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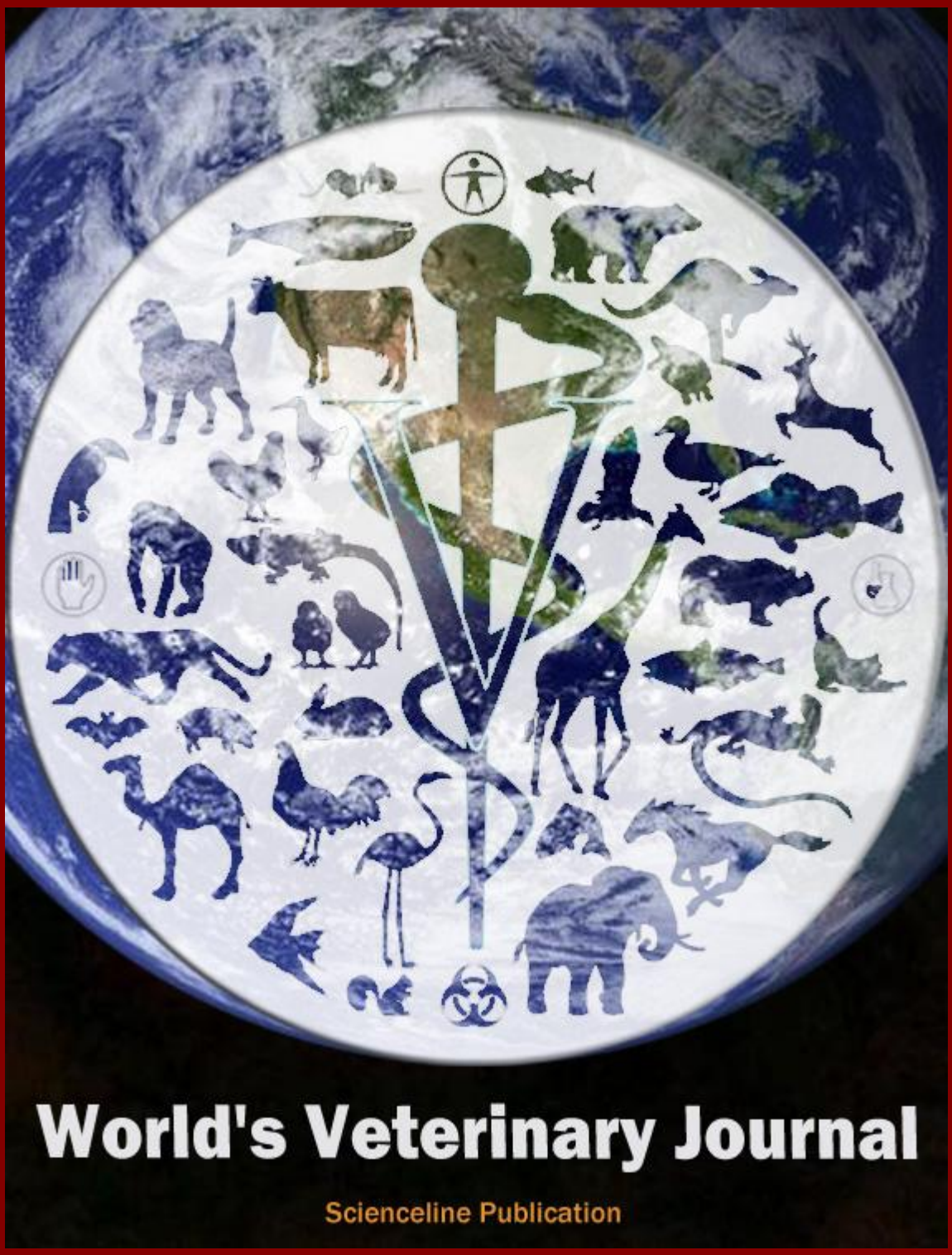

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Volume 6 (3); September 25, 2016

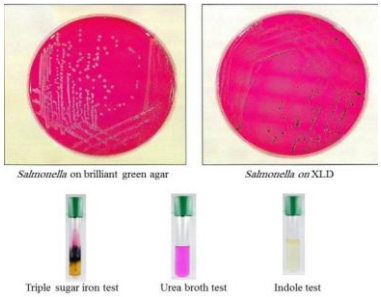
and gadB). The obtained results revealed the presence of the three virulence genes in the selected strain of *E. tarda* which gave severe lesions in the experimentally infected *Oreochromis niloticus*. *E. tarda* strain having more than one virulence gene results in more severe lesions than strains having one or even no virulence genes.
Key words: *Edwardsiella tarda*, Virulence genes, Freshwater fish
[Full text-PDF] [XML]

Research Paper

Prevalence of Antimicrobial Resistant Salmonellae Isolated From Bulk Milk of Dairy Cows in and Around Debre Zeit, Ethiopia.

Mossie T and Dires A.

World Vet. J. 6(3): 110-116, 2016; pii:S232245681600016-6



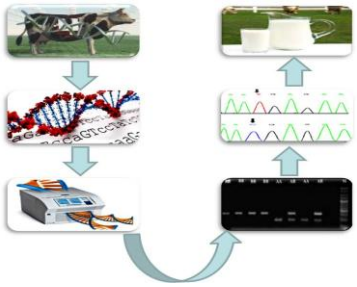
ABSTRACT
A cross sectional study to determine the prevalence and antimicrobial resistant profile of *Salmonella* isolates from 106 bulk milk of dairy cows was undertaken from December 2013 to April 2014 in supermarket, large and small holder's dairy farms in Debre Zeit, Ethiopia. The bacteria was isolated and identified according to standard methods and sensitivity tests were done by the Kirby-Bauer disc diffusion method. The study revealed an overall prevalence of 23.6% (25/106). The occurrence of *salmonella* in large and small scale farm was 20.4% and 27.3% respectively. The isolated *Salmonella* spp. was resistant to at least two or more antimicrobials which were used in this study. A higher proportion of the isolates (96%) were resistant to ampicillin and the lowest resistance was recorded for streptomycin (8%). Assay of antimicrobial resistance revealed that 96% of *Salmonella* isolates were resistant to two or more of the nine antimicrobials tested whereas 4% of the isolate was sensitive. The most common resistance was to Ampicillin 24 (96%), oxytetracycline 21 (84%), amoxicillin 12 (48%), Chloramphenicol 10 (40%). A significant proportion has developed resistance for routinely prescribed antimicrobial drugs both in veterinary and public health sectors. This poses considerable health hazards to the consumers unless prudent antimicrobial usage, adequate heat treatment, improvement of standards of hygiene and development and enforcement of suitable legislation, which safeguard consumers, are urgently instituted.
Key words: Isolates, *Salmonella*, Prevalence, Antimicrobial resistance, Bulk milk sample
[Full text-PDF] [XML]

Research Paper

Association of β –Lactoglobulin Gene Polymorphism with Milk Yield, Fat and Protein in Holstein-Friesian Cattle.

Wageh Zaglool A, Awad A, El sayed El Araby I and Mohamed El-Bayomi Kh.

World Vet. J. 6(3): 117-122, 2016; pii:S232245681600017-6



ABSTRACT
Improving the efficiency of milk production and its constituents without increasing the size of the dairy herd is the foremost goal of the selection in dairy industry. The use of polymorphic genes as detectable molecular markers is a promising alternative to the current methods of trait selection once these genes are proven to be associated with traits of interest in animals. Beta-lactoglobulin (B-LG) is one of the most important genes that play a crucial role in the milk quality and coagulation process of cheese and butter. Identification of different B-LG genotypes and association with different milk performance traits in Egyptian Holstein cattle was performed through Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and DNA sequencing of two hundred blood samples. Digestion of 447bp PCR product with Hae III restriction enzyme revealed three genotypes (AA, AB, BB), with higher frequency of B allele (64%) than A allele (36%). Nucleotide sequence analysis of different genotypes revealed two point mutation at exon four, one of them (T301C) corresponding to the same amino acid asparagine and the other Single Nucleotide Polymorphism (SNP) (C390T) represented a non-synonymous mutation producing amino acid change of alanine to valine. Animals with genotype AA had more milk yield and protein % (11461kg and 3.45) respectively, while BB genotype recorded higher fat % (3.85). The information given in the present study will be extremely helpful for improving milk production traits in dairy cattle by marker-assisted selection and outlined a strategy to avoid long and costly traditional selection methods for dairy purposes in Holstein cattle
Key words: B-LG polymorphism, PCR- RFLP, Milk production, Holstein Friesian cattle
[Full text-PDF] [XML]

Research Paper

Rabies in Animals with Emphasis on Dog and Cat in Ethiopia, Sindh, Pakistan.

Kidane AH, Sefir D, Bejiga T, Deressa A and Pal M.

World Vet. J. 6(3): 123-129, 2016; pii:S232245681600018-6

ABSTRACT
Rabies is a major viral anthropozoonosis of public health significance. The animals play a crucial role in maintenance and circulation of the rabies virus. Determining the occurrence of rabies in animals was implicated as a fundamental step to guide prevention and control efforts. The study involved cross sectional study by retrieval previously recorded cases between September 2009 and January 2012, Ethiopia. Suspected animal rabies cases were quarantined for 10 days observation period and brain samples were tested for confirmation using direct fluorescent antibody test. The statistical analysis was performed using SPSS program and Chi-square and analysis of vari¬ance (ANOVA) was used to assess the significance difference. Domestic and wild animals were associated with human exposure and death cases. However, dogs were the culprit for the highest human fatal (97%) and human exposure cases (89.5%). Only 2% of human deaths were associated with cats and wild animals. Higher rabies positivity was noted in owned dogs

138 (74.2%) than ownerless ones 49 (25.8%). The difference was statistically significant (p=0.0001). Further, all positive cases in cats were apparent in those believed to be owned. Majority of positive results associated with dogs were aged above 12 months 70 (81.4%), 10 (11.6%) were from the age between 6 and 12 months followed by 3 to 6 months 6 (7%). Positive cases in cats were more common among those aged above 12 months (60%) followed by 6 to 12 (20%) and 3 to 6 months (20%) of age. Of the animals with positive results, 4 (1.6%) were vaccinated against rabies. Overall, majority of the dogs had no vaccination history 247 (96.1%), only 10 (3.9%) were found to have been vaccinated. On contrary, none of the cats were vaccinated against rabies. The number of submitted samples had a direct correlation with the number of positive results (p< 0.05), however, no season variation was encountered. Five sub-cities that border with another region showed a significantly higher occurrence (p< 0.05) of exposure and confirmed cases and post exposure prophylaxis. Thus, integrated implementation of compulsory animal management, immunization and creation of awareness is highly imperative.

Key words: Addis Ababa, Cat, Dog, Immunization, Rabies, Zoonosis

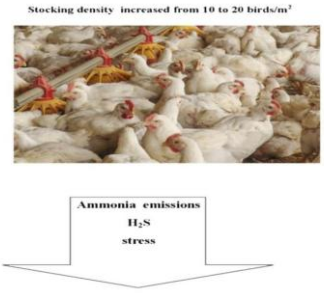
[Full text-PDF] [XML]

Research Paper

Effect of Stocking Density and Bird Age on Air Ammonia, Performance and Blood Parameters of Broilers.

Ali Abouelenien F, Khalf-Alla F, Mousa-Balabel T, El-Midany S and Abd el-Latif Nasser M.

World Vet. J. 6(3): 130-136, 2016; pii:S232245681600019-6



ABSTRACT

Current study was conducted to recompense this deficiency through detection of ammonia (NH3) in air samples taken from different groups at different SD and different ages. Additionally the effect of stocking density on stress indicators and broiler performance were studied. A total of 200 unsexed one day old chicks, purchased from a commercial hatchery at Kafr El-Sheikh city, Egypt, were used in this study. The birds were randomly divided into four groups according to SD. These experimental groups were divided as follows: the first group (G1) contains 10 bird/ m2, the second group (G2) contains 14 bird/ m2, the third group (G3) contains 17 bird/ m2 and the fourth group (G4) contains 60 chicks was kept in an area of 3 m2 with SD of 20 bird/ m2. The chicken in all groups (from day 7 to day 42) was raised on deep litter system and received the same standard management, hygienic and environmental conditions. Comparing ammonia concentrations of different SD, the highest ammonia concentration was found in G4 (maximum of 31.4 mgL-1 at 36 day age) while the lowest were found in G1 (10 bird/m2) (0.7, 3.1, 3.5 and 3.5 mgL-1 at 14, 21, 28 and 36 day age respectively). Increased SD tended to reduce the final Body Weight and Body Weight Gain significantly. On the other hand, the relative weights of spleen and bursa were increased significantly (P < 0.05) with increasing SD. In the current study there was a significant difference (P < 0.05) in Total Leukocitic Count, Heterophils %, Lymphocytes %, and H/L ratio between different SD. H/L ratio was highest in G4 (0.192%) and lowest in G1 (0.018%) which showed Increasing in H/L ratio with increase in SD that reflects increase in stress level.

Key words: Broiler, Socking density, Air ammonia, Boiler performance, Bood parameter

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Research Paper

Prevalence of *Cysticercus Tenuicollis* in Small Ruminants Slaughtered at Addis Ababa Abattoir, Ethiopia.

Bejiga T, Haile A, Solomon T, Sefir D and Pal M.

World Vet. J. 6(3): 137-142, 2016; pii:S232245681600020-6

ABSTRACT

Parasitic diseases in general and *Cysticercus tenuicollis* in particular are responsible for the low productivity of livestock. A study to determine the prevalence of *C. tenuicollis* in sheep and goats at Addis Ababa abattoir, Ethiopia. A cross sectional study was conducted and a stratified random sampling technique was employed where by sheep and goats to be slaughtered were categorized into two groups based on their sex (male and female) and also based on their age (young and adult) only in case of sheep. The statistical analysis was performed using SPSS program and Chi square. The overall prevalence of *C. tenuicollis* from 900 shoats was found to be at 355 (39%). Out of the 600 sheep inspected *C. tenuicollis* was detected in 223 (37.2%) and from 300 inspected goats *C. tenuicollis* was observed in 132 (44%). The age wise prevalence *C. tenuicollis* showed that the prevalence was higher in adult sheep (46%) than young sheep (28.3%). The prevalence of *C. tenuicollis* based on their sex was higher in female goats (45.3%) and sheep (37.8%) than in male goats (42.7%) and sheep (35.7%) with no significant statistical difference (P> 0.05). Although organ wise infection rates of both goats and sheep were 5.7% and 3.7% (mesentery), 0.7% and 0.2% (diaphragm), 0% and 0.2% (uterus), and 0.3% and 0% (rectum) with no significant statistical difference. However, in other infected organs, there was a significant statistical difference 39% and 34.2% (omentum), 14.7% and 7.8% (liver) and 3% and 0.8% (lung) in goats and sheep, respectively. The overall percentage of *C.tenuicollis* was higher in adult sheep (10.7%) than in young sheep (5%) with significant statistical difference (P< 0.05).

Keywords: Addis Ababa, *Cysticercus tenuicollis*, Goat, Prevalence, Sheep, Visceral organ

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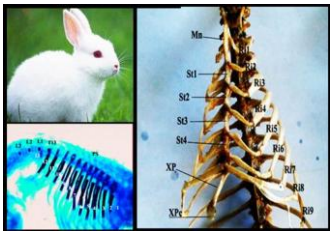
Research Paper

Development of Sternum and Ribs in White New Zealand Rabbit (*Oryctolagus cuniculus*).

Mohamed Kamal B, Farag Rashed R and Mohamed Erasha A.

World Vet. J. 6(3): 143-150, 2016; pii:S232245681600021-6



ABSTRACT

The bone development and the assessment of the fetal skeletal improvement turn into a basic segment in the behavior of a prenatal toxicity study. The sternum is a unique bone

in the distinctive shape and development. This study focuses on the development of the sternum and the ribs attached in the whit New Zealand rabbit, Specimens were collected prenatally (n=30) and postnatally (n=30). A group of specimens were double stained for bone and cartilage using Alizarin Red and Alcian blue. Another group was scanned by CT. The sternum was consisted of manubrium, four sternebrae and xiphoid cartilage. The sternum was in communication with 6 pairs of ribs. 7 ossification centers appeared in the sternum, one for each segment except the fourth one which has two. The first group centers appeared in the manubrium and the first two sternebrae on 22 days old embryos. All primary centers seen before birth. There are no secondary ossification centers for the sternum. Concerning ribs, there are four ossification centers for each except the last two ribs. The ossification centers of the body came from the extension of the transvers process of the thoracic vertebrae and it appears as early as two weeks of gestation. By the end of the third week of pregnancy, the ribs show primary center of ossification from the second to the last one. The second center was designed for the head and it appeared two weeks after birth. The third and fourth centers for the tubercle appeared a month after birth. Complete fusion between these centers takes place in three months old rabbit.
Key words: Rabbit, Sternum, Ribs, Development, Double staining, CT
[Full text-PDF] [XML]

Research Paper

Effect of Zinc Supplementation on some Physiological and Growth Traits in Local Male Rabbit.

Amen MHM and Sulaiman Muhammad S.

World Vet. J. 6(3): 151-155, 2016; pii:S232245681600022-6

ABSTRACT

Dietary supplementation of rabbits with zinc (pure zinc) was carried out to determine its effect on some physiological, reproductive performance and growth rate of rabbit during the period from1st March, 2014 to 1st May, 2014. Eighteen locally male rabbits (10weeks age) were randomly assigned to three groups (6 rabbits per group), the control group (T1) was not supplemented with zinc (0 mg zn/kg feed) while treatment groups T2(100mg/kg zn) and T3(200 mg /kg feed) were supplemented with zinc for eight weeks. The results indicated that the T2 and T3 treatments achieved the best significant (P≤0.05) results in terms of increasing the body weight gain. While no significant differences were observed among T2, T3 and the control group regarding the WBC, RBC, weight and relative weight of testes count. Significant (P≤0.05) decreases were recorded in FCR in treated animals as compare with the control group. In conclusion, supplementation of pure zinc to the diets of local rabbit acts as an ameliorative tool of some productive traits of rabbits.

Key words: Zinc, Rabbit, Male, Growth, Feed conversion rate

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Research Paper

Identification of Brucellaspp and Assessing the Impact of Brucellosis Control Programme on Ruminants and Human in Gharbia Governorate, Egypt.

El-Midany SA, El-Tras WF, Eltholth MM, Seada AS and Zaki HM.

World Vet. J. 6(3): 156-165, 2016; pii:S232245681600023-6

ABSTRACT

The aim of the present study was to assess the temporal impact of brucellosis control programme on ruminants and human and to identify Brucella spp. in Gharbia governorate, Egypt. Data for brucellosis in ruminants were collected from the active surveillance programme for brucellosis. Blood and tissue (lymph nodes and spleen) samples from positive animals were also collected. Data for human cases were obtained from the Ministry of Health, Gharbia governorate, Egypt. Statistical analyses were conducted to allow the comparison between different years and ruminant species. To compare between seropositive proportions for different years for each species, a univariate binary logistic regression model was used. There was no consistency in sampling and testing of animals and less than 3% were tested in any given year and the highest proportion of animals tested were cattle. There were variations in seropositive proportions in different species of tested animals and between districts. The number of reported cases of brucellosis in humans was increasing and there was a positive association with that in ruminants. About 36% and 50% of lymph nodes and spleen samples were culture positive, respectively. All isolated strains were identified as B. melitensis biovar 3. Brucellosis is an endemic disease in the study area and the current control programme (test and slaughter) doesn't seem to be effective. Further studies are required for assessing the social and economic impacts of brucellosis. This study indicated that the impact of the current control programme of brucellosis in an endemic area of Egypt. The outcomes of this study would help policy makers to rethink about the control of brucellosis and look for alternative strategies.

Keywords: Brucellosis, Ruminants, Human, Nile Delta, Egypt

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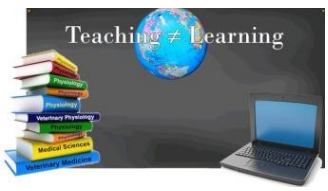
Research Paper



New Challenges of Knowledge Transfer in Veterinary Physiology in a Changing Educational Environment: An Overview of Physiology Teaching in USA and Non-USA Colleges and Schools.

Gyorffy A, Vilmos Frenyo L and Nour A.

World Vet. J. 6(3): 166-184, 2016; pii:S232245681600024-6

ABSTRACT

Veterinary physiology education, faces new challenges in a rapidly changing information technology-based world. The main factors interacting and affecting the veterinary medical education are: the subject matter itself, the new generation of students, new definitions available for knowledge, and the different teaching methods. The objectives of this work were triple. Firstly, to review the factors that impact teaching and learning; Secondly, to provide insights based upon more than twenty years of experience in teaching veterinary physiology to veterinary students at Purdue University, USA,

and the University of Veterinary Medicine, Budapest. Thirdly, a) To gain an understanding of the physiology teaching in USA and non-USA Colleges through analyzing veterinary physiology topics as well as related subjects and factors; b) To examine the contents of the widely used textbooks. Physiology courses data were collected from schools` public websites of the 28 accredited USA veterinary schools, and selected colleges outside the USA, and analyzed. . Course comparisons included the topics taught in the veterinary physiology courses, number of credit hours, signs of tracking, presence or absence of neurophysiology, clinical physiology, pathophysiology, comparative physiology, and the presence or absence of laboratory practical sessions. The contents of most popular text book were analyzed and compared. Our results showed that there were substantial differences in teaching veterinary physiology and related subjects, such as neurophysiology, pathophysiology, comparative physiology, biochemistry, and clinical sciences within and outside the USA. It was observed that not all (only 36%) physiology courses are coupled with labs, especially wet labs. It worth mentioning here that the order of topics within each physiology textbook is not the same and the depth of coverage of different chapters vary, and some of the topics are underrepresented. Interestingly, veterinary students in developed countries are committed to become veterinarians and are highly motivated. This motivation is reflected on better learning. In addition, these students have the opportunity to select during their studies a career path or track such as small animal. Currently, no track-related physiology courses exist in most curricula. Physiology education could benefit from new technologies and interactive learning approaches such as case-based and g, team-based learning, peer instruction, and the flipped classroom. In addition, the use of e-learning management systems would facilitate the learning process, and the interactions between peers and with the instructor. Further, aligning and integrating basic medical sciences and providing clinical correlations would encourage the application of physiological concepts to solving clinical cases.
Keywords: Veterinary physiology, Veterinary schools, Y Generation, Meaningful learning, Learner-centered teaching, Institutional design, Interactive learning, Case-based learning.

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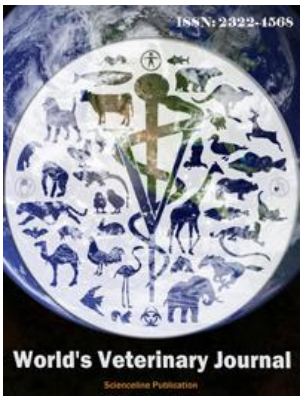

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Serological Surveillance of Caseous Lymphadenitis in Sudanese and Somali Camels Slaughtered at Al-warraq Abattoir, Giza, Egypt

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[2] *Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt*
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ABSTRACT

Caseous lymphadenitis is an economically important bacterial disease of camels and small ruminants worldwide. This study is designed for the surveillance of caseous lymphadenitis in Sudanese and Somali camels slaughtered at Al-Warraq abattoir, Giza, Egypt during the period from January to November 2015. A total of 93 camels were subjected to clinical, postmortem examinations and tested by two enzyme-linked immunosorbent assays based on *Corynebacterium pseudotuberculosis* exotoxin and sonicated whole cell antigens. In addition, the validity of bovine tuberculosis gamma interferon assay to diagnose caseous lymphadenitis in camels was tested which is reported previously in small ruminants, but not in camels. Suspected caseous lymphadenitis lesions were detected in 33 (35.4%) camels compared to seropositivity percentage of 58.06% and 61.29% by exotoxin and sonicated whole cell antigen enzyme-linked immunosorbent assays, respectively. All lesion-affected camels were seropositive except for one animal (3.03%). On the contrary, only 25% of the lesion free camels were seronegative. There was no increase in gamma interferon assay optical density values of four caseous lymphadenitis confirmed cases in response to increased concentration of the stimulating exotoxin antigen. In conclusion, caseous lymphadenitis is prevalent among Sudanese and Somali camels imported for meat consumption in Egypt. Presence of a detectable lesion is highly indicative for seropositivity, but its absence does not indicate seronegativity. In addition, bovine tuberculosis gamma interferon assay has no value to diagnose caseous lymphadenitis in camels.

Key words: Caseous, Lymphadenitis, Camels, Serological, Survey.

ORGINAL ARTICLE
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INTRODUCTION

Camels are important animals reared for transportation, meat and milk production in tropical and subtropical regions. In Egypt, camels exist either as a native herd in the western and eastern deserts or imported from some African countries to diminish the gap between meat production and consumption. Even though camels are tolerant animals, they are susceptible to many infectious diseases. Caseous lymphadenitis (CLA) is considered as one of the most important bacterial diseases of camels (Dioli, 2007). In addition to its zoonotic importance, the disease has negative economic effects on camel production and trading represented in progressive weight loss of the affected camel in addition to carcass trimmings at abattoirs. In contrast to small ruminants, *Corynebacterium pseudotuberculosis* serovar ovis is not the single pathogen responsible for CLA in camels, Many Corynebacteria, *Streptococcus* and *Staphylococcus* spp. may be involved (Aljameel et al., 2013 and Zidan et al., 2013).

The disease has superficial and visceral forms which may show detectable signs or remain subclinical. Clinical signs appear as abscessation of the superficial lymph nodes, chronic weight loss or other symptoms according to the visceral organs affected (Hawari, 2008). For surveillance against CLA, serological tests are usually used to overcome bacterial isolation and visceral lesions sampling difficulties (Oreiby, 2015). Enzyme-linked Immunosorbent Assay (ELISA) is an ideal test for serological surveillance because of its testing capacity, economic and of acceptable reliability (Hoelzle et al., 2013). Other tests such as bovine tuberculosis Gamma Interferon (γ-IFN) Assay has been used to diagnose CLA in small ruminants depending on cross reactivity between bovine, ovine and caprine γ-IFN monoclonal antibodies (Prescott et al., 2002 and Paule et al., 2003). Bovine tuberculosis γ-IFN assay has not been used previously to

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diagnose cameloid CLA cases. In Egypt, studies have been performed on CLA in native camel population and there are no studies involving the imported animals.

This study was conducted for serological surveillance against CLA in Sudanese and Somali camels slaughtered at Al-Warraq abattoir, Giza, Egypt which is not reported previously and additionally to determine the relationship between the presence or absence of detectable CLA lesions and serostatus of the examined animals. Finally, to test the validity of bovine tuberculosis γ-IFN assay to diagnose CLA in camels.

MATERIALS AND METHODS

Animals and sampling

A total of 93 camels (*Camelus dromedaries*) slaughtered at Al-Warraq abattoir, Giza, Egypt during the period from January to November 2015 were randomly selected and used in the present study. The animals' age ranged from 5 to 7 years. A blood sample was collected aseptically, vein puncture site was sterilized by ethanol and sterile vacutainer tubes were used, from each animal before slaughtering and sent to the lab (Veterinary Infectious Diseases Lab, Kafrelsheikh University). Serum was separated by centrifugation at 5000 rpm for 5 min and kept at -20 °C till be used for ELISA. Heparinized blood samples were collected from four CLA positive camels for γ-IFN assay.

Solid phase antigens

Two types of antigens, exotoxin and Sonicated Whole Cell (SWC) were prepared using a nitrate negative *C. pseudotuberculosis* strain which was isolated from CLA lesion of a native sheep. Exotoxin and SWC antigens were prepared according to Sting et al. (1998) and Binns et al. (2007), respectively. Antigens were standardized by Bradford method (Bradford, 1976).

ELISA

Both of exotoxin and SWC ELISA techniques were performed according to Seyffert et al. (2010) with some modifications. Briefly, coating of each well by 0.5 µg antigen in 50 µl bicarbonate buffer (pH 9.6) was performed at 4 °C for 12 h. After two washing steps by Phosphate Buffer Saline (PBS) containing 0.05% tween 20, wells were blocked by 2% Bovine Serum Albumin (BSA) followed by incubation at 37°C for 2 hours. ELISA plates were washed three times, 50 µl of 1:50 diluted serum samples were added and incubated at 37°C for 1 h. Three washing steps were performed and 50 µl 1:50 (according to the producer; Bio-X-Diagnostics, Belgium) diluted Horseradish Peroxidase (HRP)-conjugated polyclonal guinea pig anti-camel conjugate was added. Washing was repeated and 50 µl Tetramethylbenzidine (TMB) substrate was added to each well. The plates were incubated in a dark place at room temperature for 15 min. The reaction was stopped by 25µl/well 5% H_2SO_4. The plates were read at 450 nm and results were validated after blank correction. Cut-off value for each ELISA was calculated as the mean of negative control according to Hoelzle et al. (2013).

γ-IFN assay

A commercially available γ-IFN assay for bovine tuberculosis, BOVIGAM®2G (PRIONICS AG, Switzerland) was used to diagnose CLA in four positive camels. The animals were showing lesions and their seropositive status was confirmed by exotoxin ELISA. Exotoxin antigen was used to stimulate cameloid White Blood Cells (WBCs) in serial double fold concentrations. The assay was conducted according to the producer illustrations. The plate was read at 450 nm with 620 nm as a reference wave length.

Statistical analysis

The degree of association between the clinical status and the results of serological tests was assessed using chi-square (SPSS 21). The significance level was set at $P< 0.05$.

RESULTS

Exotoxin and SWC ELISAs cut-off points were 0.308 and 0.217, respectively. Accordingly, seropositivity percentage was reported to be 58.06% for exotoxin ELISA and 61.29% for SWC ELISA. Optical Density (OD) values of each ELISA are shown in Figures 1 and 2.

Out of the examined 93 dromedary camels, 33 (35.4%) had superficial and/or visceral abscesses suspected to be CLA as shown in figure 3 and figure 4. Considering CLA suspected cases, 18 (54.54%) were positive by both exotoxin and SWC ELISA, one (3.03%) was negative by both ELISAs, 8 (24.24%) were positive by exotoxin ELISA and 6

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(18.18%) were positive by SWC ELISA. Consequently, the existence of CLA lesions is highly indicative for seropositivity (P <0.026).

Out of the 60 clinically and postmortem (PM) negative animals, 16 (26.66%) were positive by both exotoxin and SWC ELISAs, 15 (25%) were negative by both ELISAs, 12 (20%) were positive by exotoxin ELISA and 17 (28.33%) were positive by SWC ELISA.

Concerning γ-IFN assay, it was of no value to diagnose CLA in camels. OD values of four CLA positive camels did not increase upon increasing the concentration of exotoxin antigen. Moreover, there was not a significant difference between OD values of blank sample, to which no antigen was added, and that of different antigen concentrations. Consequently, there was no cross reaction between bovine and cameloid γ-IFN monoclonal antibodies. Results of γ-IFN assay of the tested camels are illustrated in table 1.

Table 1. Gamma interferon assay optical density values of 4 confirmed CLA-affected camels slaughtered at Al-Warraq abattoir, Egypt in relation to the increased antigen concentration

Camel	OD of blank sample	OD at different antigen concentrations				
		5 µg	10 µg	20 µg	40 µg	80 µg
1	0.126	0.201	0.148	0.129	0.150	0.154
2	0.100	0.169	0.157	0.210	0.136	0.122
3	0.007	0.133	0.100	0.089	0.090	0.112
4	0.077	0.055	0.093	0.078	0.076	0.081

OD: Optical Density

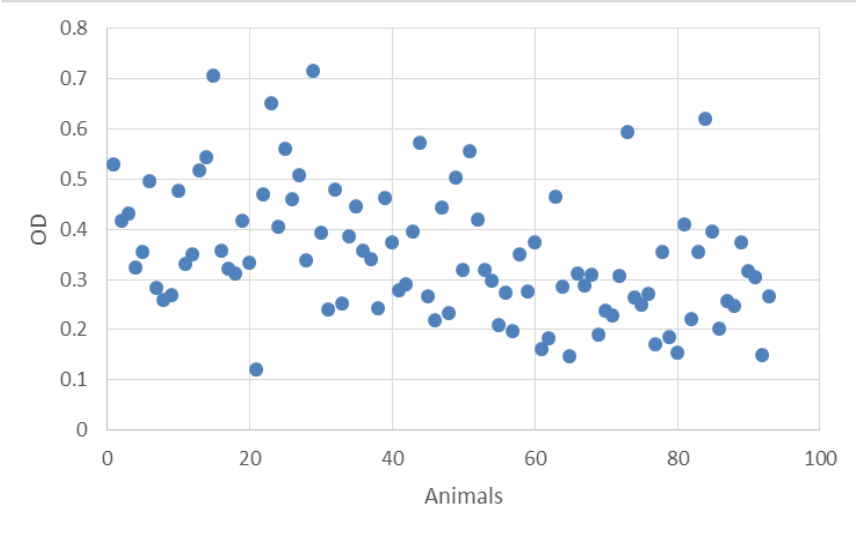

Figure1. Optical density (OD) values of exotoxin antigen enzyme-linked immunosorbent assay in tested camels

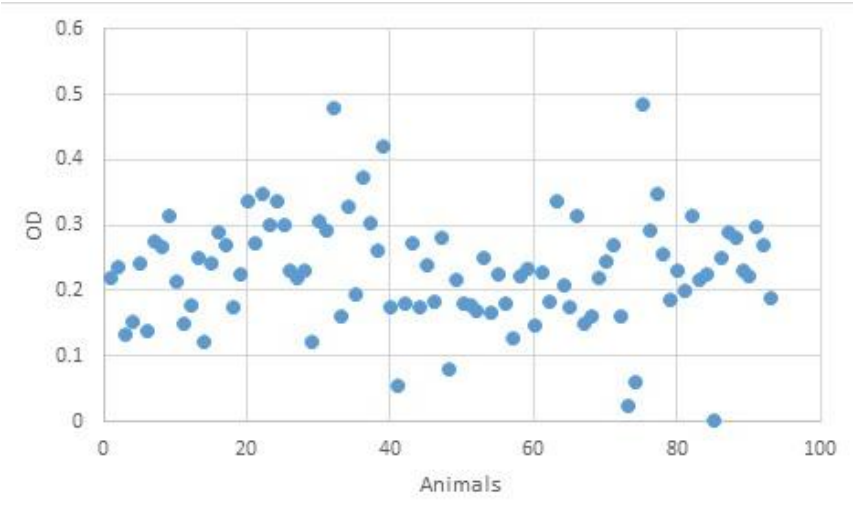

Figure 2. Optical density (OD) values of sonicated whole cell antigen enzyme-linked immunosorbent assay in tested camels

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Figure 3. A dromedary camel with bilateral inferior cervical lymph node abscesses

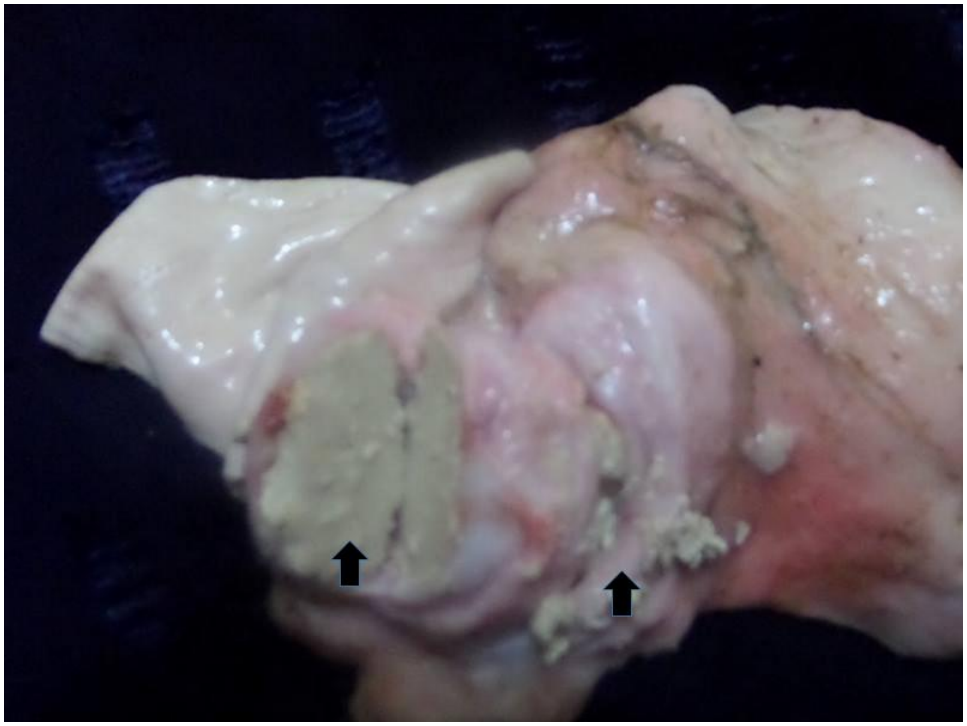

Figure 4. Bronchial lymph node of a dromedary camel showing abscess

DISCUSSION

Corynebacterium pseudotuberculosis and many other members of the order Actinomycetales have been reported as etiological agents for CLA in camels (Aljameel et al., 2013 and Zidan et al., 2013). Antigenic relationship between these bacteria facilitates serological screening against CLA in camels (Komala et al., 2008). Being a common cause for the disease in camels, antigens of *C. pseudotuberculosis* were used. Exotoxin and SWC antigens based ELISA tests revealed seropositivity of 58.06% and 61.29%, respectively. Due to the absence of other serological studies on the disease in Egypt, comparison between different seropositivity percentages could not be held. Higher seropositivity percentage of SWC ELISA than exotoxin ELISA is mainly due to wider cross reactivity between somatic antigens of the causative bacteria than that of their exotoxin antigens.

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Clinical and postmortem examinations had showed lesions in 35.4% of camels compared to seropositivity percentages of 58.06% by exotoxin ELISA and 61.29% by SWC ELISA. Consequently, depending only on the detection of visible lesions in live and slaughtered camels will yield an underestimation of the disease. This is mainly due to subclinical cases or animals with small non-progressive lesions which may escape postmortem examination. Studies conducted in some eastern African countries such as Sudan and Ethiopia reported a prevalence of 12% and 10%, respectively (Domenech et al., 1977 and Aljameel et al., 2013). The higher percentages of affected and seropositive animals comparing to that of other studies is mainly because of it's being an abattoir based study conducted on aged animals.

Concerning the relationship between existence of a suspected CLA lesion and serological status of the examined animal, only 3.03% of CLA lesion affected animals were negative by both exotoxin and SWC ELISAs. Therefore, presence of a lesion is highly indicative for the seropositive status of the examined animal. On the other hand, 25% of the lesion free camels were negative by both exotoxin and SWC ELISAs which mean that the absence of suspected lesions does not indicate seronegative status of the tested animal. Similar findings were reported in new world camels, 40% of the tested llamas and alpacas were seropositive in spite of the absence of detectable lesions (Wernery, 2012). Variations in seropositivity percentages of affected and apparently normal animals using exotoxin and SWC ELISAs may be due to the causative bacterial species and/or infection stage of the disease.

Although bovine γ-IFN assay had been used successfully to diagnose CLA in sheep and goat (Prescott et al., 2002 and Paule et al., 2003), it was of no value in camels as there were no change of OD values of four CLA confirmed cases with increased concentration of the stimulating antigen. Consequently, there is no cross reaction between bovine and cameloid γ-IFN monoclonal antibodies.

In conclusion, CLA is prevalent in Sudanese and Somali imported camels. Presence of a suspected CLA lesion in alive or slaughtered camels is highly indicative for seropositive status and finally bovine γ-IFN assay has no value to diagnose CLA in camels.

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Competing interests

The authors have declared that no competing interest exists.

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The Effect of Very Virulent Infectious Bursal Disease Virus on Immune Organs of Broilers Fed *Moringa Oleifera* Supplemented Feed

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ABSTRACT

A study was conducted to evaluate the immune modulating effect of *Moringa Oleifera* Leave (MOL) feed supplementation in broilers. 240 day old Ross 308 hybrid broiler chicks were assigned into four groups (A, B, C and D) of 60 chicks each in a deep litter house. Groups A and B were fed with formulated broiler starter and broiler finisher feeds containing 5% MOL for 28 and 21 days, respectively, while groups C and D were fed with formulated broiler starter and broiler finisher feed without MOL for 28 and 21 days, respectively. Broilers in groups A and C were vaccinated intramuscularly with 0.5 ml of an inactivated vaccine of intermediate strain of Infectious Bursal Disease (IBD) at 14 and 21 days of age, respectively. Broilers in groups A, B and C were challenged intraocularly at 35 days of age with 0.05 ml of a live very virulent Infectious Bursal Disease Virus (vvIBDV). The Thymus to Body Index (TBI) of birds in group A was 1.09, 1.05 and 1.03 at 35, 38 and 42 days of age respectively, while those in group B had a TBI of 0.84, 1.02 and 0.89 at 35, 38 and 42 days of age respectively. The TBI of birds in group C were 1.04, 1.22 and 1.29 at 35, 38 and 42 days of age respectively, however, there was significant difference between group B and C ($P < 0.02$). *Moringa oleifera* leaves feed supplementation improved the bursa, spleen, harderian and thymus to body weight index of broilers. The MOL feed supplementation and inactivated vaccine did not prevent the atrophy of bursa, spleen and harderian gland against the negative effect of vvIBDV 7 days post infection.

Keywords: Broilers, Immune organs, Infectious bursal disease virus, Organ to body weight index, *Moringa oleifera*

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INTRODUCTION

Infectious Bursal Disease Virus (IBDV) has been reported to be one of the very important immunosuppressive agents in modern poultry production. Infection with IBDV may induce a temporary or permanent destruction of the bursa cloaca and other lymphoid tissues (Sharma et al., 2000; Lukert and Saif, 2003 and Khatri et al., 2005). Therefore, the main targets of the IBD virus are the lymphoid organs and the immune cells (Faragher, 1972). IBD has been reported as a disease of economic significant to the poultry industry worldwide (Hamoud et al., 2007) due to the high mortality, reduced weight gain and condemnation of carcasses as a result of marked haemorrhage in the skeletal muscle (Kaufer and Weiss, 1976 and Van den Berg, 2000) and secondary losses due to immunosuppression (Anderson et al., 1977 and Lukert and Saif, 1997).

The measurement of the lymphoid organ weight plays an important role in providing information on the body's ability to provide lymphoid cells in time of immune response (Heckert et al., 2002). Therefore, one of the measures of immunity that have been commonly used in assessing poultry health is the lymphoid organ weights (Pope, 1991). Any change that may occur during the developmental stages in these organs in response to possible lymphotrophic agent will result in the alteration of the immune function that is associated with lymphoid cells (Cooper et al., 1966 and Boehm and Bleul, 2007).

Bioactive constituents of feed that interact with the immune response have considerable potential of reducing susceptibility to infectious diseases (Kogut, 2009). Due to the growing population of the poultry industry, the use of nutritional dietary intervention strategies may be a cost effective means of preventing specific infectious diseases and

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maintaining the health status of poultry (Kogut, 2009). To gain immunity, the animal needs energy and proteins for the manufacture of antibodies and cells, minerals (zinc, copper, iron and selenium) and vitamins (A and E) for communicating messages in parts of the animal's body in other to fight infections (Conroy, 2005).

Interestingly, *Moringa Oleifera* Leave (MOL) has been reported to possess all the above mentioned carbohydrate, proteins, minerals, vitamins and amino acids (Makkar and Becker, 1999; Kakengi et al., 2003 and Odura et al., 2008). *Moringa oleifera is* in high demand for their medicinal values as they have been reported to have the potential of boosting the immune system (Ramachandran et al., 1980; Atawodi et al., 2008; Sreelatha and Padma, 2009). The absence of available literature on evaluating the bursa of Fabricius, spleen, thymus and harderian gland to body weight index of broilers fed with MOL feed supplementation, vaccinated with an inactivated IBD vaccine and challenged with a very virulent IBDV was the reason for this study.

MATERIALS AND METHODS

Study location

The study was conducted at the poultry research unit of the faculty of veterinary medicine, Ahmadu Bello University Zaria, Nigeria.

Collection and processing of *Moringa oleifera* leaves

Moringa oleifera leaves were harvested from orchards at an early flowering stage in Potiskum, Yobe State, Nigeria. The stem and branches were cut from the Moringa trees and spreads out to shade dry under room temperature for five days. The MOL were then removed manually by hand and grounded into powder using a milling machine.

Mineral analysis

Mineral analysis of MOL was carried out according to the procedure of Association of Official Analytical Chemist (AOAC, 1990) to determine the calcium, phosphorus, magnesium, iron, sodium, zinc, copper, selenium, potassium and manganese components.

Phytochemical analysis

Qualitative and quantitative analysis of MOL was carried out according to the method described by Sofowora (1993), to determine the presence of tannins, phytates, saponins, oxalates, cyanides, alkaloids, carbohydrates, flavonoids, steroids, terpenoids, phenols and phlobatannins.

Proximate analysis

The standard methods of the AOAC (1990) for the proximate analysis of the MOL was used to determine the percentage carbohydrates, crude protein, fats, fibre, ash, moisture and metabolizabal energy.

Feed formulation and analysis

Following shade drying of MOL, it was milled with a hammer mill and sieved with 3 mm mesh to obtain *Moringa oleifera* leaf meal. 22% and 20% of crude protein for broiler starter and broiler finisher mash respectively were formulated [with 5% MOL (Olugbemi et al., 2010a) forming part of the feeds ingredients for group A and B] using pearson square and milled in a toll mill in Zaria, Nigeria. The feed was subjected to analysis based on the method described by the AOAC (1990) in the feed analysis laboratory of the department of animal science, Ahmadu Bello University Zaria, to determine the level of metabolizable energy, crude protein, crude fibre, moisture, ash content, and dry matter.

Experimental chicks and housing

A total of 240 day old Ross 308 hybrid broiler chicks were obtained from a reputable commercial hatchery located in Yola, Nigeria. The chicks were brood in a conventional open-sided house which was properly disinfected before the arrival of the chicks (deep litter system of management with wood shavings as litter material, feeders and drinkers were provided) with cyclic temperatures. The chicks were individually weighed and assigned in a complete randomised design into four different groups A, B, C and D of 60 chicks each (each pen has a floor space of 3 × 4 m). A 100 watt bulb was provided in each of the compartment to supply light and heat during brooding. The broilers were fed with broiler starter for 28 days and broiler finisher for 21 days. Feed and water were provided ad libitum (Table 1).

Vaccines and vaccination

Inactivated vaccines against IBD (Virsin 122, oil emulsion, Biovac limited, Isreal, Batch 1- 382222) and inactivated killed vaccines against Newcastle Disease (ND) (oil emulsion Komorov strain, Biovac limited, Isreal, Batch 1- 422222)

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were obtained from a reputable veterinary pharmaceutical supplier in Jos, Nigeria. Broilers in groups A and C were vaccinated intramuscularly with 0.5 ml of killed IBD vaccine on 14 and 21 days of age respectively, while vaccination against ND was done with an inactivated ND vaccine (0.5 ml) on the thigh muscles intramuscularly on 18 days of age (Table 1).

Challenge with infectious bursal disease virus

At 35 days of age, all the broilers in groups A, B and C were challenged intra ocularly with 0.05 ml of a live vvIBD virus. The IBD virus used for the challenge was a field strain of vvIBDV obtained from previously vaccinated layers that died of natural outbreak of IBD. One millilitre of bursal suspension (v/w) in phosphate buffered saline (pH 7.4) contained 10^{-976} chick infective dose (CID_{50}) of IBDV.

Collection of immune organs

Five birds were randomly selected from each group on the 35, 38 and 42 days of age (Table 1). The selected birds were euthanized and the bursa of fabricius, thymus, spleen and harderian gland were removed for the evaluation of organ body weight index (Lucio and Hitchner, 1979). The organ body index was obtained by employing the formula:

$$\text{Organ: body index} = \frac{\text{Organ to body weight ratio of groups}}{\text{Mean organ to body weight ratio of control}}$$

$$\text{Where, organ to body weight ratio} = \frac{\text{organ weight in grams}}{\text{body weight in grams}} \times 100$$

Table 1. Experimental design for evaluating the effect of *Moringa oleifera* feed supplementation on immune organs of broilers challenged with a very virulent infectious bursal disease virus at 35 days of age.

Group (MOL%)	Age (days)					
	14	**18**	**21**	**35**	**38**	**42**
A (5%)	IBD killed vaccine	NDV killed vaccine	IBD killed vaccine	Collection of Immune organs, Challenged with IBD virus	Collection of Immune organs	Collection of Immune organs
B (5%)	No vaccination	No vaccination	No vaccination	Collection of Immune organs, Challenged with IBD virus	Collection of Immune organs	Collection of Immune organs
C (0%)	IBD killed vaccine	NDV killed vaccine	IBD killed vaccine	Collection of Immune organs, Challenged with IBD virus	Collection of Immune organs	Collection of Immune organs
D (0%)	No vaccination	No vaccination	No vaccination	No challenge with IBD virus	Collection of Immune organs	Collection of Immune organs

MOL = *Moringa Oleifera* Leave; IBD = Infectious Bursal Disease; NDV = Newcastle Disease Virus

Data analyses

The values of the immune organs were expressed as organ to body index and compared with the organ to body index of the control. Values obtained were further subjected to one way analysis of variance (ANOVA) followed by tukeys post-hoc test for multiple comparism. Values of $p < 0.05$ were considered significant using GraphPad Prism version 4.0 for windows.

RESULTS

The Bursa to Body Index (BBI) of broilers in group A was 1.4, 1.57 and 0.71 at 35, 38 and 42 days of age respectively. Group B had a BBI of 1.4, 0.71 and 0.86 at 35, 38 and 42 days of age respectively. Birds in group C had a BBI of 0.86, 1 and 0.71 at 35, 38 and 42 days of age respectively, though no statistical significance between all the groups ($P > 0.05$) (Table 2).

The Spleen to Body Index (SBI) of birds in group A was 1.3, 0.9 and 0.8 at 35, 38 and 42 days of age respectively. Birds in group B had a SBI of 1.1, 0.9 and 1.1 at 35, 38 and 42 days of age respectively, while those in group C had a SBI of 0.77, 1, and 0.8 at 35, 38 and 42 days of age respectively, though no statistical significance between all the groups ($P > 0.05$) (Table 3).

Harderian Gland to Body index (HBI) in birds of group A was 2, 2.5 and 0.3 at 35, 38 and 42 days of age respectively. Birds in group B had a HBI of 2, 2 and 0.66 at 35, 38 and 42 days of age respectively, while those in group

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C had a HBI of 1.25, 1.5 and 0.66 at 35, 38 and 42 days of age respectively, though no statistical significance between all the groups ($P > 0.05$) (Table 4).

The Thymus to Body Index (TBI) of birds in group A was 1.09, 1.05 and 1.03 at 35, 38 and 42 days of age respectively, while those in group B had a TBI of 0.84, 1.02 and 0.89 at 35, 38 and 42 days of age respectively. The TBI of birds in group C were 1.04, 1.22 and 1.29 at 35, 38 and 42 days of age respectively, however, there was significant difference between group B and C ($P < 0.02$) (Table 5).

Table 2. Bursa to body weight index of broilers fed 5% *Moringa oleifera* leave feed supplement, vaccinated with inactivated infectious bursal disease vaccine at 14 and 21 days old and inoculated at 35 days old with a very virulent infectious bursal disease virus

Groups / Age (days)	A 5% MOL, IBD vaccine, vvIBDV	B 5% MOL, vvIBDV	C 5% MOL, IBD vaccine, vvIBDV	D 0% MOL
35	1.4	1.4	0.86	1
38	1.57	0.71	1	1
42	0.71	0.86	0.71	1

MOL= *Moringa oleifera* Leave; IBD = Infectious Bursal Disease; vvIBDV = Infectious Bursal Disease Virus

Table 3. Spleen to body weight index of broilers fed 5% *Moringa oleifera* leave feed supplement, vaccinated with inactivated infectious bursal disease vaccine at 14 and 21 days old and inoculated at 35 days old with a very virulent infectious bursal disease virus

Groups / Age (days)	A 5% MOL, IBD vaccine, vvIBDV	B 5% MOL, vvIBDV	C 5% MOL, IBD vaccine, vvIBDV	D 0% MOL
35	1.3	1.1	0.77	1
38	0.9	0.9	1	1
42	0.8	1.1	0.8	1

MOL= *Moringa oleifera* Leave; IBD = Infectious Bursal Disease; vvIBDV = Infectious Bursal Disease Virus

Table 4. Harderian gland to body weight index of broilers fed 5% *Moringa oleifera* leave feed supplement, vaccinated with inactivated infectious bursal disease vaccine at 14 and 21 days old and inoculated at 35 days old with a very virulent infectious bursal disease virus.

Groups / Age (days)	A 5% MOL, IBD vaccine, vvIBDV	B 5% MOL, vvIBDV	C 5% MOL, IBD vaccine, vvIBDV	D 0% MOL
35	2	2	1.25	1
38	2.5	2	1.5	1
42	0.33	0.66	0.66	1

MOL= *Moringa oleifera* Leave; IBD = Infectious Bursal Disease; vvIBDV = Infectious Bursal Disease Virus

Table 5. Thymus to body weight index of broilers fed 5% *Moringa oleifera* leave feed supplement, vaccinated with inactivated infectious bursal disease vaccine at 14 and 21 days old and inoculated at 35 days old with a very virulent infectious bursal disease virus

Groups / Age (days)	A 5% MOL, IBD vaccine, vvIBDV	B 5% MOL, vvIBDV	C 5% MOL, IBD vaccine, vvIBDV	D 0% MOL
35	1.09	0.84	1.04	1
38	1.05	1.02	1.22	1
42	1.03	0.89	1.29	1

MOL= *Moringa oleifera* Leave; IBD = Infectious Bursal Disease; vvIBDV = Infectious Bursal Disease Virus

DISCUSSION

The higher bursal, spleen, harderian and thymus to body weight index observed in the birds of group A and B before the challenge with vvIBDV (35 days of age) could be an affirmation to the immune properties of MOL that has been reported (Jayavardhanan et al., 1994 and Olugbemi et al., 2010a) and could also be that MOL included in the diet of birds in group A and B should have stimulated the infiltration of more lymphoid cells into the various organs. Increase in

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the BBI and HBI observed in group A at 38 days of age indicates the production of more B cells by these organs and also signify the importance of MOL with respect to immune stimulation. The decrease in the BBI and HBI observed at 42 days of age indicates that both the MOL in the diet of birds in group A and B and the inactivated IBD vaccine given to birds in group A and C could not prevent the atrophy of these organs.

An increase observed in the TBI of birds in group A may suggest that both the inactivated IBD vaccine and MOL in the diet of the birds may have been responsible for the increase. This is because of the immune modulatory properties of the MOL (Olugbemi et al., 2010b) and the immune response due to the vaccination with inactivated IBD vaccine. The lower TBI of birds in group B could either be due to the non-vaccination of the birds with inactivated IBD vaccine or that the immune modulatory properties of MOL alone (without vaccination with IBD vaccine) could not cause an increase in the TBI. Very virulent infectious bursal disease virus is known to cause the destruction of the B lymphocytes and has little or no effect of the T lymphocytes and the thymus is responsible for the production of T cells (Cooper et al., 1966; Boehm and Bleul, 2007). This was observed from the findings of present study where the vvIBDV was shown not to cause atrophy of the thymus in birds of group A, B and C.

CONCLUSION

Moringa oleifera leaves feed supplementation improved the bursa, spleen, and harderian to body weight index of broilers of group A and B respectively. The MOL feed supplementation and inactivated vaccine did not prevent the atrophy of bursa, and harderian gland against the negative effect of vvIBDV 7 dpi. The challenge with vvIBDV did not cause a reduction in the TBI of birds in group A, B and C 3 dpi. Supplementing broiler feed with MOL and vaccinating against IBD increased the TBI of birds in group A at 35, 38 and 42 days of age.

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Competing interests

The authors have no competing interest.

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Insight into the Virulence-Related Genes of *Edwardsiella Tarda* Isolated from Cultured Freshwater Fish in Egypt

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ABSTRACT

One of the most serious fish pathogens infecting both cultured and wild fish species was found to be *Edwardsiella tarda* which contains important virulence factors that enhance bacterial survival and pathogenesis in hosts. The present study aims to isolate, identify and characterize *E. tarda* in freshwater fishes in Kafr El-Sheikh governorate, Egypt using selective differential cultural medium (Rimler Shotts agar), morphological and biochemical tests (oxidase, catalase, methyl red, voges proskauer, indole, citrate utilization, gelatine hydrolysis, H_2S production, oxidation-fermentation, nitrate reduction and sugar utilization tests). Pathogenesis of *E. tarda* was checked by experimental infection to *Oreochromis niloticus* fish together with screening of the highly virulent three virulence genes (esrB, mukF and gadB). The obtained results revealed the presence of the three virulence genes in the selected strain of *E. tarda* which gave severe lesions in the experimentally infected *Oreochromis niloticus*. *E. tarda* strain having more than one virulence gene results in more severe lesions than strains having one or even no virulence genes.

Key words: *Edwardsiella tarda*, Virulence genes, Freshwater fish

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INTRODUCTION

Edwardsiella tarda (E. tarda) is a predominantly enteric pathogen of both fresh water and brackish water fishes (Plumb, 1994). It is mainly responsible for causing a serious systemic bacterial disease, the edwardsiellosis, having a worldwide distribution among variety of fish taxa (Austin and Austin 1999; Maiti et al., 2009). The bacterium is also a pathogen for wide range of hosts rather than fish namely, reptiles, birds and mammals including humans (Plumb, 1993 and Park and Oh, 2008). *E. tarda* is a Gram-negative bacterium that causes a systemic suppurative disease in fish and humans. Recently, the complete genome sequence of a highly virulent and multidrug resistant *E. tarda* strain isolated in China indicated that this microorganism harbor a number of virulence and toxin secretion system related genes that explain in some extent it capacity to survive within phagocytic cells and to infect a variety of hosts (Verjan et al., 2013).

E. tarda is widely distributed in aquatic environments (Pitlik et *al.*, 1987 and Wyatt et al., 1979). It is one of the serious fish pathogens, infecting both cultured and wild fish species. Research on edwardsiellosis has revealed that *E. tarda* has been considered as a common guest of a high variety of animals including fish, reptiles, amphibians, chickens and other warm-blooded animals as humans (Castro et al., 2016), and contains important virulence factors that enhance bacterial survival and pathogenesis in hosts (Park et al., 2012). *E. tarda* is an intracellular, rod-shaped, Gram negative, non-capsulated, motile, facultative anaerobic bacterium that was first isolated from a pond-cultured eel (Hoshino, 1962).

The basis of pathogenicity depends on the number of virulence factors in *E. tarda*, including the ability to invade epithelial cells (Janda et al., 1991 and Ling et al., 2000), resist serum and phagocyte-mediated killing (Ainsworth and Chen, 1990 and Srinivasa et al., 2001), and production of toxins, such as, haemolysins and dermatotoxins for disseminating infection (Ullah and Arai, 1983 and Hirono et al., 1997). *E. tarda* virulence is believed to be a multifactorial process not yet completely understood. Several potential pathogenic properties have been suggested to contribute to the infection process of *E. tarda*, including secretion of degradative enzymes, adhesions, type III secretion system, type VI secretion system and the ability to survive and replicate in phagocytes. It has been established that the

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pathogenic strains of *E. tarda* have virulence genes which might be absent in nonpathogenic strains (Rao et al., 2003 and Castro et al., 2016). Now, many virulence-related genes in *E. tarda* have been cloned or reported, which included catalase (katB), TTSS regulator (esrB), putative killing factor (mukF), Fimbrial operon **(**fimA), glutamate decarboxylase **(**gadB), Citrate lyaseligase (citC), pstS, pstC, type III secretion system (ssrB), astA, isor, $ompS_2$, Hemolysine A (hlyA) and ATPase domain of DNA Gyrase (gyrB) (Rao et al., 2003; Rao et al., 2003; Rao et al., 2004; Tan et al., 2005; Choresca et al., 2011 and Abd El-Kader, 2015). Abd El-Kader, 2015 studied that the Nile Tilapia (*Oreochromis niloticus*) could be used as the animal model for studying the pathogenicity of *E. tarda*.

This study was carried out to isolate, identify and characterize *E. tarda* from freshwater fish in Kafr El-Sheikh governorate, Egypt, and using various culture media, morphological and biochemical tests. In the present study we screened the presence of three virulence-associated genes of *E. tarda* isolated from diseased cultured fishes.

MATERIALS AND METHODS

Ethical approval

Animal ethics committee, faculty of veterinary medicine, Kafr El-Sheikh University, Egypt, approved the protocol and conducting of the study.

Isolation and identification of *E. tarda*

A total number of 120 *Oreochromuis niloticus* fish were collected from Kafr El-Sheikh governorate farms over the seasons of the year 2015. The collected fish showed hemorrhagic patches on different parts of the body and eye exophthalmia externally. Internally, abundant ascetic fluid was found in the abdominal cavity and small white nodules were observed in liver, kidney, spleen and gills.

For bacteriological examination, swabs were collected from the infected parts of the fishes. The collected swabs were firstly pre-enriched on Tryptic Soy Broth (TSB, Oxoid). After which (pre-enrichment on TSB) they were streaked on Rimler Shotts agar (RS) for presumptive identification and incubated at 28 °C for 24 hours. Well-differentiated single bacterial colonies were further streaked onto Tryptic Soy Agar (TSA, Oxoid) for obtaining pure culture.

Biochemical analysis

For phenotypic identification, pure cultures were subjected to Gram staining and viewed microscopically. Further biochemical tests like motility, oxidase, catalase, methyl red, voges proskauer, indole, citrate utilization, gelatine hydrolysis, H_2S production, Oxidation-fermentation, nitrate reduction and sugar utilization tests, were performed for the identification as well as strain differentiation of bacteria and results were compared with the reference strain of *E. tarda* (obtained from the food analysis center, faculty of veterinary center, Banha university, Egypt).

Molecular identification by PCR

DNA Extraction using QIA amp kit: Genomic DNA was extracted from every isolate of *E. tarda* using DNA extraction kit (QIAamp: Qiagen inc., USA). Isolated DNA samples were checked for purity and quantified in ND-1000. The samples were then resolved on agarose gel (0.8%) with 4 µl of template DNA mixed with 1 µl of loading dye (xylene cyanol + bromophenol blue) and electrophoresed at 120 volts for 70 min. DNA samples showing intact bands were used for Polymerase Chain Reaction (PCR) amplifications (Shah et al., 2009).

Primer sequences of E. tarda used for PCR identification system: Detection of the virulence factors of *E. tarda* represented by esrB (TTSS regulator), mukF (putative killing factor) and gadB (glutamate decarboxylase) was carried out using the peimers which is introduced in Table 1.

DNA amplification of E. tarda: The amplification was performed on a thermal cycler (master cycler, eppendorf, Hamburg, Germany). The targeted genes of *E. tarda* isolates were esrB, mukF and gadB. To amplify the genes, 25 µl of reaction mixture was made containing 20ng of template DNA, 20 pM of primers, 160 µM of dNTP mix, 1.25 U Taq polymerase, 1×Taq buffer, and 0.5 mM MgCl2. The three genes were amplified using the specific primers with 32 cycles of denaturation at 94°C for 1 min, annealing at 55°C, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR amplified products were analyzed by 1.5% of agarose gel (Sigma, USA), stained with ethidium bromide and visualized as well as captured on UV transilluminator, then compared with the marker DNA ladder (100 bp) (Wang et al., 2012).

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Table 1. The primers used for the amplification of different virulence associated genes

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Reference
esrB (F)	**5′** TCGTTGAAGATCATGCCTTGC **′3**	311	Wang et al. (2012)
esrB (R)	**5′** TGCTGCGGGCTTTGCTT **′3**		
mukF (F)	**5′** TTGCTGGCTATCGCTACCCT **′3**	357	
mukF (R)	**5′** AACTCATCGCCGCCCTCTTC **′3**		
gadB (F)	**5′** ATTTGGATTCCCGCTTTGGT **′3**	583	
gadB (R)	**5′** GCACGACGCCGATGGTGTTC **′3**		

Experimental infection

Total Bacterial count: Using the drop plate method for estimation of *E. tarda* strain per 1 ml, was used in demonstration of the inoculum dose for the experimental studies according to Cruickshank et al. (1975).

Lethal Dose 50: A total number of 70 apparently healthy *Oreochromis niloticus* fish weighting (50±0.5 gm), were divided into 7 groups, 10 fishes per each group and the seventh group was kept as a control group. All fishes were kept for 15 days under observation prior to injection for accommodation and to confirm that they are free from diseases.

24 hours colony culture of *E. tarda* strain on TSA was used, the colonies were picked up and suspended in sterile saline in a ten fold serial dilution with subsequent incubation at 28 °C for 24 hours for plate counts on TSA. Only the dilutions (10^2-10^7 cfu) were used. Each group was intraperitoneally injected with 0.5 ml/fish of each bacterial dilution. The fishes in the control group were injected with 0.5 ml PBS/fish (Phosphate Buffer Saline). All the injected fish were kept for 1 week post-inoculation for observation. The mortalities were recorded twice/ day according to Ibrahim et al. (2011). The freshly dead fishes were moved for further pm examination. The LD_{50} (the dose which kill 50% of the injected fish) was calculated according to Reed and Muench (1938).

Pathogenicity test: Experimental infection was carried out to determine the pathogenicity of *E. tarda* strain using intra-peritoneal route injection according to Ibrahim et al. (2011). A total number of 60 apparently healthy *O. niloticus* fishes weighting (50±0.5 gm), were divided into 3 groups; 20 fishes per each. Each fish in the first group was intraperitoneally injected with 0.2 ml/fish of LD_{50} dose *E. tarda* strain which was determined previously (1.8×10^5 cfu). Each fish in the second group (control negative group), was intraperitoneally injected with 0.2 ml/fish of PBS (Phosphate Buffer Saline). Each fish in the third group (Control positive group), was intraperitoneally injected with 0.2 ml/fish of obtained reference *E. tarda* strain. All injected fishes were observed for a period of 28 days post-inoculation. Mortalities were recorded daily and freshly dead fishes were moved for further pm examination and histopathological studies.

Histopathological examination

Specimens from liver, kidney and spleen of experimentally infected fishes were taken. Specimens were fixed immediately in 10% neutral buffered formalin, dehydrated and embedded in paraffin blocks. Paraffin blocks were sectioned at 4-5 µm thickness and stained with Hematoxilin & Eosin (H&E) and examined under light microscope (Leica) using ×200 and ×400 magnification power according to Bancroft and Gamble (2007).

Table 2. Comparison of the phenotypic and biochemical characteristics between the isolated *E. tarda* strain and the reference strain

Biochemical tests	Isolated *E. tarda* strain	Reference strain
Motility test	+	+
Oxidase test	-	-
Catalase test	+	+
Gram staining	-	-
Indole test	+	+
Methyl red test	+	+
Voges proskaeur test	-	-
Citrate utilization test	-	+
Arginine dehydrolization test	-	-
Acid production from glucose	+	+
Acid production from fructose	+	+
Acid production from maltose	+	+
Acid production from lactose	-	-
Acid production from rhaminose	-	-
Acid production from sucrose	-	-
Acid production from mannitol	-	-
Lysine decarboxylase test	+	+
Ornithine decarboxylase test	+	+
H_2S production	+	+
Lecithinase test	-	-

+ = positive reaction, - = negative reaction

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RESULTS AND DISCUSSION

E. tarda, the causative agent of Edwardsiellosis, is a serious systemic disease of both cultured and wild fishes, has been recognized as one of the most dangerous pathogens causing high economical losses among aquaculture industries worldwide with expanded host range (Hou et al., 2009 and Park et al., 2012) including humans (Xie et al., 2014).

External examination of naturally infected *Oreochromis niloticus* revealed hemorrhagic patches on different parts on the body, eye exophthalmia (Figure 1 and Figure 2) and cutaneous ulcers (Figure 3). The gross lesions displayed in the current study are similar to those reported by several authors (Han et al., 2006; Padros et al., 2006; Shabaan, 2007 and Ibrahim et al., 2011).

However, the postmortem findings exhibited the presence of abundant ascetic fluid in the abdominal cavity together with the enlarged congested gall bladder, liver, kidney and spleen (Figure 4). The postmortem findings displayed in the present study are similar to those reported by Shabaan (2007) and Carlos et al. (2012). The current postmortem findings may be due to the action of bacterial toxins. *E. tarda* secretes Haemolysin and Dermotoxins, Exotoxins Extracellular Products (ECP) and Endotoxin Intracellular Components (ICC) (Suprapto et al., 1995; Hirono et al., 1997 and Mathew et al., 2001). However, liver and kidney congestion might be due to hepatic and nephric virulence factors of *E. tarda* (Miwa and Mana, 2000 and Mathew et al., 2001).

Figure 1. *Oreochromis niloticus*, collected from Kafr El-Sheikh farm, Egypt in summer, naturally infected with *E. tarda* showing hemorrhagic patches (arrow) on different parts of the body

Figure 2. *Oreochromis niloticus*, collected from Kafr El-Sheikh farm, Egypt in autumn, naturally infected with *E. tarda* showing exophthalmia (arrow)

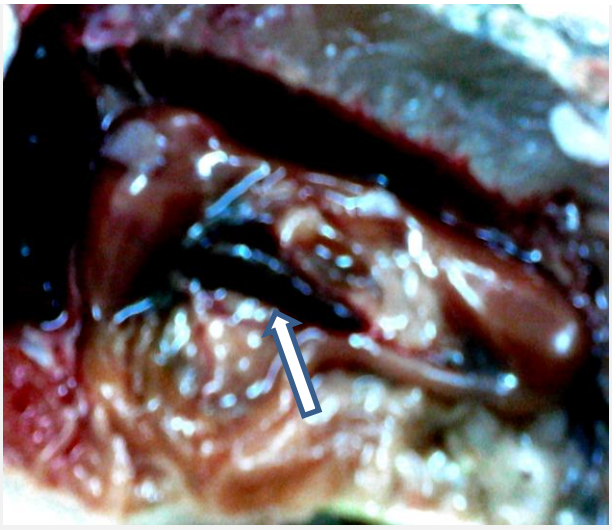

Figure 3. *Oreochromis niloticus*, collected from Kafr El-Sheikh farm, Egypt in summer, naturally infected with *E. tarda* showing cutaneous ulcers on the body (arrow)

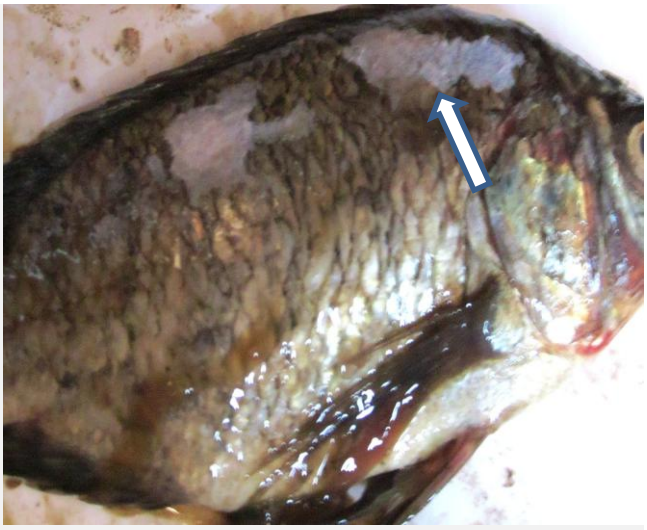

Figure 4. *Oreochromis niloticus*, collected from Kafr El-Sheikh farm, Egypt in autumn, naturally infected with *E. tarda* showing enlarged and congested spleen (arrow)

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The presumptive identification of the bacteria in the recent study was carried out from colony morphology over Rimler Shotts (RS) medium which acts as a selective medium for *E. tarda* (Buller, 2004; Das et al., 2014 and Achrya et al., 2007). They showed typical greenish colonies with black center over RS plates. Microscopically, the bacteria were observed as Gram negative short rod shaped organism. Biochemical characterizations have proven to be a valuable method for typing and differentiation of bacterial fish pathogens (Tison et al., 1982 and Austin et al., 1997). In the present study, based on 20 biochemical tests, a total number of thirty isolates were positively identified as *E. tarda*. All strains were positive for motility, catalase, indole, methyl red, production of gas and acids from glucose, fructose, maltose, H_2S production on TSI agar and lysine and ornithine decarboxylation. But, showed negative results for oxidase, voges proskauer, arginine dehydroxylation production of acid from lactose, rhamnose, sucrose and mannitol. All the obtained biochemical results were compared with the reference strain (Table 1). Most of the phenotypic characteristics of the isolates were similar as reported in Bergey's manual of determinative bacteriology (Holt et al., 1994). Based on the biochemical tests results, all obtained isolates were similar to *E. tarda* reference strain. However, a low degree of heterogeneity was observed in citrate utilization test. All biochemical obtained results were similar to findings of Amandi et al., (1982); Wei and Musa (2008) and Kumari (2011). However, negative results to citrate utilization test was similar to Coles et al. 1978) and Baya et al. (1997).

Pathogenic bacteria may have virulence genes that are absent in non-pathogenic bacteria, making them virulent. Virulence genes may also be present in both pathogenic and non-pathogenic bacteria but may be functional only in pathogenic ones (Rao et al., 2003; Wang et al., 2012 and Castro et al., 2016). One of *E. tarda* positive strains was screened for the presence of three virulence genes (TTSS regulator (esrB), putative killing factor (mukF) and glutamate decarboxylase (gadB) by PCR technique. The results revealed that the selected strain had the three screened virulence genes (esrB, mukF and gadB) (Figure 5). It has been reported that the three virulence genes (esrB, mukF and gadB) obtained in the current study were specific to pathogenic *E. tarda* (Rao et al., 2003; Choresca et al., 2011 and Wang et al., 2012). These genes can therefore be used as biomarkers to perform diagnosis of pathogenic *E. tarda*.

The median lethal dose fifty (LD_{50}) experiments in the present study revealed that the concentration 10^5 cfu was the most potent dilution causing 50% mortalities within (24-48 hr). This result is similar to Shaaban (2007) but, disagrees with Ibrahim et al. (2011) who obtained LD_{50} at concentration of 10^4 cfu. The differences in LD_{50} between different authors may be due to the number of the virulent genes in *E. tarda* strain, pathogen properties as well as size, weight of fish species and temperature difference (Amandi, 1982 and Ibrahim et al., 2011).

The (gadB) recorded in the current study, provides resistance to bacteria towards phagocytes-mediated killing inside the host. The gad system neutralizes acidity and enhances the survival under extreme acid conditions, the glutamate-dependant acid resistance system requires the glutamate decarboxylase gene for protection under acidic condition. Mutation in gadB gene of *E. tarda* resulted in attenuation of the mutant in vivo and acid sensitivity in vitro indicating that the mutant was unable to survive and cause infection inside the host (Srinivasa Rao et al., 2003). Bacteria fight against serum and phagocyte-mediated killing by gadB.

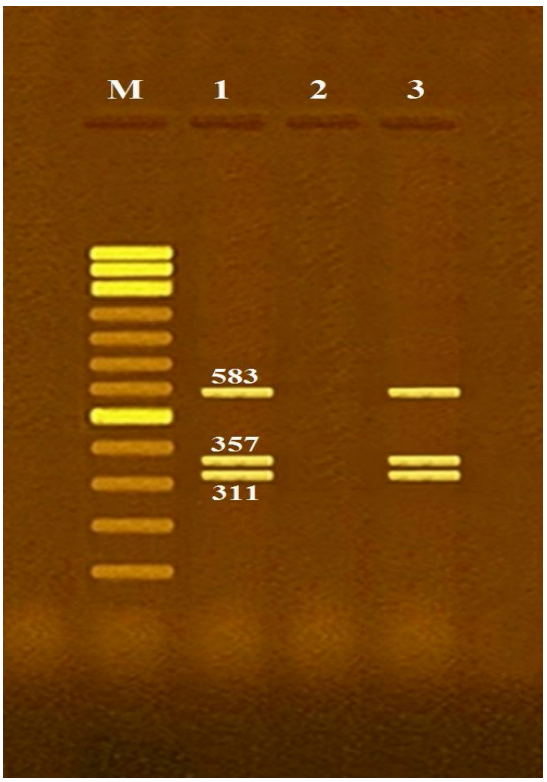

Figure 5. Agarose gel electrophoresis of multiplex PCR of esrB (311 bp), mukF (357 bp) and gadB (583 bp).virulence genes for characterization of *Edwardsiella tarda*

Lane M: 100 bp ladder as molecular size DNA marker; **Lane 1:** Control positive of *E. tarda* for esrB, mukF and gadB genes; **Lane 2:** Control negative; **Lane 3:** Positive *E. tarda* strain for esrB, mukF and gadB genes.

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The clinical signs of experimentally infected *O. niloticus* with current *E. tarda* strain revealed that fishes were lethargy showing abnormal swimming behavior, loss of escape and defensive reflexes, floating near water surface with gasping atmospheric air followed by fish death with opened mouth. This might be due to stress response and/or development of anaemia leading to insufficient oxygen supplements inside tissues and compensatory increased respiration as a result (Meyer and Bullock, 1973; Walters and Plumb, 1980; Miyazaki and Kaige, 1985; Mohanty and Sahoo, 2007 and Vasquez-Pineros et al., 2010). The Pathogenesis of edwardsiellosis is complex and associated with multiple virulence factors that might be encoded in the chromosome or in conjugative plasmids (Wang *et al.*, 2009). The recorded mortalities of the experimentally infected fishes may be attributed to the presence of more than one virulent gene in the same strain as well as the synergistic effects conferred by combination of several virulence genes including gadB (Shen and Chen, 2005). Besides, the *esrB* and mukF play an important role in virulence of *E. tarda* (Rao et al., 2003; Rao et al., 2004 and Zheng et al., 2005), that appear to confer survival and replication within macrophages (Okuda et al., 2009) or causes pore formation in the host cell membrane (Tan et al., 2005).

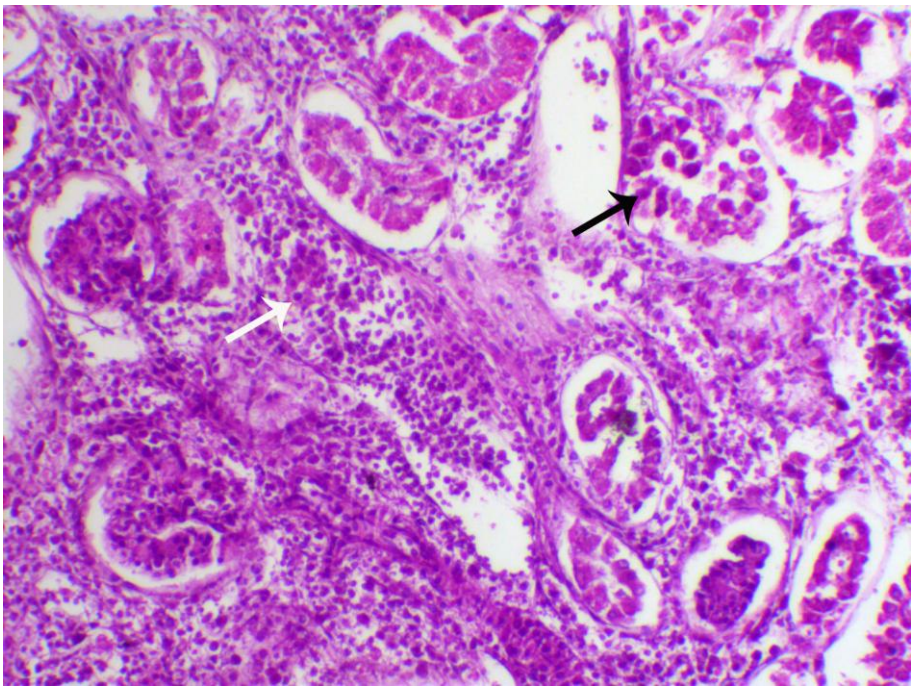

Figure 6. Posterior Kidney of *Oreochromis niloticus* infected with *Edwardsiella tarda* showing interstitial nephritis represented by marked leukocytic infiltration (White arrow) and degenerative tubules (Black arrow), H&E, bar=100µm, ×200

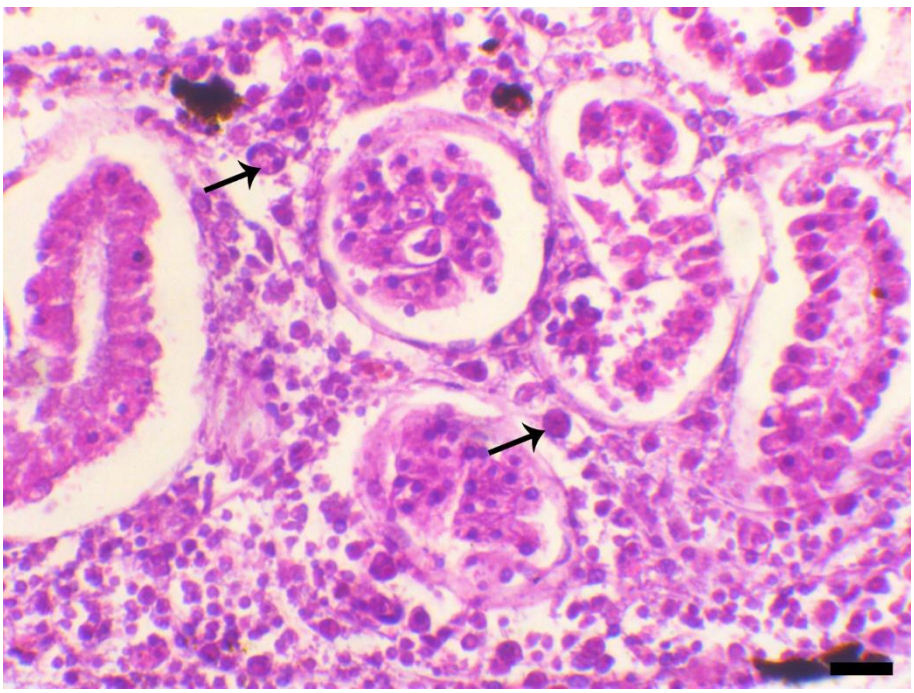

Figure 7. Posterior Kidney of *Oreochromis niloticus* infected with *Edwardsiella tarda* showing leukocytic infiltration consistent mainly from macrophages-laden bacteria (Black arrow), H&E, bar=50µm, ×400

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The histopathological findings in the experimentally infected *Oreochromis niloticus* in the present study were obviously seen in the kidney. The kidney showed multifocal interstitial nephritis accompanied with infiltration of mononuclear inflammatory cells and macrophages-laden bacteria (Figure 6 and figure 7). While the liver showed also multifocal necrotic areas associated with inflammatory cells infiltration. These findings were nearly similar to those reported by Darwish et al., 2000; Miwa and Mana, 2000; Shabaan, 2007 and Ibrahim et al., 2011. The findings of histopathological changes could be attributed to the potential virulence factors of *E. tarda* as cell adhesion factors, cell invasion activity, hemolysin, cytotoxin and enterotoxin which might induce the necrosis and degenerative changes in most organs as well as the hepatic and nephric virulence factors (Chen et al., 1996; Miwa and Mana, 2000 and Mathew et al., 2001).

CONCLUSION

E. tarda is a gram negative, rod-shaped, motile bacterium that exhibits a broad geographical distribution and host range, causing significant economic losses to the aquaculture industry. The results of the present work, the three tested virulence genes were correlated with *E. tarda* pathogenicity. The more the number of virulence genes the more the pathogenecity of *E. tarda*. This could be of importance in formulating novel therapeutics and in vaccine development to protect fish against systemic edwardsiella infection.

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Competing interests

Authors have declared that there is no competing interest.

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Prevalence of Antimicrobial Resistant Salmonellae Isolated From Bulk Milk of Dairy Cows in and Around Debre Zeit, Ethiopia

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ABSTRACT

A cross sectional study to determine the prevalence and antimicrobial resistant profile of *Salmonella* isolates from 106 bulk milk of dairy cows was undertaken from December 2013 to April 2014 in supermarket, large and small holder's dairy farms in Debre Zeit, Ethiopia. The bacteria was isolated and identified according to standard methods and sensitivity tests were done by the Kirby-Bauer disc diffusion method. The study revealed an overall prevalence of 23.6% (25/106). The occurrence of *salmonella* in large and small scale farm was 20.4% and 27.3% respectively. The isolated *Salmonella* spp. was resistant to at least two or more antimicrobials which were used in this study. A higher proportion of the isolates (96%) were resistant to ampicillin and the lowest resistance was recorded for streptomycin (8%). Assay of antimicrobial resistance revealed that 96% of *Salmonella* isolates were resistant to two or more of the nine antimicrobials tested whereas 4% of the isolate was sensitive. The most common resistance was to Ampicillin 24 (96%), oxytetracycline 21 (84%), amoxicillin 12 (48%), Chloramphenicol 10 (40%). A significant proportion has developed resistance for routinely prescribed antimicrobial drugs both in veterinary and public health sectors. This poses considerable health hazards to the consumers unless prudent antimicrobial usage, adequate heat treatment, improvement of standards of hygiene and development and enforcement of suitable legislation, which safeguard consumers, are urgently instituted.

Key words: Isolates, *Salmonella*, Prevalence, Antimicrobial resistance, Bulk milk sample

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INTRODUCTION

Salmonellosis is the most common food borne zoonotic disease in both developing and developed countries with different incidence rates (Addis et al., 2011). *Salmonella* species are the major pathogenic bacteria in humans as well as in animals which are leading causes of acute gastroenteritis in several countries (Hussein et al., 2014**)**. Dairy cows are reservoir for Non Typhoidal *Salmonella* in industrialized countries and large outbreaks of *Salmonella* infection have been associated with foodborne transmission from milk and other dairy products (Threlfall, 2000). Some studies have reported that animal origin foods are considered to be the primary source of human salmonellosis (Acha and Szyfers, 2001). *Salmonellae* are infrequent cause of mastitis in dairy cows but several species of *Salmonella* have been documented to colonize udders and shed at levels of up to 2000 Organisms /ml (Liyuwork et al., 2013). According to Jayarao and Henning (2001) *Salmonella* was isolated from 6.1% of bulk tank milk samples from dairy herds in eastern South Dakota and western Minnesota, United States of America.

Members of the genus *Salmonella* are gram negative and rod shaped short bacilli bacteria belonging to the family *Enterobacteracae* (Ellermeier and Slauch, 2006). *Salmonella* are comprised of two central species, *Salmonella enterica* and *Salmonella bongori* (Brenner *et al*., 2000). Presently, six subdivisions of *Salmonella enterica* subspecies exist with over 2500 serovars currently identified and several common serovars to human clinical infections (Coburn, 2007). *Salmonella* is primarily intestinal bacteria that widespread in the environment and commonly found in farm effluents, human sewage and in any material subject to fecal contamination. Salmonellosis has been recognized in all countries but appears to be most prevalent in areas of intensive animal husbandry, especially poultry, dairy and swine production

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(Wray and Davies, 2003). The distribution of *Salmonella* can vary greatly depending on the Serovars. Generalist species such as *Salmonella enterica* serotype *Enteritidis* and *Salmonella enterica* serotype *Typhimurium* have established global niches (Ellermeier and Slauch, 2006).

Salmonella is a leading cause of foodborne mortality and morbidity in worldwide (Morbidity and Mortal Weekly Report, 2008). The severity of disease depends heavily on host susceptibility and the virulence of the serovar. *Salmonella* virulence requires the coordinated expression of complex arrays of virulence factors that allow the bacterium to evade the host's immune system regardless of source and host status (Ohl and Miller, 2001). Pathogenicity is mediated by certain factors such as strain virulence, infectious dose, route of infection, and host susceptibility (Wray and Davies, 2000).

The World Health Organization (WHO) has reported as increase in the incidence of antibiotic resistant strains of *Salmonella* due to the use of antibiotics as treatment and prophylaxis frequently around the world WHO (2010). Resistance of *salmonella* to commonly used antimicrobials is increasing due to the use of antimicrobial agents in food animals irrationally both in the veterinary and public health sectors and has become worldwide problem (Tauxe, 1991 and D'Aoust et al., 1992). This is partly due to the use of antimicrobial agents in food animals at sub therapeutic level or prophylactic doses that may promote growth and markedly increase the human health risks associated with consumption of contaminated milk and meat product (Zewdu and Cornelius, 2009).

Development of antimicrobial resistant *Salmonella* has economic as well as public health importance. Studies made elsewhere indicated that dairy items are important vehicles of salmonella for raw consumers (Jay, 2000). In Ethiopia, limited studies were carried out on milk and milk products. The objectives of this study were to determine the prevalence and antimicrobial resistant profile of *Salmonella* isolated from bulk milk of dairy cows.

MATERIALS AND METHODS

Study area

The study was carried out in100 dairy farms found in Debre Zeit and the surrounding areas from December 2013 and April 2014. Debre Zeit town is located Misraq shewa zone of the oromia region, some 47.9 km south east of Addis Ababa and has total human population of 95000. It is located at latitude and longitude of 10° 35' 0" N and 35° 48' 0" E respectively. The area lies at an altitude of 1850 meter above sea level and experiences the mean annual rainfall, maximum and monthly temperatures ranges between 801.3mm, 25.5 0 C, 23.7 in July & 27.70 C in may, respectively. The mean annual minimum temperature is 10.50 C, monthly values ranges between 7.4 in December and 12.10 C in July and August.

Study design

The study was cross sectional and the targets were government and private holdings dairy farms and supermarkets. The list of all the currently operational large dairy farms registered in Debre Zeit was collected from the city municipality. The minimum sample size was calculated by using the following formula according to Thrusfield (2007): $n = Z^2 p \exp(1\text{-}Exp\text{xp})/ d^2$ where n = sample size, Exp (expected prevalence) = 2.1% (Tesfaw *et al*., 2013), d (absolute precision) = 0.05 at 95% confidence interval. Accordingly the sample size was calculated to be 32. To maximize the precision of the estimate the calculated sample size was inflated more than three times and a total of 106 samples were considered.

About 30 ml of milk from bulk milk was collected by using sterile screw capped labeled universal bottles and transported to the microbiology laboratory of the college of veterinary medicine and agriculture, Addis Ababa university, Debre Zeit, by using an ice box. Upon arrival, the samples were immediately processed or stored over night in a refrigerator at 4^{o}c until processed in the following day.

Isolation and identification of *Salmonella*

A total of 106 bulk milk sample from cross and local breed cows were included in the study from different households/small holders, supermarket, and large sized dairy farms found in Debre Zeit and the surrounding area, Ethiopia. A sampling unit consisting of 30ml of bulk milk was taken aseptically at random from milk tank container using screw capped universal bottles. The isolation of *Salmonella* was performed according to the standard operating procedure set by the global *salmonella* surveillance and laboratory support project of the WHO and the National Health Services for Wales (NHS), in which both procedures use ISO-6579 (ISO, 2002). Overnight frozen samples were allowed to thaw for 3-5 hours at room temperature before analysis. The bacteriological media used for the study were prepared following the instructions of the manufacturers.

Five ml of each sample were pre-enriched in 45ml Buffered Peptone Water (BPW) (Himedia) in a ratio of 1:9 and incubated for 16 to 24 hours at 37^{o}c. Following this, 1 ml and 0.1 ml aliquot of the pre-enrichment broth was transferred

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aseptically into 10 ml of selenite- F(Himedia) and 10 ml of Rappaport-Vassilliadis (RV) broth(oxiod), mixed and then incubated for 24 and 48 hours at 37ºC and 42ºC respectively. Following incubation, a loopful of each culture was streaked onto one plate of Brilliant Green Agar (oxiod) and another plate of Xylose Lysine Deoxycholate (XLD and BGA) medium and incubated at 37ºC for 24 to 48 hours. Most *Salmonellae* give an alkaline reaction in BGA medium and have pink colonies. On XLD (Himedia) medium the majorities of *Salmonella* serotypes produces hydrogen sulphide and have red colonies with a black (H2S) center (Quinn *et al.,* 1994). A total of four suspected colonies from each sample were plated on nutrient agar for further biochemical tests.

Biochemical tests

Colonies from the nutrient agar were tested by the following biochemical tests: indole, methyl red, Voges proskauer, urea broth, lysine iron agar (oxiod) and Triple Sugar Iron (TSI) (oxiod) (ISO 6579, 2002). All four suspected pure culture isolates colonies were inoculated on TSI agar, lysine iron agar, urea broth, Tryptone broth, methyl red Voges proskauer(MR-VP)broth (Himedia) and incubated for 24 or 48 hours at 37^{o}c. Colonies producing an alkaline slant with acid (yellow color) butt on TSI with hydrogen sulphide production and gas formation, alkaline slant, butt hydrogen sulphide production on lysine iron agar, negative for urea hydrolysis (yellow color), negative for tryptophan utilization (indole test) (yellow-brown ring), negative for Voges-Proskauer (yellow color of the media) were considered to be *Salmonella* positive (ISO 6579, 1998 and Quinn et al., 1994).

Antimicrobial tests

The antimicrobial susceptibility test was performed according to the National Committee for Clinical Laboratory Standards (NCCLS, 2007) by using the Kibry-Bauer disk diffusion method. The antimicrobials included Streptomycin (STR, 25µg), Ceftriaxone (CRO, 30µg), Oxytetracycline (OT, 30µg), Sulfamethoxazole (SXT, 25µg), Chloramphenicol (C, 30µg), Ampicillin (AMP, 10µg), Nalidixic acid (NA, 30µg), Amoxicillin (AMC, 30µg) and Kanamycin (KA, 30µg) (Oxoid). From each isolate, four to five biochemically confirmed well isolated colonies grown on nutrient agar were transferred into tubes containing 5ml of brain heart infusion broth (Oxoid, England). The broth culture was incubated at 37ºC for 8hrs until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension and the bacteria were swabbed uniformly over the surface of Muller Hinton agar plate (Oxiod CM 0337 Basingstoke, England). The plates were held at room temperature for 30minutes to allow drying. Antibiotic discs were placed on the inoculated agar surface at about two cm apart. The plates were incubated at 37ºc overnight and diameter of the zones of inhibition was measured. The measurements were compared with zone size interpretative chart furnished by Clinical Laboratory and Standard Institute (CLSI) guideline M100-S17 and the zones were graded as sensitive, intermediate and resistant.

Statistical analysis

The data were entered into Microsoft office excel spread sheet and analyzed using SPSS statistics (Version 20.0) software. The Chi-square test was used to assess the significant differences among breed, source of milk sample and amount of milk. Effects were reported as statistically not significant since the P value is greater than 5% since P value was 0.77.

RESULTS

Prevalence of *Salmonella*

Of the 106 bulk milk samples 25 (23.6 %) were positive for *Salmonella*. Ninety eight of the samples were from intensive farms and *Salmonellae* were isolated from 23(23.5%) of the samples. The occurrence of *Salmonella* in large and small scale farms was 20.4% and 27.3 % respectively. The bacteria was isolated from 18(22.0%) and 2(25%) bulk milk samples of cross bred and local cows respectively. The occurrence of the bacteria were not significantly different among variables/factors such as sample source/farm size, breed and milk yield/day since P value is greater than 5 % (Table 1).

Mono drug resistance

The mono drug resistant features of the isolates are presented in table 2. Only one isolate was sensitive to all of the drugs tested. A higher proportion of the isolates (96%) were resistant to ampicillin and the lowest resistance was recorded for streptomycin (8%). Resistance to ceftriaxone and nalidixic acid were observed in 7(28%) and 4(16%) of the isolates respectively. Except for ampicillin and co-trimoxazole intermediate resistance was recorded for all antimicrobials.

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Multi drug resistance

The Multi Drug Resistance (MDR) profiles of the isolates are shown in Table 3. All isolates were resistant to two or more drugs except one (Table 3). Of the 14 MDR profiles, the AMP OT AMC phenotype was recorded in 5 isolates, seven isolates displayed resistance to three drugs, one isolate was resistant to six drugs and two isolates were resistant to seven drugs.

Table 1. Prevalence of *salmonellae* isolated from bulk milk of dairy farms in Debre zeit, Ethiopia

Factors	Number of sample examined	Positive (%)	X^2	df	P value
Source					
Large farm	49	10 (20.4)	0.608	2	0.738
Small holder	44	12(27.3)			
Supermarket	13	3(23.1)			
Breed					
Cross	82	18(22)	0.652	2	0.77
Local	8	2(25)			
Mixed	16	5(31.2)			
Milk amount					
8-50	46	12 (26.1)	0.646	2	0.724
51-150	13	2(15.4)			
>150	47	11(23.4)			

X^2=chi-square; df=degree of freedom

Table 2. Antimicrobial sensitivity test results of *Salmonella* isolates from bulk milk of dairy farms in Debre zeit, Ethiopia

Antimicrobials	Resistant No. (%)	Intermediate No. (%)	Susceptible No. (%)
AMC	12(48)	9 (36)	4(16)
AMP	24(96.0)	-	1(4)
C	10(40)	5(20)	10(40)
CRO	7(28)	9(36)	9(36)
K	5(20)	5(20)	15(60)
NA	4(16)	5(20)	16(64)
OT	21(84)	1(4)	3(12)
STR	2(8)	2(8)	21(84)
SXT	9(36)	-	16(64)

AMP=Ampicillin; CRO=ceftriaxone; K=kanamycin; NA=Nalidixic acid; STR=Streptomycin; OT=Oxytetracycline; SXT= Trimethoprim-sulfamethoxazole; C=Chloramphenicol; AMC=Amoxicillin

Table 3. Multi drug resistance profiles of *Salmonella* isolates (n = 24)

Number of Antimicrobials	Resistance Pattern	No. of isolates (%)
Two	AMP OT(5)	5(20.8)
Three	AMP NA SXT (1)	7(29.2)
	AMP OT AMC(5)	
	AMP K AMC(1)	
Four	AMC CRO OT C(3)	5(20.8)
	AMP NA OT AMC(1)	
	AMP OT SXT C(1)	
Five	AMP OT AMC SXT C(1)	4(16.7)
	AMP K NA SXT C(1)	
	AMP S OT SXT C(1)	
	AMP OT C AMC SXT(1)	
Six	AMP CRO STR OT SXT C(1)	1(4.2)
Seven	AMP K NA OT SXT C AMC(1)	2(8.4)
	AMP CRO K OT SXT C AMC(1)	

AMP=Ampicillin; CRO=ceftriaxone; K=kanamycin; NA=Nalidixic acid; STR=Streptomycin; OT=Oxytetracycline; SXT= Trimethoprim-sulfamethoxazole; C=Chloramphenicol; AMC=Amoxicillin

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DISCUSSION

In this study an overall prevalence of 23.6% was estimated. The present estimates are higher than reports from finding of 8.9% estimate in east tennessee and southwest virginia (Rohrbach et al., 1992) and the 6.1% estimate in south eastern dakata and western minnesota according to Jayaroo and Henning (2001). Previous studies on milk samples collected from lactating dairy cows at sebeta, Ethiopia (Abrham et al., 2013) and Addis Ababa (Addis et al., 2011) reported prevalence estimates of 16% and 28.6% respectively. According to Liyuwork et al (2013) and Robert S Barlow et al (2015) the prevalence estimate of salmonella in milk of lactating dairy cows and fecal samples from beef, dairy and veal calf of cattle were 2.1% and 14.4% in Addis and Ababa, Ethiopia and Australia respectively. The difference in prevalence between different studies might be associated with difference in the hygienic and farm management practices. Epidemiological patterns of Salmonella differ between geographical areas depending on climate, population density, farming practice, food harvesting and processing technologies and consumer habits (Radostits et al., 1994). The prevalence of foodborne pathogens in milk is influenced by numerous factors such as farm size, hygiene, farm management practices, variation in sampling and types of samples evaluated, differences in detection methodologies used, geographic location, and season (Oliver et al., 2005). The higher occurrence of *Salmonella* poses a significant health risks to humans. It is generally accepted that the occurrence of any *Salmonella* isolate in food items should be regarded as a risk human being (Fathi et al., 1994). Thus raw milk from farms and markets predispose the household and the community to *Salmonella* infections and could result in food poisoning outbreaks. All *Salmonella* isolates but one was resistance to two or more antimicrobials. Resistance to most these drugs was also reported in earlier studies in Ethiopia (Addis et al., 2011; Teshome and Anbessa, 2012; Abrham et el., 2013 and Tesfaw et al., 2013) and elsewhere (D'Aoust et al., 1992 and White et al., 2001). The isolates were resistant to antimicrobials commonly used in veterinary and public health settings. The reasons for the emergence of antimicrobial resistant *Salmonella* isolates was most likely due to the indiscriminate use of antimicrobials (Guthrie, 1992), medications without prescriptions and administration of sub therapeutic dose of antimicrobials (Acha and Szyfers, 2001).

CONCLUSION

The occurrence of *Salmonella* in bulk milk samples is considerable and a potential source of food borne salmonellosis. Assay of antimicrobial resistance revealed that almost all isolates of *Salmonella* were resistant to two or more of the antimicrobials tested whereas only a few isolate was sensitive. The significantly high proportion of multidrug resistant isolates poses a serious threat to raw milk consumers and suggests the need to consume only cooked milk. The currents study indicated the necessity of a further investigation on the prevalence and antimicrobial susceptibility pattern of *Salmonella*, by considering it as a potential food borne pathogen, starting from the farm to table.

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Competing interests

The authors have no competing interests to declare.

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Association of β –Lactoglobulin Gene Polymorphism with Milk Yield, Fat and Protein in Holstein-Friesian Cattle

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ABSTRACT

Improving the efficiency of milk production and its constituents without increasing the size of the dairy herd is the foremost goal of the selection in dairy industry. The use of polymorphic genes as detectable molecular markers is a promising alternative to the current methods of trait selection once these genes are proven to be associated with traits of interest in animals. Beta-lactoglobulin (B-LG) is one of the most important genes that play a crucial role in the milk quality and coagulation process of cheese and butter. Identification of different B-LG genotypes and association with different milk performance traits in Egyptian Holstein cattle was performed through Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and DNA sequencing of two hundred blood samples. Digestion of 447bp PCR product with Hae III restriction enzyme revealed three genotypes (AA, AB, BB), with higher frequency of B allele (64%) than A allele (36%). Nucleotide sequence analysis of different genotypes revealed two point mutation at exon four, one of them (T301C) corresponding to the same amino acid asparagine and the other Single Nucleotide Polymorphism (SNP) (C390T) represented a non-synonymous mutation producing amino acid change of alanine to valine. Animals with genotype AA had more milk yield and protein % (11461kg and 3.45) respectively, while BB genotype recorded higher fat % (3.85). The information given in the present study will be extremely helpful for improving milk production traits in dairy cattle by marker-assisted selection and outlined a strategy to avoid long and costly traditional selection methods for dairy purposes in Holstein cattle.

Key words: B-LG polymorphism, PCR- RFLP, Milk production, Holstein Friesian cattle

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INTRODUCTION

There is a significant enthusiasm for the utilization of molecular genetics technologies to recognize specific DNA markers that are connected with the economically important traits in order to make breeding program more effective through early selection of young animals as future breeding stock (Archana, 2013). The selection efficiency of complex quantitative traits in dairy cattle relies on the identification of candidate genes responsible for these traits and in addition the determination of causative DNA polymorphism in these genes. The genetic variability offers an open door for enhancement of cattle milk production through usage of genetic improvement programs (Abu Khaizaran, 2013).The candidate gene approach is a standout amongst the most vital ways to deal with quest for genetic markers related to production traits and in investigating polymorphism of structural and protein coding genes. At present, genetic markers research applied to animal breeding and production is focused mainly on analyzing mutations located within candidate genes and their relationship with economically important production traits (Oikonomou et al. 2011). Molecular technologies have been developed to recognize alleles and frequencies within milk protein genes, including specific PCR sequences, restriction enzymes and actually single nucleotide polymorphism (Ren et al., 2011).

Milk is an important source of essential nutrients for lactating calves and a key raw material for human food preparations (Reinhardt et al., 2012). Everywhere throughout the world, individuals satisfy around 13% of their protein requirement from milk and milk products. Milk proteins including casein and whey proteins have a crucial role in contributing to the nutritional qualities and properties of milk. The total milk protein composition unequivocally relies on the expression and secretion of individual proteins (Caroli et al., 2009). Beta-Lactoglobulin (B-LG) is the significant

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whey protein in the milk of cattle, sheep, dogs, and pigs but not found in humans, mice and other mammalian species. It could have a role in metabolism of phosphate in the mammary gland and the transport of retinol and fatty acids in the gut (Kusza et al., 2015). It has been mapped on bovine chromosome 11, spans 4.7Kb which is arranged in seven small exons, six introns and encodes 162 amino acid residues. Polymorphism of B-LG gene was firstly recognized in 1955 by Aschaffenberg and Drewry and a total of 15 alleles are known. Common alleles are A, B, C and D, with alleles A and B being the most incessant (Farrell et al., 2004). The bovine B-LG A allele differs from B allele by two amino acids substitution at positions 64 (aspartate → glycine) and 118 (valine → alanine) and also has a higher B-LG protein concentration than allele B. It is likely that this difference in amount of B-LG protein is not caused by the amino acid substitutions, but instead by different levels of expression of the corresponding A and B alleles of the B-LG gene (Hill, 1993). While, BB genotype is connected with higher casein and fat contents, which are ideal properties for cheese making additionally it might improve the quality of milk (Ren et al., 2011).

In light of critical role of B-LG gene in milk related traits and their genetic trends in dairy cattle, the point of this study was to identify B-LG gene polymorphisms and to determine the impacts of these variants on milk traits in Holstein Friesian cattle reared under Egyptian condition.

MATERIAL AND METHODS

Experimental and sample collection

This study was carried out on 200 randomly selected Holstein Friesian cattle from El-Shazly farm, Egypt. The phenotypic data including date of birth, date of calving, lactation, milk yield and lactation length were collected from daily farm records. The blood samples (5 ml) were collected under a sterile condition by jugular vein puncture into sterilized vacutainer tubes containing EDTA as an anticoagulant and then brought to the laboratory in ice box containing gel cool packs and stored at -20° C until DNA extraction.

Two hundred milk samples (10 ml) were collected during morning milking in order to represent the whole milking of each animal for detecting the milk constitution (fat, total protein ,solid not fat and lactose) by using ultrasonic portable milk analyzer (milko tester model- master mini).

DNA extraction

Genomic DNA extraction was performed with DNeasy Blood &Tissue Kit (QIAGEN, Germany) following the manufacturer's protocol. The quality and quantity of DNA was evaluated by 0.7 % agarose gel electrophoresis and by UV spectrophotometer, respectively.

PCR amplification and gel electrophoresis

Amplification of a 447bp fragment of B-LG gene covering intron III, exon IV and intron IV was done using a pair of forward (5′GCC TCA GAC TCA GTG GTGA 3′) and reverse (5′ACC ACA CAG CTG GTC TCC 3′) primers. The primers were designed using primer 3.0 software and the published nucleotide sequence of the Bos taurus B-LG gene (GenBank Accession No X14710.1). PCR reactions were done in a total volume of 25μL, consisting of 12.5µl master mix (Thermo Scientific, Fermentas), 2µl DNA template, 1µl of each primer (10pmol/µl) and deionized water up to 25µl. Amplification was carried out in a thermal cycler (Biometra, Germany) with the following conditions: initial denaturation at 94^{o}C for 2 min, followed by 35 cycles of 94^{o}C for 20 sec, 61^{o}C for 20 sec and 72^{o}C for 45sec with a final extension of 8 min at 72°C. The amplified fragments were separated on 1.5% agarose gel electrophoresis, imagined under UV transilluminator. The size of the amplified product was compared with the 100-bp ladder DNA marker.

PCR-RFLP analysis

The 447bp PCR products were digested by FastDigest Hae III restriction enzyme (Fermentas) at 37°C for 5 min. The digested products were resolved in 1.5% agarose gel containing ethidium bromide as the staining agent in 1×TAE buffer. A 100bp marker ladder was also run alongside the samples to ascertain the size of the amplified products. The digested products were visualized under UV light on a transilluminator and scored in the gel documentation system.

DNA sequencing

PCR products were purified with Gene JET PCR purification kit (Thermo Scientific Fermentas) following the manufacturer's guidelines and were straightforwardly sequenced utilizing both the forward and reverse primers of PCR amplification. The sequencing procedure was done by European Custom Sequencing Centre (GATC Biotech AG, Germany). The obtained sequences were edited manually utilizing Chromas Lite Ver. 2.01, (http://www.technelysium.com.au/chromas.html) and aligned with CLC Main Workbench 7 and Clustawl Omega software.

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Statistical analysis

The alleles and genotypes frequencies were estimated according to Falconer and Mackay (1996). The Pearson's chi-square ($\chi 2$) (P value < 0.001) was used to check whether the population is in Hardy-Weinberg Equilibrium (HWE) or not. Marker-trait association analysis was conducted by least square method of the General Linear Model (GLM) procedure of the statistical packages for social science using the following linear model.

$Yij=\mu+Gi+eij$

Where, Yij= Observation of the target trait, μ=Overall mean, Gi =Fixed effect of ith genotype, eij =Random error.

Duncan's multiple range tests had been used for comparing the means. Additive effects (allele substitution effect) were estimated through adding an additional regression covariate with value 0, 1 and 2 to account for number of AA, AB and BB genotypes, respectively.

RESULTS AND DISCUSSION

The study of candidate genesis one of the vital procedures to figure out if particular genes are connected with the economic important traits in farm animals. In marker assisted selection of dairy cattle, some genes are proposed as potential candidates associated with milk performance traits. Among the different candidates, B-LG gene that influences the milk production parameters and quality of milk protein. Their polymorphisms mostly clarify the genetic variance and enhance the estimation of breeding value.

B-LG gene fragment was successfully amplified using polymerase chain reaction technique for all samples and resulted in a single product of 447bp (Figure 1A). Digestion of this fragment with Hae III restriction enzyme revealed three genotypes which are designated as AA (236, 90, 74 and 47 bp), AB (310, 236, 90, and 74 and 47 bp) and BB (310, 90 and 47 bp) (Figure 1B). PCR products representing different genotypes were sequenced and two sequences revealing A and B alleles were submitted to the GenBank and deposited under accession numbers KR732930 and KR732929, respectively. The nucleotide sequence analyses revealed the presence of two SNPs in exon four (T301C and C390T) and two in the intronic region (T226C and C276T) of B-LG gene (Figure 2). The T301C SNP was silent base substitutions corresponding to asparagine amino acid (aa) and the other C390T SNP was a non synonymous mutation leading to substitution of alanine by valine. These results were similar to that reported by Getachew (2010), Piatkowska et al. (2011), Hristov et al. (2013) and Mir et al. (2014) in Holstein cattle.

The genotype frequencies were 0.50 (100) for BB, 0.22 (44) for AA and 0.28 (56) for AB with allele frequencies of 0.36 for A and 0.64 for B among animals that were genotyped for this polymorphism. Chi-Square ($\chi 2$) value was 30.78 (P value=0.001). This means that the genotype distributions within cattle population deviated from HWE (P<0.05). These data are in the context with Ren et al. (2011) and Singh et al. (2014) who reported the highest frequency of allele B and BB genotype in Holstein cattle.

The polymorphism of B-LG gene was studied in other cattle breeds such as Girolando cows (Botaro et al., 2008),Turkish cattle breeds (Dinc et al., 2013), Sahiwal cattle (Mir et al., 2014 and Kishore et al., 2014), and Mexican Jersey cattle (Batista et al., 2015), they found that BB genotype and B allele were the most frequent in all studied breeds. In contrast to these findings Ivanković et al. (2011) and Lukac et al. (2013) reported the predominance of AB genotype in Estonian dairy cattle and Holstein cattle respectively. This can be explained by different history of the breeds, long-term geographical isolation and selection towards high fat and protein percent of milk in some breeds such as Holstein Frisian cows.

Association of AA, AB, and BB genotypes of B-LG gene with the milk traits were analyzed in Holstein Frisian cattle. The results indicated that animals with AA genotype showed higher milk yield, protein % and solid % (11461 kg, 3.45% and 9.27%) respectively. The differences were more obvious for the fat % as animals with BB genotype were higher (3.85%) than that of AA and AB genotypes (2.94% and 2.97%) respectively and there is no significant (P<0.05) effect on lactose % (Table 1). Similar effect of B-LG genotypes had been observed by Heidri et al. (2009), Getachew,(2010) and Piątkowska et al. (2011) they noted the highest milk yield for cows with AA genotype, whereas milk of B-LG BB cows exceeded milk obtained from cows with AA and AB genotypes as regards fat %.

In contrast Ahmadi et al. (2008) reported strong association between BB genotype and protein % while there was no association between B-LG genotypes and milk yield or milk fat %. Mir et al. (2014) found no differences in milk compositional characteristics among different genetic variants of B-LG in Holstein, Girolando, Czech Fleckvieh and Sahiwal cattle breeds. Hirstov et al. (2013) showed that the BB genotype determines higher milk production, Singh et al. (2014) found that AB and BB genotypes of B-LG had a significant (P< 0.05) effect on total milk yield and peak yield compared with AA.

Substituting the A allele with the B allele decreased the milk yield, protein % and solid % with (4503.7kg, 0.271 %, 0.318 %) respectively this demonstrates the superiority of the A allele with respect to quantitative milk traits but substituting the A allele with the B allele increased the percentage of milk fat by 0.525 % (Table 1).

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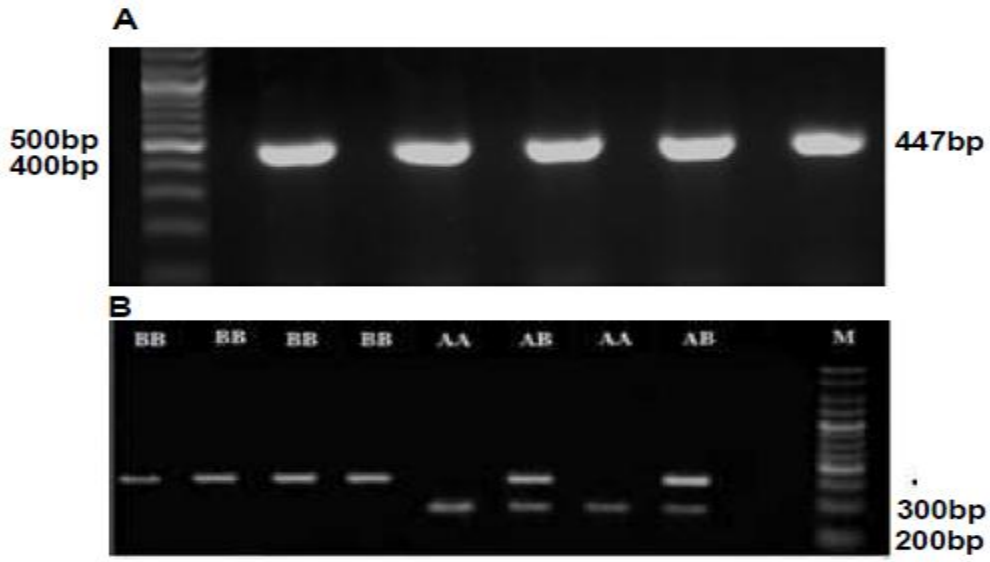

Figure 1. **A**: PCR amplification of β-lactoglobulin gene in Holstein Frisian cattle. **B:** Restriction fragment patterns of β-lactoglobulin gene after digesting with Hae III. M: 100bp ladder

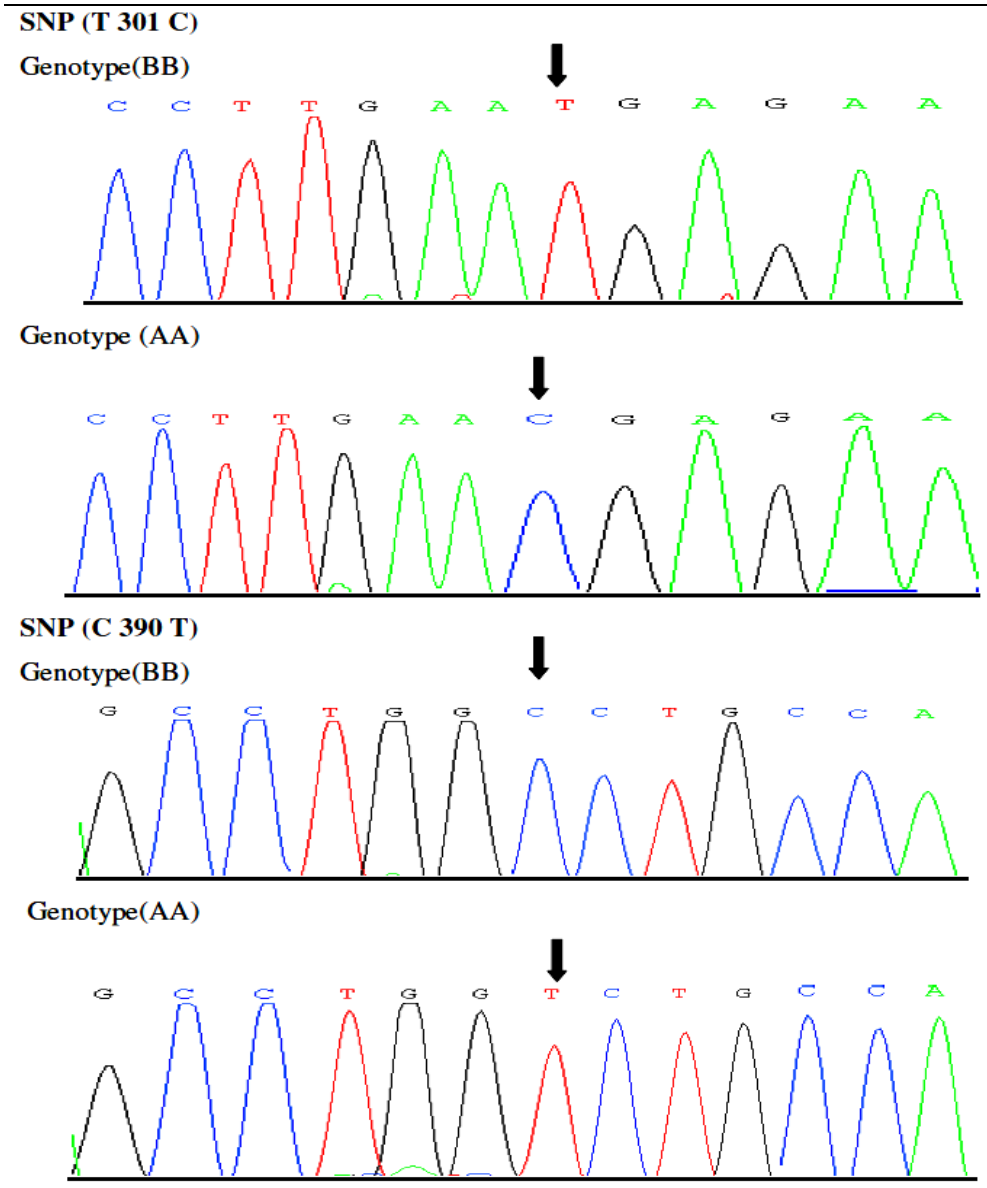

Figure 2. Relative sequenced peaks of β-lactoglobulin genotypes AA and BB in Holstein Frisian cattle. Arrows refers to site of base change

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Table 1. Least square means ± SE for milk production traits with different B-LG genotypes and allele substitution effect in Holstein Frisian cattle

Trait	Genotype (mean ± SE)				Allel substitution effect (B allel)
	AA	AB	BB	F-value	
Milk yield	11461 ± 494[a]	8475 ± 675[b]	2784 ± 176[c]	122.10[*]	- 4503.7[*]
Fat %	2.94 ± 0.122[b]	2.97 ± 0.082[b]	3.85 ± 0.045[a]	64.03[*]	0.525[*]
Protein %	3.45 ± 0.092[a]	2.86 ± 0.117[b]	2.87 ± 0.071[b]	11.39[*]	0.271[*]-
Lactose %	4.12 ± 0.033[a]	4.11 ± 0.059[a]	4.17 ± 0.034[a]	0.688[NS]	0.029
SNF %	9.27 ± 0.111[a]	9.13 ± 0.092[a]	8.60 ± 0.051[b]	25.11[*]	- 0.318[*]

* = Significant at (P< 0.05); NS= non-significant; SNF= solid not fat

CONCLUSION

In the present study PCR amplification and RFLP analysis were found to be a fast and sensitive method to recognize B-LG genotypes directly at the DNA level. It may be stated that Holstein Friesian cattle breed was polymorphic for B-LG gene producing 2 types of alleles and 3 types of genotypes. The frequency of B allele was comparatively higher than A. The genotype AA produced significantly higher milk yield, protein % whereas genotype BB yielded higher Fat % in Holstein Friesian cattle. Hence, B-LG genotyping can be used in selecting superior genetic structures for milk production in young females in shorter time than the traditional selection could. The selection of these superior individuals in early age and culling of the lower ones based on their genotype could take an interest in enhancing milk production of animals.

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Competing interests

The authors have declared that no competing interest exists.

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Rabies in Animals with Emphasis on Dog and Cat in Ethiopia

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ABSTRACT

Rabies is a major viral anthropozoonosis of public health significance. The animals play a crucial role in maintenance and circulation of the rabies virus. Determining the occurrence of rabies in animals was implicated as a fundamental step to guide prevention and control efforts. The study involved cross sectional study by retrieval previously recorded cases between September 2009 and January 2012, Ethiopia. Suspected animal rabies cases were quarantined for 10 days observation period and brain samples were tested for confirmation using direct fluorescent antibody test. The statistical analysis was performed using SPSS program and Chi-square and analysis of variance (ANOVA) was used to assess the significance difference. Domestic and wild animals were associated with human exposure and death cases. However, dogs were the culprit for the highest human fatal (97%) and human exposure cases (89.5%). Only 2% of human deaths were associated with cats and wild animals. Higher rabies positivity was noted in owned dogs 138 (74.2%) than ownerless ones 49 (25.8%). The difference was statistically significant (p=0.0001). Further, all positive cases in cats were apparent in those believed to be owned. Majority of positive results associated with dogs were aged above 12 months 70 (81.4%), 10 (11.6%) were from the age between 6 and 12 months followed by 3 to 6 months 6 (7%). Positive cases in cats were more common among those aged above 12 months (60%) followed by 6 to 12 (20%) and 3 to 6 months (20%) of age. Of the animals with positive results, 4 (1.6%) were vaccinated against rabies. Overall, majority of the dogs had no vaccination history 247 (96.1%), only 10 (3.9%) were found to have been vaccinated. On contrary, none of the cats were vaccinated against rabies. The number of submitted samples had a direct correlation with the number of positive results ($p<0.05$), however, no season variation was encountered. Five sub-cities that border with another region showed a significantly higher occurrence ($p<0.05$) of exposure and confirmed cases and post exposure prophylaxis. Thus, integrated implementation of compulsory animal management, immunization and creation of awareness is highly imperative.

Key words: Addis Ababa, Cat, Dog, Immunization, Rabies, Zoonosis

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INTRODUCTION

Rabies is a highly fatal zoonosis, which affects humans as well as a wide variety of animals (Pal, 2007), and is reported from many countries including Ethiopia (Pal, 1991 and Deressa et al., 2011). The disease is caused by a virus of the genus *Lyssavirus* of the family *Rhabdoviridae* (Pal et al., 2013). The disease is mainly transmitted from rabid animals to humans through close contact with infected saliva via bite or scratch and invariably results in death (Pal et al., 2013). Despite the preventable nature of the disease and existence of effective and economical control strategies (Lembo et al., 2010), rabies remains as one of the major public health problem resulting in an estimated loss of approximately 60000 lives worldwide each year which almost all of the cases belong to Africa and Asia (Pal et al., 2013; WHO, 2013 and Hampson et al., 2015).

Ethiopia was among the high burdened country where 10,000 people per annum were estimated to have died (Fekadu, 1997). In world rabies survey report, it was found the second leading country. In 2012, over 1400 deaths were estimated to occur due to rabies annually (Ali, 2012). A very recent global burden estimate showed over 2700 annual human lives are lost in the country (Hampson et al., 2015). Domestic and wild animals can potentially transmit the disease to humans mainly through biting (Pal, 1991). However, dog mediated rabies is known to account for over 90% of human exposure and death cases in Ethiopia and elsewhere (WHO, 2005; Ali, 2012 and WHO, 2013). Next to canine, feline species appears to be the second most affected animal in Ethiopia (Yemer et al., 2002; Deressa et al., 2010 and Ali et al., 2012). In some case, incidence of rabies in cats can exceed as compared to dogs. Further, the tendency of victims to visit health centers during a cat related incident is much lower than a dog inflicted incidents (Eidson and Bingman, 2010) making the animal vulnerable and a potential source of rabies to humans along with dogs. Meanwhile, rabies

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affects domestic animals and endangered animals like the Ethiopia Wolf (Deressa et al., 2016). Overall, rabies is a public health, animal health and livelihood concern in Ethiopia.

The general prevention of human exposure to rabies relies on taking Post Exposure Prophylaxis (PEP). Globally, close to 15 million people receive PEP annually which is considered as costly and not accessible for developing countries like Ethiopia (WHO, 2013 and Hampson et al., 2015). Whereas dog population management and dog mass vaccination are the shown be cost effective measures in rabies prevention and control (Escobar, 1988). In Africa, majority (98%) of dogs is believed to be owned and feasible strategies for successful mass dog vaccination were recommended (Jibat et al., 2015). However, the impetus towards the implementation of these strategies is highly hampered due to socio-economic and political factors in developing countries (WHO, 2005). Lack of information on the extent of the burden and risk factors significantly led to rabies being overlooked and thus, reduced collaboration including financing for implementation of successful intervention strategies such as mass dog vaccination (Hampson et al., 2015). In this regard, assessing the burden and risk factors and giving special emphasis to animals in diseases is imperative to act as a guide to cost effective intervention.

MATERIALS AND METHODS

The study was conducted in Addis Ababa city administration, the capital of the federal democratic republic of Ethiopia from October 2012 to March 2013. The city covers an area of 530.14 $km^2$and is subdivided into 10 sub-cites. Addis Ababa lies at an altitude of 2,500 meters above sea level, located between 9.03° North 38.74° east, latitude and longitude, respectively. All 10 sub cities under Addis Ababa city administration were included. The study population was animals residing in and around Addis Ababa and specifically those reported during the study period to the Ethiopian Public Health Institute (EPHI) rabies counseling and diagnostic center. The sample size was determined on the basis of previously reported incidents between September 2010 and August 2011. Furthermore, all admitted animals were subjected to quarantine for a 10 days period at EPHI and followed during the observation period for survival or death. Dead animals were tested for confirmation while those animals were given back to the submitter. In many cases, live animals are home quarantined by owners and the brain samples were submitted by local veterinarians to national rabies laboratory at EPHI. The laboratory diagnosis was applied using the direct fluorescent antibody test to confirm rabies cases in animals on brain sample impression using anti rabies conjugate.

The data collected was entered in a Microsoft excel sheet and analyzed with SPSS statistical package version 16. Descriptive statistics such as frequency were used where determined necessary. Chi-square and analysis of variance (ANOVA) was used to assess the significance difference. Additionally, linear regression was found relevant in assessing the correlation amongst the reported incident, the number of positive cases and Post Exposure Treatment (PEP). Dependent variables were exposure (incidents) and confirmed cases where independent variables were month and sub-city. Moreover, incidence was calculated using Microsoft excel. The statistically significance of P-value less than 0.05 was considered. The study considered secondary data and no animal was involved in any kind of medical procedure that may lead to suffering and all personal identifier were not used. Nevertheless, ethical clearance on scientific soundness and justification for the need to do the study was addressed by the Addis Ababa University College of Veterinary Medicine.

Ethical approval

The study considered secondary data and no animal was involved in any kind of medical procedure that may lead to suffering and all personal identifier were not used. Nevertheless, ethical clearance on scientific soundness and justification for the need to do the study was addressed by the Addis Ababa University College of Veterinary Medicine.

RESULTS

Of the human death cases reported from September 2009 to March 2013, from a total of 96 deaths reported, nearly all 93 (97%) fatal cases were attributed to dogs where only one (1%) was due to cats and the rest (1%) were due to wild animal. Of the suspected animals presented alive and dead to EPHI, 69 (20.7%) were from outside and 265 (79.3%) incidents were reported during 2010-2011 in Addis Ababa. Domestic animals such as dog, cat, cattle and wild animals like apes and foxes were associated with human exposure.

Of the laboratory examined animals, 246 (92.8%) and 15 (5.7%) were dogs and cats, respectively. The remaining 4 (1.6 %) were related to domestic animals such as bovine and equine and to wild animals such as apes. Subsequently, 186 (75.6%) canine and 9 (60%) feline brains were positive for rabies virus. Three (0.9%) of samples submitted were associated with invalid results. Higher proportion of owned 197 (80.1%) than stray dogs 49 (19.9%) was reported. In feline species, 2 (13.3%) and 13 (86.7%) stray and owned, respectively were reported. Higher positivity was noted in

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owned dogs 138 (74.2%) than ownerless 49 (25.8%). The difference was statistically significant (P=0.0001). Further, all positive cases in cats were apparent in those believed to be owned. Overall, majority of the dogs had no vaccination history 247 (96.1%), only 10 (3.9%) were found to have been vaccinated. Of these, four (1.6%) were positive for rabies despite immunization history. On contrary, none of the cats were immunized. The difference between vaccination status and positivity was statistically significance (P<0.05).

Higher number of male dogs 168 (78.5%) were submitted for laboratory examination than females 46 (21.5%). Although high proportion of males were found to be positive for rabies 123 (77.4%) than females 36 (22.6%), no significant difference (P>0.05) was noted amongst the gender and positivity. Male felines 3 (21.4%) were conversely outnumbered by females 11 (78.6%). Majority of positive results associated with dogs were aged above 12 months 70 (81.4%). 10 (11.6%) were reported from 6 to 12 months followed by 3 to 6 months 6 (7%). Positive feline cases were more common among those aged above 12 months (60%) followed by 6 to 12 (20%) and 3 to 6 months (20%) age categories.

The incidence of rabies in dogs and cats during 2010 and 2011 was calculated considering an estimated total population of 230000 to 300000, an average estimated population with 265000 both owned and stray dogs (Abegaz, 2012). Similar, estimate was used for feline species. A total of 186 positive canine cases were found over a year, hence 7 per 10000 population was calculated. A total of 9 feline brains were positive for rabies virus hereby the incidence was found 3.4 per 100000 of the population over year. According to this study, the annual incidence in dogs and cats indicated an increase between 2009 and 2012 (Figure 1). Of the 259 total confirmed cases in 2009 and 2010, 243(93%) was canine and 16 (7%) feline. In the consecutive year of 2010 and 2011, 246 (94%) and 15 (6%) of the total 261 reported cases, while in 2011 and 2012, 345 cases were reported from dogs and cats, 321 (93%) and 21 (7%), respectively.

Majority of cases between 2008 and 2012, were evident during April (10.5%) followed by February (10.2%), May (9.4%) and September (9%). The remaining months showed a slight variation indicating equal distribution as indicated in figure 2. Furthermore, significant variation was observed (F=29.7 P=0.0001, P < 0.05) between months. However, no seasonality was encountered.

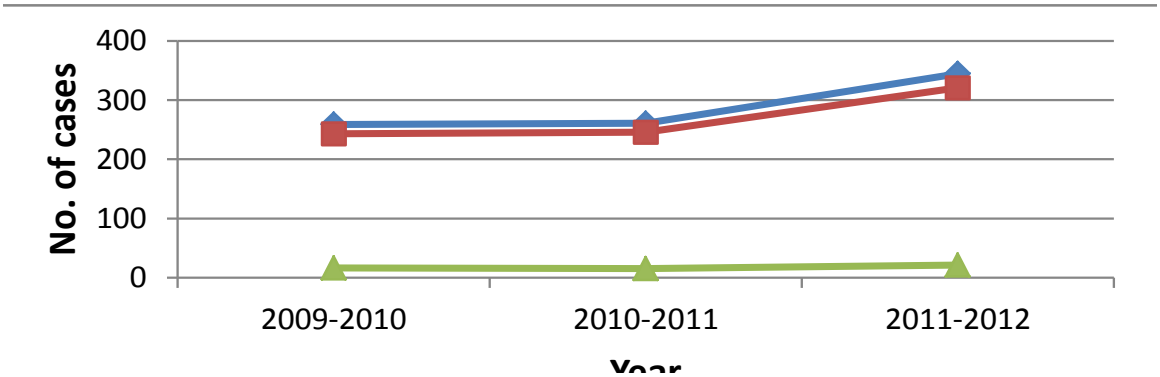

Figure 1. Pattern of confirmed dogs and cat cases between 2009 and 2012, Addis Ababa, Ethiopia

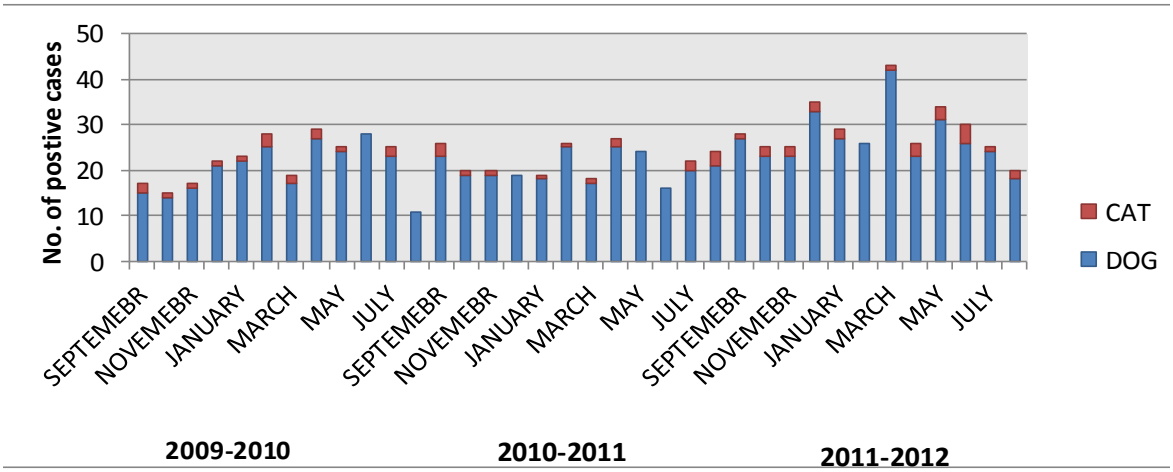

Figure 2. Annual incidences in canine and feline rabies and also monthly trends between 2009 and 2012, Addis Ababa, Ethiopia

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As shown in figure 3 and figure 4 the laboratory confirmed animal cases amongst the ten sub cities was revealed to be as Yeka 49 (18.5%), Kolfe 47 (17.7%), Gullele 42 (15.8%), and Lafto 34 (12.8%) reported the highest while Bole 22 (8.3%), Addisketema 18 (6.8%), Kirkos 16 (6%), Arada 16 (6%), Lideta 13 (4.9%), Akaki 8 (4%) constituted the remaining during the period of 2010 to 2012. Significance difference was noted (P=0.003, P < 0.05). Positive case distribution among sub-cities was analyzed where the highest positive cases 39 (20.3%) were found to have been at Yeka sub-city followed by KolfeKeranyo 35 (18.2%) and Gullele 32 (16.7%). Furthermore, the least cases were encountered from Akaki 6 (3.1%). The remaining moderate reported cases were 19 (9.9%) at NefasilkLafto, Bole 15 (7.8%), Kirkos 13 (6.8%), Addis Ketema 12 (6.2%), Arada 11 (5.7%) and Lideta 10 (5.2%). In the capital, suspected animals rabies cases were more common in sub cities namely, Gullele (17%), KolfeKeranyo (16.2%), Yeka (12.1%), NefasilkLafto (12%), Bole (10%), and AddisKetema (9.2%). Moreover, Arada (7.9%), Kirkos (6.9%), Lideta (5.5%), and AkakiKaliti (2.6%) were amongst the least reported. The number of submitted samples had a direct correlation with the number of positive results (t=20.962, P=0.0001, P<0.05).

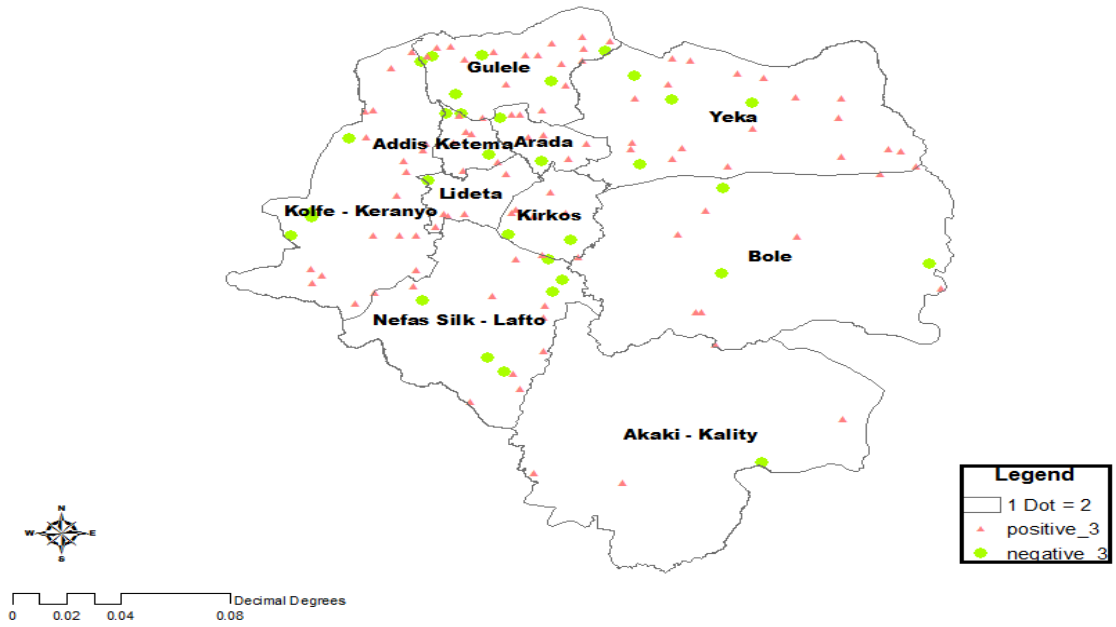

Figure 3. Laboratory confirmed animal rabies cases in the ten sub cities under Addis Ababa during 2010 and 2011, Addis Ababa, Ethiopia

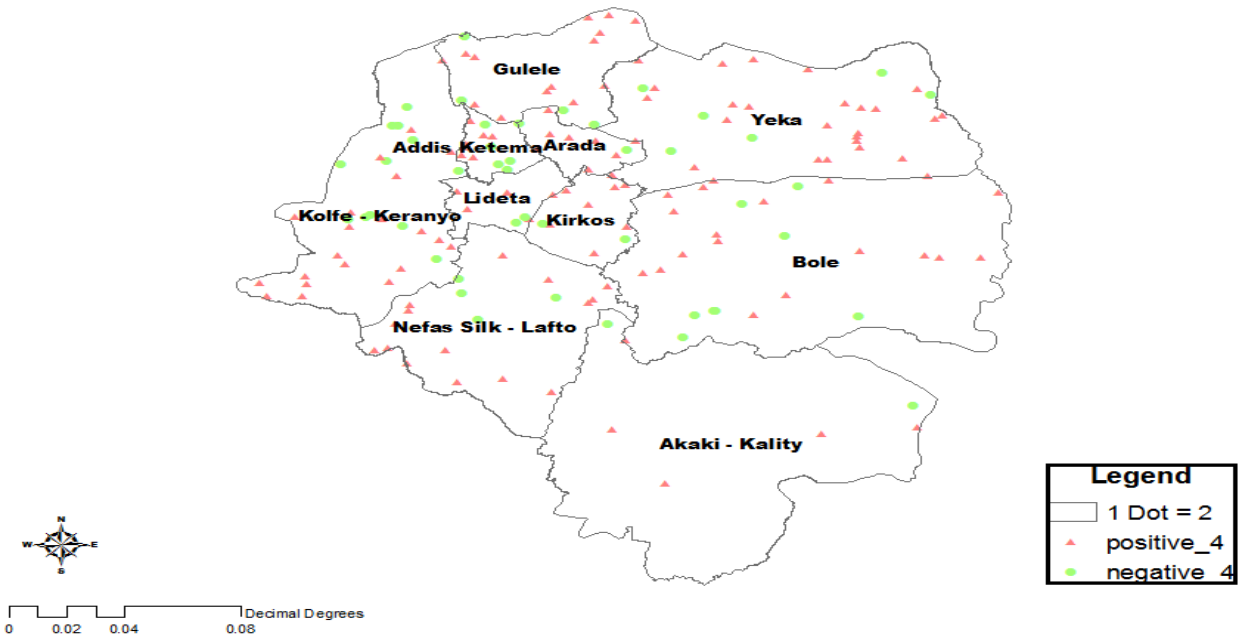

Figure 4. Laboratory confirmed cases in the ten sub cities under Addis Ababa, Ethiopia during 2011 and 2012

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DISCUSSION

According to this study, the annual incidences in dogs and cats indicated an increase between 2009 and 2012. This can be conjectured to the haphazard intervention strategy in the case of stray dog control and immunization (Ali et al., 2010). Likewise, lack of institutional contribution gave opportunity for the persistent prevalence. The need for inter-sectoral integration towards rabies elimination is indicated as the only alternative (Escobar, 1988). Herein, majority of animal owners failed to immunize their pets where only 4.2% of dogs were reported to have been immunized. According to global estimate, a very limited finace is allocated to the veterinary sector for dog vaccination (Hampson et al., 2015). Nevertheless, 1.6% was positive for rabies despite immunization history. Poor vaccination practice was described elsewhere (Ali et al., 2010). Eng et al. (1993) reported rabies in animals with prior vaccination history 3(1%) two cats and one dog. This is associated with factors such as the poor quality of vaccines due to inappropriate cold chain management. On the other hand, all cats were not subjected to immunization (Newayeselassie et al., 2012). The difference between vaccination status and positivity showed statistical significance ($P<0.05$). Legislation, education, and mass vaccination of dogs were ideal intervention strategies (Escobar, 1988). Moreover, consideration of mass immunization of 70% of dogs is believed to eliminate the disease by developing herd immunity (Mitmoonpitak et al., 1998). This study, however, found only 4% of dog owners practiced vaccination. This vaccination practice is much lower than reported by other studies (Jemberu et al., 2013 and Kitala et al., 2000). The differences could be due to the fact that present study was used retrospective whereas both studies were active surveys. Meanwhile, the free of cost accessibility of vaccine is believed to maximize the vaccination coverage close to 70% in Africa context (Jibat et al., 2015).

Animal brains submitted for laboratory test were mostly from dogs 92.8% and cats 5.7%. As a result 75.6% of canine and 60% feline brains were positive for rabies virus. This is supported by previous studies that indicated higher proportion of canine and feline (Yemer et al., 2002; Ali et al., 2010 and Deressa et al., 2010). Most (80.1%) brain samples from owned dogs were commonly submitted as opposed to stray ones 19.9%. As a result, higher positivity was noted in owned dogs 74.2% than ownerless 25.8%. This is complementary to Ali et al.,,(2010) where stray were indicated to higher positivity among owned dogs. Similarly, 13.3% and 86.7% stray and owned cats were reported where all under ownership were positive and none feral cats were positive. This could be due to the better chance of owned animals being available for diagnosis. The poor management of pets is conjectured to have a role in contracting the infection while roaming for food and for breeding. Additionally, this implicates the equal probability of acquiring risk in owned to stray dogs the latter was known as principal player for circulation where in our context animals are not leashed, properly sheltered, and immunized (Yemer et al., 2012).

Male dogs outnumbered female dogs in 78.5% and 21.5% of positive cases respectively. On the contrary, females were 78.6% outnumbered by counterpart 21.4%. This can be justified by the fact that the variation in ownership trend of canine and feline species among Ethiopian custom where male dogs are privileged for avoiding overpopulation. In line with this, Yemer et al. (2012) reported that 78.4% of informants were found to keep male dogs in majority of cases for the sake of security. About 81.4% of positive samples were from dogs aged above 12 months followed by among those age between 6 and 12 months (11.6%) and 3 to 6 months (7%). Similarly, cats aged above 12 months 60% was highly positive than 6 to 12 months 20% and 3 to 6 months 20% age categories. On contrary, higher incidence of rabies was reported in dogs less than 12 months of age (Morters et al., 2015). The discrepancy could be attributed to the fact that older dogs were usually reported perhaps due to their chance of being suspected to rabies in the study area while puppies were disregarded as if they might not contract the infection (Kaare et al., 2009). White et al. (2007) described that even clinicians were steered away from suspect and diagnosis of pups despite their higher vulnerability to infection than older dogs.

Seasonal variation was assessed, however no seasonal influence was observed to influence occurrence. Monthly variation was assessed where majority of cases were evident during April 10.5% followed February 10.2%, May 9.4% and September 9%. The remaining months had showed a slight variation. Similarly, Yemer et al. (2002) did not observe any seasonal association. On contrary, Ali et al. (2010) found rabies varying along season where an increase of positive cases was common from June to September. This paper however indicated the existence of rabies through whole year despite breeding influence. In country where the awareness level is very much limited, the issue of seasonality may significantly influence the treatment seeking behavior of individuals and thus, careful explanation on the seasonality of rabies is required while dealing with awareness raising efforts. Further, variation in rabies cases in sub-cities could be associated with several factors such as variation in dog population size; however, this study does not answer to actual burden in relation to animal population. The possibility of sample submission to the central rabies laboratory is significantly rare leaving the true burden is undisclosed.

As for limitations, the present study was retrospective and cases were subjected to under reporting since animals suspected of being rabid were brought to the EPHI by animal owners and in few cases by veterinarians. The Institute

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serves mostly Addis Ababa inhabitants and thus, the information was very much to be representative of Addis Ababa and its surrounding.

CONCLUSION

Rabies is still imposing a great risk to the animals. The lack of compulsory animal management, poor intersectoral and haphazard trend of dog control and immunization strategy are the associated factors described. Canines remain the scapegoat for almost all deaths and post-exposure treatment and felines to some extent were also part of the burden. Positive cases among vaccinated animals and both among owned and stray dogs were identified. Distribution of exposure, vaccine use and confirmed cases amongst those sub-cities were found border to other districts. A multisectoral and inter agency collaboration including funding for mass vaccination of dogs, and investigation of the actual burden and risk factors of rabies in animals and humans through an integrated surveillance system is recommended for efficient and effective rabies prevention and control in the country.

Competing Interests

The authors have declared that there are no competing interests exist.

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Effect of Stocking Density and Bird Age on Air Ammonia, Performance and Blood Parameters of Broilers

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ABSTRACT

Current study was conducted to recompense this deficiency through detection of ammonia (NH_3) in air samples taken from different groups at different SD and different ages. Additionally the effect of stocking density on stress indicators and broiler performance were studied. A total of 200 unsexed one day old chicks, purchased from a commercial hatchery at Kafr El-Sheikh city, Egypt, were used in this study. The birds were randomly divided into four groups according to SD. These experimental groups were divided as follows: the first group (G1) contains 10 bird/ m^2, the second group (G2) contains 14 bird/ m^2, the third group (G3) contains 17 bird/ m^2 and the fourth group (G4) contains 60 chicks was kept in an area of 3 m^2 with SD of 20 bird/ m^2. The chicken in all groups (from day 7 to day 42) was raised on deep litter system and received the same standard management, hygienic and environmental conditions. Comparing ammonia concentrations of different SD, the highest ammonia concentration was found in G4 (maximum of 31.4 mgL^{-1} at 36 day age) while the lowest were found in G1 (10 bird/m^2) (0.7, 3.1, 3.5 and 3.5 mgL^{-1} at 14, 21, 28 and 36 day age respectively). Increased SD tended to reduce the final Body Weight and Body Weight Gain significantly. On the other hand, the relative weights of spleen and bursa were increased significantly ($P < 0.05$) with increasing SD. In the current study there was a significant difference ($P < 0.05$) in Total Leukocitic Count, Heterophils %, Lymphocytes %, and H/L ratio between different SD. H/L ratio was highest in G4 (0.192%) and lowest in G1 (0.018%) which showed Increasing in H/L ratio with increase in SD that reflects increase in stress level.

Key words: Broiler, Socking density, Air ammonia, Boiler performance, Bood parameter

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INTRODUCTION

Chicken has become one of the most famous meats consumed in the world (Watt Executive Guide, 2010). In Egypt the poultry industry is one of the main agricultural industries, where investment in this industry is estimated to be worth LE18 billion. The size of the labor force is about 1.5 million permanent workers and about one million temporary workers Elnagar et al (2007). The industry contributes a large part of the country's supply of animal protein (national agricultural income, Economic Affairs Sector (EAS) ministry of agricultural and land reclamation) Poultry production differs from other animal production activities in several ways, as broilers shows high growth rates and feed efficiency (Duclos et al., 2007).

Although the use of high Stocking Densities (SD) can adversely affect the individual health and performance (Sorensen et al., 2000; Feddes et al., 2002; Al Homidan et al., 2003; Dawkins et al., 2004 and Buijs et al., 2009), this has not always been a motivation for producers to lower the stocking densities because the economic benefit per square meter is often still higher if the chickens are stocked more densely (Cravener et al., 1992 and Feddes et al., 2002).

Intensive production with high stocking density usually associated with many environmental pollution problems. These problems include reduced air quality with high concentrations of organic and inorganic dust, pathogens and other micro-organisms as well as harmful gases such as ammonia, nitrous oxide, carbon dioxide, hydrogen sulphide, and methane (Ellen, 2005 and Gates et al., 2008).

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So, the poultry producers must make tremendous adjustments to meet the increasing demand for cheap and safe supply of meat and eggs. Increasing stocking density of broilers is a management practice used for reducing costs associated with labor, housing, fuel and equipment. Intensive broiler chicken production increased from year to year over the world attracting accusations of poor welfare.

Air pollutants have public health and economic importance for livestock production. When both the emission rate and the concentration level is high in livestock buildings, it causes health problems among housed animals. The public health importance of air pollutants is predicated on the diseases they may cause in livestock workers when their levels are high in the livestock pens. Most of studies in harmful gases of livestock buildings have concentrated on ammonia because of its toxicity and its role in acid rain formation (Okoli et al., 2004).

Aerial pollutant emissions in poultry farms may influence on bird performance that Threaten health of birds as a source of environmental pollutants. The sustainability of poultry production has to determine through monitoring of aerial pollutant emissions (Wathes, 1998). Effect of density on broiler mortality was not significant (Skomorucho et al., 2009).

Under the conditions used for the broilers industry in Egypt, the air released from the production buildings is polluted by microorganisms, dust, toxic gases and substances with an unpleasant odor (Baykov and Stoyanov, 1999). Most of the previous studies focused on the effects of stocking density on broiler performance and welfare parameters (Shanawany1988; Cravener et al., 1992; Martrenchar et al., 1997; Feddes et al., 2002; Adeyemo et al., 2016 and Shailesh et al., 2016). Ravinderan et al. (2006) studied the effect of adding in-feed antibiotic in relation to stocking density. None of these studies has examined the effects of stocking density in relation to age and air ammonia concentration. So, this study was carried out to make focusing on the relationship between different Stocking Densities (SD), bird ages and air hygiene. This aim could be achieved through, detection of ammonia (NH_3) in air samples taken from different groups at different SD and different ages, broiler performance of experimental broiler farm under different SD and finally monitoring of some blood parameters and hormones related to stress.

MATERIALS AND METHODS

The experiment was started on February 23, 2015 and lasted for 6 weeks at the research poultry farm unit, faculty of veterinary medicine, Kafr El-Sheikh University, Egypt.

Animal ethical approval

Animal ethics committee, faculty of veterinary medicine, Kafr El-sheikh University, Egypt, approved the protocol and conduct of the study.

Experimental birds

A total of 200 unsexed day-old (avian 48 type breed) chicks, purchased from a commercial hatchery at Kafr El-Sheikh city, Egypt, were used in this study. The birds were randomly divided into four groups according to SD. The first group (G1) contains 47 chicks were kept in an area of approximately 4.7 m^2, with SD of 10 bird/ m^2; the second group (G2) contains 42 chicks were kept in an area of 3 m^2, with SD of 14 bird/ m^2; the third group contains (G3) 51 chicks were kept in an area of 3 m^2, with SD of 17 bird/ m^2; the fourth group (G4) contains 60 chicks were kept in an area of 3 m^2 with SD of 20 bird/ m^2.

Housing and management

All chicks were brooded for seven days at the same room (deep litter system) and under the same condition at 33°C by using gas heater before they divided into four groups. The chicken in all groups (from day 7 to day 42) were raised on deep litter system and received the same standard management, hygienic and environmental conditions. The ventilation was provided with inlet and outlet ventilators and 1m ×1 m in size side windows, heating with a central thermogen, and artificial lighting with 16 regularly distributed bulbs.

Feed and water were provided *ad labitum* using manual plastic feeders and drinkers. Used ration was formulated (Table 1) to meet the nutrient requirements for broilers according to NRC (1994). Water was changed three times daily (at 8 AM, 4 PM and 12 PM).

Chickens were vaccinated against New Castle disease by oral administration in drinking water at days seven and eighteen using Hitchner and LaSota strains according to manufacturer recommendations, respectively. The light program was set as 24h continuous light during the first three days and twenty three hours light and 1h dark (23L/1D) till the end of the experiment using red bulb.

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Table 1. Composition of the diet used in the experiment from the day one to the day 42.

Ingredient	Experimental diet%
Yellow corn	55.5
Soybean meal	32.6
Sun flower oil	5.35
Fish meal	3.25
Dicalcium phosphate	1.5
Lime salt	1.0
L.lysine	0.1
Dl.methionine	0.1
Salt (NaCl)	0.3
Premix	0.25
Nutrient composition of ration	
Energy(kcal/kg)	3200.71
Crude protein (%)	21

Sampling and analysis

Air sampling and analysis: The air samples for ammonia measurement were collected by liquid impinger containing diluted sulfuric acid yielded aqueous solution of NH3. Ammonia was determined by the colorimetric method using Nessler's reagent according to Marr and Cresser (1983).

Blood sampling and analysis: From each group, five blood samples (5 ml each) were collected from the brachial vein at day 28th of the experiment and from bleeding during euthanize at the day 42. For making total and differential leucocytes count and to estimate of Heterophil / Lymphocyte (H/L), from each set of samples, 1ml whole blood was taken in Ethylenediaminetetraacetic acid (Edta) tubes ratio (Dein, 1984). Serum was prepared from the other 4 ml blood by centrifugation at 3000 rpm for 10 min. Serum samples were stored at -20°C until used for stress enzymes (Lactate Dehydrogenase (LDH) and Alkaline Phosphatase (APK)) assessments. Stress enzymes were measured. The analyses were performed using commercial analytical kits according to the manufacturer's recommendations (Jian Cheng Bioengineering Institute, Nanjing, China).

Broiler performance

It was recorded from day 0 to 42, chickens were individually weighed weekly. Average live Body weight (BW) and Body Weight gain (BWG) were calculated. Feed Intake (FI) and Water Intake (WI) were calculated daily for each group. Feed Conversion Ratio (FCR) and WI/FI ratio were calculated weekly for all groups.

At day 42, five birds of an average BW from each group humanly euthanized via exsanguinations (from a neck cut that severed the carotid artery and jugular vein). The birds were kept for five mins for bleeding and then dipped in a hot water bath for two mins to facilitate the process of de-feathering. Manual evisceration was performed to obtain carcass, gizzard, heart, liver, spleen and bursa weight. Absolute weight and relative weight of carcass and internal organs to final BW were calculated.

Statistical analysis

Data were tested for distribution normality and homogeneity of variance. Data was reported as mean ± standard error of the mean and analyzed by ANOVA using SAS (Statistical Analysis Software), Institutes INC (2005). The significance of difference among the different treatments was evaluated by Tukey's test. The significance level was set at $P<0.05$.

RESULTS AND DISCUSION

Effect of different stocking density on gaseous pollutants of broiler house

Ammonia: Ammonia is the most famous pollutant in poultry houses. Elevated concentrations harmfully affect bird performance, welfare and human health (Costa et al., 2012). Figure1a and figure 1b illustrate the effect of SD on ammonia concentration in air of experimental farm at different ages. Figure1a: Ammonia concentration was 0.733, 1.434, 1.838, and 1.95 mg/L in G1, G2, G3, and G4 respectively at age 14 days. Figure1b showed no significant difference between G2, G3, and G4, but they are significantly differs from G1 ($P<0.05$). Figure1a indicated ammonia concentration was 3.13, 3.55, 5.41, and 6.86 mg/L in G1, G2, G3, and G4 respectively at age 21 days. Fgure1b showed no significant difference between G1 and G2, but they are significantly differs from G3 and G4 ($P <0.05$).

Ammonia concentration was at 3.53, 4.2, 7.5, and 20.26 in G1, G2, G3, and G4 respectively at age 28 days: Figure 1b showed no significant difference between G1 and G2, but they are significantly differs from G3 and G4 ($P<0.05$). Figure1a indicated that ammonia concentration was 3.47, 9.04, 11.1, and 31.4 in G1, G2, G3, and G4 respectively at age 36 days. Figure1b showed no significant difference between G2 and G3, but they are significantly differs from G1 and G4 ($P < 0.05$). Comparing ammonia concentrations of different SD. Highest ammonia concentration was found in G4 (SD 20 bird/m^2) (1.95, 6.86, 20.28 and 31.4 mgL^{-1} at 14, 21, 28 and 36 day age respectively) while lowest ones were found in G1 (10 bird/m^2) (0.7, 3.1, 3.5 and 3.5 mgL^{-1} at 14, 21, 28 and 36 day age respectively).

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From the obtained results, it was found that there was an increase in ammonia emission with increasing SD and with age. This result was in agreement with Harper et al. (2010) and Meda et al. (2011) who stated that ammonia emission was increased after the third week of broiler age. Ammonia concentration obtained in this study was much higher than that obtained by other surveys of ammonia concentrations in different poultry systems showed mean concentrations of 12.3 mgL^{-1} in perchery systems (Wathes, 1998; Groot Koerkamp et al., 1998; Kristensen and Wathes, 2000). Additionally Lima et al. (2011) stated that the concentrations of ammonia in poultry houses are usually around 20 ppm. As ammonia is the product of microbial mineralization of urea and uric acid in poultry litter mixed with chicken manure (Nahm, 2003). The obtained result could be explained as follows; by increase in SD and age, the amount of chicken manure increased and concomitantly ammonia emission increased. The obtained results are in agreement with those obtained by Tasistro et al. (2007); Zhao et al. (2015) and Brouček and Čermák (2015).

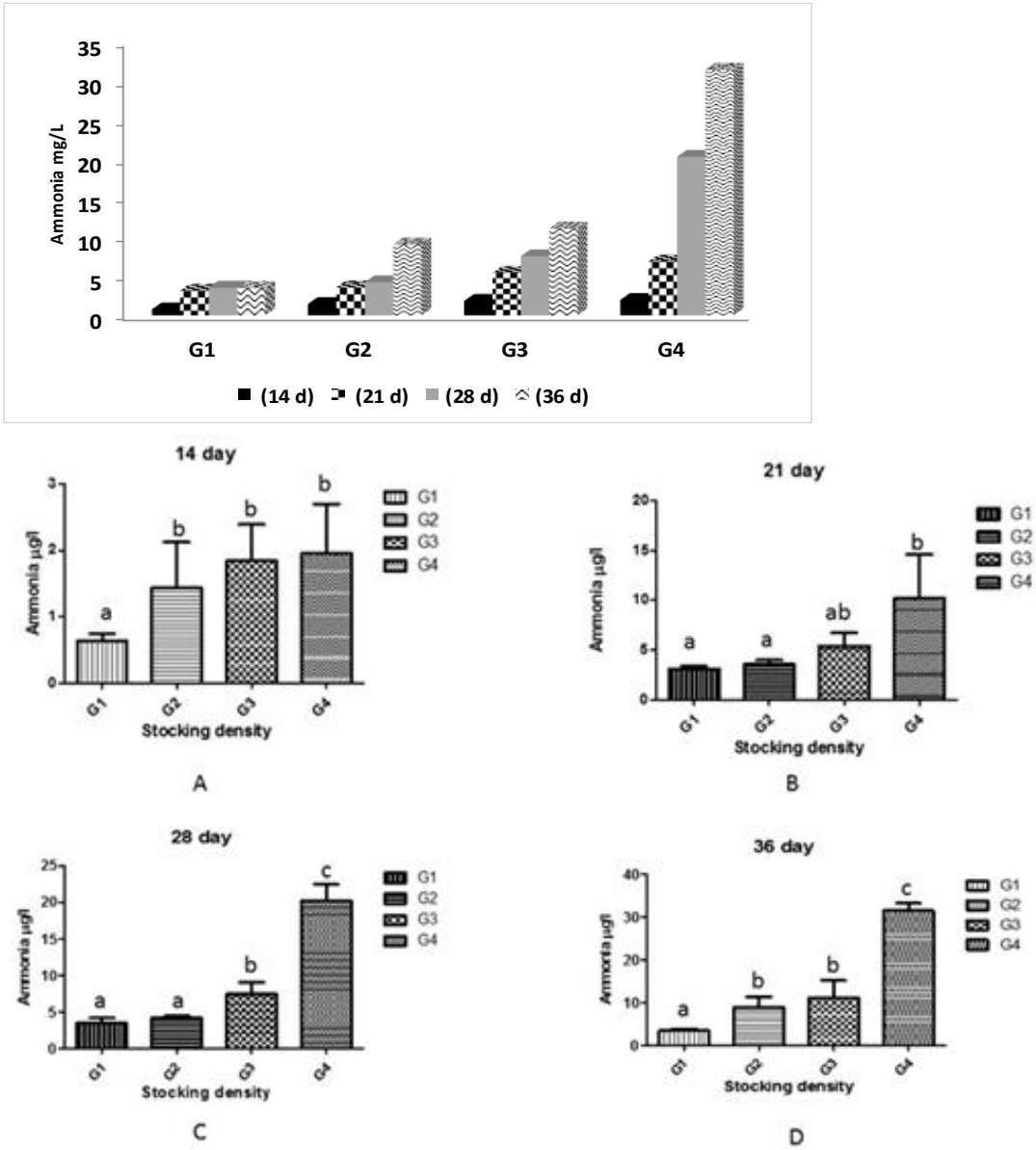

Figure1. a: Ammonia concentration in air samples of the broiler farm during day 14, 21, 28 and 36 in different groups; **b**: Detailed ammonia concentration (mgL^{-1}) with statistical differences between groups in air of the broiler farm in different ages (14 day, 21 day, 28 day, and 36 day). *Means ± Standard error which superscripts with different small letters (a-c) within the same diagram differ significantly at P<0.05.

Effect of stocking Density on boiler performance

Only two birds died during the experiment at 10 days age (data not shown). There were no significant differences in the initial BW among the experimental groups. However, increased SD tended to reduce the final BW and bodyweight gain significantly ($P < 0.05$) (Table 2).

The effect of SD on performance of broiler chickens over the 42 days trial period is shown in table 2. Stocking density had effect on the BW of birds. We found that birds raised at SD of 10 and 14 birds m^{-2} had significantly higher BW of 2530±41.44 and 2395±103.2 respectively than reared at stocking density of 17 and 20 birds m^{-2} 2178±46.63 and 2214±91.52 respectively ($P < 0.01$) The results are in contrast to Feddes et al. (2002), who reported similar BW of birds

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reared at densities of 12, 18, and 24 birds/m^2. Also Buijs et al. (2009) found no difference in final BW at 39 days of age as SD increased.

FCR was at 2.15, 1.74, 1.77 and 1.87 % for 10, 14, 17 and 20 birds m^{-2} respectively (Table 2). It was found that there is a significant difference between G1 and other groups ($P < 0.01$) but no significant difference between other 3 groups (14, 17 and 20 birds m^{-2}). Even though daily feed intake decreased significantly ($P< 0.05$) (Table 2) as SD increased. The result was similar to the report in another experiment (Dozier et al., 2005), who found a depression in daily weight gain, and the cumulative feed consumption was decreased as the SD increased.

Table 2. Effects of stocking density on body weight gain, feed intake, water intake, eviscerated weight and feed conversion ratio from first day to day 42 in broiler chicken

Parameter	G1	G2	G3	G4	P- value
BW (g)	2530±41.44^{a}	2395±103.2^{ab}	2178±46.63^{b}	2214±91.52^{b}	0.0155
BWG (g)	2460.11	2960.11	2264.71	2208.81	
Eviscerated Weight. (g)	1908±91.47^{a}	1715±58.74^{a}	1555±44.94^{b}	1526±60.88^{b}	0.0031
FI / week (g)	337.4±31.36^{a}	326.3±15.00^{ab}	255.2±11.50^{b}	263.1±13.38^{b}	0.0050
WI/ week (ml)	155.9±6.633^{a}	183.6±8.259^{b}	139.1±5.311^{a}	142.2±6.618^{a}	0.0001
FCR	2.15	1.74	1.77	1.87	

G1, 10 bird/m2; G2, 14 bird/m2; G3, 17 bird/m2; G4, 20 bird/m2; BWG=Body weight gain; FI=Feed intake; WI=Water intake; FCR=Feed conversion ratio; *Means ± Standard error which superscripts with different small letters (a-c) within the same row differ significantly at P<0.05.

Lymphoid organs

Liver, spleen and bursa are used as anatomical indicators of stress (Pope, 1991and Freire et al., 2003) as it is well known that the average weights of the lymphoid organs (spleen and bursa) change in response to stress (Ravindran et al., 2006). In the present study, the relative weights of spleen 2.42, 2.79, 2.85 and 3.69 g in G1, G2, G3 and G4 respectively and bursa (1.01, 1.8, 1.49 and 2.44 g in G1, G2, G3 and G4 respectively) were increased significantly ($P < 0.05$) with increasing SD (Table 3). These findings are in agreement with Muniz et al. (2006) who stated that higher SD cause detrimental effects on the lymphoid tissue. But the current results are different than that obtained by Heckert et al., (2002); Onbaşılar et al. (2008) and Tong et al. (2012) who concluded that increasing SD did not significantly affect the spleen and bursa weights. Additionally liver weight increased with increase in SD and stress level, this result was in line with Puvadolpirod and thaxton, 2000 and Sekeroglu et al. (2011).

Table 3. Effects of stocking density on body weight of lymphoid organs from first day to day 42 in broiler chicken

Parameters	G1	G2	G3	G4	P- value
Liver Weight (g)	52.26±2.922^{a}	56.80±3.108^{ab}	56.18±3.297^{ab}	66.26±1.843^{b}	0.0202
Spleen Weight (g)	2.422±0.1603^{a}	2.792±0.2910^{a}	2.848±0.1211^{a}	3.692±0.1428^{b}	0.0018
Bursa Weight (g)	1.010±0.1720^{a}	1.802±0.1498^{b}	1.490±0.2441^{a}	2.442±0.1899^{c}	0.0007

*Means ± Standard error which superscripts with different small letters (a-c) within the same row differ significantly at P<0.05.

Effect of stocking Density on Blood parameters

The effects of SD on some blood parameters are shown in table 4 it was found that there is a significant difference in the amount of blood hemoglobin and RBCs. This result was in contrast to that obtained by Sekeroglu et al. (2011). On the other hand significant differences were found in other blood parameters (PCV%, MCV (fl), MCHC (g/dl)) ($P > 0.05$). The results were in agreement with Thaxton et al. (2006), who found that SD did not result a significant difference in some blood parameters.

Table 4. Effects of stocking density on blood parameters and stress enzymes from first day to day 42 in broiler chicken

Parameters	G1	G2	G3	G4	P- value
Haemoglobin (g/dL)	11.60±0.2608^{a}	11.18±0.1463^{a}	10.82±0.1428^{b}	10.80±0.2168^{b}	0.0365
RBCs (×10^6/µl)	3.004±0.1966^{a}	2.918±0.03169^{ab}	2.560±0.04393^{ab}	2.524±0.09563^{b}	0.0151
PCV (%)	36.74±2.167	37.92±0.9173	36.04±1.059	35.80±1.305	0.7325
MCV (fl)	141.0±2.481	137.8±2.918	140.7±2.204	137.7±2.060	0.6601
MCHC (g/dL)	30.02±0.4224	29.98±0.2354	30.06±0.4250	30.44±0.4057	0.8164
TLC (x10^3/cmm)	106.1±3.261^{a}	108.4±2.948^{a}	103.3±2.134^{ab}	91.42±4.557^{b}	0.0114
Heterophils (%)	1.6±0.245^{a}	3.4±0.245^{a}	9.6±0.511^{b}	14.2±0.860^{c}	0.0001
Lymphocytes (%)	91.40±1.400^{a}	84.60±2.657^{a}	76.40±1.077^{b}	74.00±1.225^{b}	0.0001
H/L ratio	0.018±0.003^{a}	0.041±0.004^{a}	0.126±0.007^{b}	0.192±0.012^{c}	0.0001
LDH (IU/L)	41.00±4.461^{a}	506.2±19.62^{b}	601.0±47.52^{b}	872.8±44.61^{c}	0.0001
APK (IU/L)	3946±714.2^{a}	4632±314.2^{a}	5751±298.7^{b}	6091±286.6^{b}	0.0119

*Means ± Standard error which superscripts with different small letters (a-c) within the same row differ significantly at P<0.05.

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In the current study there was a significant difference ($P < 0.05$) in TLC, hetrophils %, lymphocytes % and H/L ratio. Mohammed et al. (2014) stated that TLC and H/L ratio are considered the most important parameters that used for assessing physiological stress in birds. Results in table 4 showed that, there is a significant difference ($P < 0.05$) in TLC, heterophil (H%), lymphocyte (L%) and H/L ratio among the experimental groups. From these results, it was found that G4 (SD, 20bird/m^2) was represented by the lowest values of TLC (91.42×10^3/cmm), and highest (108.4×10^3/cmm) at G2 (SD, 14bird/m^2). In table 4 Heterophil H% was increased with increase in SD to reach highest percent in G4 (14.2%), on contrast and lymphocyte L% was decreased by increase in SD with highest percent in G1 (91.4%). H/L ratio was highest in G4 (0.192%) and lowest in G1 (0.018%), which showed increasing in H/L ratio with increase in SD which reflects increase in stress level, this result was in line with Puvadolpirod and Thaxton (2000) and Onbaşılar et al. (2008).

LDH is generally associated with cellular metabolic activity, which is inhibited or elevated under oxidative stress (Das et al., 2004). LDH and APK activity observed in the present study were increased laniary with increase in SD (Table 4). This result could be explained as increasing in SD resulted in increase in air pollution especially ammonia which increase level of stress and consequently increase the activity of LDH (Wang et al., 2014).

CONCLUSION

In conclusion, this study has declared that ammonia emissions from broiler house increased significantly with increasing in SD and age. This results pays attention to the need for improved ventilation and managemental procedures especially with increase in SD. Increased SD tended to reduce the BW and BWG significantly. On the other hand, the relative weights of spleen and bursa were increased significantly ($P < 0.05$) with increasing SD. H/L ratio was highest in G4 (0.192%) and lowest in G1 (0.018%), which showed increasing in H/L ratio with increase in SD which reflects increase in stress level.

Competing interests

The authors have no competing interests to declare.

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Prevalence of *Cysticercus Tenuicollis* in Small Ruminants Slaughtered at Addis Ababa Abattoir, Ethiopia

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ABSTRACT

Parasitic diseases in general and *Cysticercus tenuicollis* in particular are responsible for the low productivity of livestock. A study to determine the prevalence of *C. tenuicollis* in sheep and goats at Addis Ababa abattoir, Ethiopia. A cross sectional study was conducted and a stratified random sampling technique was employed where by sheep and goats to be slaughtered were categorized into two groups based on their sex (male and female) and also based on their age (young and adult) only in case of sheep. The statistical analysis was performed using SPSS program and Chi square. The overall prevalence of *C. tenuicollis* from 900 shoats was found to be at 355 (39%). Out of the 600 sheep inspected *C. tenuicollis* was detected in 223 (37.2%) and from 300 inspected goats *C. tenuicollis* was observed in 132 (44%). The age wise prevalence *C. tenuicollis* showed that the prevalence was higher in adult sheep (46%) than young sheep (28.3%). The prevalence of *C. tenuicollis* based on their sex was higher in female goats (45.3%) and sheep (37.8%) than in male goats (42.7%) and sheep (35.7%) with no significant statistical difference (P>0.05). Although organ wise infection rates of both goats and sheep were 5.7% and 3.7% (mesentery), 0.7% and 0.2% (diaphragm), 0% and 0.2% (uterus), and 0.3% and 0% (rectum) with no significant statistical difference. However, in other infected organs, there was a significant statistical difference 39% and 34.2% (omentum), 14.7% and 7.8% (liver) and 3% and 0.8% (lung) in goats and sheep, respectively. The overall percentage of *C.tenuicollis* was higher in adult sheep (10.7%) than in young sheep (5%) with significant statistical difference (P<0.05).

Keywords: Addis Ababa, *Cysticercus tenuicollis*, Goat, Prevalence, Sheep, Visceral organ

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INTRODUCTION

Ethiopia with its great variation in climate and topography possesses one of the largest small ruminant populations in the world, which is mostly kept by small holder farmers and managed under extensive husbandry system (ILCA, 1993). Their ability to utilize wider variety of plant species, their short generation cycle and high reproductive performance make sheep and goats complementary to cattle and camel production (Ibrahim, 1998). Sheep and goat are major source of income (cash) for the poor rural farmers in most parts of tropics including Ethiopia (Devendra and Mcleory, 1990; Ibrahim, 1998). However, the full exploitation of these resources is hindered in the tropical environment, due to a combination of factors such as drought, poor genetic potential and backward animal husbandry practice and due to the prevalence of diseases (Schillorn van Veen, 1985 and Ibrahim, 1998).

Parasitic diseases are significant causes of morbidity and mortality in humans as well as in animals (Pal, 2007 and et al., 2014), and are mostly found in warm tropical and sub-tropical regions of the world (Hadush and Pal, 2016). Many parasitic diseases are prevalent in Ethiopia, which are responsible for the low productivity of livestock besides contributing to reduced meat production due to carcass or organ condemnation (Abebe, 1995; Kebede et al., 2013; Nasr and Pal, 2016). In Ethiopia, parasitic diseases including *C. tenuicollis* in small ruminants were implicated as cause of organ condemnation in Abattoir enterprise leading to significant economic loss (yehualashet et al., 2012). *C. tenuicollis* is the cystic stage of *Taenia hydatigena*, which is found in the small intestines of dogs and cats. Cysts of *C. tenuicollis* are responsible for a high degree of morbidity and mortality in livestock (Abidi et al., 1989). Ova passed with dog feces are ingested by intermediate hosts such as sheep and goat with pasture contaminated with the eggs. After ingestion the larvae, which develop penetrate the small intestine and disseminate to various tissues, especially the liver, omentum, mesentery, and peritoneum. If the larvae reach the liver surface, they develop into thin walled fluid filled bladders and if

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they fail, they degenerate and become calcified. Migration through the liver leaves grayish white tortuous tracts. Heavy infestation in young animals causes liver damage and hemorrhages or peritonitis (Soulsby, 1989).

When many embryos migrate simultaneously through the liver, clinical signs may be seen. The migration may cause severe destruction of the liver tissue and the pathology seen in the liver may be similar to that observed in liver fluke infection (Jorgan and Brian 1994). A serious condition known as" hepatitis cysticercosis" caused by *C. tenuicollis* in different animals was described by many workers (Pathak et al., 1982 and Soulsby, 1989). In extremely heavy infection, the viscera may become knotted together and organ function may be impaired. Degenerated cysts are replaced by caseous and calcareous debris and may damage tissue. If large numbers of larvae migrate through the liver parenchyma, tissue will be damaged and acute and fatal hepatitis may be the result (Pathak et al., 1982).

Even though various investigations have been conducted to determine the prevalence of parasitic diseases resulting in organ condemnation in Ethiopia (Jemberie, 2002; Yimam, 2003 and Asefa, 2005), most of the survey paid little attention to the study of *C. tenuicollis*. Therefore, the objectives of the abattoir survey were to determine the prevalence of *C. tenuicollis* in sheep and goats slaughtered at Addis Ababa abattoir, to find out the distribution of *C. tenuicollis* in visceral organs of sheep and goats and also to assess the relationship between some risk factors and prevalence of *C. tenuicollis* in sheep and goats.

MATERIALS AND METHODS

The study was conducted from November 2008 to March 2009 at Addis Ababa abattoir, Ethiopia. Adds Ababa is located at an elevation of about 2,400 meters above sea level and receives an annual rainfall of 1800 mm in bimodal pattern. The long rainy season extends from June to September followed by a dry season ranging from October to February. The short rainy season lasts from March to May. The average minimum and maximum temperatures are 10.7^0C and 23^0 C, respectively (AACA, 2004). The animals included in the study were male and female, young and adult sheep and goats of local breeds brought from different localities around Addis Ababa, Ethiopia.

In this study, animals were classified into two species: sheep and goats, two age groups (young and adult) two sexes (male and female) and two origins (highland and lowland). Age was determined by observation of the erupted permanent incisors and classification in to two age groups was performed according to Gatenby (1991) and Steel (1996). Areas above 1500 MASL was considered as highland and lower than 1500 MASL was considered as lowland. Sex was determined by observation. On Postmortem inspection, visceral organs such as liver, omentum, mesentery, diaphragm, uterus and rectum were examined visually for the presence of *C. tenuicollis.* Transparent cyst filled with fluid and presence of white dot indicating scolex was considered as *C .tenuicollis.* The raw data generated during postmortem meat inspection were entered into MS Excel program and the statistical analysis was performed by using SPSS program for windows (version 15.0, 2008). Descriptive statistics such as percentage was used to determine the rate of infection. The variation between infection rates of specific organs, age, and species of animals were evaluated by Pearson's chi-square (X^2) and differences were regarded as statistically significant if P-value is less than 0.05.

Ethical Approval

The study considered direct observation of slaughter animals in the abattoir and no animal was subjected to suffering as a result of the study. Nevertheless, ethical clearance on scientific soundness and justification for the need to do the study was addressed by the Addis Ababa University College of Veterinary Medicine.

RESULTS

During the study period, a total of 900 shoats were slaughtered and inspected for the presence of *C. tenuicollis*. The overall prevalence of *C. tenuicollis* was 355 (39 %) and the prevalence in sheep and goats were 37.2% and 44%, respectively with a significant statistical difference (P<0.05) between the two species (Table 1).

Table 1. The prevalence of *Cysticercus tenuicollis* in sheep and goats slaughtered at Addis Ababa abattoir, Ethiopia during November 2008 -March 2009

Species	No examined	Prevalence N (%)	P–value
Sheep	600	223(37.2)	0.008
Goats	300	132(44)	
Total	900	355 (39)	

N= Number of infected animals

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Out of 300 sheep inspected, *C. tenuicollis* was detected in 107 (35.7%) and 116 (38.7%) male and female sheep, respectively. *C. tenuicollis* was found more in adult male sheep 66 (44%) than in young male sheep 41 (27.3%) with a significant statistical difference in infection rates between the two age groups (P <0.05). The prevalence of *C. tenuicollis* was higher in adult females 70 (46.7%) than in young female sheep 46 (30.7%) with a significant statistical difference in prevalence (P <0.05). The prevalence of *C. tenuicollis* was higher in adult female goats 68 (45.3%) than in adult male goats 64 (42.7) (Table 2).

Table 3 indicates the infection rate of *C. tenuicollis* in different organ of sheep and goats. The cysts in sheep and goats had a tendency to be located more in the omentum, and it was lower in other organs. The infection rate in goats and sheep were comparable. Out of a total of 471 cysts counted in different internal organs of sheep and goats, 322 (68.3%), 39 (8.28%), 91 (19.32%), 14 (2.98%) and 3(0.63%), 1(0.2%), and 1(0.3%) were found in the omentum, mesentery, livers, lungs, diaphragm, uterus, and rectum, respectively. More cysts were found in each of the above visceral organs of goats than in the same organs of sheep (Table 3).

Table 4 indicates the percentage of *C. tenuicollis* in the visceral organs of young and adult sheep. The percentage was higher in the omentum of adult sheep 124 (41.3%) than in the omentum of young sheep 81 (27.0%). Likewise, the infection rate was higher in the liver of adult sheep 32 (10.7%) than the young sheep 15 (5%) with a Significant statistical difference (P < 0.05). The number of cysts counted in the omentum, mesentery, liver, lung, diaphragm, and uterus was higher in adult sheep than in young sheep (Table 4).

Table 5 shows that variation between infection rates in different organs of the two species. The infection rate was higher in the omentum 117 (39%), mesentery 17 (5.7%), liver 44 (5.7%) of goats than in the sheep in the same organs with a Significant statistical difference (P< 0.05). There was Significant statistical difference between the numbers of cysts found in the omentum, liver, and lung of goats and sheep. There was no significant difference between the number of cysts counted in the mesentery, diaphragm, uterus and rectum of goats and sheep (Table 5).

Table 6 shows the variation in infection rates between different organs in relation to the sex of animals. There was no significant difference in the infection rates between male and female goats in all of the examined organs. But significant difference in infection rate in the omentum of male and female sheep was noted (Table 6).

Table 2. Prevalence of *Cysticercus tenuicollis* in sheep and goats based on their sex and age slaughtered at Addis Ababa abattoir, Ethiopia during November 2008 -March 2009.

Species (N)	Sex (N)	Age (N)	Prevalence N (%)	P-Value
Sheep (600)	Male (300)	Young (150)	41(27.3)	0.003
		Adult (150)	66 (44)	
		Total =300	107(35.7)	
	Female (300)	Young (150)	46 (30.7)	0.002
		Adult (150)	70 (46.7)	
		Total =300	116 (38.7)	
Goats (300)	Male (150)	Adult (150)	64 (42.7)	0.078
	Female (150)	Adult (150)	68 (45.3)	
		Total=300	132 (44)	

N: - Number of animals

Table 3. Infection rates of different visceral organs with *Cysticercus tenuicollis* in sheep and goats slaughtered at Addis Ababa abattoir, Ethiopia during November 2008 -March 2009

	Inspected visceral organs						
Species	**Omentem**	**Mesentery**	**Liver**	**Lung**	**Diaphragm**	**Uterus**	**Rectum**
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Goats	117 (39.0)	17 (5.7)	44 (14.7)	9(3.0)	2(0.7)	0(0)	1(0.3)
Sheep	205 (34.2)	22(3.7)	47 (7.8)	5(0.8)	1(0.2)	1(0.2)	0(0)
Total	322(68.36)	39(8.28)	91(19.32)	14(2.98)	3(0.6)	1(0.2)	1(0.3)

No: Number of infected animal's organ.

Table 4. Percentage of *Cysticercus tenuicollis* in the visceral organs of young and adult sheep slaughtered at Addis Ababa abattoir, Ethiopia during November 2008 -March 2009

	Inspected visceral organs					
	Omentum	Mesentery	Liver	Lung	Diaphragm	Uterus
Age	Positive =N (%)	Positive =N (%)	Positive =N (%)	Positive =N (%)	Positive =N (%)	Positive =N (%)
Young	81(27.0)	10(3.3)	15(5)	2(0.7)	0(0)	0(0)
Adult	124(41.3)	12(4.0)	32(10.7)	3(1)	1(0.3)	1(0.3)

N: Number of animals

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Table 5. Variation between infections rates of specific organs in sheep and goats slaughtered at Addis Ababa abattoir, Ethiopia. November 2008 -March 2009.

Visceral organ	Goats (300)	Sheep (600)	P-value
	Positive = N(%)	Positive = N(%)	
Omentum	117(39)	205(34.2)	0.021
Mesentery	17(5.7)	22(3.7)	0.52
Liver	44(14.7)	47(7.8)	0.001
Lung	9(3)	5)0.8)	0.013
diaphragm	2(0.7)	1(0.2)	0.222
Uterus	-	1(0.2)	0.667
Rectum	1(0.3)	-	0.333

N: Number of infected animals.

Table 6. Variation between infection rates of specific organs in relation to sex of the animals (sheep and goats) slaughtered at Addis Ababa abattoir, Ethiopia during November 2008 -March 2009.

Visceral organ	Sex	Goats (300)	P-value	Sheep (600)	P-value
		Positive =N (%)		Positive =N(%)	
Omentum	Male	56(37.3)	0.079	97(32.3)	0.044
	Female	61(40.7)		108(36)	
Mesentry	Male	8(5.3)	0.191	8(2.7)	0.075
	Female	9(6.0)		14(4.7)	
Liver	Male	21(14.0)	0.123	23(7.7)	0.119
	Female	23(15.3)		24(8.0)	
Lung	Male	5(3.3)	0.249	1(0.3)	0.156
	Female	2(2.7)		4(1.3)	
Diaphragm	Male	2(1.3)	0.249	1(0.3)	0.5
	Female	-		-	
Uterus	Male	---	----	-	0.5
	Female	---		1(0.3)	
Rectum	Male	-	0.5	----	----
	Female	1(0.3)		----	

N: Number of infected animals.

DISCUSSION

During the study period, a total of 900 shoats were examined from November 2008- March 2009 to determine the prevalence rate and to assess the infection rates of different visceral organs with of *C. tenuicollis*. The prevalence of 37.2% and 44% was found in sheep and goats, respectively. The prevalence of *C. tenuicollis* by species was higher in goats when compared with that of sheep and this difference was found to be statistically significant. Similar observation was made by Samuel (2008) and Abdulikadir et al. (2015) who found out that goats were more infected with *C. tenuicollis* than sheep and also in According to Torgersan et, al. (1998) under condition of high infestation with C. *tenuicollis*, most sheep develop protective immunity early in life and the density dependent constrains regulates the parasite population, whereas goats develop the immunity more slowly. This considerable degree of immunity against *C. tenuicollis* infection in sheep may be the reason for the low prevalence of the parasite in sheep. High prevalence was reported in East Ethiopia by Sisay et al. (2007). The overall prevalence was higher in sheep (79%) than in goats (53%) with a significant statistical difference.

The prevalence of *C. tenuicollis* in sheep and goats in our study was relatively lower when compared with the results attained by Samuel (2008) and Abdulikadir et al. (2015) who recorded the prevalence of 40% and 45% in sheep and 46.6% and 53.9% in goats in central Ethiopia and in Bishoftu, Elfora Export Abattoir, Ethiopia respectively. But this finding is compared to the report of Endale et al. (2013) the prevalence of *C. tenuicollis* in sheep and goats in this study is relatively higher. Different investigators have also reported from other part of the world on the prevalence of *C. tenuicollis.* The prevalence of 37.03% and 27.29% have been reported in sheep and in goats, respectively in India (Pathak and Gaur, 1982) and in Nigeria, a prevalence of 21.4% in sheep and 34.2% in goats have been reported by Dada and Bellino (1978). In Germany, Hasslinger and Weber-Werrinhen (1988) recorded a prevalence of 16.7% in sheep. The prevalence of 21.4% in sheep and 34.2% in goats was reported in Iran (Solaymani-Mohammadi et al., 2003), and also similar results were obtained in Iran by Radfar et al. (2005) who described a prevalence of 12.84% in sheep and 18.04%

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in goats. Relatively lower prevalences were recorded in other countries and this may be due to the variation in temperature, environmental condition, the degree of pasture contamination and the way of raising and grazing of these animals which may favor the transmission cycle between ruminants and dogs and other wild canines. (Samuel, 2008)

C. tenuicollis was encountered 35.7% in 300 male sheep, whereas the prevalence in female sheep was 38.7%. The prevalence of *C. tenuicollis* was found to be 42.7% in male and 45.3% in female goats. In this study, the animals were categorized in two age groups (young and adult). Out of 300 slaughtered and examined young sheep, *C. tenuicollis* was detected in 28.3% of them, whereas the prevalence in adult sheep was 46% indicating high infection rate in adult sheep with a statistical difference ($P < 0.05$). Similar results were obtained in Bishoftu Elfora Export Abattoir, Ethiopia. by Abdulikadir et al., (2015) who described a prevalence of 34.1% in adult sheep and 15.3% in young sheep with a statistical difference. The difference in infection rates between young and adult sheep may be due to the fact that adult sheep lived longer and consumed larger number of eggs during grazing when compared with young sheep which had only lived for a shorter period of time. Comparison of infection rates between young and adult goats could not be made, while no young goats were delivered to the Addis Ababa Abattoir during the study period.

The current study indicated that different visceral organs and other organs can be infected with *C. tenuicollis* at different infection rates. In this study the predominant predilection site of *C. tenuicollis* was found to be the omentum. A similar observation was made by El-Azazy and Fayek (1990).The percentage of infection was higher in the omentum of goats (39%) than in the omentum of sheep (34.2%). This difference in the infection rate of *C. tenuicollis* in the omentum of goats and sheep was statistically significant ($P <0.05$). Similar observations were made by Radfar et al. (2005) and Samuel (2008). The infection rate of *C. tenuicollis* was higher in the omentum of adult sheep (41.3%) than the young ones (27.0%), and the infection rate of *C. tenuicollis* in the liver of adult sheep was higher (10.7%) than the young sheep (5%) with a statistically significant difference ($P<0.05$). The higher prevalence of *C. tenuicollis* in the slaughtered animals also indicated that the cyst causes considerable economic loss, due to condemnation of the affected organs at the slaughter house. Such losses are of particular importance for Ethiopia, which has low economic output where sheep and goat production are the major livestock industries.

CONCLUSION

In conclusion, *C.tenucollis* is prevalent in Ethiopia, in shoats, whereby goats are more infected than sheep and also the adult animals are more infected than young ones. The cyst invades most of the visceral organs, with higher frequency of being detected in the omentum, mesentery, and liver. Particularly, infection of the liver leads to condemnation of the organ which results in scarcity of edible offal and severe economic losses. Therefore, extensively studied both in the final and intermediate hosts, and appropriate control measures should be put in place to reduce the prevalence of the disease in sheep and goats at all level.

Competing interests

The authors have declared that there are no competing interests exist.

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Development of Sternum and Ribs in White New Zealand Rabbit (*Oryctolagus cuniculus)*

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ABSTRACT

The bone development and the assessment of the fetal skeletal improvement turn into a basic segment in the behavior of a prenatal toxicity study. The sternum is a unique bone in the distinctive shape and development. This study focuses on the development of the sternum and the ribs attached in the whit New Zealand rabbit, Specimens were collected prenatally (n=30) and postnatally (n=30). A group of specimens were double stained for bone and cartilage using Alizarin Red and Alcian blue. Another group was scanned by CT. The sternum was consisted of manubrium, four sternebrae and xiphoid cartilage. The sternum was in communication with 6 pairs of ribs. 7 ossification centers appeared in the sternum, one for each segment except the fourth one which has two. The first group centers appeared in the manubrium and the first two sternebrae on 22 days old embryos. All primary centers seen before birth. There are no secondary ossification centers for the sternum. Concerning ribs, there are four ossification centers for each except the last two ribs. The ossification centers of the body came from the extension of the transvers process of the thoracic vertebrae and it appears as early as two weeks of gestation. By the end of the third week of pregnancy, the ribs show primary center of ossification from the second to the last one. The second center was designed for the head and it appeared two weeks after birth. The third and fourth centers for the tubercle appeared a month after birth. Complete fusion between these centers takes place in three months old rabbit.

Key words: Rabbit, Sternum, Ribs, Development, Double staining, CT

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INTRODUCTION

The rabbit has been repeatedly used as a model to elucidate the typical and strange bone development since it takes after a fundamentally the same as example to the human infant, these factors are considered extremely valuable for the research in human models (Alberius and Selvik, 1986). The sternum is present in the mid ventral region of the thorax of the rabbit. The sternum is one of the skeleton parts with frequent variation in appearance (Jitender et al., 2015). It is made by five bony pieces called "Sternebrae". The most cranial sternebrae is called the presternum (manubrium). The sixth sternebra is the xiphisternum, which ends with xiphoid cartilage (Goodman et al., 1983; Gray et al., 1995; Graeber and Nazim, 2007; Restrepo et al., 2009). Seven ribs are articulated with sternum by their cartilage part

In human, the sternum developed over a long period of time which begins during the prenatal period and continues through the third and fourth decades of the postnatal period (Williams et al., 1989; Kozielec et al., 1973; O'Rahilly and Muller, 1992 and O'Neal et al., 1998). Moreover, the stages and pathways of appearance of the ossification centers in the skeleton during intrauterine in rodents and rabbit were considered a reliable indicators of fetal maturity in teratological experiments. In rats and mice, the ossification of the parietal and occipital bones, distal limb skeleton, sternum, and vertebral centra is particularly indicative (Strong, 1960).

The normal development of skeleton has been described for laboratory animals such as rat (Menegola et al., 2002) and hamster (Bruce and Hindle, 1994), mouse and rat (Fukuda and Matsuoka, 1980). However, there is no information available regarding normal development of ossification centers in the sternum and ribs of rabbit. This work is to determine the number and the time of the initial ossification centers for the sternum and ribs in the white New Zeeland rabbit. Moreover, the age of fusion of the resultant bony elements will be determined.

MATERIALS AND METHODS

The present study was conducted on white New Zealand rabbits (*Oryctolagus cuniculus)*, that were collected from different areas of Beheira and Cairo governorate, Egypt.

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Ethical approval

The use of the animal species was approved, before the study began, by the Institutional Animal Care and Use Committee (IACUC) of the faculty of veterinary medicine, university of Sadat city, Egypt.

Prenatal samples

Eight female rabbits were naturally mated at the same day and the day of coitus was considered as zero gestation day, then rabbits were sacrificed, the abdomen was opened the uterus to get the fetuses, which were collected on day 16th through 28th of pregnancy, n=30specimens (at least five specimens for every age). All specimens were counted and measured by metal caliber for the Crown-Rump Length (CRL) is the measurement of the length of embryos and fetuses from the top of the head (crown) to the bottom of the buttocks (rump). Fetuses were skinned, eviscerated and fixed in 95% ethyl alcohol for at least seven days.

Postnatal samples

A total of (30) rabbits were used at the ages of 1st day, 3rd day, 7th day, 15th day, 30th day, 45th day, 60th day, and 90th day. Animals were bled, skinned, eviscerated and stored in neutral buffer containing formalin 10%.

Double staining technique

The fetuses were held in 95% Ethyl alcohol for at least seven days, then placed in acetone for two days for fat removal. Maceration process was performed with different grades of potassium hydroxide(KOH) to remove muscle according to the age of the sample, in our laboratory 2% solution is used for the prenatal specimens and in the postnatal samples we used the concentration of 4% KOH solution.

For staining ossified bone, the Alizarin Red is enough. However, for staining the bone and cartilage, Alizarin Red and Alcian blue have been recommended by many researchers (Inouye, 1976; Kimmel and Trammel, 1981; Webb and Byrd, 1994 and Redfern et al., 2007). Immersion of the specimens in a mixture of Alcian blue for two days. After two days, the specimens were rehydrated in degrading series of alcohol. The specimens were immersed in this solution for 24 hours.

Transparency process

This process was performed in four successive steps:

1st step: stained fetuses were put in 1% KOH for one day. 2nd step: they were put in 80 cc 1% KOH and 20 cc 20% glycerin for five days. 3rd step: they were put in 50 cc 1% KOH and 50 cc 50% glycerin for five days. 4th step: they were put in 20 cc 1% KOH and 80 cc 80% glycerin for five days. Whole stained embryos were examined using stereoscopic microscope at a magnification of 30× and were photographed.

Computed tomography and 3D reconstruction

Settings for the Computed Tomography (CT) image technique in this study were as follows: 120 kV, 200MA and the image acquisition time was approximately 30 seconds at 2.00 mm thickness.

RESULTS

Sternum

The sternum was present as the cartilaginous bar in the ventral aspect of the chest wall at 16days old embryo. The sternum was formed of six sternebrae; the first one formed manubrium, four other sternebrae formed the mesosternum and the last one formed the xiphoid cartilage. Each sternebrae had a single ossification center. At the age of 22days old embryo, ossification centers appeared in the manubrium and the first two sternebrae. Within the next 24 hours, an ossification center appeared in the third sternebrae (Figure 1a). At the age of 25days old embryo, the fourth ossification centers appear in the fourth sternebrae and the xiphoid one. The whole sternum showed ossification centers before birth (Figures 1b, 2b).

The manubrium of the new born rabbit as well as the first three sternebrae had one primary ossification center. The fourth sternebrae had two ossification centers, primary and accessory ossification center. It was a small center distal to the primary center (Figure 1c). After one week, the manubrial ossification center was a large composite mass (Figure 2a) and the other three noteworthy essential centers of the sternebrae had lost all remnants of midline coalescence. Two weeks after birth, an increase in the size of ossified part and decrease in the cartilage between the sternebrae was observed (Figure 2c). In one-month old rabbit, ossification became increasingly evident as the ossification centers expand out toward the margins of the

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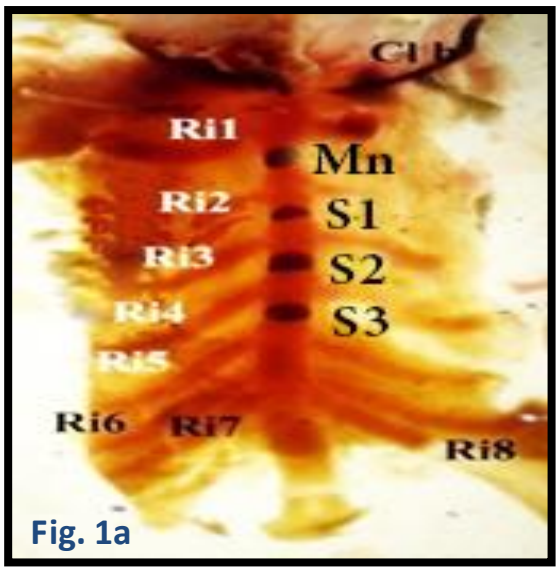
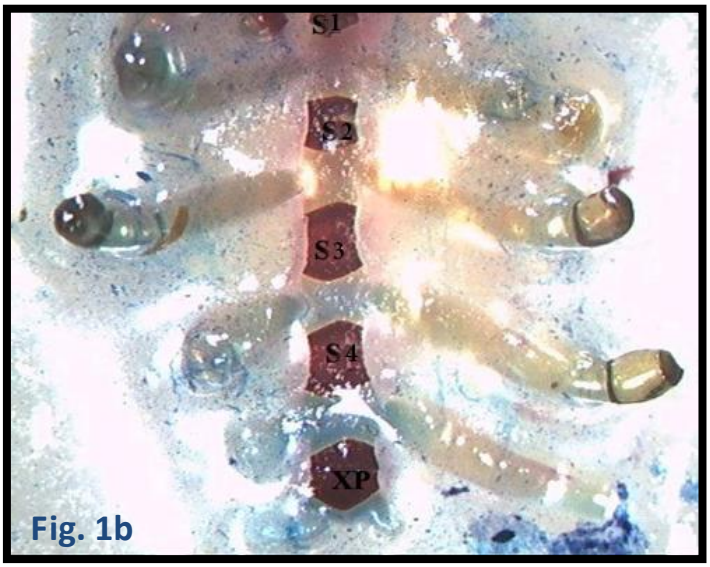
sternebrae and towards each other, they gradually developed small concavities at the junctions with the costochondral segments. 15 days later, the sternebrae developed a marginal lip at the junction of the costochondral segment with the sternum. Similarly, at the cranial end, the manubrium continued to develop. In two months old rabbit, the sternal segments two through four had undergone coalescence, although interestingly they still showed a longitudinal ossification response to the attachments of the rib costochondral segments. The original interosseous cartilage was being replaced by a bony plate. Three months after birth, complete ossification occurred in the sternum. It acquired the adult shape which formed the cartilaginous ends of the first pair of ribs articulate directly with the sides of the manubrium, the second through fifth pairs of ribs articulated with the inter sternebrae cartilages of the manubrium and the first to third sternebrae and the sixth and seventh pairs articulated with the cartilage between the fourth sternebrae and xiphoid process (Figure 2d).

Ribs

By the 15 days' post coitum, the transverse process of vertebrae in the thoracic part of the column were much longer than those of the other vertebral region. They extended ventrolaterally following the curve of the body wall forming the rib primordium. Is vertebral end formed a head, a neck, a tubercle and a shaft.

After three weeks of pregnancy in rabbit, the shaft of all ribs showed a primary center of ossification (Figure 1a 3a, 3b, 4a, 4c). All primary centers of ossification for ribs were present before birth (Figure 3c and Figure 4b).





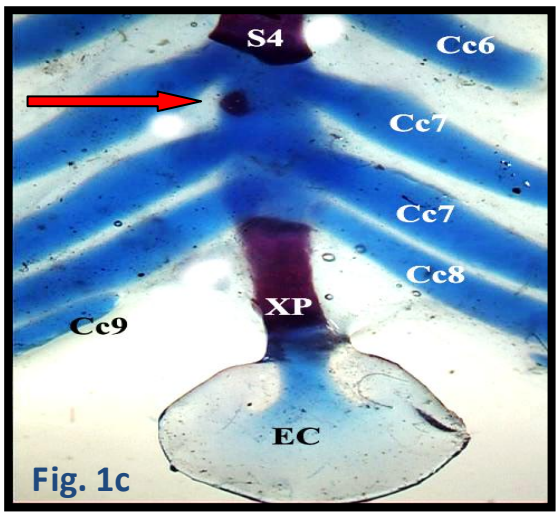


Figure 1.

A- Ventral view of rabbit sternum of 23 days' post coitum (dpc) Alcian blue and alizarin red S stained embryo show ossification centers of sternebrae 1-4 with the inter-sternal cartilage between the sternebrae attached to it the costal cartilage of ribs 1-8, the manubrium and xiphoid are still cartilaginous

B- Ventral view of the sternum of 28 days (dpc) double stained embryo of rabbit shows ossification of Sternebrae (S) 1-3 with the inter-sternal cartilage between the sternebrae attached to it the costal cartilage of ribs 1-4, the manubrium single ossification center Sternebrae (S) 1-4, Manubrium (Mn), Xiphoid Process (XP).

C- Ventral view of the sternum of new born rabbit double stained shows ossification of Xiphoid Process (XP), Ensiform Cartilage (EC), Ribs (Ri) from 6-9 costal cartilage. Red arrow; additional ossification center

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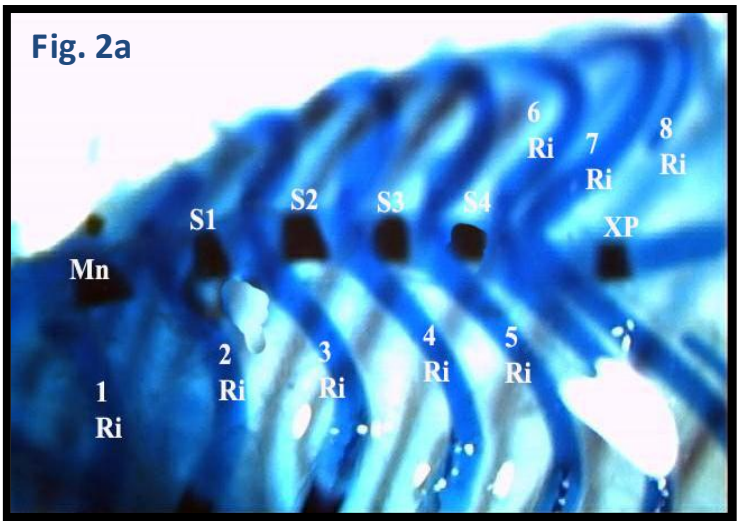

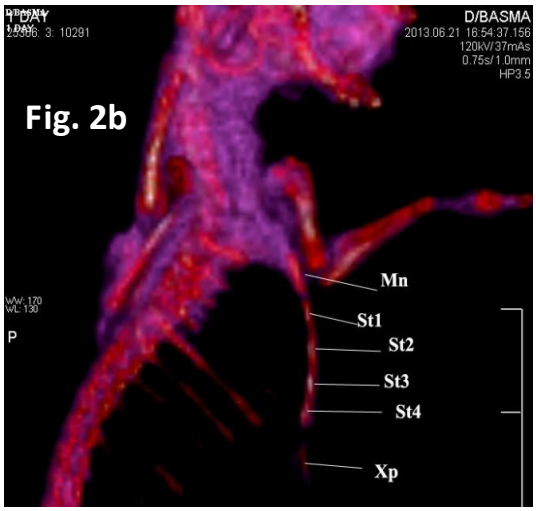

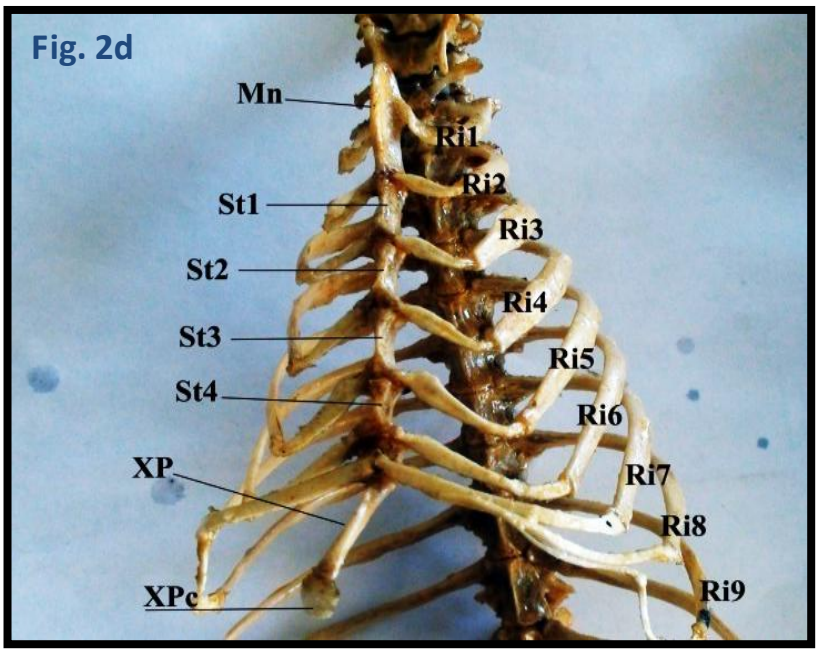

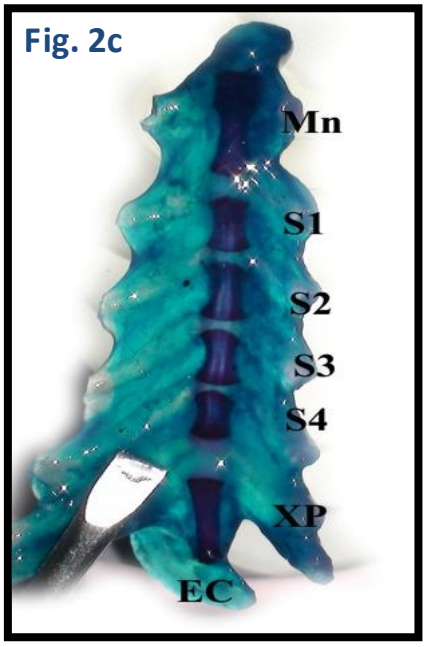

Figure 2.

A- Ventral view of the sternum of one-week old rabbit double stained shows ossification of sternebrae 2-4 attached to it Ribs (Ri) from 2-5 and in the inter-sternal cartilage between the 4th and xiphoid costal cartilage of ribs from 6-8.

B- Reconstruction of the axial skeleton lateral view of new born old rabbit showing the primary ossification center in the sternum manubrium (Mn), Sternbare1-4, (St. 1-4), and Xiphoid Process (XP).

C- Ventral view of sternum of two weeks old rabbit double stained showing ossification of Manubrium (Mn), Sternebrae (S) from 1 to 4 xiphoid Process (Xp), Ensiform Cartilage (EC).

D- Ventral view of sternum of 90-day old rabbit showing ossification of sternum parts manubrium (Mn), (St1-St4) sternebrae from 1 to 4, Xiphoid Processes (XP), Xiphoid cCrtilage (XC) attached to the sternum.

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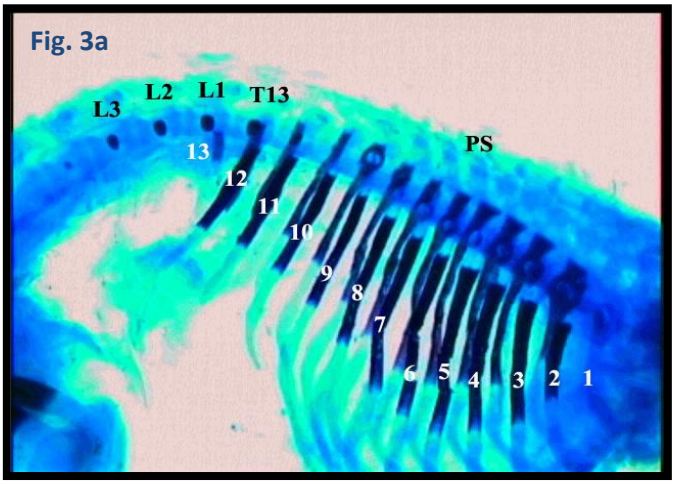


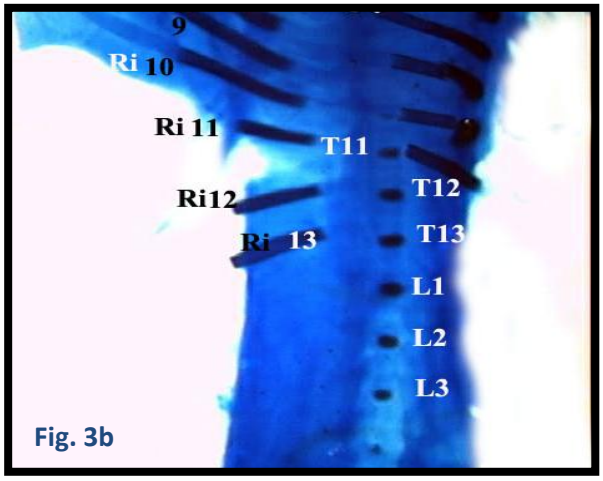
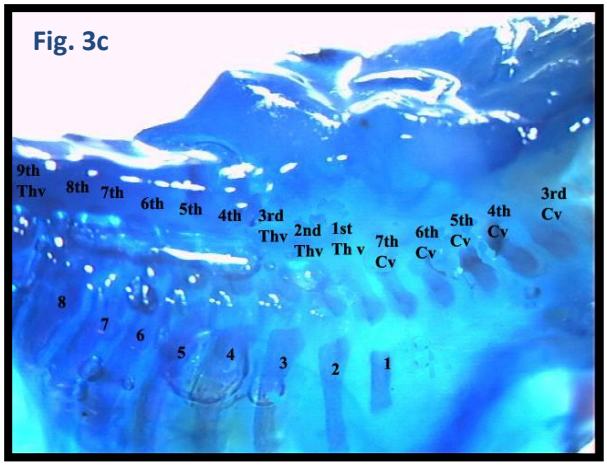


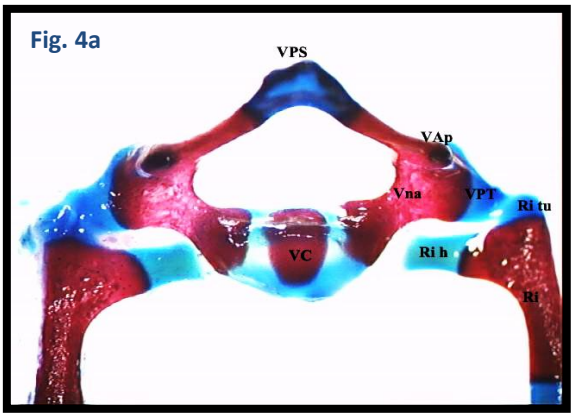
Figure 3.

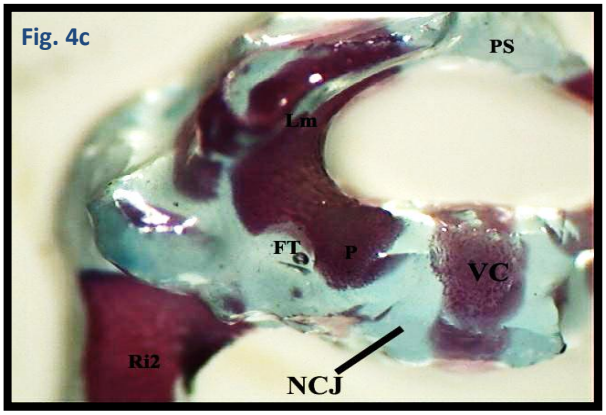
A- Lateral view of 21 days (dpc) double stained, in thoraco-lumber region of rabbit shows the ossification in the body of the ribs from (2-12) the first still cartilaginous and the last incompletely ossified, the vertebral centrum of the Thoracic (T) vertebrae 13 and first three Lumbar vertebrae, Processes Spinalis (PS) of the thoracic vertebrae are cartilaginous in nature.

B- Ventral view of 21 days (d.p.c) double stained, in thoraco-lumber region of rabbit shows the ossification in the body of the Ribs (Ri) from (9-13), the vertebral centrum of the Thoracic (T) vertebrae 11- 13 and first three lumber vertebrae.

C- Lateral view of cervico-thoracic region of 23 days (dpc) double stained of rabbit shows ossification in the body of the Ribs (Ri) from the 1-7, the cartilaginous nature of the Processes Spinalis (PS) and the ossification of the neural arch of the cervical vertebrae from the 1-7.







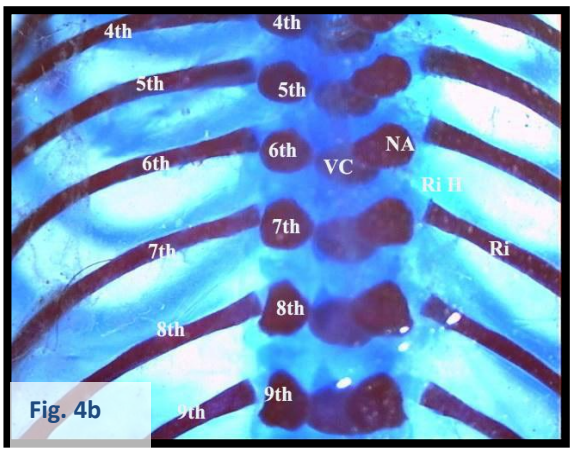


Figure 4.

A- Cranial view of typical thoracic vertebrae of 25 days (dpc) double stained of rabbit showing ossification of Vertebral Centrum (VC), Pedicle (P), Lamina (LM), Forman Transversus (FT), Processus Transversus (PT) is completely cartilaginous, Neurocentral Junction (NCJ), Processus Spinous (PS) is still cartilaginous, Rib (Ri) no.2.

B- Lateral view of typical thoracic vertebrae of 25 days (dpc) double stained of rabbit shows ossification of Vertebral Centrum (VC), Neural Arch (NA) and note that the 10th thoracic vertebrae anticlinalis (V Ac) with processus spinous is still cartilaginous.

C- Cranial view of first thoracic vertebrae of 28 days (dpc) double stained of rabbit shows ossification of Vertebral Centrum (VC), Pedicle (P), Lamina (LM), Processus Transversus (PT), Neurocentral Junction (NCJ), Processus Spinous (PS) is still cartilaginous, Rib (Ri), Rib head(Ri h), Rib tubercle (Ri tu).

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Fifteen days postnatal

An ossification center appeared in the head of the rib from the first one to the last one with cartilage collar separating the ossification center of the head from the body.

One month old rabbit

Another two ossification centers appeared in the tubercle one in the articular part and another one in the non-articular part. But in the last two ribs only single ossification center appeared as the head and tubercle was fused together.

Forty five days old rabbit

There was a marked increase in the total length of the ossified part of the ribs and appearance of ossification center at the tubercle of the rib with cartilage collar between the tubercle and the transverse processes.

Sixty days old rabbit

There was a marked decrease in the size of cartilage plate between the head and the tubercle and the body of the rib.

Ninety days old rabbit

A complete ossification occurred in the body of the ribs with bony fusion between the body, head and the tubercle (Figure 2d).

DISCUSSION

The normal development for laboratory animals, such as guinea pig (Gonzalez, 1932; Draper, 1920; Isben, 1928) and hamster (Bruce and Hindle, 1994) are depicted. In any case, there is no data accessible with respect to ordinary improvement of ossification centers in the sternum and ribs of rabbit.

The present study took after the ossification of the sternum and related ribs in the white New Zeeland rabbits prenatally and postnatally by the aide of the double recoloring procedure (Alcian blue and Alizarin red) and CT scanning.

Sternum

In human, the sternum begins to form in the lateral mesoderm plates during the sixth week of the prenatal period (Hanafi et al., 2014; Garriock et al., 2015). In orthograde (upright) Posture mammals, the sternum is typically made up of seven segments and differs from pronograde (quadrupedal) mammals in the shape of xiphoid process (Hill, 2016).

. In calf, the first focal ossification starts from the seventh sternal segment in 75 days old fetus (Lindsay, 1969). In the rabbit, the sternum begins to show up at 21 years old days old pre-birth that concurs with the consequences of Cozens (1965), who expressed that in the white New Zealand rabbit hardening begins on days 22 and 23 pre-birth. The human sternum was created from a couple of longitudinal mesenchymal buildups named sternal bars that structure in the ventrolateral body wall. The sternal bars fusion starts along the midline by the end of the 10th week of pregnancy (Hanafi et al., 2014). Fusion of the sternal bars will be finished with the formation of the xiphoid. In this study, the sternum was present as cartilaginous bar in the ventral aspect of the chest wall. Ossification of the sternal bars is an endochondral type. In human, it begins from the cranial to caudal part, producing the definitive bones of the sternum (Larsen, 1997). In this study the appearance of the ossification centers of the sternum runs in a Craniocaudal direction.

Ossification centers appear for the first two sternebrae at the age of 22 days old fetus then another ossification center appears in the third sternebrae. At the age of 25 days old fetus, an ossification centers appear in the fourth sternebrae. At the age of 28 days old fetus, the six segments of the sternum show ossification center. All previously mention data agree with the results in albino rat (Strong, 1961), in sheep (Harris, 1938) and in Sprague-Dawley rats (Alberius and Selvik, 1986) and in rabbit (Winkelmann and David, 2009).

At birth, in the human sternum, the calcified areas can only be observed in the manubrium and the mesosternum. Xiphoid process calcification appears at six years of age (O'Neal et al., 1998; Skandalakis el al., 1994; Hanafi et al., 2014). In this study, Xiphoid Processes (XP) ossification center appear before birth in rabbit. In human, three distinctive ossification models are portrayed by ossification of the manubrium and

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the body of the sternum during the postnatal period. Type one model is portrayed by one center in the manubrium and one center in the sternebrae of the body. Type two models are characterized by one center in the manubrium and in the first sternebrae of the body, two ossification centers in the other sternebrae. In the third model, the sternum has one center the manubrium and two centers in the sternebrae of the body. The type 2 ossification pattern was the most common pattern (Ashley, 956; Wong and Carter, 1988 and Hanafi et al., 2014 and Delgado et al., 2014). In rabbit the ossification pattern was similar to the type 1 ossification pattern; only two centers appeared in the fourth sternebrae of the body. At sternum of sixty days old rabbit, sternal segments two through four have undergone coalescence. At Ninety days old rabbit, complete ossification occurs in the sternum and the cartilaginous separation is replaced by bony part. In human the fusion of the manubrium and the body occurs between 6–10 years old, however as the xiphoid fusion with the body of sternum is attained between 20-25 years (Hanafi et al., 2014).

Ribs

In human, every rib, except for the last two, is ossified from four focuses. an essential focus for the body, and three epiphyseal focuses, one for the head and one each for the articular and non-articular parts of the tubercle (Louise and Sue, 2000).

In human, primary centers of ossification first appear in ribs 5-7 in the region of the posterior angle between 8-9 weeks of fetal life (Geddes, 1912; Noback and Robertson, 1951 and Ogden, 1979). Ossification centers then appear in a bidirectional manner, with the primary center for the first rib appearing before that of the last (Noback and Robertson, 1951). At the end of the third month of pregnancy, all ribs (except the last) have a single primary center of ossification (Flecker, 1932). The rib therefore commences ossification in advance of its corresponding vertebra, indicating that in terms of development it is divorced from the primitive mesenchymal vertebra, at a very early fetal age (Ogden, 1979). The epiphyses for the head and tubercle show up between the 16th and 20th years and are joined to the body during the 25 year (Gray, 1918).

In this study, there are four ossification centers for each except the last two ribs. The ossification center of the body of ribs comes from the extension of the transvers process of the thoracic vertebrae and it appears as early as two weeks of gestation. By the end of the third week of pregnancy, the ribs show primary center of ossification from the second to the last one. The second center was designed for the head and it appeared two weeks after birth. The third and fourth centers for the tubercle appeared a month after birth.

All in all, the formative pathways of the rabbit sternum and ribs were near the human model, and that could be useful in toxicological examinations as the rabbit give a decent case for the human advancement in brief time and more characteristic results.

Competing Interests

The authors declare that there are not significant personnel, professional or financial competing interest that might have influenced the presentation of the results of the study described in this manuscript.

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Effect of Zinc Supplementation on some Physiological and Growth Traits in Local Male Rabbit

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ABSTRACT

Dietary supplementation of rabbits with zinc (pure zinc) was carried out to determine its effect on some physiological, reproductive performance and growth rate of rabbit during the period from1st March, 2014 to 1st May, 2014. Eighteen locally male rabbits (10weeks age) were randomly assigned to three groups (6 rabbits per group), the control group (T1) was not supplemented with zinc (0 mg zn/kg feed) while treatment groups T2(100mg/kg zn) and T3(200 mg /kg feed) were supplemented with zinc for eight weeks. The results indicated that the T2 and T3 treatments achieved the best significant (P≤0.05) results in terms of increasing the body weight gain. While no significant differences were observed among T2, T3 and the control group regarding the WBC, RBC, weight and relative weight of testes count. Significant (P≤0.05) decreases were recorded in FCR in treated animals as compare with the control group. In conclusion, supplementation of pure zinc to the diets of local rabbit acts as an ameliorative tool of some productive traits of rabbits.

Keywords: Zinc, Rabbit, Male, Growth, Feed conversion rate

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INTRODUCTION

Zinc is an essential element in the nutrition of human beings, animals, and plants. Zinc is required in the genetic makeup of every cell and is an absolute requirement for all biologic reproduction. Zinc is needed in all DNA and RNA syntheses and is required at every step of the cell cycle (Kornberg, 1982; Rhodes and Klug, 1993 and Chrastinova et al., 2015).

Zinc is a component of various enzymes that help maintain structural integrity of proteins and regulate gene expression. Zinc is an integral component of about 200 zinc metalloenzymes include ribonucleic acid

(RNA) polymerases, alcohol dehydrogenase, carbonic anhydrase,carboxypeptidase,glutamic dehydrogenase, lactic dehydrogenase andalkaline phosphatase(King and Keen1999; McDonald et al.,2002 and Chrastinova et al., 2015), as well as hormones, such as thymulin, testosterone, prolactin, and somatomedin (Zapsalis and Beck, 1985).The biological function of zinc can be catalytic, structural or regulatory. More than 85% of total body zinc is found in skeletal muscle and bone (King and Keen, 1999).DNA is about 5000 times less susceptible to damage by Zn^{2+} ion than is RNA, suggesting its role in the predominant evolutionary selection of DNA, rather than RNA, as the bearer of the primary genetic information, as well as in cellular differentiation,synthesis and stability of DNA (Butzow and Eichhorn, 1975 and Evenson et al., 1993).Deficiencies of zinc are quite common. It is also essential in cell division and important for the production of healthy sperm. It is the most critical trace mineral for male sexual function. It is needed for testosterone metabolism, testicle growth, sperm production, motility, count, reducing excess estrogen in male reproductive tissue. Every time a man ejaculates he loses about 5 mg of zinc. Alcohol depletes zinc in the body. Folic acid, tea, coffee, high fiber intake, and iron may inhibit absorption. Vitamin B6 and C may aid absorption (Amen and Al-Daraji, 2012).

The sources of zinc are lean meat, fish, seafood, chicken, eggs, pumpkin and sunflower seeds, rye, oats, whole grains, legumes, ginger, parsley, mushrooms, brewer's yeast, and wheat germ (Amen and Al-Daraji, 2011).

About 2 grams of zinc is distributed throughout the body (average 10 to 200 mg/gm) of an adult human being(Zapsalis and Beck, 1985).Absorption of dietary zinc occurs over the duodenal and jejunal regions of the gastrointestinal tract. Active transport of zinc into portal blood is mediated by metallothionein. Zinc competes with other metals for absorption, and absorption is believed greatly retarded by ingestion of fiber and phytates(Oberleas and Harland, 1977;Zapsalis and Beck, 1985).

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Plasma zinc is compound to organic forming a complex. Zinc-albumin complexes account for about 50 percent of the zinc and the metal is readily exchangeable throughout the peripheral circulation. About 7 to 8 percent is loosely bound to amino acid constituents in plasma. The remaining 42 percentage of plasma zinc is largely bound to macroglobulins and unavailable for nutritional purposes. Serum and plasma zinc concentrations in adults range from 80 to 150 mg/dL (Zapsalis and Beck, 1985).

Zinc deficiency symptoms are nonspecific, perhaps in part because of their need in so many enzymes and their critical roles in both protein synthesis and molecular genetics. Many enzymes may become nonfunctional in the absence of zinc, even though the presence of the enzyme remains undisturbed. The integrity of cell membranes, including the integrity of red and white blood cells, depends upon loosely bound ionic zinc. Zinc deficiency stunts growth and causes serious metabolic disturbances. Inadequate intake in people and animals results in serious immunodeficiency, increased numbers of infections, increased severity of infections, stunted growth, skin lesions and delayed sexual maturation (Zapsalis and Beck, 1985). So this study was carried outto investigate the effect of dietary zinc (Pure zinc) of local male rabbit onsome Physiological traits, productive andreproductive performance.

MATRIALS AND METHODS

Experimental animals, diets and studied traits

A total of 18 local male rabbits (10 weeks age) were used in this study. They were divided randomly into 3 equal group (six rabbits per group) in a completely randomized designwere randomly allotted to three dietary zincT1=Control without zinc supplementation,T2=100ppm zinc/kg feed and T3=200ppm/kg feed. Theirweights wereranged between 965-1015gm,where has been sheltering in the housewith temperature21-24 C$^{\circ}$ and light 14hours per day,water and feed were offered *ad-libitum*. Commercial feed were providedwhich contained 15%crude protein and 3.5% crude fat,fiber23%, Nitrogen Free Extract (NFE)43% and ash 11%throughout the research(NRC,1994).Using a special room for rabbits outside the Kalar technical institute. The animals were individually housed in cleaned and sterilized metal cages prepared for this purpose,raised 60 cm above the floor, measuring 60×30×30 cm and provided with wire screened floor which permit or allow feces and urine to drop. Each animal was provided with water canal and feeder and left the rabbits for two weeks for the purpose of adjusting to environmental and experimental conditionsto stabilize them and they were later treated with dietary zinc for eight weeks. The experimental diets were offered ad-libitum. A record of feed intake and body weight changes were kept weeklyas well as Feed Conversion Ratio (FCR)was calculated as(FCR equal Feed intake / Body weight gain). At end of the experiment the animals were killed humanlyand rules of animal welfare were applied for humane handling of experimental animals throughout the study.The following parameters were measured:

Body weight gain equaldifferences in the body weight at the beginning and at the end of the experiment were determined.

Testes weight (Paired):Both testes (left and right testes) wereweighed.

Relative testes weightequalpaired testes weight divided by body weight and multiply by 100.

Hematological Parameters

Three individual blood samples were collected from each replicate for each analysis in a test tube with EDTA from the jugular vein of the rabbit, to determine the RBCs, and WBC count at four different time periods (twice a month) ofanimal age and throughout the study. Counting was done with haemocytometer chamber and according to Al-Daraji et al. (2008).

Ethical approval

The ethics regarding the handling and euthanizing of the animals have been approved by scientific committee of animal's health department and Kalar technical institute at Sulaimani Polytechnic University which have been performed according to animal welfare principles.

Statistics Analysis

Data generated from experiment was carried out in a complete randomized design (Steel and Torrie, 1980). These data were subjected to ANOVA according to general linear model procedure of statistical analysis system software (SAS, 2001**).** The significant differences among means were determined by Duncan's multiple range tests (1955) with ($P \leq 0.05$) level of significance.

RESULTS AND DISCUSSION

Some performance traits (body weight gain, feed intake and FCR) of rabbit at different levels of dietary zinc as a mean value was presented in table 1,body weight gain and final body weight of rabbits treated with dietary zinc(T2 and

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T3) were significantly (P≤0.05) increased in compared with thecontrolgroup (T1).While, there was no significant difference between T2 andT3regarding thesetraits.As well as, no significant differences were observed among T1, T2 and T3 in feed intake.Significantly (P≤0.05) decreased in FCR were observed of treated animals as compared with control. Results revealed thatno significant differences were observed among T1, T2 and T3 regarding the traits WBC and RBC in table 2 and testes and relative testes weight in table 3.

Table 1. Effect of different levels of dietary zinc on some productive traits of local male rabbits (Mean ± Se)

Traits	Treatments		
	T1(Control)	**T2(0.1 gm zinc /kg feed)**	**T3(0.2 gm zinc /kg feed)**
Initial body weight (gm)	1005	990	1015
Body weight gain (gm)	722.67±12.01[b]	916.33±5.03[a]	1067.67±14.47[a]
Total feed intake (gm/week/rabbit)	4186.33±52.82[a]	4105.02±15.01[a]	4291.65±27.54[a]
Final body weight (gm) (20weeks)	1718±36.18[b]	1915±37.75[a]	2091 ±45.37[a]
Feed Conversion Ratio (FCR)	5.79±0.02[a]	4.49±0.04[b]	4.03±0.03[b]

[ab] means along rows with different superscripts are significantly different (P<0.01)

Table2. Effect of different levels of dietary zinc on the mean of some hematological traits of local male rabbits at different periods (Mean ± Se)

Hematological traits	Periods (each2week)	Treatments		
		T1(Control)	**T2(0.1 g zinc /kg feed)**	**T3(0.2 g zinc /kg feed)**
RBC *(*10^6/mm^3)	1	6.35±0.39a	6.81±0.59a	6.72 ±0.20a
	2	6.36±0.34a	6.92±0.49 a	6.73±0.30a
	3	6.05±0.40a	6.83±0.67a	6.94±0.29a
	4	6.10±0.36[a]	6.93±0.47[a]	7.09±0.56[a]
WBC (x10^3/mm^3)	1	7.72±0.42[a]	8.03±0.90[a]	7.62±0.83[a]
	2	6.45±0.66[a]	7.32±0.31[a]	7.46 ±0.75[a]
	3	7.73. 40±0.83a	8.20±1.46[a]	8.17 ±1.84[a]
	4	7.70±0.71[a]	8.54±0.99[a]	8.62±0.94[a]

[ab] means along rows with different superscripts are significantly different (P<0.01)

Table 3. Effect of different levels of dietary zinc on the mean weight and relative weight of testes in local male rabbits (Mean ± Se)

Traits	Treatments		
	T1(Control)	**T2(0.1 g zinc /kg feed)**	**T3(0.2 g zinc /kg feed)**
Body weight (gm)	1718	1915	2091
Testes weight gm (paired)	3.90±0.13[a]	4.10±0.19[a]	4.32±0.3[a]
Relative testes weight (%)	0.23±0.17[a]	0.21±0.015[a]	0.20 ±0.005[a]

[ab] means along rows with different superscripts are significantly different (P<0.01)

The positive results obtained in the production traits may be due to zinc has role in insulin secretion by protamine zinc–insulin and globulin zinc- insulin contain Zn++ for their functioning also zinc involved in storage and secretion of insulin as well as zinc has role in growth and reproduction, zinc can improve intestinal absorption (Chatterjea, 2009).Zinc participates actively in protein synthesis and carbohydrate metabolism. The discovery that the enzyme carbonic anhydrase contains 0.33 % of zinc in its molecule is considered the first acceptable explanation of Zn mechanism of action. After that, DNA-polymerase, being fundamental in cell division process (Chrastinova et al., 2015).Large quantities of zinc were found to provide stability to the structures of RNA, DNA and ribosomes (Prask and Plocke, 1971; Dowel, 1992 and Chrastinova et al., 2015).Perhaps with the intervention of trace elements such as zinc as dietary supplement, we may improve animal protein production by regulating growth rate inrabbit.These results were in agreement with Abd El-Rahim et al,,1995; Nessrinet al., 2012; Ayyat and Maria,2000) who observed that dietary zinc levels did significantly affect (P≤0.05) body weight and Feed Conversion Ratio (FCR) when adding Zn by levels 50,100 or 200mg /kg diet. While incompatibility with Al-Khalifa(2006) who reported that supplemental dietary Zn by levels of

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50,100 or 200ppm had no significant effect on live weight gain and FCR. On the other hand, the obtained data suggests that live weight gain and FCR of growing rabbits could be improved by 12.3and 10.6 % respectively, with supplementing the diet with 100 Zn mg /kg over the content provided by the control diet (Nessrinet al., 2012).On the other hand, Chrastinova et al. (2016) concluded that growing rabbit is acceptable to excessive dietary doses of the macroelements or Zinc. Also, a supplemental Zinc in the rate of 100 mg/kg diet leads to improving live body weight gain and significantly improves feed conversion ratio of the rabbit,as well asfeeding of rabbits with inorganic or organic zinc sources had no negative effect on the rabbit growth performance.Zinc plays a crucial role in metabolism as an essential trace element with antioxidant properties (Hao and Maret, 2005). A previous study suggested that Zn acts as an intracellular signaling molecule, plays a role in communicating between cells by converting extracellular stimuli to intracellular signals, and controls intracellular events. Abnormal Zn homeostasis can lead to a variety of health problems including growth retardation, hypogonadism, immunodeficiency, and neuronal and sensory dysfunctions (Fukadaet al., 2011). For many years, zinc has been shown to play an important role in central nervous system as a neurosecretory factor and as a biofactor, is responsible for activation of numerous enzymes engaged in the metabolic processes and simultaneously in brain development from the early neonatal stage to the maintenance of brain function in adults (Frederickson and Moncrieff, 1994; Frederickson et al., 2000).

Competing interests

Authors have declared that there is no competing interest.

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Identification of *Brucella* spp. and Assessing Impact of Brucellosis Control Programme on Ruminants and Human in Gharbia Governorate, Egypt

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ABSTRACT

The aim of the present study was to assess the temporal impact of brucellosis control programme on ruminants and human and to identify *Brucella* spp. in Gharbia governorate, Egypt. Data for brucellosis in ruminants were collected from the active surveillance programme for brucellosis. Blood and tissue (lymph nodes and spleen) samples from positive animals were also collected. Data for human cases were obtained from the Ministry of Health, Gharbia governorate, Egypt. Statistical analyses were conducted to allow the comparison between different years and ruminant species. To compare between seropositive proportions for different years for each species, a univariate binary logistic regression model was used. There was no consistency in sampling and testing of animals and less than 3% were tested in any given year and the highest proportion of animals tested were cattle. There were variations in seropositive proportions in different species of tested animals and between districts. The number of reported cases of brucellosis in humans was increasing and there was a positive association with that in ruminants. About 36% and 50% of lymph nodes and spleen samples were culture positive, respectively. All isolated strains were identified as *B. melitensis biovar 3*. Brucellosis is an endemic disease in the study area and the current control programme (test and slaughter) doesn't seem to be effective. Further studies are required for assessing the social and economic impacts of brucellosis. This study indicated that the impact of the current control programme of brucellosis in an endemic area of Egypt. The outcomes of this study would help policy makers to rethink about the control of brucellosis and look for alternative strategies.

Keywords: Brucellosis, Ruminants, Human, Nile Delta, Egypt

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INTRODUCTION

Brucellosis is one of the most common zoonotic diseases worldwide. The disease is endemic and a major cause of economic losses particularly in developing countries (McDermott et al., 2013; Refai, 2002; Pappas and Memish, 2007). This may be due to the lack of resources, lack of compliance to control programmes and the attitudes of livestock owners (Hegazy et al., 2009; Holt et al., 2011 and Eltholth et al., 2015). Since brucellosis was reported in Egypt in 1939, the disease has been endemic in the country with a high prevalence. The national control programme for brucellosis in ruminants since 1981involves testing all females older than six months and slaughtering the serologically positive ones, with voluntary vaccination of calves using *Brucella abortus S19* vaccine also lambs and kids by *Rev1* vaccine (Hassanain and Ahmed, 2012;Refai, 2002). Evaluation of the impact of the disease control in El-Beheira governorate, Egypt, using data from the active surveillance programme between 1990 and 2012 indicated that brucellosis was endemic and there was no significant reduction of the seropositive animals (Eltholth et al., 2015). Further analysis of the impact of the control programme of brucellosis on the seroprevalence in ruminants all over the country between 1999 and 2011 indicated that the disease is endemic without significant reduction the seropositive proportion of ruminants (Eltholth et al., 2016).

Although data for the prevalence of brucellosis in Egypt are scarce, the following studies suggested the disease is endemic in all ruminant species, with a high prevalence. In a study where samples were collected from 126 herds from all over the country, 26.66%, 18.88% and 17.22% of sheep flocks, goat flocks and cattle herds were seropositive for *Brucella spp*. respectively (Kaoud et al., 2010). In some regional studies such as in Monufia governorate, 5.36%, 3.33%

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and 3.17% of cattle, buffaloes and goats were seropositive for *Brucella spp*. by Rivanol test and 7.14%, 4.26%, 2.47% and 6.35% of cattle, buffaloes, sheep and goats were seropositive by the buffered acidified plate antigen, respectively (Samaha et al., 2008). In the same governorate, the individual and household seroprevalence of *Brucella spp.* in cattle and buffaloes was 11.0% and 15.5%, respectively (Holt et al., 2011). In Kafrelsheikh governorate, the seroprevalence in cattle, buffaloes, sheep and goats was at 12.2%, 12%, 12.2% and 11.3%, respectively (Hegazy et al., 2011). Recently, the seroprevalence of *Brucella* was reported to be18.09% in blood samples from sheep and goat flocks from five governorates in the Nile Delta (Mahboub et al., 2013).Samples from private dairy cattle farms in Dakahlia, Damietta and Port-Said governorates, indicated that the proportion of seropositive animals was 52.2% and 4.2% in cattle with reproductive failures and apparently healthy cattle, respectively (Gwida, 2015).

However, results from the previous study were not reliable to estimate the seroprevalence of brucellosis in the study area because of the lack of study design and targeting cattle with a history of reproductive failure. The most recent study in Kafrelsheikh governorate indicated that, the true seroprevalence of brucellosis in sheep was 20 % (95 % CI 15.3–24.7) with the village prevalence, having at least one seropositive sheep, of 95.5 % (95 % CI 92.2–100) and the village flock seroprevalence ranged from 0 to 46.8 % (Hegazy et al., 2016).These studies indicated that, brucellosis is endemic in Egypt with a high prevalence. The same situation in humans, there were no accurate data for the actual prevalence and/or incidence of brucellosis. Therefore it is important to assess the impacts of the current brucellosis control programme on the disease prevalence. The aim of this study was to assess the temporal impacts of brucellosis control programme on animals and human brucellosis in one of the central governorates of the Nile Delta of Egypt, Gharbia governorate. It was also important to identify *Brucella* spp. circulating in livestock. Therefore, blood and tissue samples from brucellosis positive animals were analysed.

MATERIALS AND METHODS

Study area

This study was conducted in Gharbia governorate located in the middle of the Nile Delta, between Damietta and Rosetta branches. In the north, it is bordered by KafrElshiekh, in the south by Monufia, in the east by Dakahlia, and in the west by El-Beheira governorate, figure 1. According to the 2014 censuses, it is one of the highly populated areas in Egypt with human population of 4,648,408 and a density of 2400/Km^2, Egypt State Information Service (ESIS). This governorate is also characterised by agricultural activities and a high density of animal population. About 60% of the inhabitants live in rural community in close contact with their livestock. According to the Ministry of Agriculture and Land Reclamation (MALR, 2010), the total number of cattle, buffalo, sheep and goat was 233,007, 245,408, 200,086 and 116,604, respectively. The number of animals for the subsequent years was estimated by using a growth rate of 1.1%. The proportion of animals supposed to be tested (aged more than 6 months) according to the brucellosis control programme in Egypt was estimated as 0.7 of the total number of animals.

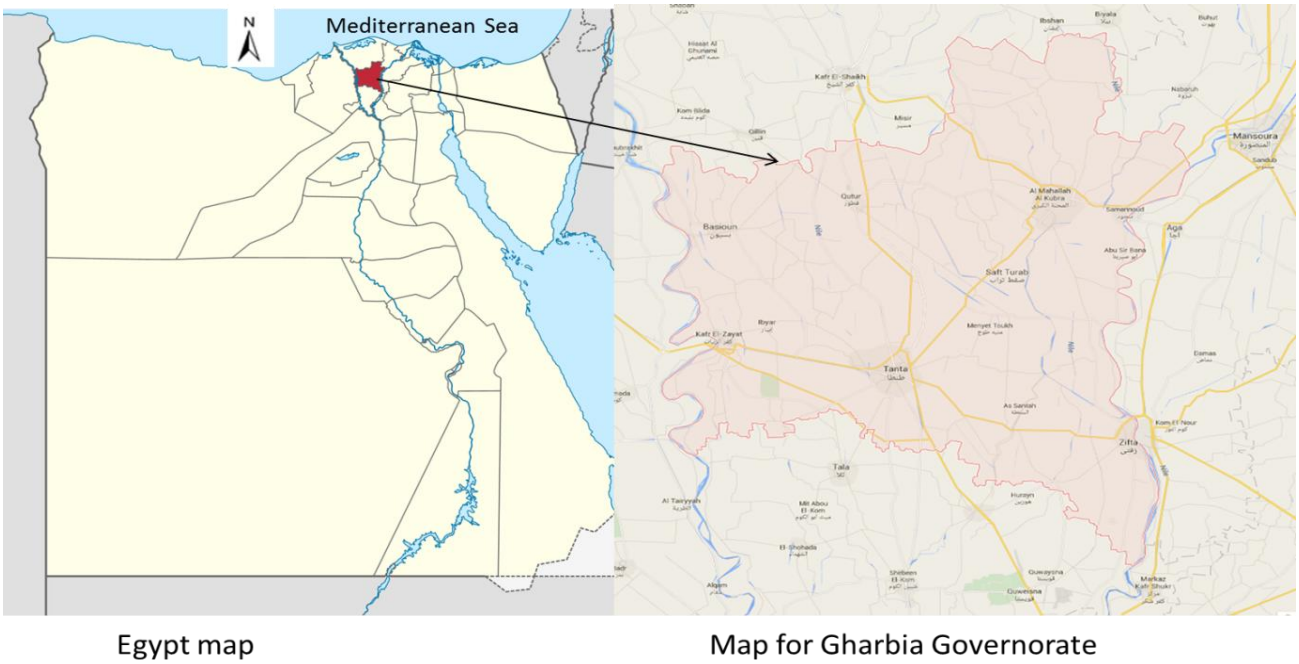

Figure 1. Study site for brucellosis in Gharbia governorate, Egypt

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Ethical approval

Handling of animals were according to the guidelines of Animal ethics committee, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt

Data for brucellosis in ruminants

The data for this study was collected from the active surveillance programme for brucellosis control conducted by the General Organisation of Veterinary Services (GOVS), Egypt. The available data were for years 2010 to 2015 for the annual number of animals tested and number positive for cattle, buffalo, sheep and goat. Detailed data per district were available only for the years 2010 to 2011.

Biological samples

Blood and tissue samples were collected from seropositive animals for brucellosis. These samples were used for Polymerase Chain Reaction (PCR) and bacteriological examination to identify *Brucella* spp. in Gharbia governorate. Blood samples (5ml) were collected from the jugular vein of the examined animals through a sterile dry needle into a sterile heparinized vacationer tube and stored at-80^{o}C until analyzed. Tissue samples (61 supramammary lymph nodes and 10 spleens) from different animal species were collected (Table 1). Tissue samples were collected and kept in sterile bags directly after slaughtering and stored at -20ºC until cultured.

Table 1. Bacteriological examination for presence of *Brucella* spp. in tissue samples of livestock in Gharbia governorate, Egypt.

Species	Lymph nodes	Spleen	Total
Cattle	23	4.0	27
Buffalo	15	2.0	17
Sheep	12	2.0	14
Goat	11	2.0	13
Total	61	10	71

Bacteriological examination and Polymerase Chain Reaction (PCR)

Isolation, identification and biotyping of *Brucella*spp. were carried out according to the recommendation of the FAO/WHO, expert committee on brucellosis(Alton et al., 1988). DNA was extracted from blood samples using blood DNA preparation kit(Jena Bioscience Cat. No. PP-205S) following the instruction of the manufacturer. DNA amplification was done by two different PCR sets of primers. Oligonucleotide primers specific for *B.abortus* were used to amplify the insertion sequences IS711(Bricker and Halling, 1994).Oligonucleotide primers P1 and P2 specific for *B. melitensis* were designed from *Brucella*omp 2 gene (Bardenstein et al., 2002). The sequences of the primers were listed in table 2. DNA amplification: the PCR 25 µL reaction volume containing 5 µLofTaq master ready–to use mixes for PCR (Jena Bioscience, Cat. No.1025), 10 PM of each oligonucleotides primer, 5 µL of DNA template and fill up to25 µL with DNAse-RNAse free water. For P1 and P2 primers, PCR was performed as follows: 35 cycles of PCR with 1 cycle consisting of 20s at 95ºC for DNA denaturation, 1 min at 50ºC for primer annealing and 1 min at 72ºC for polymerase mediated primer extension. The last cycle included incubation of the sample at 72ºC for 7 min. Samples were considered positive when there was a single band of DNA at 282 bp. For IS711 and *B. abortus* primers, after an initial denaturation at 93ºC for 5 min, the PCR profile was set as follows: template denaturation at 95ºC for 1.25 min, primer annealing at 55.5ºC for 2 min and primer extension at 72ºC for 2 min, for a total of 35 cycles, with a final extension at 72ºC. Samples were considered positive when a single band of DNA at 498 bp. All PCR were performed in a DNA thermocycler (Perken Elmer model 9600).

Table 2. Sequences of oligonucleotide primers used for polymerase chain reaction

Primers name	Sequences	Amplified product	References
P1	5`TGGAGGTCAGAAATGAAC3	282 bp	(Bardenstein et al., 2002)
P2	5` GAGTGCGAAACGAGCGC3`		
B. abortus	5'- GAC GAA CGG AAT TTT TCC AAT CCC	498 bp	
IS711	5'- TGCCGA TCA CTT AAG GGC CTT CAT		

Data for human brucellosis

Data for human cases were obtained from the EgyptianMinistry of Health, Gharbia directorate for the years2014, 2015 and the first quarter of 2013. The only available data were the number of confirmed human cases.

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Statistical analysis

Data were stored in a Microsoft Office Excel 2007. Frequency tables were used to calculate the proportions of tested and the proportions of seropositive animals for *Brucella* spp. per year for each ruminant species. Statistical analyses were conducted to allow comparison between different years and ruminant species using IBM SPSS Statistics for Windows (Version 20.0. Armonk, NY: IBM Corp). To compare between seropositive proportions for different years for each species, a univariate binary logistic regression model, with seropositive as the response variable and year 2010 was used as the reference.

RESULTS

The results (Table 3) indicated that there was no consistency in sampling and testing of animals. The highest proportion of animals tested was cattle, from about 3% to about 6% of the number of cattle supposed to be tested. The proportion of buffalo and sheep tested ranged from about 1.5% to about 2%. The lowest proportion of ruminant tested was goat about 1%. The proportion of seropositive animals per districts in 2010 and 2011 are summarised in table 4 and 5, respectively. The results showed that there were variations in seropositive proportions in different species of ruminates and between districts. In 2010, the highest proportion of seropositive cattle (1.69%) was in El-Mahalla El-Kubra and the highest proportion of seropositive buffalo (2.43%) and sheep (5.5%) was in Tanta (Figure 2).

In 2011, the highest proportion of seropositive cattle (0.72%), sheep (1.08%) and goat (33.33%) was in Tanta, while the highest proportion of seropositive buffalo (1.2%) was in Kafr El-Zayat (Figure 3). Results for the seropositive proportion of ruminants along the study period are summarised in figure 4. It showed that in 2011 and 2014 the highest proportion of seropositive animals was observed among goat. Results of regression analysis are summarised in table6. In cattle, there was a significant decrease in the proportion of seropositive animals in 2011 and 2012 followed by an increase in 2013 to 2015. The proportion of seropositive buffalo was fluctuating, there was a significant decrease in 2011 than an increase in 2012 and 2013 followed by a decrease in 2014 and 2015. Among sheep, there was a general decrease in the seropositive proportion. For goat, there were no positive cases among those tested in 2010 and 2013. In 2014, the proportion of seropositive goat was higher than in any other species along the study period. According to the health records in Gharbia Directorate, Ministry of Health, there were 54,217 and 342 reported human cases of brucellosis in the last quarter of 2013, 2014 and 2015, respectively. The distribution of cases per district (Figure 5) indicated that, the highest number was recorded in El-Mahalla El-Kubra district particularly in 2015. These figures indicated that the number of reported cases of brucellosis in humans was increasing (Figure 6). Also the number of reported cases in humans was increasing with that in ruminants (Figure 7). The monthly distribution of reported human cases (Figure 8) indicated that, the highest number of cases was in June and March for 2014 and 2015, respectively. On the other hand the highest total number of reported cases per month in ruminants within the study period was in May followed by February (Figure 9). Tissue samples from seropositive animals, 61 lymph nodes and 10 spleens were cultured for bacteriological analysis. The results (Table 7) showed that 36.07% and 50% of lymph nodes and spleen samples were positive, respectively. About 40% of samples from cattle, buffalo and sheep were positive while 30% of samples from goat were positive. All isolated strains were identified biochemically as *B.melitensis* biovar 3. All examined blood samples of infected animals reacted positively with DNA products with a molecular size of 282 pb, indicative of *B.melitensis* DNA as shown in figure10.

Table 3. Livestock census and proportion of animals tested for brucellosis control programme in Gharbia governorate, Egypt during 2010-2015

Species / Year	Cattle			Buffalo			Sheep			Goat			Ruminants		
	Total, n	Tested, n	Tested, %	Total, n	Tested, n	Tested, %	Total, n	Tested, n	Tested, %	Total, n	Tested, n	Tested, %	Total, n	Tested, n	Tested, %
2010	163105	8236	5.05	171786	2795	1.63	140060	2300	1.64	81623	535	0.66	556574	13866	2.49
2011	171950	8867	5.16	181102	3615	2.00	147656	1561	1.06	86049	470	0.55	586758	14513	2.47
2012	181276	4637	2.56	190923	2822	1.48	155664	3373	2.17	90716	868	0.96	618578	11700	1.89
2013	191106	7866	4.12	201277	2943	1.46	164106	968	0.59	95636	250	0.26	652125	12027	1.84
2014	201470	11589	5.75	212193	4320	2.04	173005	1991	1.15	100822	304	0.30	687491	18204	2.65
2015	212397	12386	5.83	223701	3799	1.70	182388	1980	1.09	106289	1115	1.05	724775	19280	2.66

N=number of animals tested/positive, %=percentage of animals tested/positive

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Table 4. Proportion of seropositive ruminates for brucellosis in Gharbia Governorate, Egypt, per district in 2010

Species / District	Cattle			Buffalo			Sheep			Goat			Total		
	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %
Tanta	1689	17	1.01	206	5.0	2.43	73	4.0	5.5	120	0.0	0.0	2088	26	1.25
Zifta	361	0.0	0.00	630	1.0	0.16	1.0	0.0	0.0	2.0	0.0	0.0	994	1.0	0.10
El-Mahalla El-Kubra	826	14	1.69	362	3.0	0.83	1218	18	1.5	2.0	0.0	0.0	2408	35	1.45
Kotoor	1263	0.0	0.00	253	0.0	0.00	120	0.0	0.0	0.0	0.0	0.0	1636	0.0	0.00
Kafr El-Zayat	1446	3.0	0.21	124	1.0	0.81	0	0.0	0.0	0.0	0.0	0.0	1570	4.0	0.25
El-Santa	1007	10	0.99	633	2.0	0.32	644	1.0	0.2	217	0.0	0.0	2501	13	0.52
Bassyoun	1335	3.0	0.22	84	1.0	1.19	194	4.0	2.1	78	0.0	0.0	1691	8.0	0.47
Samannoud	309	0.0	0.00	503	0.0	0.00	50	0.0	0.0	116	0.0	0.0	978	0.0	0.00
Total, n (%)	8236	47	0.57	2795	13	0.47	2300	27	1.2	535	0.0	0.0	13866	87	0.63

N=number of animals tested/positive, %=percentage of animals tested/positive

Table 5. Proportion of seropositive ruminates for brucellosis in Gharbia Governorate, Egypt, per district in 2011

Species / District	Cattle			Buffalo			Sheep			Goat			Total		
	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %	Tested, n	Positive, n	Positive, %
Tanta	1253	9.0	0.72	216	1.0	0.46	185	2.0	1.08	12	4.0	33.33	1666	16	0.96
Zifta	572	2.0	0.35	949	0.0	0.00	89	0.0	0.00	11	0.0	0.00	1621	2.0	0.12
El-Mahalla El-Kubra	768	0.0	0.00	447	0.0	0.00	125	0.0	0.00	0.0	0.0	0.00	1340	0.0	0.00
Kotoor	1486	0.0	0.00	276	0.0	0.00	98	0.0	0.00	0.0	0.0	0.00	1860	0.0	0.00
Kafr El-Zayat	2363	1.0	0.04	83	1.0	1.20	143	0.0	0.00	0.0	0.0	0.00	2589	2.0	0.08
El-Santa	1078	4.0	0.37	856	2.0	0.23	523	0.0	0.00	249	2.0	0.80	2706	8.0	0.30
Bassyoun	1010	1.0	0.10	164	0.0	0.00	228	0.0	0.00	198	2.0	1.01	1600	3.0	0.19
Samannoud	337	0.0	0.00	624	0.0	0.00	170	0.0	0.00	0.0	0.0	0.00	1131	0.0	0.00
Total, n (%)	8867	17	1.58	3615	4.0	1.90	1561	2.0	0.13	470	8.0	1.70	14513	31	0.21

N=number of animals tested/positive, %=percentage of animals tested/positive

Table 6. Proportion of seropositive ruminates for brucellosis in Gharbia Governorate, Egypt from 2010 to 2015

Animal spp.	Year	Tested animals	Seropositive (%)	OR	95% CI	P value
Cattle	2010	8236	0.57	Ref	-	-
	2011	8867	0.19	0.34	0.335-0.192	0.000
	2012	4637	0.13	0.23	0.096-0.528	0.001
	2013	7866	0.42	0.73	0.470-1.147	0.174
	2014	11589	0.53	0.94	0.641-1.371	0.738
	2015	12386	0.68	1.19	0.832-1.702	0.342
Buffalo	2010	2795	0.47	Ref	-	-
	2011	3615	0.11	0.24	0.077-0.728	0.012
	2012	2822	0.18	0.38	0.135-1.067	0.066
	2013	2943	0.34	0.73	0.319-1.667	0.454
	2014	4320	0.16	0.35	0.138-0.872	0.024
	2015	3799	0.21	0.45	0.187-1.091	0.077
Sheep	2010	2300	1.17	Ref	-	-
	2011	1561	0.13	0.11	0.026-0.455	0.002
	2012	3373	0.71	0.60	0.347-1.048	0.073
	2013	968	0.31	0.26	0.079-0.865	0.028
	2014	1991	0.50	0.42	0.205-0.880	0.021
	2015	1980	0.45	0.38	0.180-.819	0.013
Goat	2010	535	0.00	-	-	-
	2011	470	1.70	Ref	-	-
	2012	868	0.12	0.07	0.008-0.534	0.011
	2013	250	0.00	-	-	-
	2014	304	3.29	1.96	0.766-0.5.034	0.16
	2015	1115	0.54	0.31	0.108-0.905	0.032
All ruminant	2010	13866	0.63	Ref	-	-
	2011	14513	0.21	0.34	0.225-0.511	0.000
	2012	11700	0.31	0.49	0.331-0.721	0.000
	2013	12027	0.38	0.61	0.425-.870	0.006
	2014	18204	0.49	0.78	0.579-1.047	0.097
	2015	19280	0.55	0.88	0.665-1.174	0.394

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Table 7. Results of bacteriological analysis fortissue samples from serologically positive animals for brucellosis

Species \ Parts	Lymph nodes		Spleen		Total	
	Samples, n	+ve, n (%)	Samples, n	+ve, n (%)	Samples, n	+ve, n (%)
Cattle	23	9.0 (39.13)	4.0	2.0 (50)	27	11 (40.74)
Buffalo	15	6.0 (40)	2.0	1.0 (50)	17	7.0 (41.18)
Sheep	12	4.0 (33.33)	2.0	1.0 (50)	14	5.0 (41.67)
Goat	11	3.0 (27.27)	2.0	1.0 (50)	13	4.0 (30.77)
Total, n (%)	61	22 (36.07)	10	5.0 (50)	71	27 (38.03)

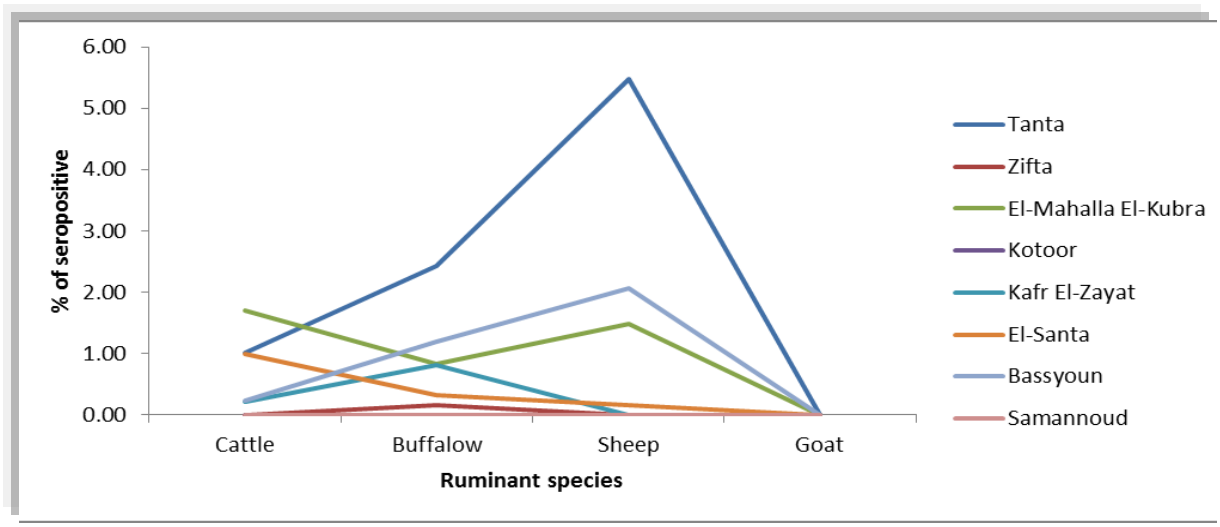

Figure 2. Distribution of seropositive ruminants per district in 2010 in Gharbia Governorate, Egypt

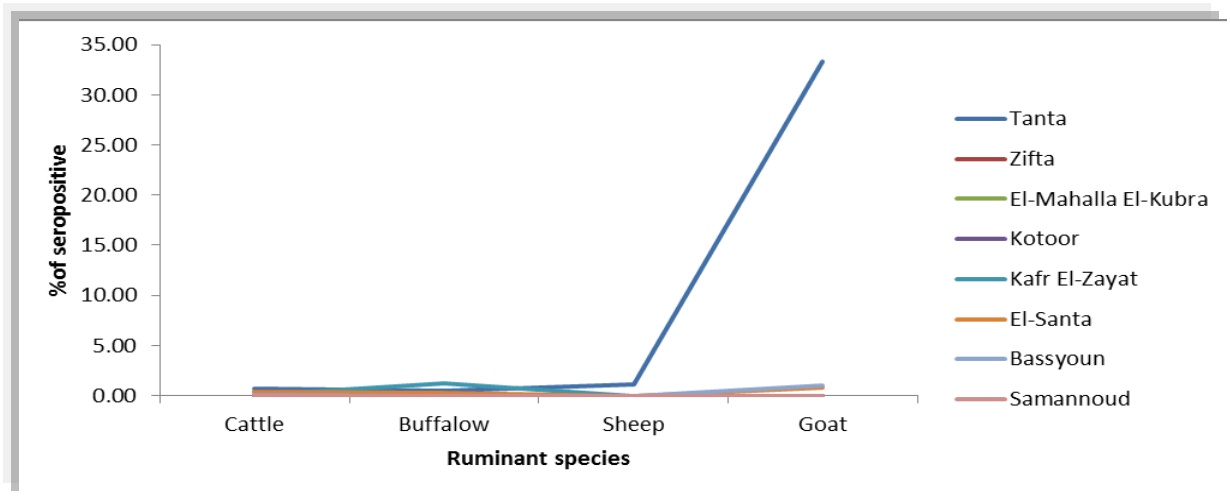

Figure 3. Distribution of seropositive ruminants per district in 2011 in Gharbia Governorate, Egypt

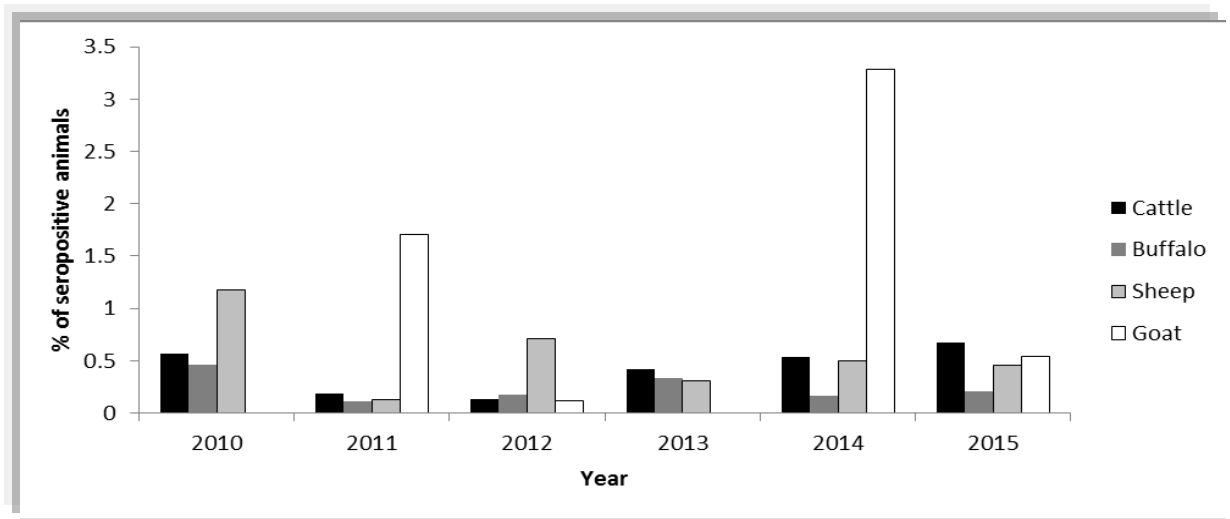

Figure 4. Seropositive ruminates for brucellosis in Gharbia Governorate, Egypt from 2010 to 2015

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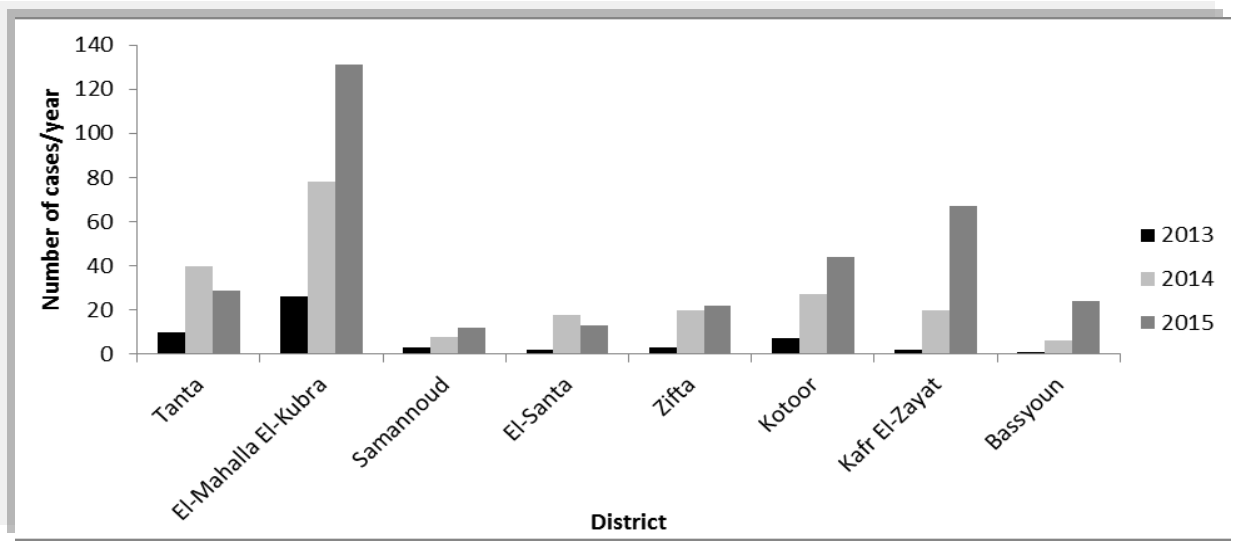

Figure 5. Reported human cases of brucellosis per district in Gharbia Governorate, Egypt from 2010 to 2015

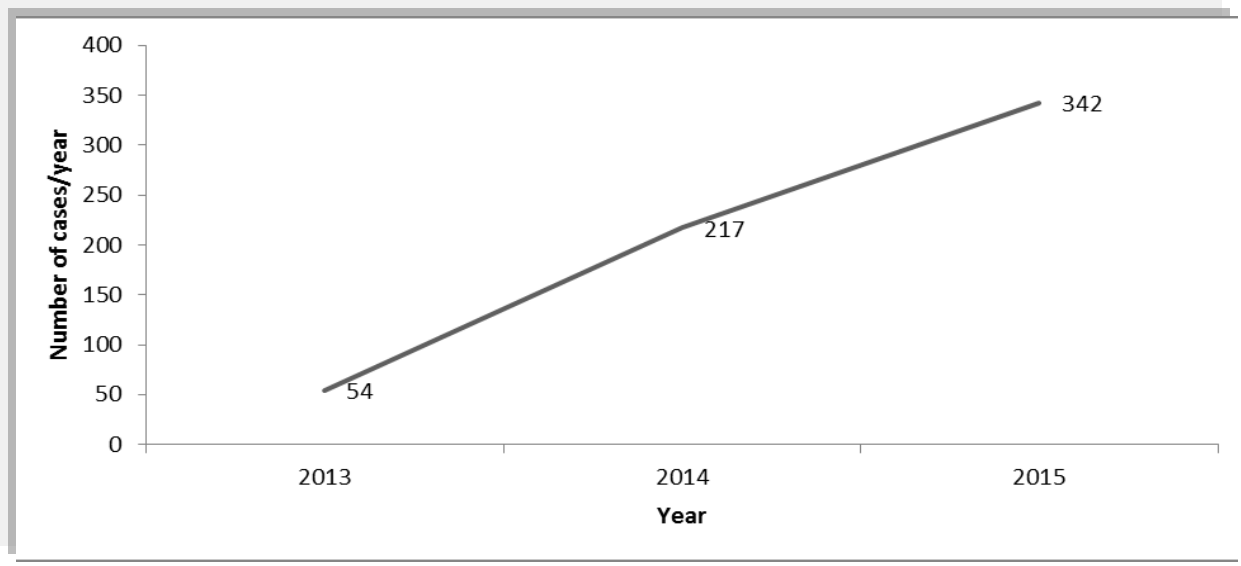

Figure 6. Reported human cases of brucellosis in Gharbia Governorate, Egypt from 2010 to 2015

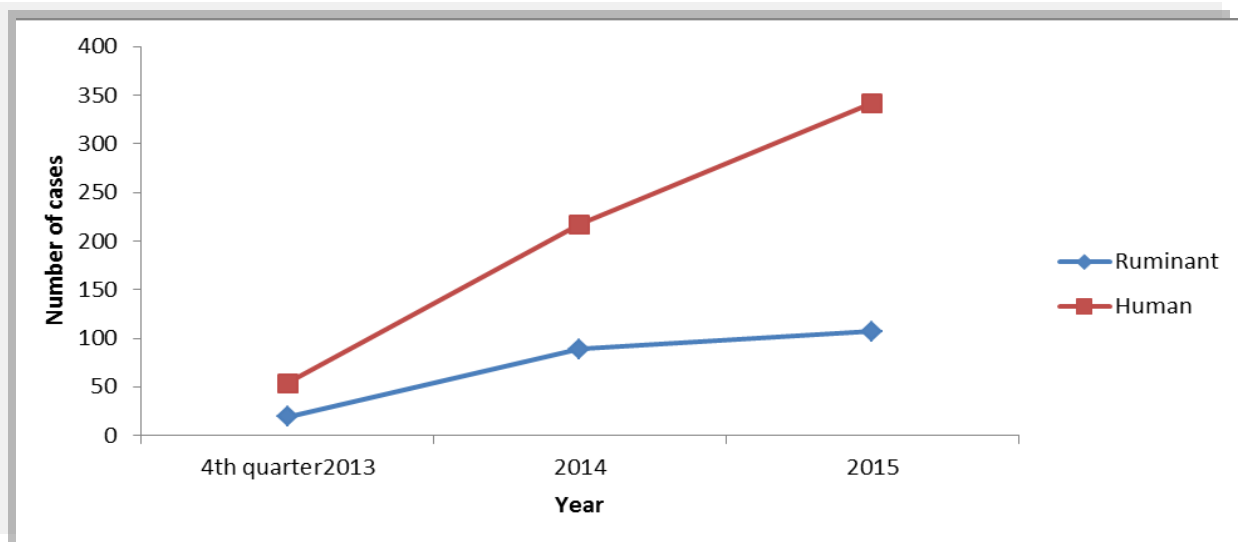

Figure 7. Reported animal in human cases of brucellosis in Gharbia Governorate, Egypt from September-2013 to December-2015

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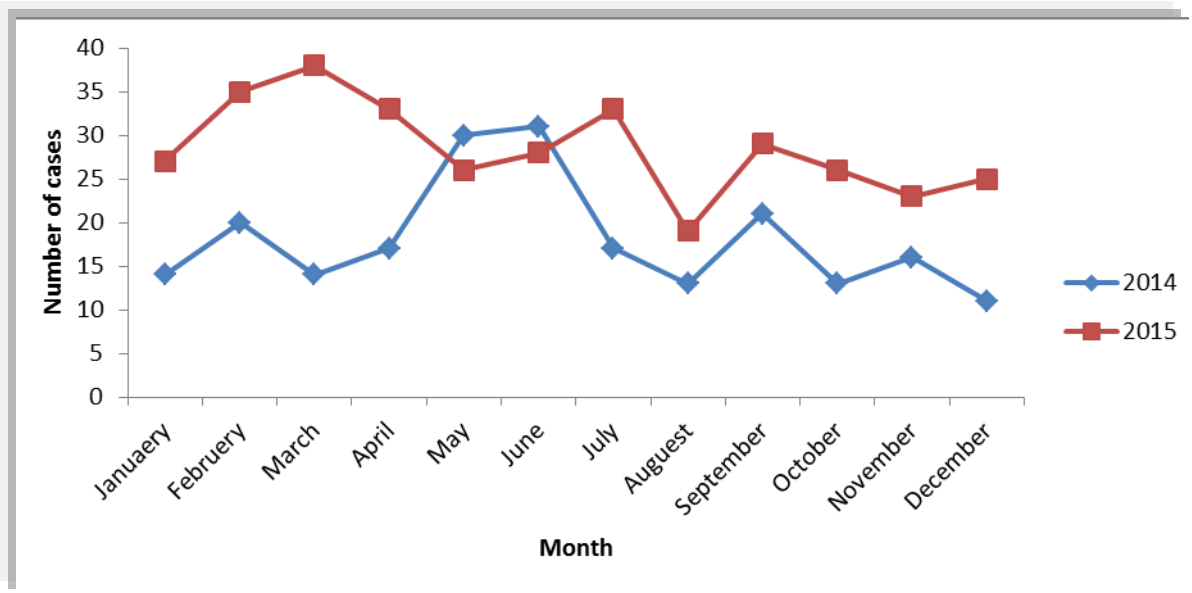

Figure 8. Monthly distribution of reported human cases of brucellosis in Gharbia Governorate for 2014 and 2015

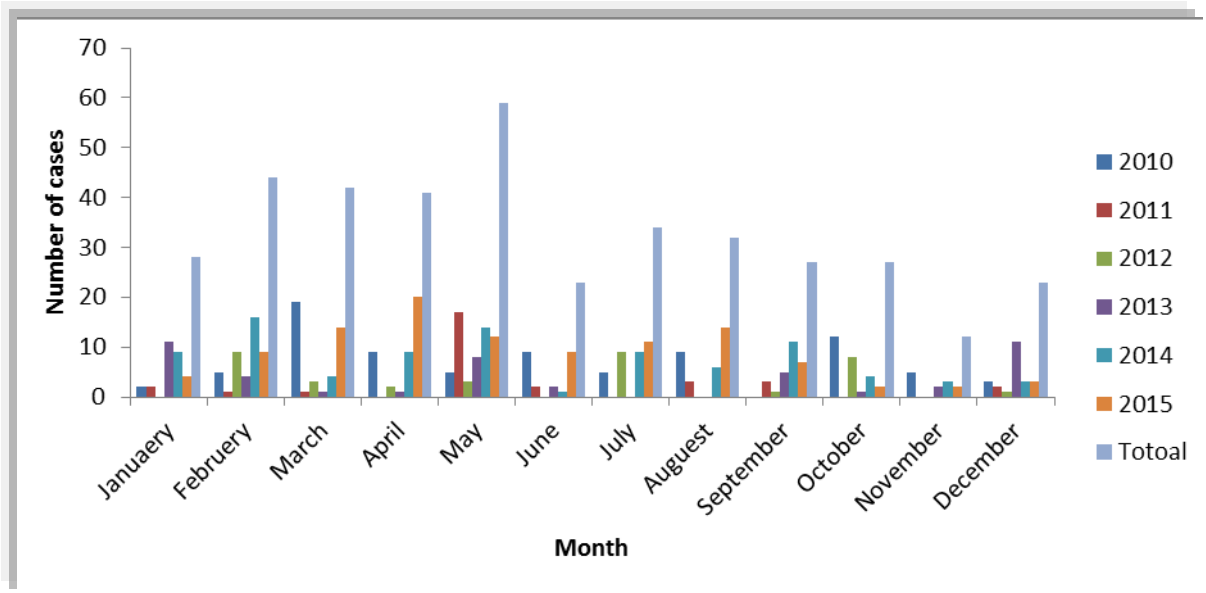

Figure 9. Monthly distribution of reported cases of brucellosis in ruminants in Gharbia Governorate from 2010to 2015

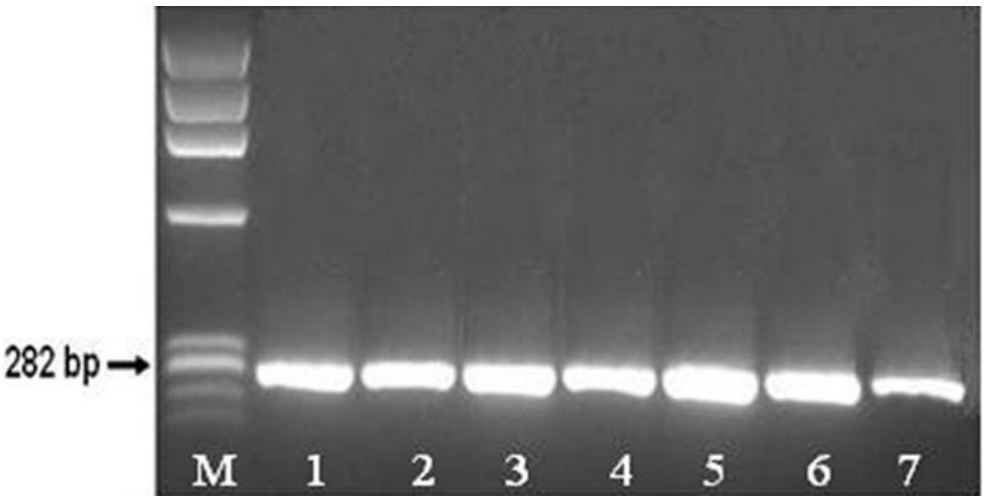

Figure 10. Agarose gel electrophoresis of PCR-amplified omp 2 gene fragments from *Brucellamelitensis* strains. The figure shows a single band 282-bp DNA fragment. M: ØX 174 RF DNA HaeIII digest marker (Biolabs). Lane, 1 *B. Melitensis*biovar 3 field strain. Lane

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DISCUSSION

The aim of this study was to assess the impact of the national brucellosis control programme on the temporal pattern of brucellosis in ruminants and human in Gharbia governorate, Egypt. The results indicated that in any given year of the study period (2010 to 2015), the proportion of tested ruminants for brucellosis was less than 3%. Cattle were the highest species to be frequently tested. There were no consistency in sampling of different ruminant species particularly goat and also there were no consistency in sampling in different districts of Gharbia governorate. These findings were similar to that in El-Behira governorate, where cattle were the highest species to be tested and for which 70% of the control programme costs were spent (Eltholth et al., 2015). Analysis of data from the brucellosis control programme in Kafrelsheikh governorate also indicated that, the fractions of ruminant sampled ranged from 2.2% to 6.5%, the percentages of sampling per species were 2.2% and 7.5% for cattle, 1.4% and 7.4%for buffaloes, 0.9% and 4.8% for sheep and 0.4%and 4.6% for goats(Hegazy et al., 2009). The results showed that the proportion of seropositive animals was low and there were variations in seropositive proportions in different species of ruminates and between districts. Given that there was no clear sampling strategy and no adherence to the programme, the results were not reliable to estimate the prevalence of the disease in the study area. For example negative results for goat in some districts for two consecutive years did not mean that goat in these districts were free from brucellosis; it was simply because goat was not sampled. Comparing with results from other studies in the same governorate and the neighbor ones, this proportion of seropositive animals was quite low (Holt et al., 2011; Hegazy et al., 2011; Mahboub et al., 2013 and Hegazy et al., 2016). A cross-sectional study was conducted in two villages in Gharbia governorate, in which the proportions of seropositive sera were 0.0 and 16% among livestock of villages I and II, respectively (El Sherbini et al., 2007). In El-Behira governorate, the proportion of seropositive cattle, buffalo, sheep and goats for brucellosis was found to be 5.86%, 5.83%, 7.20% and 11.33%, respectively(Sayour and Azzam, 2014). Results of regression analysis showed that, the proportion of seropositive ruminates fluctuated up and down. A significant increase in the proportion of seropositive animals after a significant reduction might be related to the variation in the number of tested animals for each year and non-adherence to the programme (Hegazy et al., 2009; Eltholth et al., 2015 and Eltholth et al., 2016). In our opinion if there was an actual reduction in the prevalence of the diseases due to the control programme, the proportion of seropositive animals would not have increased again unless there were other factors. The results of PCR indicated that all isolates were *B.melitensis* biovar 3 which thought to be the most common prevalent strain of brucellosis in Egypt (Refai, 2002; Montasser et al., 2012 and Ramadan et al., 2013).

The number of reported human cases of brucellosis was increasing from 2014 to 2015. This was positively associated with the increase of the proportion of seropositive ruminates in the same years. These results indicated that, controlling the disease in livestock would prevent human exposure to brucellosis. A previous study in the same governorate found that, keeping sheep in the household was a significant risk factor for human brucellosis (P=0.01) and among livestock, sheep showed the highest seropositive proportions of brucellosis(El Sherbini et al., 2007). The highest number of reported human cases in2014was in June and that for 2015 was in March. On the other hand the highest total number of reported cases per month in ruminants within the study period was May followed by February. These results indicated the association between brucellosis in animals and human and this time space could be the incubation period for infected human to develop symptoms. In another word, waves of cases in humans come after the increase of seropositive proportion in livestock. Furtherepidemiological studies should be conducted not only to assess the prevalence of disease in livestock and human but also to assess the risk factors of livestock infection for terms of prophylaxis.

Competing interests

The authors declare that there are not significant personnel, professional or financial competing interest that might have influenced the presentation of the results of the study described in this manuscript.

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New Challenges of Knowledge Transfer in Veterinary Physiology in a Changing Educational Environment: An Overview of Physiology Teaching in USA and Non-USA Colleges and Schools

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ABSTRACT

Veterinary physiology education, faces new challenges in a rapidly changing information technology-based world. The main factors interacting and affecting the veterinary medical education are: the subject matter itself, the new generation of students, new definitions available for knowledge, and the different teaching methods. The objectives of this work were triple. Firstly, to review the factors that impact teaching and learning; Secondly, to provide insights based upon more than twenty years of experience in teaching veterinary physiology to veterinary students at Purdue University, USA, and the University of Veterinary Medicine, Budapest. Thirdly, a) To gain an understanding of the physiology teaching in USA and non-USA Colleges through analyzing veterinary physiology topics as well as related subjects and factors; b) To examine the contents of the widely used textbooks. Physiology courses data were collected from schools` public websites of the 28 accredited USA veterinary schools, and selected colleges outside the USA, and analyzed. . Course comparisons included the topics taught in the veterinary physiology courses, number of credit hours, signs of tracking, presence or absence of neurophysiology, clinical physiology, pathophysiology, comparative physiology, and the presence or absence of laboratory practical sessions. The contents of most popular text book were analyzed and compared. Our results showed that there were substantial differences in teaching veterinary physiology and related subjects, such as neurophysiology, pathophysiology, comparative physiology, biochemistry, and clinical sciences within and outside the USA. It was observed that not all (only 36%) physiology courses are coupled with labs, especially wet labs. It worth mentioning here that the order of topics within each physiology textbook is not the same and the depth of coverage of different chapters vary, and some of the topics are underrepresented. Interestingly, veterinary students in developed countries are committed to become veterinarians and are highly motivated. This motivation is reflected on better learning. In addition, these students have the opportunity to select during their studies a career path or track such as small animal. Currently, no track-related physiology courses exist in most curricula. Physiology education could benefit from new technologies and interactive learning approaches such as case-based and g, team-based learning, peer instruction, and the flipped classroom. In addition, the use of e-learning management systems would facilitate the learning process, and the interactions between peers and with the instructor. Further, aligning and integrating basic medical sciences and providing clinical correlations would encourage the application of physiological concepts to solving clinical cases.

Key words: Veterinary physiology, Veterinary schools, Y Generation, Meaningful learning, Learner-centered teaching, Institutional design, Interactive learning, Case-based learning.

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INTRODUCTION

Physiology is crucial to the understanding of other important basic and applied veterinary medical sciences. In addition, physiology having an interdisciplinary character is indispensable for the teaching of biomedical sciences and biomedical engineering (Clase et al., 2008). Veterinary physiology differs from both classical mammalian physiology and medical physiology. It has to prepare students for the practice of veterinary medicine that deals with a wide range of species, specialties, and tracks. It has to help students in solving clinical cases, and understanding issues related to animal wellbeing and veterinary public health. Veterinary physiology is expanding due to the expanding research areas (Blumberg, 2005; Forrester, 2006; Murray and Sischo, 2007; Lane, 2008; Summerlee, 2010; Habtemariam, 2012; Warren and Donnon, 2013 and Salomäki et al., 2014). To cope with the new knowledge, intellectual learning must be the focus of education instead of classical methods based on gathering and memorizing facts (Hoover and Pelaez, 2008). It could be noted that college curricula are being revised to accommodate the knowledge expansion and information overload. However, it could be noted that ensuring students' in-depth knowledge in all aspects of veterinary medicine is

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getting more and more impracticable (Eyre, 2011). Traditional facts-stuffed curricula may have made pre-clinical courses boring and often perceived as irrelevant to the practice of veterinary medicine. On the other hand, lifelong learning, problem-orientated approach, communication skills, critical thinking, and information management skills have become indispensable for the practice of veterinary medicine and its specialties (Blumberg, 2005; Forrester, 2006; Murray and Sischo, 2007 and Lane, 2008). Understandably, clinical correlations and problem-based curriculum motivate students to learn basic relevant physiological concepts.The objectives of this work were triple.Firstly, to review the literature on the factors that impact teaching and learning (the new generation of students, the concepts of knowledge and meaningful learning, and the ways and means used to realize meaningful learning). Secondly, to provide insights based upon more than 20 years of experience in teaching veterinary physiology to veterinary students at Purdue University (Lafayette, Indiana, USA) and the University of Veterinary Medicine (Budapest, Hungary). Thirdly, a) to gain a better understanding of the physiology teaching in USA and non-USA Colleges through analyzing veterinary physiology curricula topics being taught in veterinary schools and colleges, as well as related subjects and factors that affect the success of teaching veterinary physiology. These factors include integration and alignment, the biochemistry factor, physiology textbook topics, and the presence or absence of laboratory practical sessions b) to examine the contents of the widely used text books in order to identify current trends in veterinary physiology teaching worldwide. The study proposed an integrated approach to knowledge transfer in veterinary physiology within the current educational environment that is characterized by curricula revisions, including learning outcomes.

MATERIALS AND METHODS

An extensive review of the literature was carried out using current and relevant publications on the following: 1) characteristics of the new generation of veterinary students, differentiated curricula (tracking) and physiology teaching, new knowledge definitions and the teaching and learning of physiology, and ways of teaching and learning, 2) veterinary physiology teaching in the USA veterinary schools and colleges, and related factors affecting the success of teaching veterinary physiology. On the other hand, physiology courses in the 28 accredited USA veterinary schools (Smith and Fenn, 2011; Roush et al., 2014 and American Veterinary Medical Association, 2016) were reviewed (Table 1). Professional curricula data were collected in 2014 from the veterinary colleges' and schools` public websites in the USA and outside the USA and comparisons were made. Course comparisons included the following: topics taught in the veterinary physiology courses, number of credit hours, signs of tracking, presence or absence of neurophysiology, clinical physiology, pathophysiology, and comparative physiology courses. In addition, the related factors affecting the success of teaching veterinary physiology were evaluated. These related factors include integration and alignment, the biochemistry factor and physiology textbook topics.

Ethical approval

The research in this study was conducted according to normal educational practices. It involved comparisons of curricula, instructional techniques, and classroom management. The research received Exempt Status from Institutional Review Board because 1) It did not involve human participants or animals; 2) It did not involve student-related data or records or face-to-face interactions with students. The research was conducted ethically in a way that promotes understanding of educational practices and veterinary curricula in different colleges and schools in the USA and outside the USA using publically available information.

Table 1A. American Veterinary Medical Association-accredited colleges of veterinary medicine in the USA

ALABAMA **Auburn University** College of Veterinary Medicine Auburn University, AL http://www.vetmed.auburn.edu **Tuskegee University** School of Veterinary Medicine Tuskegee, AL http://tuskegee.edu	**ARIZONA** **Midwestern University** College of Veterinary Medicine Glendale, AZ http://www.midwetern.edu
CALIFORNIA **University of California** School of Veterinary Medicine Davis, CA http://www.vetmed.ucdavis.edu **Western University of Health Sciences** College of Veterinary Medicine 309 E Second Street - College Plaza, Pomona, CA http://www.westernu.edu/veterinary	**COLORADO** **Colorado State University** College of Veterinary Medicine and Biomedical Sciences Fort Collins, CO http://www.cvmbs.colostate.edu
FLORIDA **University of Florida** College of Veterinary Medicine, Gainesville, FL http://www.vetmed.ufl.edu	**GEORGIA** **University of Georgia** College of Veterinary Medicine Athens, GA 30602 http://www.vet.uga.edu

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ILLINOIS **University of Illinois** College of Veterinary Medicine, 2001 South Lincoln Avenue Urbana, IL http://www.cvm.uiuc.edu	**INDIANA** **Purdue University** College of Veterinary Medicine, 1240 Lynn Hall West Lafayette, IN http://www.vet.purdue.edu
IOWA **Iowa State University** College of Veterinary Medicine Ames, IA http://www.vetmed.iastate.edu	**KANSAS** **Kansas State University** College of Veterinary Medicine Manhattan, KS http://www.vet.ksu.edu
LOUISIANA **Louisiana State University** School of Veterinary Medicine Baton Rouge, LA http://www.vetmed.lsu.edu	**MASSACHUSETTS** **Tufts University** School of Veterinary Medicine 200 Westboro Road North Grafton, MA http://www.tufts.edu/vet
MICHIGAN **Michigan State University** College of Veterinary Medicine G-100 Veterinary Medical Center, East Lansing, MI http://cvm.msu.edu	**MINNESOTA** **The University of Minnesota** College of Veterinary Medicine St. Paul, MN http://www.cvm.umn.edu
MISSISSIPPI **Mississippi State University** College of Veterinary Medicine Mississippi State, http://www.cvm.msstate.edu	**MISSOURI** **University of Missouri-Columbia** College of Veterinary Medicine Columbia, MO http://www.cvm.missouri.edu
NEW YORK **Cornell University** College of Veterinary Medicine Ithaca, NY http://www.vet.cornell.edu	**NORTH CAROLINA** **North Carolina State University** College of Veterinary Medicine 4700 Hillsborough Street Raleigh, NC 27606 http://www.cvm.ncsu.edu/
OHIO **The Ohio State University** College of Veterinary Medicine 1900 Coffey Road Columbus, OH 43210-1092 http://www.vet.ohio-state.edu	**OKLAHOMA** **Oklahoma State University** College of Veterinary Medicine Stillwater, OK 74078 http://www.cvm.okstate.edu
OREGON **Oregon State University** College of Veterinary Medicine Corvallis, OR http://www.vet.orst.edu	**PENNSYLVANIA** **University of Pennsylvania** School of Veterinary Medicine 3800 Spruce Street Philadelphia, PA 19104-6044 http://www.vet.upenn.edu
TENNESSEE **University of Tennessee** College of Veterinary Medicine 2407 River Drive Knoxville, TN http://www.vet.utk.edu **Lincoln Memorial University** College of Veterinary & Comparative Medicine 6965 Cumberland Gap Pkwy Harrogate, TN http://www.lmunet.edu	**TEXAS** **Texas A&M University** College of Veterinary Medicine & Biomedical Sciences College Station, TX http://www.cvm.tamu.edu
VIRGINIA **Virginia Tech** Virginia-Maryland Regional, College of Veterinary Medicine Blacksburg, VA http://www.vetmed.vt.edu	**WASHINGTON** Washington State University College of Veterinary Medicine Pullman, WA http://www.vetmed.wsu.edu
WISCONSIN **University of Wisconsin-Madison** School of Veterinary Medicine 2015 Linden Drive West Madison, WI http://www.vetmed.wisc.edu	

https://www.avma.org/.../Colleges/Documents/colleges_accredited.pdf ; Last accessed June 20, 2016

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Table 1B. Nationally-accredited colleges of veterinary medicine outside the USA

STATES	Colleges of veterinary medicine
MEXICO	**Universidad Nacional Autonoma de México** Facultad de MedicinaVeterinaria y Zootecnia, Coyoacan Web site: http://www.fmvz.unam.mx/
THE NETHERLANDS	**State University of Utrecht** Faculty of Veterinary Medicine Utrecht, Web site: http://www.uu.nl/faculty/veterinarymedicine/en/pages/default.aspx
NEW ZEALAND	**University College of Sciences** Institute of Veterinary, Animal, and Biomedical Sciences Palmerston North http://ivabs.massey.ac.nz/
SCOTLAND	**University of Glasgow** Faculty of Veterinary Medicine Bearsden Road Glasgow G61 1QH www.gla.ac.uk/Acad/FacVet **The University of Edinburgh** Royal Dick School of Veterinary Studies Summer Hall Edinburgh EH9 1QH www.vet.ed.ac.uk
WEST INDIES	**Ross University** School of Veterinary Medicine Basseterre, St. Kitts http://www.rossu.edu/veterinary-school/ **St. George's University** School of Veterinary Medicine Grenada West Indies 800-899-6337 http://www.sgu.edu/school-of-veterinary-medicine/index.html

https://www.avma.org/ProfessionalDevelopment/Education/Accreditation/Colleges/Documents/colleges_accredited.pdf. Last accessed June 20, 2016.

Characteristics of a new generation of veterinary students, and teaching and learning of physiology

New generation of veterinary students

Colleges teach physiology to veterinary students who aspire to become veterinarians capable of providing quality veterinary medical care, or engage in disease diagnosis and research. A question is often asked by instructors: do we really know the characteristics of veterinary students who have the desire to become veterinarians? College and university students, including veterinary students, are changing in parallel with the changing world around them, and this leaves a "generation gap" between faculty and the students. Current veterinary students, who are computer literate, belong to the so-called generation Y, the millennial or global generation, or the "digital natives" as characterized by Prensky (2001). This generation Y is used to the Internet, computer games (deBie and Lipman, 2013), "instant gratification, multitasking, and short focusing periods" (Salisbury, 2008). Oblinger and Oblinger (2005) noted that this "Net (Internet) Generation " possess the following attributes "Ability to integrate the virtual and the physical, learning better through discovery than by being told, rapidly shifting attention, quick responses and expectation of the same quick response; immediacy, social activities in the e-sphere (instant messaging, virtual games, blogging etc.), and needed structuralizing in order to be able to understand the rules". On the other hand, continuing education is important for the practice of veterinary medicine. The spirit of lifelong learning is indispensable to coping with a changing work environment, and the new knowledge that is constantly emerging. For the licensure to practice veterinary medicine in the USA, formal life-long learning is expected. With Continuous Professional Development (CPD), one can keep up with the recent developments in veterinary clinical sciences, improve his/her professional skills, and become a better board certified clinician in a specialty of veterinary medicine. Outside the USA, there are veterinary colleges, such as the Royal College of Veterinary Surgeons, RCVS, UK, that also made CPD obligatory for its members. Recently, computer-based and online resources such as videos, audio presentations, CD-ROMs, and web-based courses, as those provided by Veterinary Information Network, VIN, are increasingly used instead of the traditional methods of instruction (face to face courses, conferences, seminars, journals, textbooks). According to Short et al. (2007), RCVS has developed its own e-CPD model based on a known and effective adult distance learning method that recognizes that several steps are occurring in the learners: "self-motivation and ability to access the material, online socialization, information exchange, knowledge construction, and development". Expectedly, an increasing number of veterinarians will take part in the e-courses for CPD (Short et al., 2007).

In general, veterinary students, especially in developed countries, are highly motivated (Salomäki et al., 2014), committed to being veterinarians, and are ready to utilize their time and resources to earn their degree. However, their motivation can originate from their deep interest in their studies, as well as external factors, such as having a profession and an associated prestigious status in the society, and most importantly an interest in animals. Motivated students tend to achieve high-quality learning, while current study practices might result in superficial/rote learning (Mikkonen and

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Ruohoniemi, 2011). In developing countries, such as those in Africa, most students opt for studying veterinary medicine when their grades are not competitive for their admission into the medical or pharmacy colleges.

Differentiated curricula (tracking) and physiology teaching

By and large veterinary schools in the USA offer differentiated curricula (tracking) so students can learn disciplines that are needed for their chosen career path (i.e. small animals, equine and large animal, mixed practice etc.). Tracking is a common practice that might reduce content overload while increasing students' interest in learning specific topics (Ruohoniemi et al., 2010; Eyre, 2011; Mikkonen and Ruohoniemi, 2011). All students, regardless of their tracks, go through a core curriculum that is required for earning a Doctor of Veterinary Medicine degree (DVM). Tracking requires certain electives. Interestingly, many veterinarians who change their tracks emphasis after graduation and during their career would prefer a more general education (Summerlee, 2010). Tracking choice and change were investigated by the school of veterinary medicine of the University of California, Davis, USA. This investigation documented that, at the time of admission, veterinary school students choose a track primarily based on their previous experience and personal preferences or other factors (Chigerwe et al., 2010). Nevertheless, during veterinary school years, 27.4% of the students changed their track choice. The major causes for that change were: further experience during the first two years of the veterinary school, anticipation of better job opportunities and competitiveness, and personal factors such as interest in veterinary public health, or in different species (Chigerwe et al., 2010).Toward this end, the topics covered physiology courses are not only expected to help a student prepare for his/her core curriculum and selected track, but also support track changes as well.

A balanced coverage of important physiology concepts should provide an overview of the comparative physiology of different species during the first year of the curriculum. Later, additional specialized physiology courses that would serve specific tracks may also be offered as electives or required courses (e.g. avian or pet bird physiology, zoo and wildlife physiology, reptiles, amphibians, fish, and pet rodent physiology, laboratory animal physiology, clinical physiology). Tracks choices generally reflect jobs available in veterinary practices in a given country (i.e. equine, food animal and poultry, large animal, small animal, and exotic and aquatic animals, and wildlife). Generally in most colleges, physiology teaching supports the core curriculum and not the elective courses needed by the tracks. If available, the physiology courses for a particular track should include both the basic physiological concepts as well as physiological concepts that support the track-specific elective courses. For example, a student opting for small animal track would be expected to take the two core physiology courses plus canine and feline physiology and exotic animals or lab animal physiology courses. The elective courses may be taught as clinical physiology courses with emphasis on the track options, for example, clinical physiology of small animals.

Emerging tracks such as exotic or wild animals have recently become very important for the practice of veterinary medicine, and have been attracting students' interests. Learning the physiology of exotic or wild animals can help veterinary students become better veterinarians for the praxis market. These tracks should be taken seriously and should be emphasized in the core subjects. Currently, no such track-related physiology courses exist in most curricula. On the other hand, the new challenges that originate from the One Health/ One Medicine Initiative, which promotes linking human health, animal health and the environment, need to be addressed by designing interrelated tracks so that students could gain knowledge and experience in the ecological, social, and political aspects of veterinary medicine and its relationship to human health and the ecosystem (Chaddock, 2012 and Mor et al., 2013).

New knowledge definitions and the teaching and learning of physiology

Recognizing that veterinary physiology is a continuously evolving interdisciplinary subject that should be taught to a new generation of veterinary students, the question that arises is what to teach the students? Do students need to be taught physiology just for the knowledge sake, or for its application in solving clinical cases or answering research questions? Basically, teaching and facilitated learning should address all of the above points and provide the students with adequate coverage and in depth knowledge of physiology. However, it depends on what knowledge means. The definition of knowledge has undergone a fast evolution in the past decades and is not simply equivalent to learning the subject matter now; it is rather a mixture of factual knowledge, skills, abilities, interdisciplinary approach, and information literacy. The Association of college and research libraries set five IT-literacy requirements (Blumberg, 2005): "determining information needs, acquiring information effectively and efficiently, critically evaluating information and its sources, incorporating selected information into one's knowledge base, and using information legally and ethically." In addition, students should have the following competences: problem-solving, team work, effective communication, ability to assess themselves and their peers, and apply self-directed learning (Lane, 2008). Critical thinking, information management skills, lifelong learning ability and creative thinking have also become indispensable for the practice of veterinary medicine (Forrester, 2006 and Clase et al., 2008). The 2010-2014 strategic plan of the association of American veterinary medical colleges lists among the goals and objectives the following: "Lead efforts to review, evaluate and improve veterinary medical education in order to prepare graduates with the competencies needed to address societal needs" (AVMC, 2009). Therefore, in teaching physiology, it must be recognized that the term "knowledge" is also changing and the classical knowledge and basic literacy may no longer be enough for current and future professionals (Hodgson et al., 2013).

The Pew national veterinary education program report emphasizes that biomedical knowledge should originate from problem solving and information gathering skills instead of a comprehensive knowledge of biomedical facts (Warren and Donnon, 2013). Therefore, the new knowledge model needs a new definition for learning in the 21th century. Pelaez and Gonzalez (2002) summarized learning as a process that requires the following: "critical thinking, the

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evaluation of information, synthesis of ideas, the testing of ideas and data through comparison against pre-existing models, and the development of new models". Teachers in the 21st century, including instructors of physiology in veterinary schools and colleges, must facilitate students-centered learning instead of using teacher-centered instruction (Mayer, 2010).

Ways of teaching and learning

The criticism of classical teaching methods lists the following: preference of lectures for the lecture-based delivery, focusing on facts, lack of problem-solving exercises and small group learning, emphasis on teaching instead of learning (Abrahamson, 1990). However, the famous Confucius quote says "Tell me and I will forget; show me and I may remember; involve me and I will understand". Therefore, teaching that involves students (e.g. interactive learning) is expected to be superior to lecturing at the students. Innovations, conceptual and methodical revolutions in teaching and learning, and responses to criticism are not unique in educational history. As an example, the "chalk and board" method that is nowadays referred to as traditional or obsolete appeared in the mid-1800s in the USA as an innovation in teaching. That new method spread quickly and teachers needed only a short period of time to use it in their teaching (Abrahamson, 1990). As a result, the efficiency and dynamics of teaching then increased rapidly. Today technical changes and inventions offer an opportunity for bigger leap toward increasing learning success and efficiency. Even the idea of the good old chalkboard is not thrown away; but was transformed into the "new" interactive whiteboards (Ragan, 2007). Teaching/learning innovations does not necessarily mean throwing away the old and replacing it by the new. It should focus on what would the best approach and what technology integration is needed that would help students learn.

A question may be asked: what is the result of the classical way of teaching physiology? It was observed that students tend to see physiology as a pile of factual information that only needs cramming/memorizing instead of digestion and utilization for solving problems or clinical cases (Pelaez, 2002). However, rote learning does not provoke meaningful learning (Pelaez, 2002 and Hazel et al., 2013). There are several effects of rote memorization and consequently surface learning such as aimlessness, mere memorization, sticking to the syllabus, and focusing on passing the exam (Ryan et al., 2009).

Learner-cantered teaching

The root of the change to learner-centered-teaching is a shift from teaching-focused pedagogy to a learner-centered andragogy (Pirkelbauer et al., 2008). The role of teachers is no longer viewed as a simple one way of conveying information to students (teacher-centered) but rather facilitation of students' learning and acting as a "partner" in the learning process (Miflin, 2004). However, to make this shift to learner-centered teaching, one should remember that as teachers tend to adhere to their teaching routines, students are often resistant to changing their usual and previously successful learning habits (Pirkelbauer et al., 2008). On the other hand, pre-professional university education has a profound effect on the ability of students to adapt to the professional education environment, such as veterinary medicine, and the learning methods; since they are expected to be successful in a challenging professional (Rutland et al., 2016). It worth mentioning here that before students are accepted in DVM programs in the USA, they have to complete pre-veterinary university education (2-4 years).

Recent findings (Abrahamson, 2007) about learning principles in educational psychology give more "handholds" or guidelines to understanding what critics of the teacher-centered approach mean. These "handholds" are described as follows: "No two people learn in exactly the same way; learning is facilitated by motivation; learning is incentive, therefore, should be based on the true motivation of the learner; incentives work best (i.e., promote true learning) when they are positive not negative; and learning is much more effective if we begin where the learner is, not where we wish he or she were". Acor (2005) noted that the concept of "good teaching practice" was created and could be developed further for quality assessment in teaching in a similar fashion in which "good laboratory practice" has been used in the pharmaceutical industry). Good teaching practice is expected to "encourage student-faculty contact and cooperation among students, and active learning. It gives the learner prompt feedback, emphasizes time on task, transmits what is expected of students, considers talents, and ways of learning. "Kolb's learning cycle also points out the importance of the active and constructive mental participation of students in the learning process" (Buur, 2013). It is also highlighted that active learning takes place if the teaching is student-focused and induces students to consider what they are doing (Keegan et al., 2012).

The goal is meaningful learning

Michael (2004) defined meaningful learning as "learning with understanding". As a result of meaningful learning, students can retrieve previous knowledge, they can combine the previous and new knowledge, and they can solve new tasks and problems based on their knowledge. As for medical (either human or veterinary) students, they must recall and use their basic medical science knowledge in order to become successful in practice. Notably, meaningful learning cannot miss the accumulation of facts and the ability of using them (Michael, 2004). However, the students` previous misconceptions also interfere with successful learning (Pelaez, 2002).

The opposite of the meaningful learning is rote learning, or rote memorization, when students simply memorize facts and new information without trying to integrate them into their previous knowledge (Diwakar et al., 2007). Meaningful learning can help in setting up mental models (Michael, 2004) that resemble concept maps (Newman, 2005). Mental models help with prediction, calculation, and explanation; a reflection of student's understanding. It goes without saying that for meaningful learning to occur, an environment conducive to active learning must be created, where students can collect information, their knowledge can be facilitated, tested and refined, and where they can learn how to

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use the knowledge to solve problems (Michael, 2004). Without these possibilities, a student may seem to be active but will not undergo a meaningful learning process. Last but not least, it is important to note that the learning process occurs exclusively in the learner (Simon, 2001).

To understand the current situation of veterinary physiology education, this study examined the professional veterinary curricula in all accredited USA and in selected non-USA veterinary schools, focusing on veterinary physiology and related subjects. The curricula are at different developmental stages and revisions in order to respond to the new challenges in different ways. However, we are hoping by this analysis to promote veterinary physiology education globally. In addition, since the world's best ranked veterinary medical colleges are found in the USA, comparing USA and non-USA curricula might yield additional ideas and information for improving veterinary physiology teaching and learning.

RESULTS

Veterinary physiology teaching in the USA veterinary schools and colleges

All available USA curricula contained at least one obligatory (core), *solo* or integrated physiology course. If the physiology core is more than one semester long, the courses are either numbered sequentially or named after the sub-topics of physiology (e.g. digestive, renal, respiratory, etc.). Physiology courses were typically offered at the beginning of the professional curriculum between year 1 (Fall/1st semester) and year 3 (Fall/5th semester). Based on the available information, we found that veterinary physiology course credits/credit hours vary between 3 and 12, most commonly 4 credits. Generally, physiology is not a required course in the pre-veterinary curriculum in the USA. However, undergraduate students usually may take human or mammalian physiology offered by a biological sciences or animal science departments. Nevertheless, students taking these pre-veterinary physiology courses might not know how to relate the physiological concepts they learned to veterinary medicine. The impact of pre-veterinary physiology information and any misconception on learning veterinary medical physiology need to be further investigated.

The topics taught in the veterinary physiology courses are highly variable. Some courses cover the classical physiology content, which starts with homeostasis/nervous system/blood/cardiovascular system and ends with reproduction, renal and acid-base balance. Other courses deal with only some topics of interest, such as cardiovascular physiology, while omitting physiology of the integumentary system, bone or the immune system. However, these topics might be included in the pre-veterinary physiology courses or might be included within other subjects, such as anatomy, animal husbandry, cell biology, immunology etc.

Based on the available course descriptions, 85 professional veterinary physiology courses were investigated, from which 51 courses were available with descriptions. Classical topics in these physiology courses were analyzed. Table 2provides a summary of the physiology topics taught in schools and colleges of veterinary medicine in the USA, and the percentages of courses that address a given topic are presented. It is worth mentioning here that, in some cases course descriptions were not available at all; in other cases they were not detailed enough. In horizontally and vertically integrated curricula, the classical physiology topics were merged with those of other courses so they could not be identified clearly. The latter factor produced relatively strong limitations for comparison of course topics and resulted in relatively low percentage ratios.

Table 2. Physiology topics taught in schools of veterinary medicine in the USA and the percentage of courses that address the given topic (Year of the survey, 2014).

Topic	Percentage of courses that included the topic (total number of courses with topics: 51)
Homeostasis	14%
Blood	14%
Immunophysiology	4%
Cardiac Physiology	24%
Circulation	27%
Respiration	25%
Renal Physiology	27%
Gastrointestinal Physiology	27%
Metabolism	10%
Thermoregulation	2%
Bones	8%
Muscle Physiology	27%
Endocrinology	27%
Reproduction	24%
Nervous System	29%
Senses (vision)	8%
Acid Base Balance	6%
Integumentary System	6%

Calculation of percentages were as follows: We added up the number of physiology courses with available topic lists. (Several curricula do not contain the list of topics taught in a course just the title of the course and credit hours.) There were 51 such courses among USA ones. From the 51 courses, 15 courses (that is the 29% of 51) included neurophysiology as a topic. The same way, only one course (that is the 2% of the total 51 courses) included thermoregulation.

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The most common classical topics taught in the veterinary physiology courses were: neurophysiology (29%), endocrinology, muscle physiology, gastrointestinal physiology, renal physiology, circulation (27% each), respiration (25%), cardiac physiology (24%), and reproduction (24%). Several physiology courses deal with the blood and homeostasis (14% each). However, metabolism (10%), bone physiology, special senses (8% each), acid base balance (6%), integumentary system (6%), immunophysiology (4%), and thermoregulation (2%) are underrepresented in the USA curricula. Calculation of percentages was done as follows: The number of physiology courses with available topic lists was added up. It worth mentioning here that several curricula do not contain the list of topics taught in a course; they provide just the title of the course and credit hours. There were 51 such courses among USA ones. From the 51 courses, 15 courses (that is the 29% of 51) included neurophysiology as a topic. The same way, only one course (that is the 2% of the total 51 courses) included thermoregulation.

Most schools divide the physiology instruction into separate modules or units, where each one can be completed by passing an exam before moving to the second unit, etc. Some schools offer a set of physiology courses focusing on one or two subtopics of physiology. These courses can usually be taken in parallel. On the other hand, some course topics are offered in pairs such as muscles and bones, kidney and body fluids/acid-base balance, respiration and kidney, cardiac and vascular physiology, digestion and metabolism, homeostasis and blood, endocrinology and reproduction.

Obesity, nutritional, and metabolic disorders are emerging problems in the 21st century not only in human populations but among pet animals as well. The necessity of teaching intermediary metabolism to DVM students, is therefore an urgent issue. Some physiology courses and textbooks have separate chapters on metabolism and/or the regulation of feed intake. However, most of the books do not discuss metabolism. Naturally, metabolism can be taught not only in the frames of physiology instruction, but it can also appear in biochemistry, nutrition, or in endocrinology sections. It may be taught as part of digestive physiology section that deals with nutrients utilization during the absorptive and post absorptive states, as in the case of Purdue University, USA.

Acid-base balance rarely forms a separate chapter in most books, but it is usually mentioned (at least partly) as a part of introduction to homeostasis, kidney function, and respiratory system physiology. Interestingly enough, temperature regulation is the most common small topic taught in physiology courses and mentioned in physiology textbooks. It is hard to understand, however, why such topics as physiology of the bones and the integumentary system are often neglected when skin problems, for example, are most frequent cases seen in patients visiting small animal practices in USA, and in other parts of the world. Moreover, a basic knowledge of these two topics is important for understanding pathology or internal medicine topics. Although the structure of the integumentary system and bones are taught in gross anatomy and histology in details, their physiology is not a part of any of the physiology courses examined.

The immune system might be a part of microbiology or it can also be offered as a separate course. However, this study did not investigate this matter. Nevertheless, white blood cells, innate and acquired immunity are often briefly covered in blood physiology, as in the case of Purdue University, USA. On the other hand, metabolism, thermoregulation, and the physiology of bones would deserve more emphasis in physiology courses because they help students understand basis of nutrition, internal medicine, and animal husbandry, and are helpful to soft tissue and bone surgery. The physiology of special senses and sensation has been treated with benign neglect in physiology courses, and if it appears in the course description, it usually focuses only on vision (table 2). Among the usual physiology topics, the emphasis on reproduction could perhaps be a little bit decreased since it will be dealt with in details in theriogenology. All the other topics, however, are reasonable to be taught. For providing a solid basis for many succeeding courses such as pathology, pharmacology, medicine etc., a physiology course must overview all the main topics.

Sixteen out of the 28 schools in the USA (57%) offer a separate neuro course, such as neuroscience, neurobiology, or neurophysiology, regardless of the topics taught in veterinary physiology. In the USA, neurophysiology courses are not usually offered to make up for lack of coverage of the nervous system's physiology in a veterinary physiology course but rather offered independently to cater for the need to expose the students to neuroscience before they take courses in clinical sciences. As an example, Purdue University carved a credit hour from the freshmen' physiology and another credit hour from another course to create a neuroscience course in order to provide students with a basic understanding of nervous system physiology, functional anatomy, and the basis of neurological diseases. The course is taught by a neurologist in the department of veterinary clinical sciences, with a joint appointment with the department of basic medical sciences. Nervous system physiology does not receive additional coverage in the two mammalian physiology courses taught to DVM students at Purdue University, USA.

A separate pathophysiology course, containing pathophysiological issues such as the biology of disease or pathobiology exists only in six schools (21 %). However, pathophysiology is a very important link between physiology and internal medicine. Alternatively, pathophysiology might be incorporated in the core physiology or pathology course(s) or in integrated courses in order to enhance understanding of clinical correlations. However, this subject should be taught in cooperation with clinicians for the maximal clinical relevance. It could be argued that pathobiology is essentially learnt in case-based learning courses such as the Application and Integration (A&I) courses at Purdue University, USA.

This study could not find any classical comparative physiology courses but some physiology courses have comparative features (most common ruminant, equine, and avian physiology, but one of the courses deals with reproduction, zoo animals and fish as well). Comparative aspects are usually embedded in integrated curricula. Avian physiology and zoo medicine are offered as elective courses in some schools. In one school a "comparative biology of disease" course is offered.

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It was observed that not all (only 36%) physiology courses are coupled with labs, especially wet labs. This is quite interesting since physiology is an experimental subject, and lab work is expected to enhance the understanding of difficult physiological concepts. Indeed, some physiological laboratory classes might appear as a part of laboratory courses of other subjects. Labs may include wet labs, team-based learning exercises, and physical exam as related to physiology, and demonstrations and tutorials. It may utilize, as done at Purdue University, USA, ultrasound and endoscopy.

In spite of the fact that tracking is present in most of the USA veterinary colleges, the authors could not find any signs of tracking in the physiology courses syllabi they examined. Choosing a track might be available option for the students after the first half of their veterinary studies.

Additional physiology topics may also be considered based on the interest of students in emerging tracks or areas of veterinary medical practice or research. New topics, covered in some physiology books, include space physiology, deep-sea diving physiology, exercise physiology, avian physiology, wildlife physiology, etc. Tracking emphasis would be also enhanced by offering separate courses on small animal, large animal, or wildlife physiology, etc. Alternatively, physiology courses can be organized according to species, like horse-, ruminants, swine, poultry physiology, etc. Generally, structuring the physiology courses is a matter of concept that affects the whole institutional/curriculum design. Efforts are being made in system-based teaching or integration of anatomy, physiology, and histology in teaching different topics. Several schools are currently looking at their curriculum and making revisions to better integrate basic sciences topics. Some schools are seriously considering transformative teaching including adopting flipped classroom approach where students have more time for learning through activities in the classrooms. Students read textbooks, assignment, watch lectures online before coming to the classroom and engage in interactive sessions. Problem-Based Learning (PBL), Team-Based Learning (TBL), and Peer Instruction (PI) are approaches that use cases and clinical correlations to promote interactive learning, use of information, and retention of what is learnt.

Problem-based learning: case studies and clinical correlations in teaching veterinary physiology

In some USA veterinary schools such as Cornell University College of Veterinary Medicine and the University of Illinois Urbana-Champaign college of veterinary medicine, physiology (and the basic medical sciences) are embedded in a problem-based learning program, which includes clinical cases which discuss the anatomy and physiology of the organism in context of the case in order to demonstrate the clinical relevance of a concept (Newman, 2005). Such a combination may affect the balanced and in-depth coverage of all the physiology topics, and one might ask whether the integration of clinical examples distract students from basic medical sciences or not? Since Cornell University CVM is a top ranked college, their approach and success might answer the above question. The University of Illinois Urbana-Champaign CVM will be another good candidate to test the effects of the combined program. The results of this integration will take a few years to be evaluated.

The University of California-Davis school of veterinary medicine has recently introduced a new, integrated and comparative curriculum for the class of 2015 and beyond. It ranked among the best three veterinary schools in the USA in previous years. The effect of the introduction of the new curriculum might be seen in several years. The Western University of Health Sciences CVM follows an integrated, problem-based curriculum that comprises of self-directed study, case studies, teamwork within an interdisciplinary environment. The college is not highly ranked, however, it might be due to the fact that it is the youngest accredited USA College of veterinary medicine.

Purdue University has created application and integration courses that present students with clinical cases to help them better understand the physiology, anatomy, histology, pharmacology, pathology, and neuroscience. Basic medical sciences concepts are integrated with clinical aspects to help students solve clinical cases. The cases were designed to follow the materials covered in the basic sciences courses. Cases usually take 2-3 weeks to complete. Students work in groups of seven and each group has a tutor. On the other hand, in the fall's freshmen physiology course at Purdue University, three lab periods (2hrs each) have been, for the last eight years (2007-20015), devoted to an adapted version of TBL. TBL, a form of small group learning, is becoming increasingly popular in different disciplines including medical sciences. The topics addressed in the freshmen's veterinary course TBL sessions are: body fluids and fluid therapy; Neuro Muscular Junctions (NMJ); and associated problems clinical cases related to the pancreas. The class is randomly divided into groups, 7 students each. The format used includes giving students a reading assignment which they study before coming to the TBL session, followed by individual quiz ("Individual Readiness Assurance Test or IRAT"), after that the students, as a group, will take the same quiz("group readiness assurance test or GRAT"). When the quiz answers were discussed, each group will show a colored board with their answers. A discussion may follow each question to make sure the students fully understand the physiology basic concepts. A case will then be presented and students will be asked a question that they will answer as a group. Each group will show their answer and discussions will follow. Each case may have four to five questions, and each TBL session may have more than one case. The latter part is called application exercise. In addition, before presenting a fluid therapy case, students will have on their table samples of all types of replacement and maintenance fluids, and potassium used in the school's veterinary hospital. The session will jointly be presented by a physiologist and a clinician. Critical care clinicians are the ones involved in the fluid therapy TBL session, while a neurologist is involved in the NMJ TBL session, and the clinical pathologist is involved in the pancreas TBL session. At the end of each TBL session, students will individually fill an evaluation form. The evaluation requests the assessment of the reading assignment, the quizzes, the case presentation and its questions, as well as working as a group and the individual's contribution to the group. The lab grade is an average of their scores in the individual and group quizzes, the application exercises, and completing the evaluation form. In addition to TBL sessions, there are regular wet labs, and ultrasound demonstration of motility of stomach and intestines, and internal organs. An

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endoscopy of the normal equine gastrointestinal track (with emphasis on the esophagus, stomach and duodenum) is organized and videotapes of clinical cases are presented and discussed.

Veterinary physiology teaching in non-USA veterinary schools

Based on the latest rankings of universities, sixteen non-USA veterinary schools representing all continents (Table 3). Data were collected from the national language or English version of the selected accredited veterinary schools` public websites (American Association of Veterinary Medical Colleges, 2009). Some schools do not provide full or any public information on their professional curriculum or their course contents, just like several schools in the USA. This has resulted in certain limitations during the examination of physiology courses. The authors are aware of the fact that there are considerable structural and organizational differences between the educational systems in the USA and in other countries. However, the Bologna process that resulted in agreements between European countries has enforced that at least the educational structure (BSc–MSc–PhD pyramid) must be similar within the European Union. Nevertheless, it is believed that factors such as topics, credit hours, signs of tracking, presence or absence of neurophysiology, clinical physiology, pathophysiology, and comparative physiology courses, are independent from the above differences. In other words, the study has selected some detached features of the curricula but did not evaluate them as a part of the whole educational system.

All available non-USA curricula contained at least one obligatory (required, core) physiology course. If the physiology course is more than one semester long, the courses are either listed sequentially or named after the sub-topics of physiology. Physiology courses were offered at the beginning of the professional (DVM/BVMS/BSC-Vet/BVM) curriculum, between year 1 Fall/1st semester and year three Spring/6th semester. The course credits/credit hours vary between three and eighteen (most commonly three or eight). The topics of the courses are highly variable. Some courses cover the classical physiology content, while others deal with only some subtopics such as homeostasis, physiology of reproduction and lactation, or the physiology of productive processes. However, this might depend on the prerequisite pre-veterinary physiology courses completed before the student was admitted to a veterinary program in Australia or UK where the educational structure is similar to that in the USA.

Table 3. Selected Veterinary Medicine Schools in different continents that display curriculum on their public websites

Continent	Veterinary School (College or Faculty)
Africa	Cairo University Faculty of Veterinary Medicine, Cairo, Egypt[+] University of Cape Town Faculty of Veterinary Science, cape Town, South Africa[*]
Asia	Bombay Veterinary College Mumbai, Mumbai, India[*] National Taiwan University School of Veterinary Medicine, Taipei, Taiwan[+] Peking University, Peking, China[+] University of Tokyo, Graduate School of Agricultural and Life Sciences Veterinary Medical Science/Animal Resource Science, Tokyo, Japan[+]
Central and South America	National Autonomous University, Mexico City, Mexico[*] University of Buenos Aires Faculty of Veterinary Sciences, Buenos Aires, Argentina[*] University of Chile Faculty of Veterinary Sciences and Livestock, Santiago de Chile, Chile[*] University of Sao Paulo Faculty of Veterinary Medicine and Zootechnics, Sao Paulo, Brasil[*]
Australia and Oceania	University of Sydney Faculty of Veterinary Science, Sydney, Australia[*]
Europe	University of Veterinary Medicine, Budapest, Hungary[*] University of Cambridge Veterinary School Department of Veterinary Medicine, Cambridge, UK[*] University of Glasgow Faculty of Veterinary Medicine, Glasgow, UK[*] University of Veterinary Medicine, Hannover, Germany[*] Utrecht University Faculty of Veterinary Medicine, Utrecht, The Netherlands[+]

[*]The national language version of the school's website was investigated; [+] The English version of the school's website was investigated.

The study investigated what classical topics these physiology curricula cover. Altogether 31 courses were reviewed, of which 26 courses were given with their description (Table 4).From table4 that presents the physiology topics taught in schools/colleges of veterinary medicine outside the USA, and the percentage of courses that address the given topic, the following observations could be made. The most common topics taught are neurophysiology (50%), circulation, reproduction, (42% each), cardiac physiology, respiration, renal physiology, gastrointestinal physiology, endocrinology (35% each), homeostasis, and muscle physiology (31% each). Several physiology courses deal with thermoregulation (23%), blood, metabolism (19%), senses (15%), and acid base balance (12%). Immunophysiology, bone physiology, and the integumentary system appears in the 8% of the available course topic.

Some schools offer unique topics as a part of their core physiology course such as exercise physiology, body growth, physiology of meat, wool and fur production, physiology of milk production, and physiology of behavior in domestic animals. Only one school (6%) offers a separate neurophysiology course (the curriculum is not detailed enough

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so authors do not know whether neurophysiology is a topic in the regular physiology courses in that school). Immunophysiology might be a part of microbiology or the immunology course, or is offered as a separate course. The topics of non-USA physiology courses are more inclusive than those in the USA veterinary schools. Interestingly acid-base balance, metabolism, and thermoregulation are more emphasized in non-USA veterinary schools than in the USA ones. However, USA curricula might incorporate the above topics in other subjects, (e.g. acid-base balance issues might be a part of kidney physiology). It must be remembered that, in contrast to non-USA colleges, USA colleges and schools teach veterinary medical sciences and graduates earn a DVM. A pre-veterinary curriculum is covered in 2-4 years before students are admitted into the DVM program. Therefore, it would be interesting to know that the topics not covered in the DVM physiology courses are not usually covered in the pre-veterinary courses.

In non-USA colleges, the vast majority (92%) of physiology courses are coupled with wet labs as opposed to USA-ones. Laboratory work, however, can enhance understanding of major physiological concepts. As one of our Hungarian students commented on physiology teaching at the university of veterinary medicine, Budapest, Hungary that well-structured and on-line lecture materials make possible learning physiology at home, even without the face to face lectures. However, authors believe, neither lectures nor labs should be thrown away but rather lecture time should be used more creatively for interactive learning such as peer instruction or TBL. Physiology labs are better platforms for building concepts, showing clinical correlations, offering hands-on experiences, and stimulating active learning than classical lectures alone. Similarto USA physiology courses, there are no signs of track-serving physiology courses innon-USA courses. In addition, no core clinical or comparative physiology subjects exist in the investigated and available veterinary curricula neither in USA nor in non-USA colleges.

Separate pathophysiology courses containing pathophysiological issues exist in two (13%) schools outside the USA. From a European perspective, it appears that the US veterinary schools and colleges have more interdisciplinary inclination (e.g. integrating clinical and basic courses) and less coordination (aligning courses, vertical and horizontal integration) in their curriculum. The non-US schools investigated, on the other hand, offer rather classical curricula, where marked distinction can be found between basic, preclinical, and clinical courses. There are only two institutions (University of Cambridge, Cambridge veterinary school, department of veterinary medicine, Cambridge, UK, and University of Sydney, faculty of veterinary science, Sydney, Australia) among the non-USA schools investigated that show certain similarity to the US integrated system. A possible reason for that perhaps is the Anglo-Saxon character of both systems.

However, taking into account the results of university ranking, we must admit that such an integrated curriculum offered by USA veterinary schools are worth consideration by non-USA schools and colleges.

Table 4. Physiology topics taught in schools of veterinary medicine outside the USA and the percentage of courses that address the topic in the veterinary curriculum.

Topic	Percentage of courses that include the topic (total number of courses with topics: 26)
Homeostasis	31%
Blood	19%
Immunophysiology	8%
Cardiac Physiology	35%
Circulation	42%
Respiration	35%
Renal Physiology	35%
Gastrointestinal Physiology	35%
Metabolism	19%
Thermoregulation	23%
Bones	8%
Muscle Physiology	31%
Endocrinology	35%
Reproduction	42%
Nervous System	50%
Senses (vision)	15%
Acid Base Balance	12%
Integumentary System	8%

Calculation of percentages were as follows: We added up the number of physiology courses with available topic lists. (Several curricula do not contain the list of topics taught in a course just the title of the course and credit hours.) There were 51 such courses among USA ones. From the 51 courses, 15 courses (that is the 29% of 51) included neurophysiology as a topic. The same way, only one course (that is the 2% of the total 51 courses) included thermoregulation.

Central European experience (the Budapest example)

The University of Veterinary Medicine, Budapest in Hungary is one of the oldest veterinary schools in Europe following primarily conservative, continental European traditions with a quite rigorous and rigid curriculum offered to the veterinary students. It does not provide possibility for case-based learning in the first four semesters. Instruction begins with solid basic science courses throughout the first two years, followed by preclinical and clinical subjects. One of the reasons is the genuine structural difference between the continental European and the Anglo-Saxon higher

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education prior to the Bologna process and the structure that recently being introduced in Europe. It means that veterinary students start their university education right after they graduate in high school because there is no pre-veterinary requirement before a student starts veterinary studies. Therefore, clinical studies must be introduced in the first semesters when teaching basic courses such as Chemistry, Biochemistry, Physics etc.. This makes the foundational courses in basic sciences curricula rather crucial. In addition, the quite traditional primarily classroom-based teaching methods, and the oral exams which are almost exclusively required, create an atmosphere with very little interaction and flexibility. Students, on the other hand, frequently demand getting involved in clinical aspects as early as possible, while the faculty teaching physiology and other basic subjects has very little or no clinical experience. Integration of PBL in basic sciences to provide meaningful learning is yet to be established. The department of physiology and biochemistry currently provides a two semester-long veterinary physiology course based on a comprehensive curriculum that presents the physiology of each organ system in details (including physiology of the nervous system). The physiology course that two of the authors taught for years in Hungary consists of theoretical and practical parts, 2×2 hours lecture and 1.5 hours laboratory session in a week. Animal experiments are no longer the backbone of the laboratories as the practical classes they are supplemented by computer-based learning in Hungary.

Related factors affecting the success of teaching veterinary physiology

When addressing the issue of facilitating meaningful learning of veterinary physiology, related factors affecting the learning success must be considered. Some of these factors, such as integration and alignment, availability of biochemistry courses in the veterinary curriculum, physiology textbook contents, and teaching platforms, are important to consider.

Integration and alignment

To relay on students' previous knowledge as a foundation for learning physiology, the first step is to know and harmonize the topics and materials of prerequisite subjects with that covered in physiology to avoid unnecessary repetitions and contradictions, and to find out the topics that have not previously been covered. Then by highlighting the contact points between the physiology material and those previous subjects, students could be helped to retrieve what they have learned earlier, and gaps in their knowledge base could be revealed. This integration should be done very watchfully because it can easily break down the thread of logic within the material covered and students would end up in a hardly understandable mixture of contents. However, the advantage of this integration process is that students can flourish in a single learning environment.

Alignment, as well as integration, is highly dependent on the institutional structure and interest and willingness of the faculty. If the departments/units are organized according to the classical basic subjects, a very strong and well-coordinated interdepartmental cooperation can be achieved. The major subjects that can be aligned and/or integrated with physiology are: anatomy, histology, and animal husbandry. For explaining clinical correlations, pathophysiology, clinical pathology, pharmacology, and internal medicine can also be partially incorporated, at least in cased-based learning modules.

The biochemistry factor

In the USA, biochemistry is almost exclusively taught in pre-veterinary programs. However, this study could identify only seven of USA veterinary schools that have biochemistry, physiological chemistry, nutritional chemistry, and animal physiological chemistry courses in their professional curriculum. Biochemistry has been pushed out of the DVM curriculum into the pre-veterinary curriculum to make room for new courses (e.g. Integration and application or neuroscience as in the case of Purdue University, USA). This is quite interesting since knowing and understanding the biochemical processes are indispensable for understanding the majority of physiological concepts. DVM students often struggle to recall and adapt what they learned in general biochemistry courses, which include plant and animal biochemistry, to understanding mammalian biochemistry and physiology concepts. On the other hand, in 13 (81%) of non-USA universities, biochemistry is included in the veterinary curriculum. In the USA DVM professional curricula. Biochemistry courses reviewed focus on the basic biochemical structures and mechanisms and intermediary metabolism. Non-USA veterinary biochemistry courses have a much wider scope, spanning homeostasis, enzymes, ribonucleotides, intermediary metabolism, DNA and gene expression, metabolism of ruminants, and vitamins. At the University of Veterinary Medicine, Budapest, Hungary, veterinary students learning basic biochemistry have two semesters to develop a solid foundation for further studies in pharmacology, toxicology, and animal nutrition.

Physiology textbook topics

Physiology materials are traditionally textbook-based although electronic materials are becoming more and more available as well. The authors, however, believe that it is important to compare the contents of the most-known and available textbooks required in the physiology courses. This study investigated the contents of 7 commercial, widely used physiology textbooks (Colville and Bassert 2002; Sjastaad et al., 2003; Berne et al., 2004; Reece, 2004; Reece, 2005; Cunningham and Klein, 2007 and Hall, 2010) written in English (Table 5A, 5B, and 5C).The order of topics within each textbook is not the same and the depth of coverage of different chapters vary. Chapters included: Introduction, cellular, molecular, and chemical bases of physiological function, and the nervous system are usually discussed in the first chapters. However, some books put muscle, blood, respiratory system, kidneys, acid-base regulation, membrane physiology, and body fluids in the first or second chapter. In the middle of the book, cardiovascular physiology is found in most of the books (5 out 7 books, 71%). Among the last chapters, endocrinology

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(in 2 books, 29%), reproduction, clinical correlations, thermoregulation, biogenesis and growth, neuromuscular system, avian anatomy and physiology are found.

Table 5A. Topics covered by seven veterinary textbooks widely used in the world

Sections/units (from all books)	Cunningham JG and Klein BG (eds.).Text book of Veterinary Physiology, 4th edition (11 sections)	Hall JE (ed.) Guyton and Hall Textbook of Medical Physiology, 12th edition (15 sections)	Berne RM, Levy MN, Koeppen BM and Stanton BA (eds.). Berne and Levy Physiology, 5th edition (8 sections)
Basic Chemistry and Physics	–	–	–
Cells and Tissues	X (1)	X (1)	X (1)
The Nervous System	X (2)	X (2, 9, 10, 11)	X (2)
The Senses	–	X (9, 10)	–
The Endocrine System	X (5)	X (14)	X (8)
Bone Tissue and Mineral Metabolism	–	–	–
Muscles	–	X (2, 11)	X (3)
Blood and its Function	–	X (6)	–
Immunology	X (10)	X (6)	–
The Cardiovascular System	X (3)	X (3, 4)	X (4)
The Respiratory System	X (8)	X (7)	X (5)
The Kidneys and the Urinary Tract	X (7)	X (5)	X (7)
Acid-Base Regulation	–	–	–
The Digestive System	X (4)	X (12)	X (6)
Metabolism of Carbohydrates, Proteins, and Lipids	X (4)	X (15)	–
The Skin	–	–	–
Regulation of Body Temperature	–	X (15)	–
Reproduction	X (6)	X (14)	–
Lactation	X (6)	–	–
Biogenesis and Growth	–	–	–
Homeostasis	X (9)	–	–
Clinical Correlations	X (11)	–	–
Introduction to Physiology	–	X (1)	–
Membrane Physiology	–	X (2)	–
Body Fluids	–	X (5)	–
Blood Coagulation		X (6)	–
Aviation, Space, and Deep-Sea Diving Physiology	–	X (8)	–
Sports Physiology	–	X (13)	–
Joints and Synovial Fluid	–	–	–
Lymph	–	–	–
Avian Anatomy and Physiology	–	–	–

X: indicates the presence of the section/unit; (number): indicates number of chapter that deals with the section/unit

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Table 5B. Topics covered by seven veterinary textbooks widely used in the world.

Sections/units (from all books)	Sjastaad OV, Hove K and Sand O. Physiology of Domestic Animals, 1st edition (20 sections)	Reece WO (ed.). Duke's Physiology of Domestic Animals, 12th edition (6 sections)
Basic Chemistry and Physics	X (1)	–
Cells and Tissues	X (2)	–
The Nervous System	X (3)	X (6)
The Senses	X (4)	X (6)
The Endocrine System	X (5)	X (5)
Bone Tissue and Mineral Metabolism	X (6)	–
Muscles	X (7)	X (6)
Blood and its Function	X (8)	X (1)
Immunology	X (9)	–
The Cardiovascular System	X (10)	X (3)
The Respiratory System	X (11)	X (2)
The Kidneys and the Urinary Tract	X (12)	X (2)
Acid-Base Regulation	X (13)	X (2)
The Digestive System	X (14)	X (4)
Metabolism of Carbohydrates, Proteins, and Lipids	X (15)	X (4)
The Skin	X (16)	–
Regulation of Body Temperature	X (17)	X (6)
Reproduction	X (18)	X (5)
Lactation	X (19)	X (5)
Biogenesis and Growth	X (20)	–
Homeostasis	–	–
Clinical Correlations	–	–
Introduction to Physiology	–	–
Membrane Physiology	–	–
Body Fluids	–	X (1)
Blood Coagulation	–	–
Aviation, Space, and Deep-Sea Diving Physiology	–	–
Sports Physiology	–	–
Joints and Synovial Fluid	–	–
Lymph	–	–
Avian Anatomy and Physiology	–	–

X: indicates the presence of the section/unit; (number): indicates number of chapter that deals with the section/unit

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Table 5C. Topics covered by seven veterinary textbooks widely used in the world

Sections/units (from all books)	Reece WO. Functional Anatomy and Physiology of Domestic Animals. 3rd edition (16 sections)	Colville T and Bassert JM (eds.).Clinical Anatomy and Physiology for Veterinary Technicians. 21st edition (16 sections)
Basic Chemistry and Physics	–	–
Cells and Tissues	–	X (2, 3)
The Nervous System	X (4)	X (6)
The Senses	X (5)	X (12)
The Endocrine System	X (16)	X (13)
Bone Tissue and Mineral Metabolism	X (6)	X (4)
Muscles	X (7)	X (11)
Blood and its Function	X (3)	X (8)
Immunology	–	X (8)
The Cardiovascular System	X (8)	X (7)
The Respiratory System	X (9)	X (9)
The Kidneys and the Urinary Tract	X (10)	X (14)
Acid-Base Regulation	–	–
The Digestive System	X (11)	X (10)
Metabolism of Carbohydrates, Proteins, and Lipids	–	–
The Skin	–	X (5)
Regulation of Body Temperature	X (12)	–
Reproduction	X (13, 14)	X (15)
Lactation	X (15)	–
Biogenesis and Growth	–	–
Homeostasis	–	–
Clinical Correlations	–	–
Introduction to Physiology	X (1)	X (1)
Membrane Physiology	–	–
Body Fluids	X (2)	–
Blood Coagulation	–	–
Aviation, Space, and Deep-Sea Diving Physiology	–	–
Sports Physiology	–	–
Joints and Synovial Fluid	X (6)	–
Lymph	–	X (8)
Avian Anatomy and Physiology	–	X (16)

X: indicates the presence of the section/unit; (number): indicates number of chapter that deals with the section/unit

DISCUSSION

Physiology teaching platforms

The traditional ways of teaching physiology are lectures and practical classes (physiology labs). Physiology laboratories are also called laboratory-based practical classes. These practical classes are designed to illustrate the theoretical concepts covered in lectures. Labs are relatively expensive to run due to the high costs of reagents and other materials, and the high staff to student ratio (Ryan et al., 2009). Another serious concern is the use of live animals in physiology labs and the invasive experiments that have been conducted. Wet labs that involve sacrificing rats, guinea pigs or rabbits are significantly being reduced. Yet hands-on experience is indispensable to improve learning outcomes (Salomäki et al., 2014).

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Although traditional lectures are widely used in teaching physiology (Keegan et al., 2012; Warren and Donnon, 2013), interactive classrooms are being recognized as important tools for meaningful learning. Interactive classrooms reflect the dynamic interaction characteristic of clinic training and related work. It has a positive impact on the students` thinking, problem-solving skills, and motivation (Brown, 2004). The characteristics of an interactive classroom, according to Brown (2004) are summarized as follows: "Students are attentive and intellectually committed to the lecture; students ask questions frequently; the lecturer tries to motivate the students and make them reach their maximum potential but expects excellence; students often prepare for the lecture in advance; students answer the lecturer's questions freely and thoughtfully; students are expected to attend the class and to arrive on time; students must have willingness for learning; constructive criticism appears on both sides; students often ask for additional material and more challenge; veterinary medicine focused concepts and principles have a privilege over details; exams are challenging and relatively difficult, including so-called open-ended questions such as essays; students may obtain old exams and asking the students for posing questions at the end of a lecture."

Since a course must suit the Instructional Design (ID) of the school's curriculum, changing teaching strategies within a course cannot be done without changing/reforming the school's ID and curriculum expected outcomes. However, there are ways to engage students even in a traditional classroom. This includes flipped classroom learning activities, and Peer Instruction (PI). In these two learning strategies students study the lecture material at home and come to school for engaging sessions focused on applications and solving problems. Students usually work in groups as small as two students per group as in PI. In PI, students try first to answer the question alone, select the answer and send using clickers. If < 70% of the students answer correctly, then the student discusses the question with his/her peer and agrees on and answer and votes again. More weight in the grades is given to the peers answers.

The very diverse composition of veterinary professional curricula and the recent curricular reforms makes curricula and courses almost incomparable. Our results have their limitations. However, we believe that this study succeeded in revealing the main trends, and provided a good overview that may help in future comparisons.

Instructional design

According to Reagan (2007), Instructional design (ID) is the "arrangement of learning events within an educational context"), in other words, ID is about "how to help people learn"(Mayer, 2010), while decreasing "extraneous cognitive load" (Khalil et al., 2010). ID can either refer to the developmental process of a course or the organization of a learning event. Teaching faculty who has face to face classroom activities are already applying ID. However, in an online educational environment, there are no ready and consistent teaching protocols. Nevertheless, ID can take place on various platforms, and can start with PowerPoint slides made available on the institutional web sites. PowerPoint is good for including both text and pictures; however, it is not practical for presenting multimedia elements. The website can be designed using HTM or Java languages; however, these require more IT skills. Therefore, the learning or course management systems (Blackboard, Moodle, WebCT, Angel, Desire to Learn etc.) are increasingly used for online instruction ((Bernardo and Malinowski, 2005; Ragan, 2007). On the other hand, we must not forget about social media that has got key role in the life of the millennial generation (Coe et al., 2012). The use of social media in teaching on a Facebook group model was investigated (Kustritz, 2013). Ragan (2007) found that ID has a leading role in the so-called learning effect and the usefulness of incorporating social media in teaching.

Teaching faculty must, therefore, focus on the five primary elements of a teaching and learning: "how content is delivered, how instruction of that content occurs, the role of interactions and communications between class participants, student activities, and evaluation and assessment strategies" (Ragan, 2007). In addition, faculty must avoid the common pitfalls of e-learning and the interesting IDs that some online courses use that tend not to contribute to learning. Bogert et al. (2016) reported that although satisfaction of veterinary students increased with interactive learning their performance in cranial nerves anatomy exams decreased.

Understandably, several teaching tools can be applied in an ID for online courses: synchronous meetings, document sharing, participant video imaging, voiceover internet protocol, group collaboration software, virtual office hours, etc. Ragan, 2007). It goes without saying that, for a successful ID, the natural abilities of the students should also be known and considered. The vast majority of veterinary students use his/her design memory as a primary learning channel while tonal (voice) learning is the least preferred one (Brown et al., 2011). At the North Carolina State university college of veterinary medicine, the learning style of a cohort of 150 veterinary students was investigated; and it was concluded that veterinary students were similar in their learning styles to engineering and health sciences students (Neel and Grindem, 2010).

Practical classes (laboratory sections)

Physiology practical classes play an important role in the successful teaching and learning. Pre-clinical veterinary education surveys revealed a positive correlation between deep learning strategies and the application of practical classes. On the contrary, a negative correlation was found between practical teaching and surface learning (Ryan et al., 2009).

During a laboratory class, unexpected results are obtained and their evaluation mimics real science (Pelaez and Gonzalez, 2002). Practical classes can encourage discovery, critical thinking, and scientific reasoning. The national research committee's BIO2010 report noted that "Students should be taught the way scientists think about the world and how they analyze a scientific problem in particular"(Clase et al., 2008). This enhances another important skill which is scientific literacy (Pelaez and Gonzalez, 2002).When the national research council's committee on high school labs allocated the desirable goals of a typical instructional lab, Clase et al. (2008) believe that these goals can also apply to

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veterinary medical education, and to physiology learning in particular: Development of interest in the science of living things and in learning it, the mastery of subject matter, development of scientific reasoning skills and familiarity with how to design experiments, ability to collect and analyze data, the development of teamwork competences, and recognition of the mechanisms of how the complex systems body work. In addition, Clase et al. (2008) found that good undergraduate research laboratories have a several positive outcomes including as increasing interest in opting for a research career and to start graduate studies, increased science knowledge, and acquisition of hands on experiences and ability to work cooperatively in a team, and the motivation to dig deeper in the scientific phenomenon and increase one's understanding of scientific principles. It would be interesting to evaluate the relationship between physiology laboratory practicals and clinical/research skills of veterinary graduates.

CONCLUSION

Although all curricula teach physiology, physiology teaching vary among institutions in the USA and outside the USA. Instructors in veterinary schools and colleges in USA and outside the USA are faced with the dilemma whether they should teach physiology to provide factual knowledge or facilitate its learning by their students. To get a better perspective, the above dichotomy in teaching physiology should be looked at globally. The rather traditional institutions still argue about the necessity of classroom-based teaching based on extensive factual knowledge, while others are introducing different ways to facilitate meaningful learning, where student/faculty interaction and the use of technology receives more emphasis. Another school may prefer passing knowledge to students (knowledge transfer) as a process that would help in adding to existing knowledge and retention of information. Without arguing on the superiority of one over the other, one must realize that rapidly changing IT and students' attitudes towards technology cannot be ignored. Moreover, the globally available advantages of the latest high-tech developments should become an integral part of knowledge providing institutions, and the knowledge transfer process. This certainly requires a paradigm shift in academia. While applying the results of new technology in higher education does not necessarily mean a higher quality instruction, ignoring these opportunities will certainly put the students of such institutions at an unfavorable situation. Here again, global competition is going to sort out which of the models are worthwhile to follow.

When strategic planning for teaching and learning of the revised curricula is on the table, one must take into consideration that new learning systems must be designed for a generation who is not only IT literate, but almost IT addicted (not in any negative sense). Therefore, any veterinary institution determined to fulfill its mission of serving society through teaching the next generation of professionals must reconsider its relationship with the new technologies, and focus on intensive faculty development as well as on redesigning its curriculum that allows for more engagement of students in the learning process. The globally changing educational environment must be taken into consideration in future strategic planning within knowledge-providing institutions of higher learning, and must evoke intensive, information technology-conscious schools/colleges, and faculty development. While applying the results of new technology in higher education does not necessarily mean a higher quality instruction, ignoring these opportunities will certainly put the students of such institutions in an unfavorable situation. Physiology education can benefit from the new technologies and approaches that would make learning more interactive and fun. Case-based learning, including the use of team-based learning, has been credited for improving learning in medical schools, and can certainly enhance learning in veterinary schools and colleges. The same can be said about peer instruction and the flipped classroom. In addition, the use of e-learning management systems would facilitate the process of learning by making the materials available for students before they come to class, and facilitate online interaction with their peers and with the instructor. Further, aligning and integrating the teaching of basic medical sciences and providing clinical correlations that encourage the application of physiological concepts to solve clinical cases would facilitate students' engagement and would improve their learning and competences.

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Competing interests

The authors declare that there are no significant personnel, professional or financial competing interest that might have influenced the presentation of the results of the study described in this manuscript.

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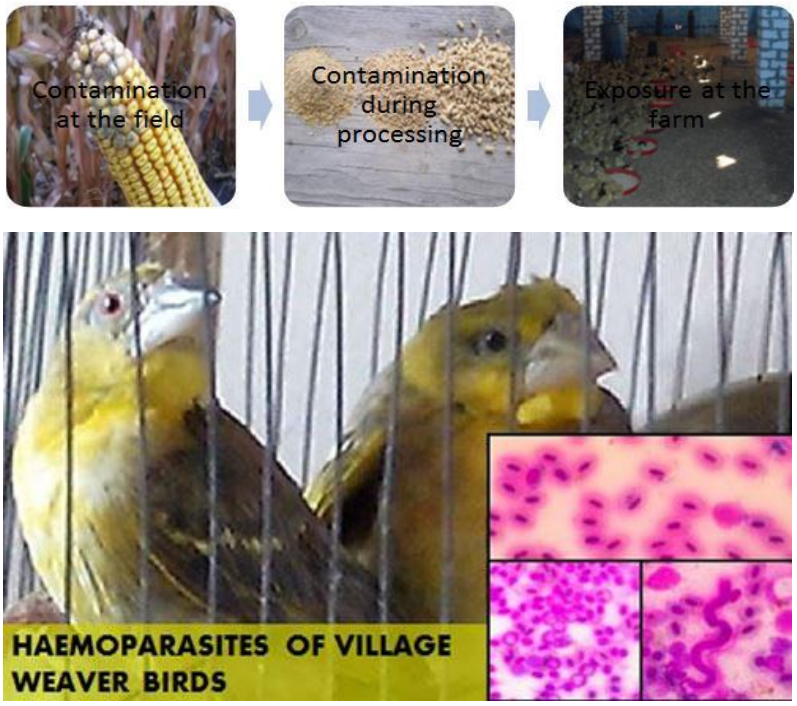

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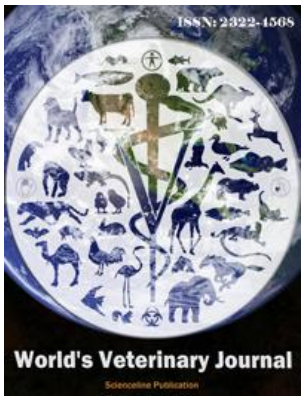

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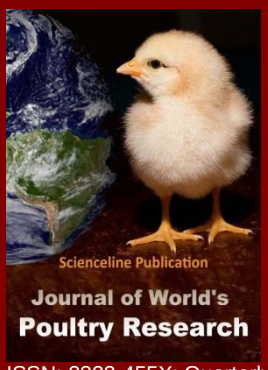

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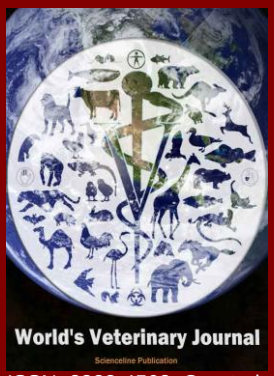

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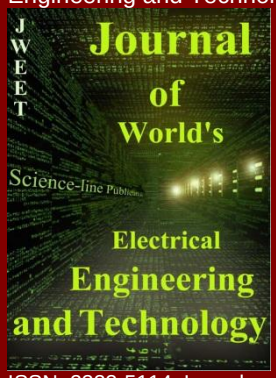

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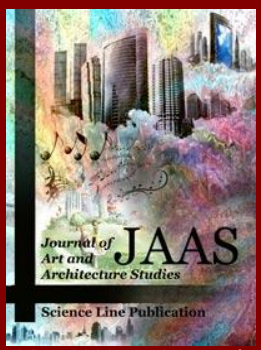

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