Romania Dolj county
7	Linden 2nd quality	38	Southern Romania Mehedinţi county
8	Linden 2nd quality	40	Southern Romania Olt county
9	Polyfloral 1st quality	38	Southern Romania Teleormancounty
10	Polyfloral 1st quality	37	Southern Romania Prahova county
11	Polyfloral 2nd quality	45	Southern Romania Dolj county
12	Polyfloral 2nd quality	48	Southern Romania Ialomiţa county

Table 2 shows the results obtained for all the physicochemical analyses on the 10 samples of honey. All samples met the requirements in the EU legislation, except for HMF (≤40 mg/kg).

Table 2 Physicochemical parameters of honey samples (average values)

Sample	Moisture (%)	Conductivity (mS/cm)	Ash (%)	pH -	Acidity (meq AC/kg)	HMF (mg/kg)
1	15,81	0,21	0,05	3,7	17,13	19,24
2	16,75	0,24	0,47	4,0	21,84	20,08
3	17,04	0,28	0,08	3,8	25,58	25,31
4	15,54	0,62	0,34	3,8	22,65	67,44
5	16,87	0,36	0,28	4,2	25,39	48,68
6	17,62	0,27	0,14	4,2	25,46	41,37
7	17,28	0,43	0,19	4,2	26,72	32,52
8	15,31	0,55	0,28	4,1	18,94	38,48
9	15,27	0,24	0,32	4,4	20,55	59,12
10	15,59	0,37	0,30	4,2	18,37	74,05

The levels of microbial contamination for the 10 honey samples are presented in table 3.

Table 3 The levels of microbial contamination

Sample	Aerobic mesophiles	Moulds and yeasts	Fecal coliforms	Sulphite-reducing clostridia	Salmonella spp.
	CFU/g	CFU/g	MPN	in 0,01 g	in 25 g
1	< 10	< 10	<1	Negative	Negative
2	< 10	< 10	<1	Negative	Negative
3	< 10	< 10	<1	Negative	Negative
4	2×10^1	< 10	<1	Negative	Negative
5	< 10	$1,9 \times 10^1$	<1	Negative	Negative
6	$1,5 \times 10^1$	$1,7 \times 10^1$	<1	Negative	Negative
7	< 10	< 10	<1	Negative	Negative
8	$1,3 \times 10^1$	< 10	<1	Negative	Negative
9	< 10	$2,3 \times 10^1$	<1	Negative	Negative
10	$2,1 \times 10^1$	< 10	<1	Negative	Negative

DISCUSSIONS

Moisture contents ranged from 15, 27 for sample 9 to 17,62 for sample 6. This reveals that these types of honeys were stored in good conditions, because fermentation would have occurred if otherwise. High moisture contents would have accelerated crystallization in certain types, these converting themselves into very good media for yeasts' development.

The highest acidity was achieved for sample 7 (26, 72 meq AC/kg) while the lowest one was specific to sample 1 (17, 13 meq AC/kg). The values of acidity show that the honey samples are fresh.

HMF analysis revealed the fact that only samples 1, 2, 3, 7 and 8 presented values under 40 mg/kg, meeting the requirements for this parameter, included in EU Legislation. The other samples presented values between 41, 37 mg/kg and 74, 05 mg/kg (sample 10). HMF parameter shows the freshness of honey, being absent in fresher honeys and increasing during processing or aging of this product. A series of factors are known to influence directly the HMF content of the honey, such as: temperature and time of heating, storage conditions, pH and floral source (figure 1).

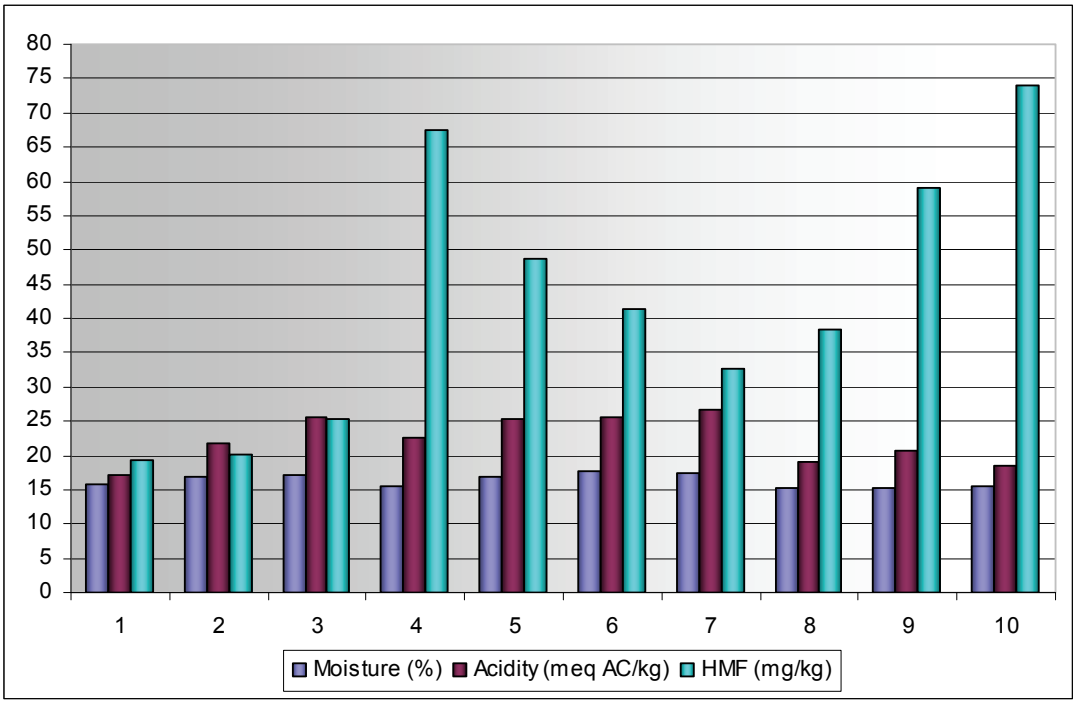

Fig. 1 – The moisture content, the acidity and HMF content.

The pH is quite important, as a low pH inhibits the growth of microorganisms. This parameter is used during the extraction and storage of honey, influencing the texture, stability and shelf life of honey. The lowest pH was the one specific to sample 1 (3, 7), while the highest was found for sample 9 (4, 4).

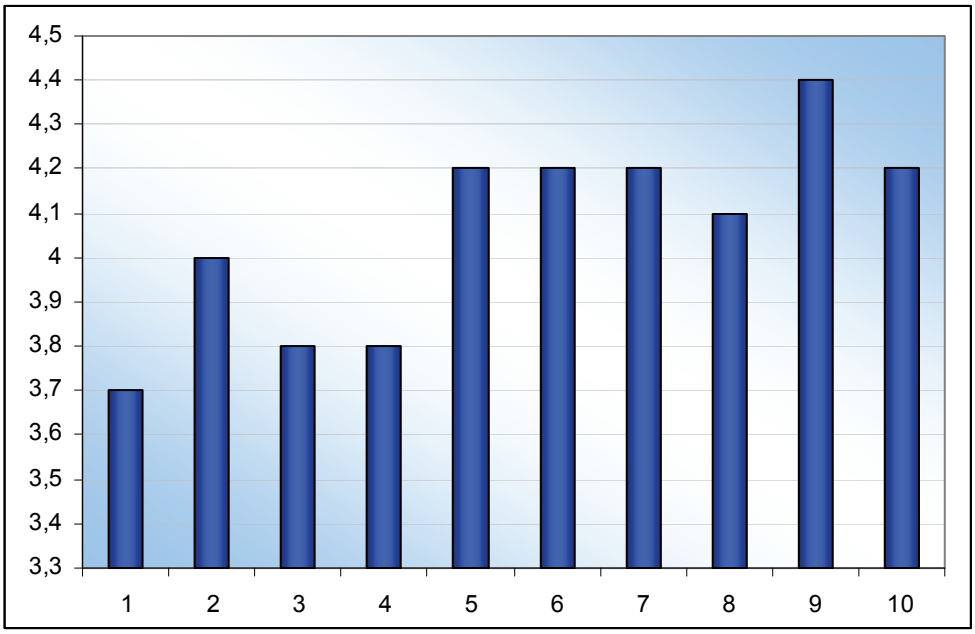

Fig. 2 – The pH values for the ten samples analyzed during the study

The ash content was highest in sample 2 (0, 47%) and the lowest in sample 1 (0, 05%). The conductivity is also an important parameter, due to the fact that any honey with values above 0,8 mS/cm is a nectar honey, others with values under this range being considered adulterated. Conductivity presented values between 0, 21 mS/cm (sample 1) and 0, 62 mS/cm for sample 4 (figure 3).

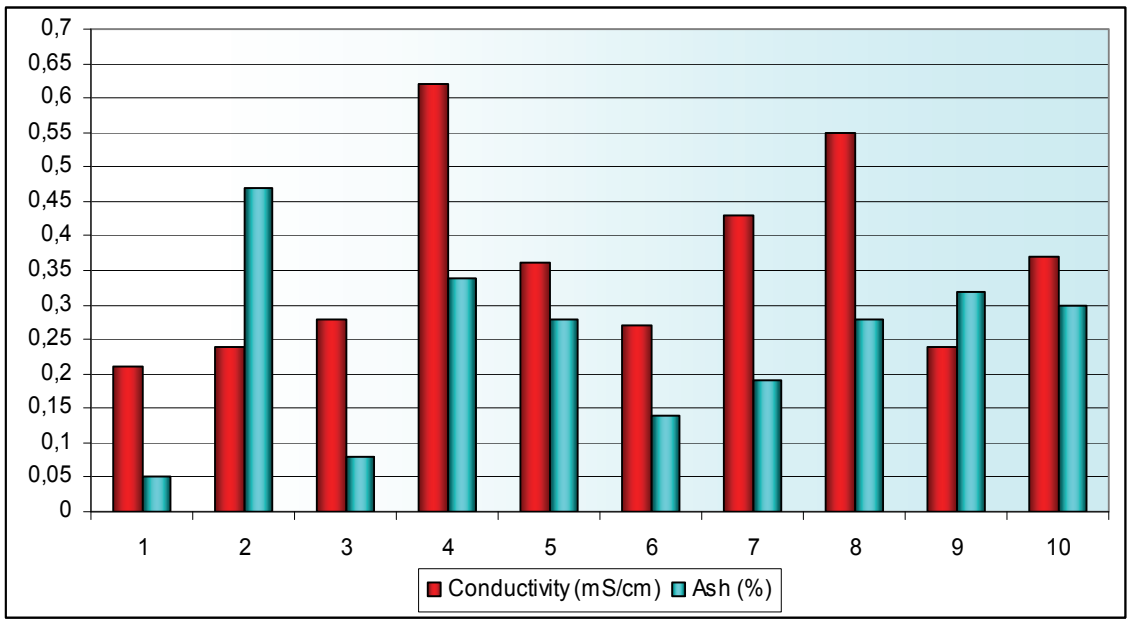

Fig. 3 – Conductivity and ash content for the honey samples.

The levels of quantification for aerobic mesophiles, moulds and yeasts were low, while the sanitary quality, (fecal coliforms) and safety (sulphite-reducing clostridia and *Salmonella* spp.) all samples were negative.

CONCLUSIONS

The declared quality was confirmed, consisting of specific Pfund values for each type of honey, this way the customer knowing for sure that the ratio price:quality is the real one.

Concerning the moisture contents of all types of honey, these show that the storage was realized in adequate conditions, the producers taking care of this stage with great attention. Also, the acidity revealed the fact that all types of honey were fresh, and the HMF values were situated under the maximum limit according to the EU legislation, meeting the specific requirements. In addition, the specific conductivity values showed that all types of

honeys were nectar honeys, not even one being modified due to adulteration.

Considering the microbiological quality, the samples showed that the honey is safe for the consumer, the sulphite reducing clostridia and Salmonella spp. being absent.

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DETECTION OF *LEPTOSPIRA* SPP. IN SLAUGHTERED SHEEP FROM BRAZIL

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SUMMARY

Leptospirosis is a zoonotic disease with worldwide distribution that affects several species of animals and men. Sheep, once infected, can became ill and act as reservoirs, eliminating the bacteria by their urine in the environment. The aim of the study was to detect the infection by *Leptospira* spp. in slaughtered sheep from Brazil. Blood, kidney and liver samples were collected from 465 sheep. The sera were tested by the Microscopic Agglutination Test (MAT), using 25 serovars. Kidney and liver of all seropositive animals were individually cultured in Fletcher medium enriched with rabbit sera, using the serial dilution technique. Another fraction of the same material was submitted to the Polimerase Chain Reaction (PCR). The culture and PCR was also performed in 15 seronegative animals chosen aleatory. In the MAT 21 animals were positive (4.5%) to the serovars Hardjo (n=12), Hebdomadis (n=5), Sentot (n=2), Wolffi (n=1), and Shermani (n=1). In the PCR six animals were positive, all from kidneys samples. None of the liver samples were positive. All the PCR-positive animals were seropositive and presented high titers in the MAT (200, n=1; 400, n=4, 1600, n=1) for the serovar Hardjo. None of the animals were positive in the bacteriological culture. The present study confirms that sheep can act as reservoir of *Leptospira* spp. in Brazil; the PCR is more sensitive than the bacteriological culture to confirm the infection in tissue samples.

INTRODUCTION

Leptospirosis is a disease that affects several species of animals and men. It is caused by bacteria of genus *Leptospira*, which is widely distributed in the world, usually associated with high levels of humidity. The infection is mainly by oral route or direct contact, and can vary from asymptomatic to severe illness, leading to death. The animals, once infected, can act as reservoirs, eliminating the bacteria by their urine and contaminating the environment (2). Sheep flocks affected by leptospirosis can develop serious health problems (3), especially abortion. Besides, animals eliminating leptospires can became a threat to other animals and men (1). Thus, the diagnosis of leptospirosis in sheep is important to detect infected animals and prevent such hazards. The objective of the present study was to confirm the role of sheep in the epidemiology of leptospirosis in Brazil, and to detect the infection by *Leptospira* spp. using different methods.

MATERIAL AND METHODS

Blood, kidney and liver samples were collected from 465 sheep in a slaughterhouse located in São Manoel Municipality, São Paulo State, Brazil. The sera were tested by the Microscopic Agglutination Test (MAT), using 25 serovars kept in EMJH medium: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Djasiman, Sentot, Gryppotyphosa, Hebdomadis, Copenhageni, Icterohaemorraghiae, Javanica, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi, Andamana and Patoc. Animals presenting titers equal to or greater than 100 were considered positive. Kidney and liver of all seropositive animals were individually macerated and added to a sterile solution containing water, neomycin and furazolidone. This mixture was cultured in Fletcher medium (DIFCO Laboratories, Detroit, USA) enriched with rabbit sera, using the serial dilution technique, and analized after 60 days in a dark field microscopy. Another fraction of the same mixture was submitted to the Polimerase Chain Reaction (PCR), using the GFX™ Genomic Blood DNA Purification Kit, and primers previously described (6). The amplification was performed in a DNA thermal cycler (Mastercylcer® ep eppendorf, Hamburg, Germany), and the amplicons were visualized by electrophoresis in 2% agarose gel stained with ethidium bromide. The culture and PCR was also performed in 15 seronegative animals chosen aleatory.

RESULTS

In the MAT 21 animals were positive (4.5%) to the serovars Hardjo (n=12), Hebdomadis (n=5), Sentot (n=2), Wolffi (n=1), and Shermani (n=1). Titers ranged from 100 to 1600. In the PCR six animals were positive, all from kidneys samples. None of the liver samples were positive. All the PCR-positive animals were seropositive and presented high titers in the MAT (200, n=1; 400, n=4, 1600, n=1) for the serovar Hardjo. None of the animals were positive in the bacteriological culture. The results are sumarized in the table 1.

DISCUSSION

Most of the animals were positive to serovar Hardjo. However, it is not possible to affirm that this serovar was responsible for the infection, because cross-reaction with other serovars can occur. It is correct to say that serovars belonging to Sejroe serogroup were responsible for most of the infection in the studied animals. Previous studies described similar results, demonstrating that this serogroup is responsible for a significant number of infected sheep (3,8).

The bacteriological culture of leptospires is considered a method of low sensitivity, due to the high susceptibility to contamination and the fastidious growth of these bacteria (4,5). In the present study it was not possible to isolate leptospires using this method. The PCR is considered a sensitive test (7), and were efficient to confirm the infection in the studied animals, especially in those with high titers in the MAT. Probably animals with active infection maintain high levels of antibodies due to frequent imunologic stimulation.

The negative PCRs in seropositive animals were probably because of the low or abscent bacterial DNA, which is common in animals that did not develop chronic infection, but still has antibodies against the bacteria. *Leptospira* spp. usually can be detected in the liver only during acute infection, while the kidney is the organ where they remain during chronic infection. Because of the short time of the acute infection, the detection of leptospires in the liver is more difficult to obtain. Probably all the PCR-positive sheep were chronically infected when the samples were collected, or the small quantity of bacteria in the liver was not sufficient to be detected in the PCR

Table 1: Results of the MAT, bacterial culture and PCR of seropositive slaughtered sheep from Brazil. Botucatu, 2011.

Animal	MAT[a]	Culture	PCR
A-112	Hardjo (100)	-	-
A-150	Hardjo (1600)	-	+
A-171	Hardjo (400)	-	+
A-173	Hardjo (400)	-	+
A-174	Hardjo (400)	-	+
A-213	Hardjo (1600)	-	-
A-215	Hardjo (400)	-	+
A-229	Hardjo (200)	-	-
A-237	Hardjo (100)	-	-
A-247	Hardjo (200)	-	+
A-256	Hebdomadis (100)	-	-
A-259	Shermani (100)	-	-
A-245	Hardjo (400)	-	-
A-323	Hebdomadis (100)	-	-
A-344	Hebdomadis (100)	-	-
A-346	Wolffi (100)	-	-
A-350	Hebdomadis (100)	-	-
A-414	Hebdomadis (400)	-	-
A-460	Sentot (100)	-	-
A-461	Sentot (100)	-	-
A-467	Hardjo (1600)	-	-

[a]Serovar (titer)

CONCLUSIONS

The present study confirms the role of sheep as reservoirs of *Leptospira* spp. in Brazil; serovars that belong to Sejroe serogroup are the most prevalent in sheep; the PCR is more sensitive than the bacteriological culture to confirm the infection in tissue samples of sheep; the liver is not a suitable tissue to detect *Leptospira* spp. when compared to the kidney; animals presenting high titers in the MAT have more probability to harbor *Leptospira* spp..

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OFFAL YIELDS OF SPRINGBOK, GEMSBOK, KUDU, RED HARTEBEEST AND ELAND FROM NAMIBIA (Abstract)

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INTRODUCTION

Namibia has a number of regulations that apply to the sustainable use of game animals for commercial meat production. Food business operators have to maintain hygiene control procedures and measures based on HACCP principles before export of game meat to international countries are approved. Offal items from game are often discarded as it undervalued as an alternative protein source in local and export markets.

ANIMALS, MATERIALS AND METHODS

This investigation determined the offal yields of male and female springbok (*Antidorcas marsupialis*), gemsbok (*Oryx gazella*), kudu (*Tragelaphus strepsiceros*), red hartebeest (*Alcelaphus buselaphus*) and eland (*Tragelaphus oryx*) harvested from the highland savannah in Namibia. Animals were harvested and eviscerated according to standard operational procedures approved by the Namibian Veterinary Authorities.

RESULTS

No differences were observed between male and female springbok for heart, liver, kidney, spleen, lung or intestine weights. The head and skin of springbok males were significantly heavier than the females when compared as a percentage of the carcass weight. Offal items contributed 40 – 43% to the live weight of the springbok. Male gemsbok did not differ significantly from females in the weight of the heart, kidney, spleen, feet or hide. The weight of the lungs differed significantly for gender. Offal items contributed 42 - 46% of the live weight of the gemsbok. The head of male kudu contributed significantly more (5.6%) to the live weight of the male than the female 4.1%. Offal items contributed between 37 - 39% to the live weight of the kudu. The contribution of all offal items to the live weight of the red hartebeest was between 40 - 44%. The head of the red hartebeest did not differ with gender. Only the spleen of the eland differed significantly with gender. Calculated p-values showed significant species differences for all offal items, except for intestines.

CONCLUSIONS

The investigation concluded that there would be minimal justification for sorting and pricing of offal for commercial meat markets on a gender basis of weight or yield alone. If harvested hygienically, game offal could contribute to the economic value of the carcass.


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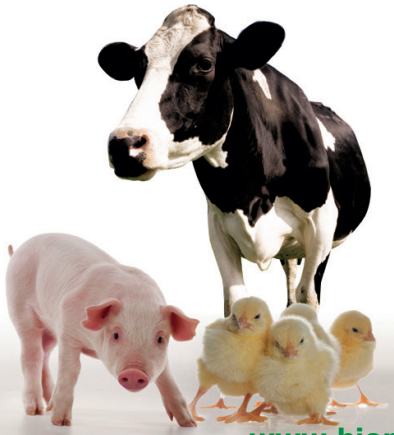

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