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The Acknowledgments of people, grants, funds, etc should be brief.

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Cole (2000), Steddy et al. (2003), (Kelebeni, 1983), (Bane and Jake, 1992), (Chege, 1998; Cohen, 1987a,b;Tristan, 1993,1995), (Kumasi et al., 2001)

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Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds), Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International. pp 181-190.

Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for Striga suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

Furmaga EM (1993). Pharmacist management of a hyperlipidemia clinic. Am. J. Hosp. Pharm. 50: 91-95

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Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

On-farm diagnosis of contagious bovine pleuropneumonia in nomadic herds using latex agglutination test (LAT)

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Three outbreaks of contagious bovine pleuropneumonia (CBPP) in Kafur Local Government Area of Katsina State, involving a total of 250 animals were reported. All the animals were examined thoroughly and based on the clinical signs exhibited, 120 (group A, n= 51, B, n=29 and C, n=40) animals were selected for latex agglutination test (LAT), using serum from each of the animal. The results indicate infection rates of 11.8% (6/51) in adult animals and 19.6% (10/51) in calves in farm A, while in farm B the infection rate were 27.6% (6/29) for the adult animals and 13.8% (4/29) for calves. While in farm C, 15.0% (6/40) of the adult animals and 17.5% (7/40) of the calves are positive, as demonstrated by strong (+++) agglutination in cell 1 (Plate 2). Overall infection rate of 16.7 and 17.5% was observed for adult and calves respectively. Although this diagnostic test LAT is not new, this is the first time it's being conducted in the area of study for the diagnosis of CBPP.

Key words: *Mycoplasma mycoides* sub sp. *Mycoides,* nomadic cattle, latex agglutination test.

INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is a highly contagious disease of cattle caused by *Mycoplasma mycoides* subspecies *mycoides* small colony (MmmSc) (Osiyemi, 1981; Provost et al., 1987; Taylor et al., 1992; Terlaak et al., 1992). The disease is characterized clinically by severe coughing, weakness, emaciation and sometimes by elevated body temperature (Provost et al., 1987; Egwu et al., 1996). Transmission occurs from direct and repeated contacts between sick and healthy animals. The first incidence of the disease in Nigeria was recorded in 1924 when reliable records were first available (Foluso, 2004). As at today the disease is endemic in Nigeria, West, Central, East and parts of Southern Africa (Tambi et al., 2006). In Nigeria, "alive with the disease'' attitude has prevailed in the last few years, farmers hardly report cases but resort to treatment with antibiotics like any other bacteria disease (Chima, 1999, 2001). Inadequate funding of cattle annual mass vaccination program, lack of a rapid on farm screening test to aid sero–monitoring, and refusal of some farmers to allow vaccination of their animals due to post-vaccinal cellulitis, have largely contributed to the spread of the disease rendering data on infection of the disease within the country inaccurate and subjective (Chima, 2001; Molokwu, 2003). Diagnosis of CBPP in most developing countries of Africa is

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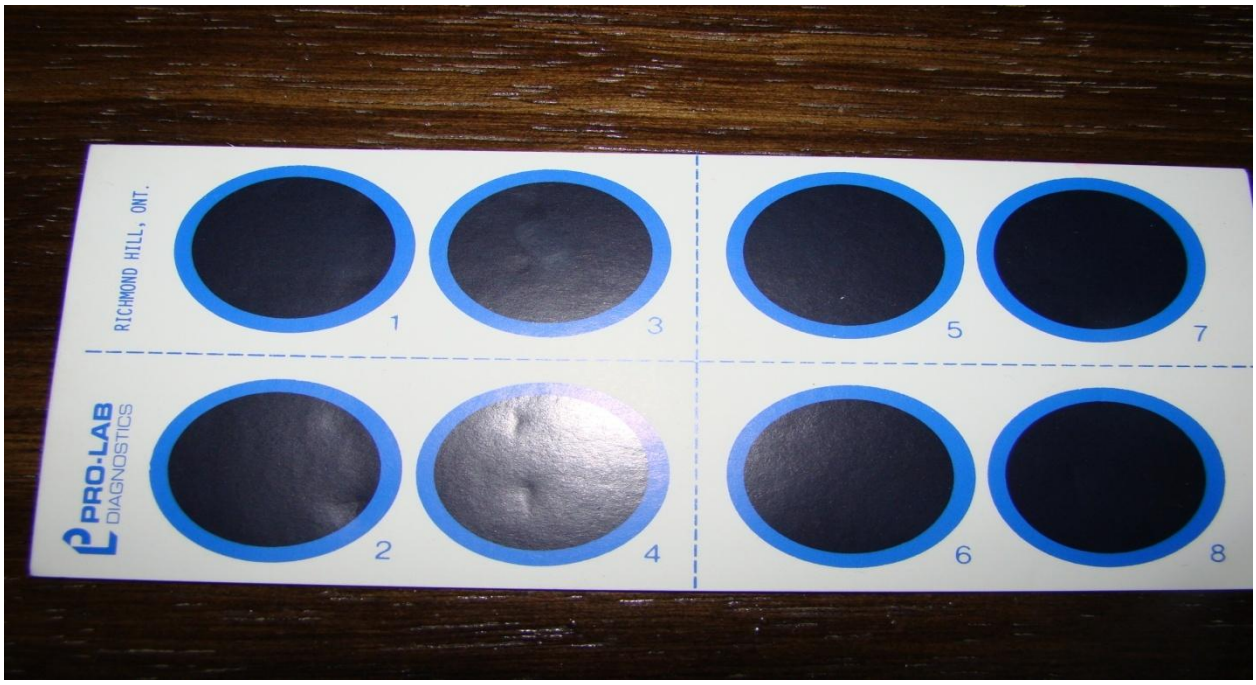

Plate 1. Showing the individual cells for the agglutination.

Presently based on culture and isolation of the causal agent (which is fastidious and slow growing), serology. And post-mortem (PM) examination of lungs of affected animals. Though the compliment-fixation test (CFT) is commonly used as a diagnostic method in most CBPP-endemic countries of Africa, its sensitivity in detecting chronically affected animals is low (Provost et al., 1987; Nicholas et al., 1996). The inability of CFT to discriminate between natural and vaccinal exposures in animals has led to a greater reliance on PM examination of lung lesions for monitoring and surveillance of CBPP in Nigeria.The current Office International des Epizooties-prescribed test for the diagnosis of CBPP is the modified CFT (Camphell, A.D., and Turner, 1953; OIE, 2002). Although the test is highly specific, it is relatively expensive to perform, it is slow, and it requires trained personnel and laboratory facilities. In addition, it is less effective at diagnosing animals in the early stages of the disease or of animal with chronic lesions (OIE, 2002). A number of more modern tests have recently been described, including biochemical (Rice et al., 2000), indirect and competitive enzyme-linked immunosorbent assay (ELISA) (Le Goff Thiaucourt, 1998; Nicholas et al., 1996), immunoblotting (Nicholas et al., 1996; Regalla et al., 2000), and PCR (Bashiruddin et al., 1994; Miserez et al., 1997).

Efforts currently being made in Nigeria for the eradication of CBPP involve vaccination backed up with abattoir surveillance. The test and slaughter policy recommended by the Office of Internal Epizootics (OIE) for seropositive animals is currently not practicable because of the cost involved in the payment of compensation to livestock owners. Presently, in Nigeria, the extent and pattern of CBPP prevalence is largely unknown. The aim of this paper is to report on-field-diagnosis of CBPP using latex agglutination test (LAT). LAT (Developed by Veterinary Laboratories Agency, New Haw, Addlestone. Surrey, KT15 3NB United Kingdom). This test is a rapid test for the diagnosis of CBPP. It has the advantage of not requiring electricity so quite suitable for third world countries. It is believed that this test can be beneficial in determining the extent of CBPP in Nigeria.

MATERIALS AND METHODS

Three herds A, B and C with 99, 58 and 93 Bunaji breeds of cattle respectfully located in Kafur Local Government Area (LGA) of Katsina State, Northern Nigeria were used for these studies following a reported outbreak. One hundred twenty cattle that showed clinical signs similar to CBPP (fever mucopurulent nasal discharge, shallow, grunting respiration, painful coughing, arched back, head extended towards the direction of wind with abducted forelimbs and exercise intolerance) were clinically examined out of a total of 250 cattle in the three herd. Five milliliters of blood were collected from 120 cattle of mixed sex, and placed in a vacutainers without anticoagulant for serum extraction, the separated sera were screened on-farm for the presence of antibodies to *Mycoplasma mycoides* subspecies *mycoides* small colony (MmmSC) using LAT–PA6223 (LAT) as described by Ayling et al. (1999). Plate 1 shows the agglutination slides.

Recording and interpretation of results

Slide test result will be recorded as follows after three minutes:

Table 1. Detection of antibodies to MmmSC in Nomadic Cattle using LAT in Kafur L.G.A., Katsina State.

Herd	Herd size	No. screened	Calves	Adults	No.+ve (Calves%)	No. +ve (Adults%)
20 A	99	51	17	34	10(19.6)	6(11.8)
B	58	29	10	19	4(13.8)	8(27.6)
C	93	40	15	25	7(17.5)	6(15.0)
Total	250	120	42	78	21	

Calves = 3-12 Months, Adults =>12 Months.

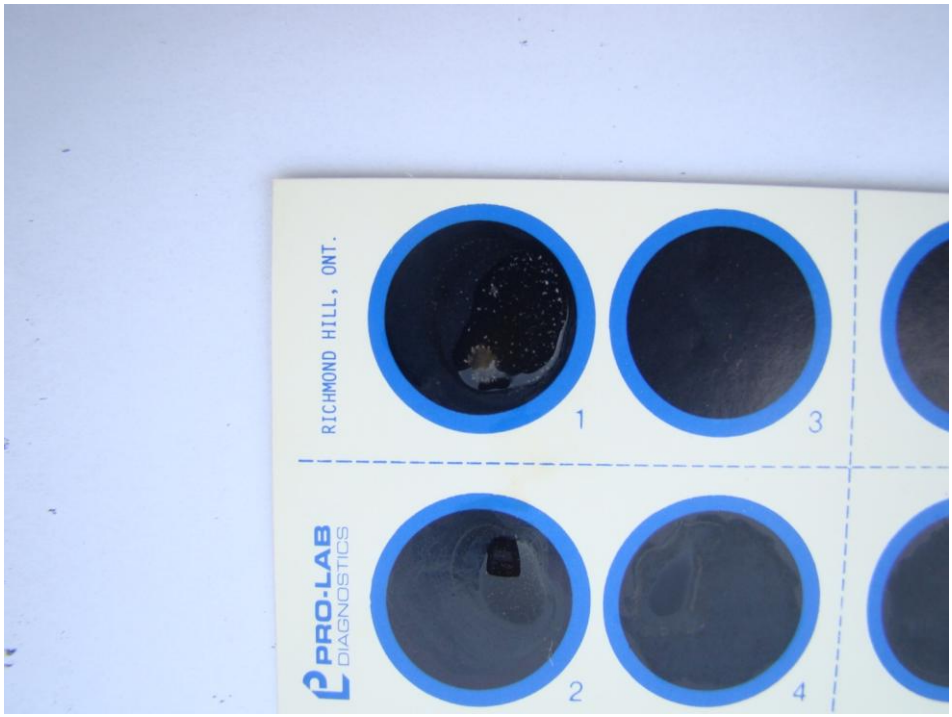

Plate 2. Showing positive (+++) strong clumping of latex beads in cell 1.

i) Positive (+++). Strong clumping of latex beads. Agglutination should begin within one minute.
ii) Positive (++). Clear agglutination of the latex beads. Agglutination should begin within one and two minute.
iii) Positive (+). Fine agglutination of the latex beads between two and three minutes.
iv) Negative (-). No agglutination within three minutes (Plate 1).

RESULTS

Fifty one cattle were screened in herd A, made up of 34 adults and 17 calves, 11.8% (6/51) of the Adult animals were positive while 19.6% (10/51) of calves were also positive for contagious bovine pleuropneumonia (CBPP) using LAT. In herd B, a total of 29 cattle, consisting of 19 adult animals and 10 calves were screened for CBPP antigen out of which 27.6% (8/29) adults animals were positive, and 13.8% (4/29) of calves were also positive. In herd C, out of a herd of 40 cattle (25 adult and 15 calves) screened 15.0% (6/40) of the adults were positive and 17.5% (7/40) of the calves tested positive (Table 1). The agglutination observed in cell 1 (Plate 2) is due the fact that when the latex beads coated with capsular polysaccharide purified from *Mycoplasma macoides* subspecies *mycoides* cells are mixed with blood or serum from animal suspected or suffering CBPP, antibodies recognizing the capsular polysaccharide will bind and cross-link the latex particles causing agglutination (Plate 2).

DISCUSSION

M. mycoides subsp. *mycoides* small colony is the etiological agent for a potentially lethal lung disease of cattle called CBPP (Thomson, 2005). The high economic impact of this disease on the cattle industry necessitates the development of rapid, sensitive and specific diagnostic assays. Ayling et al. (1999) developed the LAT for rapid diagnosis of CBPP on the field. The dynamics of

infection of CBPP in herds of cattle in endemic areas appears to be more complicated than would be presumed for a directly transmitted infection with a single host species. In this study an overall infection rates of 16.7% in adults and 17.5% in calves out of 120 animals screened indicates probably a low infection rates for a disease that is transmitted through contact, ingestion and aerosol. This report is at variant with that of McDermott et al. (1987) and Dasho (2001) were they reported serological values of 8.1 to 9.2% infection rates in southern Sudan and Ethiopia. The low values observed in this study could also be attributed to the inability of the test LAT to diagnose convalescent animals, this is partly because high level of circulating capsular polysaccharide antigen can lead to false-negative due to antibody masking effects (March et al., 2003). This study observed a slight increase though not statistically significant ($P<0.005$) in the susceptibility of calves to CBPP infection over adult, this findings is in agreement with the report of Masiga and Windsor (1978) where they reported that during an epidemic, the morbidity and mortality rates were higher in calves than adult animals, and that where the infection had been present for some time, morbidity was higher in adult animals but mortality was higher in calves. In naïve populations, calves do not appear to possess a higher level of resistance than older animals (Thiaucourt et al., 2004).

The continued presence of this important disease in Nigeria is attributed to diminished control due to the incomplete and irregular vaccination programme over the years as well as steady illegal introduction of infected cattle into these areas across the control barriers (particularly through transhumance and nomadism). Moreover, the presence in some herds of carrier animals, which might not be detected clinically or serologically could enhance the maintenance of the disease in these areas (Aliyu et al., 2000), therefore, in a third world country like Nigeria, the test LAT will allow for rapid and inexpensive primary herd screening prior to confirmatory laboratory diagnosis (using PCR or ELISA) and this will help in early recognition of the disease and allows appropriate control measures to be swiftly implemented, for example, quarantine or movement restriction.

ACKNOWLEDGEMENT

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Full Length Research Paper

Histomorphology of the digestive system of red deer (*Cervus elaphus* L.) in Latvia

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The present study described the histo-morphological and immuno-histochemical characteristics of the digestive and neuroendocrine systems of captive wild domesticated red deer (*Cervus elaphus*) in Latvia. Results revealed that four (4) out of the five (5) animals studied showed keratinization of the rumen mucosa, with patchy loci of parakeratotic basal cells in the epithelium of the rumen wall. Neutrophilic leukocytes, lymphocytes and rare macrophages had infiltrated the mucosa and muscle layers of the rumen wall. Vacuolization of gangliocytes was seen in the intermuscular nerve plexus. It was observed that red deer's small and large intestines were more innerved than the rumen, despite the variations in some parts of the enveloped mucosa. Small intestines were characterized by abundant expression of serotonin. Focal appearance of neurofilaments (NF)-containing nerve fibers was characteristic of the tissues of the rumen in the direction of the large intestine. Prominent apoptosis was also seen in the rumen. Moderate hepatocyte activity, a small number of apoptotic cells in the *vena centralis* area, with simultaneously distinct expression of interleukins and limited expression of degeneration enzymes was observed in the liver of the animals studied.

Key words: Diffuse neuroendocrine system, digestive tract, red deer, morphology, Latvia.

INTRODUCTION

The digestive tract is an important system in living organisms, and plays a vital role in food processing and absorption (Hill et al., 2008). In food animals the digestive process ensures safety and healthiness of the animal product consumed by humans. Investigation of digestive tract of deer is related to health control issues for the protection of their gene pool in Latvia (Skriba, 2011).

Red deer is a ruminant belonging to herbivorous animals which prefer leguminous food: bushes, hard grass, weeds and tree scions as well as grass and fodder (Fulbright and Ortega, 2006). Fulbright and Ortega (2006) have classified deer as "intertype" animals focusing on leguminous food unlike other herbivorous animals, choosing only herbs or solely grass. Deer eats legumes, as well as hard to digest plants and their parts containing starch, protein and fat rather than grass. It is able to digest cellulose in plant cell walls, although this ability is restricted.

Feed processing starts in the rumen (the largest structure in the digestive tract of deer) with consecutive passage through the small and large intestines. The process in the whole digestive tract is closely connected with the functioning of the diffuse neuro-endocrine system (DNES), described by Ceccarelli et al. (1995) in fallow deer *(Dama dama)* and Franco et al. (2004) as well as Masot et al. (2007) in red deer *(Cervus elaphus)*. Mallard et al. (1998) and Zabielski et al. (1998) suggested impact of neuropeptide-containing

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innervations on the intrauterine development of the digestive system, breaking down and absorption of the nutrients, contractions of the walls of hollow organs of the digestive system, as well as local immune responses. Bueno and Fioramonti (1994) found correlations between the changes of neuro-peptide innervations and metabolic disorders of the body. Although, Münnich et al. (2008) have studied the role of DNES in the digestive tract of based on feed selection, there is scarcity of information on the morpho-functional structure of the digestive tract of wild red deer under captivity in Latvia.

Wholesome investigations of the deer digestive tract require concurrent studies of the homeostasis of the liver cells in this animal, as liver is the biochemical laboratory of the body where various processes such as essential metabolic functions, protein synthesis, detoxification of the body, bile secretion, etc occur (Barrett, 2011). Function of the digestive tract in the body is closely related to function of the liver: the absorbed nutrients are carried to the liver where they are processed and stored (Barrett, 2011). According to Junqueira and Carneiro (2003), liver is a coordinator between the digestive tract and blood. Histological studies by Soveri (1993) revealed that in winter, when a deer under natural circumstances is forcefully starved, the parenchyma of its liver changes due to reduction in the size of the liver cells and sinusoidal cavities. According to Knolle and Gerken (2000) and Kmieč (2001), mutual functioning of separate cells of the liver tissues is strictly connected in both healthy and sick deer.

Previous histological studies in red deer (Hartwig and Hartwig, 1975; Hals and Cato Olsen, 1984; Soveri, 1993; Shabadash and Zelikina, 2003), revealed scanty or no data on morpho-functional structures of the digestive system of wild captive species of in Latvia. Studies are needed in order to justify conditions of captivity to comply with the physiological requirements of the bodies of red deer, ensuring their well-being and high quality of the acquired meat. The main goal of our study was to carry out histological and immunohistochemical (IHC) investigations of the morphological structures and neuroendocrine system of the digestive system of captive red deer (*C. elaphus* L.).

MATERIALS AND METHODS

Animals and tissue preparation

The study included five clinically healthy 18 months old red deer (*C. elaphus* L.) farmed in Sigulda municipality of Latvia, in captivity. Each of the animals over the winter period had received daily feed ration of 7 kg of haylage and kg of rolled grain; containing 2.63 kg of dry matter and 121 g (per kg of dry matter) crude protein. The amounts were estimated by means of "RationPro MVP" (USA) software on the bases of the feeding-stuff analysis. Additionally, animals fed themselves on bushes growing within their fence as supplements.

Animals were slaughtered at the end of the experiment, and 0.5-1 × 1 cm tissue samples were taken from the back part of *saccus caecus ventralis* of rumen, the medium sections of *duodenum* and *colon* and from the liver. Rumen and intestinal samples were rinsed with warm 0.9% NaCl solution. Subsequently, all the tissue samples were placed in 10% neutral formalin at room temperature for 48 h and then dehydrated in tissue processor (TISSUE-TEKII) and embedded in paraffin blocks. Tissues were processed according to standard procedures (Kiernan, 2008; Carson and Hladik, 2009).

For general histological assessment, the tissue samples were de-paraffinized with xylene and ethyl alcohol, and then stained as described by (Carson and Hladik, 2009).

Immunohistochemistry

Samples for immunohistochemical (IHC) investigation were fixed for twenty-four hours in a mixture consisting of 2% formaldehyde, 0.2% picric acid and 0.1 M phosphate buffer (pH 7.2) (Aughey and Frye, 2001). Then the pieces of tissues were rinsed for 12 h in a thyroid buffer containing 10% sucrose, embedded in paraffin and cut with a microtome in 8 µm thick layers. Prior to immune-staining, sections were de-paraffinized and rehydrated. Sections were processed in a microwave for 20 min in 4% citrate buffer (pH 10), quenched for 10 min with 3% H_2O_2 to block endogenous peroxidase activity, rinsed in a phosphate–buffered saline (pH 7.4), pretreated with a non-immune goat serum for 10 min to block the nonspecific antibody binding and then incubated for 2 h with the primary antibodies (Hsu et al, 1981).

The primary antibodies used in the IHC investigation of gastrointestinal samples were protein gene peptide 9.5 (PGP 9.5) (rabbit polyclonal, working dilution 1:600, code Z5116, DACO, Denmark), serotonin (mouse monoclonal, code M758, working dilution 1:10, DACO, Denmark), neuro-filaments (NF) (mouse monoclonal, code M0762, working dilution 1:160, *Euro-Diagnostika*, Denmark),neuro-peptide Y (NPY) (rabbit polyclonal, code B48-100, working dilution 1:100, Peninsula, Sweden).

Expression of hepatocyte growth factor (HGF) in the liver samples was evaluated by means of HGF antibody (mouse monoclonal, code AF294NA, working dilution 1:300, R&D Systems, Germany). Expression of interleukin 6 (IL-6, mouse monoclonal, code NYRhIL6, working dilution 1: 50, Santa Cruz Biotechnology,, USA) and interleukin 10 (IL-10), rabbit polyclonal, code ab34843, working dilution 1: 400,Abcam, UK) and matrix metalloprotease 13 (MMP13),*Escherichia coli*, code sc 81547, working dilution 1:100, Santa Cruz, USA) were also studied in the liver cells.

Immune reaction was visualized by the avidin-biotin immune-peroxidase method using an LSAB kit (Dako Cytomation, DK) and diaminobenzidine (DAB) solution (Dako Cytomation, DK) was used as chromogen, and hematoxylin was used as the counter-stain.

Apoptosis was detected by the means of TUNEL assay, described by Negoescu et al. (1998) in all the tissue samples from the gastrointestinal tract. *In situ* Cell Death Detection kitPOD (1684817, Roche Diagnostics) and Vector DAB Peroxidase substrate kit were used to perform the TUNEL assay according to the manufacturers' instructions, briefly: deparaffinized sections (xylol 2×4 min, 99% ethanol 2×2 min, 95% ethanol 2×2 min and 70% ethanol 2×2 min) were rinsed with water for 7 to 10 min and transferred to PBS (pH 7.5) for 10 min. Subsequently slides were placed into 50 ml PBS solution with 500 µl 30% hydrogen peroxide for 30 min on shaker to block the endogenous peroxidases. Afterwards, tissue samples were washed with PBS (3×5min), placed into 0.2 M boric acid (pH 7.0), and then placed in microwave (700 W) for 10 min to fix antigen. Content was cooled to room temperature and rinsed with PBS. Subsequently, slides were kept in refrigerator in 0.1% bovine serum albumin (BSA) solution with PBS for 19 min and incubated in TUNEL mix (Tdt – mix of terminal deoxynucleotide transferase and DIG-ladeled deoxynucleotide) for 1 h at 37°C. Then the slides were rinsed with PBS 1:10 and incubated for 30 min at 37°C with POD (anti-fluorescein antibody,

Fab fragment from sheep, conjugated with horse-radish peroxidase). Then the slides were washed with PBS, covered with DAB (diaminobenzidine chromogen) for 7 min, and then rinsed with running water for 5 min. Finally, haematoxylin and eosin staining was performed on each sample. Sections were covered with a polystyrene-based medium and coverslip.

Samples were viewed by Leica DM 5000B microscope (×250 and ×400), and pictures were obtained by Leica DC 300F digital camera using software *Image Pro Plus* version 6.3.0.512 for Windows XP Vista, serial number 41N63000-58567 (Media Cybernetics, USA).

Statistical analysis

The mean values and standard deviations were determined statistically using statistical package SPSS 17.0. Pearson correlation test was applied for determination of the correlation among parameters (Arhipova and Balina, 2006).

Wilcoxon's T-criterion test was applied for interdependent groups of samples and statistically significant differences were evaluated at $p<0.05$ (Sheskin, 2011). Quantitative method was applied to count HGF positive structures and apoptotic cells within three freely chosen visual fields around the central vein of the liver and portal tract area at magnification ×400. The expression of cytokines was determined by simplified qualitative counting method, and presence or amount of positive immune structures were evaluated as low (+), medium (++) or high (+++) using a method described by Pilmane et al. (1998).

RESULTS

Examination of the sampled tissue sections under light microscope revealed characteristic keratinization of the *fundus* of rumen mucosa in four of the five animals studied, clear distinctions in two cases. Also some areas of the tissue samples from the rumen wall revealed more or less distinct basal cell hyperplasia of para-keratotic epithelium (Figure 1). Infiltration of patchy inflammatory cells in mucosa and muscular layer was represented by neutrophilic leukocytes, lymphocytes and rare macrophages. Vacuolization of gangliocytes was observed in some areas of the inter-muscular nerve plexus. Changes in the small intestines of four animals were relatively similar: walls, especially mucosa, were infiltrated by high or medium-high number of inflammatory cells. Inflammatory cells were represented by lymphocytes, neutrophilic and eosinophilic leukocytes, macrophages and plasmocytes. In two cases, proliferating connective tissue were detected in the small intestinal villi. Inflammation of the mucosa of the large intestine was prominent in two cases (Figure 2) and of small intestines - in one case. One of the animals had evident atrophy of mucosa in some areas of the large intestine.

The present study showed that the small and large intestines of red deer (*C. elaphus* L.) bred in Latvia was more innervated than the rumen although in some areas mucosal envelope presented variations.

General marker PGP 9.5 for the DNES indicated nerve fibers around arteries; the largest number of peptide-containing nerve fibers and gangliocytes was found in sub-mucosa and inter-muscular nerve plexus of the small and large intestinal walls (Table 1). Wilcoxon's T-criterion test for interdependent groups of samples did not reveal statistically significant differences ($p>0.05$) between the expression of PGP 9.5 in the mucosa of rumen, small and large intestines. Correlation was also insignificant ($p>0.05$) between the expression of PGP 9.5 in the sub-mucosa and the muscle layers of rumen as well as small and large intestines.

A few patchy loci of NPY containing nerve fibers were found in the deer's rumen and the small intestinal wall muscle layer. Some NPY-containing fibers were found in the muscle layer of the wall of large intestines. The insignificant expression of NPY confirmed the minor role of this neuro-peptide on the functions of digestive system of deer. NF-containing nerve fibers were widely found in the basal and muscle layers of the rumen and the small intestines and in all of the wall layers of the large intestine, though they were patchy and fragmentary in some areas. A high amount of serotonin-containing neuroendocrine cells were found in the epithelium of the small intestine while medium-high amount of those cells was found in the epithelium of the large intestine (Figure 3); no cells of this type were found in the epithelium of the rumen. Index of apoptosis was higher in the rumen mucosa (0.17), but was lower in the mucosa of the small intestines and that of the large intestine (Figure 4, Table 2).

Wilcoxon's T-criterion test for interdependent groups of samples revealed significantly higher level of apoptosis in the rumen mucosa comparing to mucosa of the small intestine ($p<0.05$ or $T=0 < T_{0.05;\ 15}=30$). The difference between the levels of apoptosis in the mucosa of the small and large intestine was insignificant ($p>0.05$ or $T=30 > T_{0.05;\ 15}=25$).

Histological investigations of the liver samples of deer revealed well-formed liver structure: a liver lobe with *V. centralis* and portal tracts. Inflammation with lymphocytes was detected in the walls of the blood vessels of portal tracts. Expression of HGF was more distinct in the cytoplasm of the hepatocytes of the portal tracts and less – around *V. centralis.*

As shown in Table 3, 34.4 ± 17.47 HGF positive hepatocytes were found on average in 3 visual fields near the acinus. The average number of apoptotic cells in the portal tract was 5.2 ± 1.09, apoptotic index – from 0.04 to 0.07, in *V. centralis* area - 13.2 ± 2.59, apoptotic index: 0.1 to 0.2. Distinct expression of IL-6 was detected around the blood vessels and bile ducts. IL-10 positive cells were found in the whole liver parenchyma. Weak expression of MMP13 was observed around the blood vessels.

DISCUSSION

Animals included in the study were given 7 kg of haylage and 1 kg of rolled grain during the winter period thus

Table 1. Relative distribution of protein gene peptide 9.5 in separate parts of deer's digestive tract.

Animal	Tissue layer	Rumen	Small intestine	Large intestine
	Mucosa	++	++	+
1.	Submucosa	++	++++	++++
	Muscle layer	++	++	++++
	Mucosa	++	++	+++
2.	Submucosa	++	++++	+++
	Muscle layer	++	+++	+++
	Mucosa	+/-	++++	++++
3.	Submucosa	++	++++	++++
	Muscle layer	+/-	++++	++++
	Mucosa	+/-	++	+
4.	Submucosa	+	++	++
	Muscle layer	+/-	++	+++
	Mucosa	+	+++	++
5.	Submucosa	+	++++	+++
	Muscle layer	+	++++	++++

+/- Rare positive structures in visual field; + few containing structures in visual field; ++ moderate number containing structures in visual field; +++ numerous containing structures in visual field; ++++ very high number of containing structures in visual field.

Table 2. Number of apoptotic cells in digestive tract wall of red deer.

Parts of red deer's digestive tract in mucosa	Average number of positive cells from 100 cells in visual field and standard deviation	Index of apoptosis
Rumen	14.7±3.06	0.17
Small intestine	8.7±2.52	0.10
Large intestine	4.7±2.52	0.05

Table 3. Results of immunohistochemical investigationsof red deer (*Cervus elaphus* L.) liver.

Animals	HGF positive cells	Portal tract		*V. centralis* area		Interleukin expression		MMP13 expression
		Number of apoptic cells	Apoptic index	Number of apoptic cells	Apoptic index	IL-6	IL-10	
1.	20	5	0.05	10	0.1	+++	+++	+
2.	29	5	0.05	13	0.15	+++	+++	+
3	35	7	0.07	12	0.14	+++	+++	+
4.	64	5	0.05	17	0.20	++/+++	+++	+
5.	24	4	0.04	14	0.16	++/+++	+++	+
Average	34.4±17.47	5.2±1.09		13.2±2.59				

Positive immune cells are marked by: (+) – few, (++) – moderate, and (+++) – high number of positive cells.

providing 2.63 kg of dry matter and 318.2 g of crude protein as well as sufficient ration of energy (46.7 MJ ME per day). Our findings agree with the study of Josefsen (1997), which reported that changes in the mucosa of

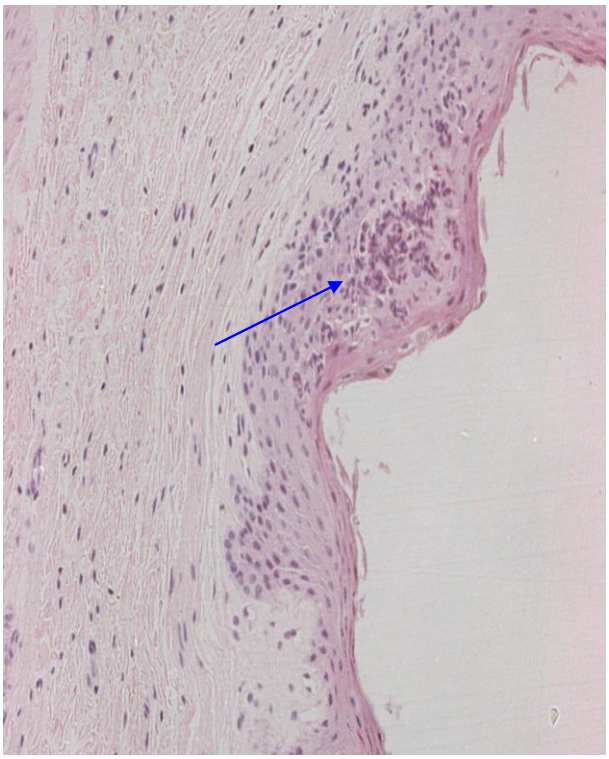

Figure 1. Keratinization of ruminal mucosa (H&E, magnification ×200).

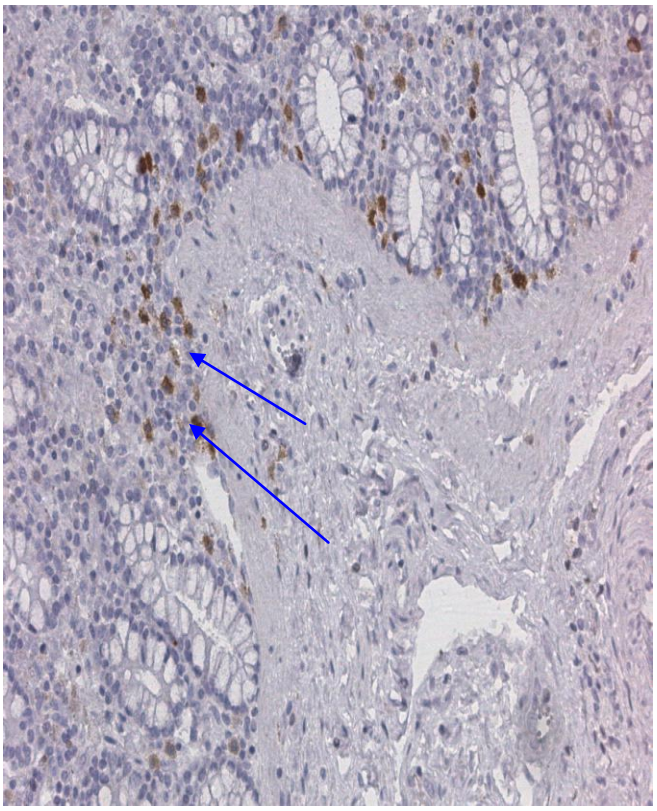

Figure 3. Serotonin expression in colon wall (IHC, magnification ×250).

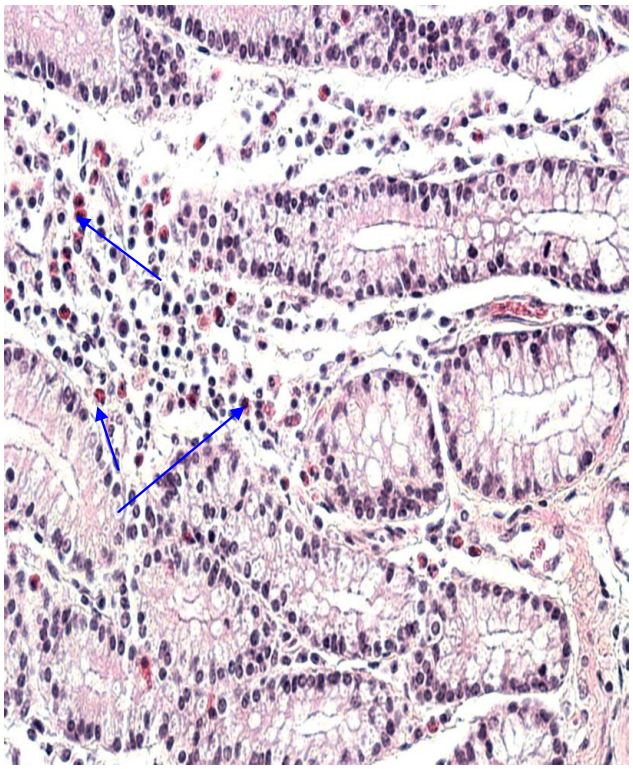

Figure 2. Inflammatory process in large intestines wall (H&E, magnification ×400).

digestive tract of deer (inflammation, para-keratosis and atrophy) may be caused by the influence of local solid forage particles. Atrophy of the mucosal villi was observed in the digestive tract of one of the deer in our study because the deer preferred grazing on bushes. Atrophy of the mucosal villi, found in the digestive tract of deer, according to Mallard et al. (1998), is related to the changes of the local immune system and the DNES. Műnnich et al. (2008) have investigated innervations of the DNES in the rumen wall of fallow deer and found that the rumen of these animals is less controlled in terms of cholin-ergic nerves in comparison with that of cattle, sheep and goats, despite the fact that the majority of neuron sub-populations were found in the inter-muscular nerve plexus of the rumen wall. Development of the DNES in the digestive tract was investigated also for red deer (*C. elaphus* L.) at the stage of prenatal development; no neuro-endocrine cells were found in *rumen, reticulum, omasum* or *abomasum* until the 67th day of fetal development (Franko et al., 2004a, b; Redondo et al., 2005; Masot et al., 2007). In the consecutive days of fetal development they were found in sub-mucosa - *lamina propria* and muscle layer - *tunica muscularis* (Franko et al., 2004 a, b; Redondo et al., 2005; Masot et al., 2007). DNES was not found in the epithelium. Prenatal development of the DNES in deer is

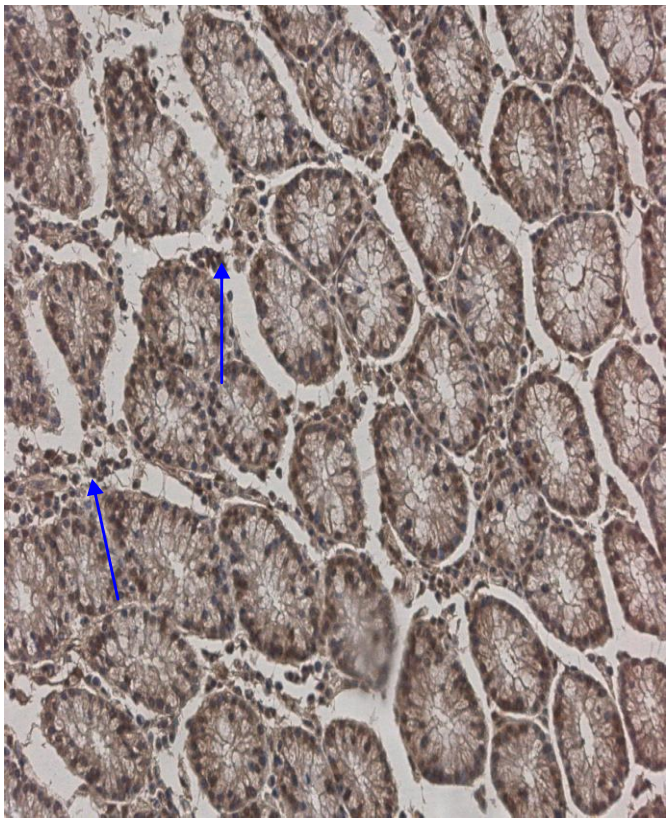

Figure 4. Apoptosis in large intestines wall (TUNEL, magnification ×250).

slower in comparison with that of other ruminants: sheep, goats and cattle (Redondo et al., 2005). Activity of DNES of adult red deer digestive tract (*C. elaphus* L.) is related to its physiological functions by consuming different feed in winter and summer periods (Sibbald and Milne, 1993; Freudenberger et al., 1994). The growth factors and peptide hormones of DNES regulate the splitting up and absorption of the nutrients and contractions of the muscle membrane of whole wall of the digestive tract (Zabielski et al., 1998).

A few patchy loci of NPY-containing nerve fibers found in the muscle layer of the deer rumen and the wall of small intestines as well as some NPY-containing fibers in the muscle layer of the wall of the large intestine could be related to the antibiotic-like ability to disintegrate penetrability of microorganism surface and change the lumen of blood vessel wall as described by Vouldoukis et al. (1996). Although some studies (Franco et al., 2004 a,b; Redondo et al., 2005; Masot et al., 2007) have reported the presence of distinct NPY in submucosa and muscle layer of *rumen, reticulum, omasum* and *abomasum* of red deer embryos before birth, the present study revealed that NPY does not play a significant role in the digestive function of adult deer. The finding in the present study also contradicts those of Allen et al. (1987) which showed that NPY can be found in the nerve fibers of the whole digestive tracts of pigs, rats and guinea-pigs, but are lowly expressed. Low expression of NPY in the digestive tract of the studied animals basically excluded this neuro-peptide from the list of important regulators of digestive system functions of deer.

According to Ulfig et al. (1998) NF are characteristic structural differentiation of the structure of nerves. Pathological changes in NF have a significant impact on the regeneration of motor and sensory axons (Rao et al., 1998). Our results showed that NF-containing nerve fibers are widely seen only in mucosa and muscle layers of the rumen and the small intestines, however some focal points of these nerve fibers were found in all the layers of the large intestine wall. Appearance of patchy loci of NF-containing nerve fibers from the rumen towards the large intestine is a base for patchy loci of cytoskeleton damage and therefore proves the reduced quality of the nerve fibers. Previous study reports that changes in both the cytoskeleton of nerve fibers and the DNES may occur during active metabolic processes (Bueno and Fioramonti, 1994), which was also the assumption in the present study.

Results of the present study also showed the presence of high amount of serotonin-containing cells in the epithelium of the small intestine, and medium-high amount in the epithelium of the large intestine. According to Camilleri (2009), serotonin is involved in many physiological functions of the digestive tract: Its primary role being the prevention of acid secretion in the stomach and may serve as an endogenous enterogastron. Other functions include stimulates the production and secretion of mucus in the stomach and the large intestine, influencing the innervation of intestinal smooth muscle by focusing directly on the mesenteric vascular smooth muscle or through intestinal nerves and exerting impact on the blood flow in the stomach and the intestinal tract. Thus, it be can concluded that abundant expression of serotonin in the epithelium of the small intestine makes these cells the most intense place of metabolic processes in the digestive tract.

Programmed cell death or apoptosis appears when death of the cell is caused by the factors in the cell itself and is also fostered by the expression of *bcl2* gene outside the cell, activating caspase and simultaneously initiating activation mechanism of mitochondrion and lysosomes, as well as, plant oxidative stress (Brunner and Mueller, 2003). In comparison with other parts of the deer's digestive tract, the present study observed the most distinct apoptosis in its largest part– the rumen where the most intense decomposition of feed and absorption processes occurs. These probably increase cell stress and initiate the cascade of programmed cell death.

The walls of the vessels of the portal tract area revealed inflammation processes probably related to the transport of nutrients absorbed in the small intestine.

According to the studies published by Watanabe et al. (2003) and Hironobu et al. (2006), the liver portal tract area shows higher apoptotic index and statistically significantly higher number of HGF positive cells that ensure regeneration ability of the liver. Maher (1993) described that in a healthy liver HGF is found in the cells of sinusoidal endothelium and Kupffer cells. However, during regeneration process of the liver, it is produced by the liver stellate cells or *Ito* cells (Hironobu et al., 2006), as well as fibroblasts, epithelial and endothelial cells, hepatocytes and Kupffer cells (Maher, 1993). Studies by Miyazawa et al. (1996) and Ishikawa et al. (2001) reports that HGF in a healthy liver is found in an inactive-single chain form, but in the cases of pathology it turns into an active-heterodimeric form. By reducing cell apoptosis, HGF participates in the angiogenesis and morphogenesis (Funakoshi and Nakamura, 2003), which corresponds with the findings of the present study: whenever there appears an inflammation in the walls of blood vessels, HGF expression increases and apoptotic index decreases and vice versa in the *V. centralis*.

Matrix metalloproteases (MMP13) are enzymes of cell surface that demolish the majority of peri-cellular substrate (collagen, gelatine, fibronectin, laminin and proteoglycan) participating in many physiological and pathological processes (Nagase and Woessner, 1999). The expression of MMP13 in the parenchyma of the deer's liver was very low. Investigation in to MMP13 revealed that their function in the majority of body development processes and homeostasis, and in a disease process were still uncertain (Birkedal-Hansen et al., 1993; Sternlicht and Werb, 2001). Our study did not reveal any significant expression of MMP13 in the digestive tract of red deer and neither a significant role in their functions.

Our study also revealed distinct expression of interleukins: Expression of IL -6 was mainly found around the blood vessels and bile ducts, and IL-10 markedly expressioned throughout the whole liver parenchyma. Previous studies (Cressman et al., 1996; Heinrich et al., 2003; Coelho et al., 2007) have explained that distinct IL-6 expression around the blood vessels relies on the regeneration processes in the liver. Expression of IL-10 in the liver is related to the immune system and IL-10 is the main regulator of the immune system with its key role in providing anti-inflammatory effect (Howard and O'Garra; 1992; Abbas et al., 1994). Considering the findings of the above studies we concluded that small number of apoptotic cells around *V. centralis* area, high expression of interleukins and simultaneously limited expression of degeneration enzymes on the background of medium distinct hepatocyte activity in the liver of the animals in the present study indicate normal liver activity.

Conclusions

1) The small and large intestines of captive red deer (*C. elaphus* L.) are richly innervated although there are variations of the nerve supply in some places.
2) Abundant expression of serotonin in the epithelium of the small intestines makes this part of the digestive system to be the most intense place of metabolic processes in the digestive tract, while the low NPY expression basically exclude this neuro-peptide from the list of important regulators of the deer's digestive system functions.
3) Focal appearance of NF-containing nerve fibers from the rumen towards the large intestine is a base for patchy loci of cytoskeleton damage and therefore reduces the quality of nerve fibers.
4) Intense processes of apoptosis in the rumen probably rely on the intense food cleavage and absorption processes and could be evaluated as a compensatory reaction on the side of tissues.
5) The small number of apoptotic cells around *V. centralis* area, the distinct expression of interleukins and at the same time limited expression of degeneration enzymes on the background of medium hepatocyte activity in the livers of the animals studied most probably indicate normal liver tissue activity.

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Full Length Research Paper

Occurrence of ectoparasites and gastro-intestinal helminthes infections in Fayoumi chickens (*Gallus gallus* Fayoumi) in Debre Zeit Agricultural Research Center Poultry Farm, Oromia region, Ethiopia

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A study for ectoparasites and gastrointestinal (GI)-helminthes was conducted on 212 Fayoumi chickens raised under semi-intensive management system at Debre Zeit Agricultural Research Center (DZARC) poultry farm from October, 2009 to May, 2010. The results of study indicated that 52 (24.53%), 34 (16.04%) and 5 (2.35%) of the examined chickens were harbouring one species of nematode (*Ascaridia galli*), three species of cestodes (*Raillietina echinobothrida, Raillietina tetragona* and *Raillietina cesticillus*) and two species of ectoparasites (*Menacanthus stramineus* and *Menopon gallinae*), respectively. There was no statistically significant difference (p > 0.05) in prevalence infection of cestodes between sex (males and females) and age groups. Similarly, there was no statistically significant difference (p > 0.05) in prevalence of infection with nematode sex (males and females) and age groups. The present study clearly indicated that Fayoumi chickens kept under semi-intensive management system in DZARC poultry farm were exposed to ectoparasites and GI-helminthes. Due attention is required to the control the parasitism.

Key words: Debre Zeit Agricultural Research Center (DZARC) poultry farm, ectoparasites, Fayoumi chickens, gastrointestinal (GI)-helminths, prevalence.

INTRODUCTION

Poultry production is becoming one of the most highly developed segments of food animal production globally. Accordingly, to gain the maximum profitability out of the industry, greater efforts have been put to make changes in the methods of production (Ensminger, 1992). In developing countries, apart from traditional back yard methods of raising chickens, the more commercialized poultry production is increasingly intensified in large scale (FaBiyi, 1980; Alemu, 1985). Despite the presence of large number of chickens in Ethiopia, contribution to the national economy or benefit exploited for domestic chicken is very limited due to disease and nutritional limitation (Smith, 1990).The main constraints to the development of indigenous chicken production in rural Ethiopia include diseases, predation, lack of feed, poor housing, and to a lesser extent financial problems and management (Alemu, 1985; Permin et al., 2002). Among the disease of poultry, ectoparasites and gastrointestinal (GI)-helminthes plays an important role in reducing the total poultry production potential of the country.

Parasites are common in tropics, where the standard of poor husbandry practices and climatic conditions are favorable for the development of the parasites (Abebe et al., 1997). Most ectoparasites (for example, lice), stay

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close to the host during their entire life cycle while others move from one host to the next quite frequently as ticks and mites (Yacob et al., 2009). Ectoparasites cause intense pain, irritation, slow weight gain, decreased egg production, and general poor and ill health (Urquhart et al., 1996, Kaufman, 1996). Lice are the most common ectoparasites of poultry, causing major economic loss to the productivity of animals (FaBiyi, 1980; Alamargot et al., 1985).Lice normally eat feather products except *Menacanthus stramineus*, which consumes blood. The virus of equine encephalomyelitis has been isolated from *M. stramineus* while the intracellular bacterium *Chlamydia psittaci* that causes an infection of birds known as orinthosis is isolated from *Menopon gallinae* (Calnek, 1991; Kaufman, 1996).

Raillietina echinobothrida and *Raillietina tetragona* are highly pathogenic while other species are not normally harmful unless the infection is extremely heavy, which cause significant decrease in production (Kaufman, 1996). *Ascaridia galli* is the most important nematode species of considerable economic importance. The large size of these nematode parasites may cause intestinal occlusion (Whit man and Brikford, 1989). The objectives of the present study were therefore, to identify the different species of ectoparasites and GI helminths of Fayoumi chickens in Debre Zeit Agricultural Research Center (DZARC) poultry farm.

MATERIALS AND METHODS

Description of the study site

The farm is situated in Debre Zeit town which is located at 9°N latitude and 4°E longitudes, at altitude of 1850 m above sea level in the central Oromia region. The area has an annual rainfall of 86.6 mm, of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26 and 14°C, respectively, with mean relative humidity level of 61.3% (National Metrological Service Agency (NMSA), 2011).

Study design

The study design employed was a cross-sectional type, with the objective of determining the prevalence and level of infection of the major ectoparasites and GI-helminthes of Fayoumi chickens in DZARC poultry farm.

Study animals and management

A total of 212 apparently healthy and clinically sick and/or dead Fayoumi chickens, between the age of 6 ± 12 months (100) and $12\pm24^{+}$ months (112), raised under semi-intensive management system, were considered for the study purpose. Any suddenly dead found chicken was also collected and examined. All chickens were allowed for partial scavenging, being protected from scavengers by open air wire mesh construction. A total of 80 males and 132 females Fayoumi chickens were selected randomly and then transported to the Faculty of Veterinary Medicine (FVM) Parasitology laboratory for detailed parasitological and necropsy examination.

Examination procedure

The birds were sacrificed by the method of cervical dislocation and subjected to parasitological examination according to the procedure described in Hansen and Permin (1998).

Postmortem examination

Examination for ectoparasites

Immediately after sacrificing, the skin from each individual bird was medially incised and detached from the underlying tegument together with the feathers. The legs and featherless areas of the body with any seborrhea or crustation were scraped for microscopic examination. Visible ectoparasites were collected using thumb forceps. Minute ectoparasites were detected using magnifying glass and collected by scraping from live chickens. The detected external parasites were preserved in 70% alcohol for identification. Collection of ectoparasites from the chickens was carried out by careful examination of the entire external body parts according to method of Yacob et al. (2009).

Gastrointestinal parasites isolation and identification

The viscera were detached from the mesentery and the GI tract was separated into smaller pieces. The oesophagus with crop, gizard with proventriculus, and caeca with the rest of the intestine were kept in three separate containers. Each piece was identified and incised longitudinally. The worms were collected from the different intestinal pieces by washing with physiological saline in separate trays and placed in different beakers containing physiological saline. The parasites were examined in a stereomicroscope. The identification of GI helminths was carried out using the characters described by Olsen (19) and Soulsby (1982). Ectoparasites were identified based on the criteria set by Kaufman (1996), Calnek et al. (1991) and Soulsby (1982).

Data analysis

Appropriate data were collected from individual birds and stored in Micro Soft Excel spread sheet. Data analysis was carried out by using computer based Statistical package for social sciences (SPSS 11.5 statistical package). Pearson chi-square test was used for comparison of the possible variation between the study areas, and $p < 0.05$ was considered significant**.**

RESULTS

Ectoparasites prevalence

Out of the total 212 Fayoumi chickens examined from DZARC poultry farm, 2.35% were found harboring two

ectoparasites species: *M. stramineus* was found in three birds (1.41%) and *M. gallinae* was found in two birds (0.94 %). The difference in the prevalence of ectoparasites on the basis of sex (3.75% in males and1.52% in females) and age groups (3% in 6 ± 12 months and 1.79% in 12 ± 24^{+} months), were found to be statistically not significant ($p > 0.05$) (Table 1).

Nematodes prevalence

The difference in the prevalence of nematode infection (*A. galli*) on the basis of sex (23.75% in males and 12.5% in females) and different age groups (37% in 6 ± 12 months and 13.99% in 12 ± 24^{+} months), were not statistically significant ($p > 0.05$) (Table 2).

Cestodes prevalence

Out of the total 212 Fayoumi chickens examined from DZARC poultry farm, 34 (16.04%) were found harboring different species from genus *Raillietina*. *R. echinobothrida* (8.49%) was the most prevalent cestode species followed *R. tetragona* (7.07%) while *R. cesticillus* (0.47%) was the least (Table 3). The difference in the prevalence on the basis of sex (16.25% in males and 13.36% in females) and age groups (12% in 6 ± 12 months and 19.64% in 12 ± 24^{+} months), were found to be statistically not significant ($p > 0.05$) (Table 3).

DISCUSSION

The present study showed the occurrence of only one nematode parasite, three cestodes species and two different ectoparasites in semi-intensive farm, with prevalence of 24.53, 16.04 and 2.35%, respectively.

In this study, a low prevalence of two ectoparasites species *M. stramineus* (3%) and *M. gallinae* (2%) was seen as compared to the previous findings in backyard chickens (Bresabeh, 1999; Hagos 2000) and scavenging chicken (Yacob et al., 2009). This is mainly because of the difference in management system, where a higher prevalence is expected in backyard scavenging system of production. *M. stramineus* and *M. gallinae* were also reported as the most prevalent and pathogenic arthropod parasites in Nigeria (FaBiyi, 1980). There were no statistically significant differences ($p > 0.05$) in the prevalence of ectoparasite infection between sex. However, the intensity of infection was relatively higher in males than females. The increased contact due to the fact that male chickens mate with several females, may lead to high degree of infestation (Kaufman, 1996). Generally, the result clearly indicated that a very low prevalence of ectoparasite infection in the study farm was mainly due to the fact that ectoparasites were minimized by thorough cleaning of the house between batches of birds, whole flock replacement, smooth house construction, and use of mesh to keep out wild birds and keeping manure dry.

In this study, only one species of nematode that is, *A. galli* was identified with a prevalence of 24.53% (Figure 1). There was no statistically significant difference ($p > 0.05$) in the prevalence of *A. galli* infection in male (23.75%) and females (25%). However, the difference in the prevalence of *A. galli* in the two age groups (37% in 6 ± 12 months and 13.39% in 12 ± 24+ months) was found to be statistically significant ($p < 0.05$).

In previously conducted studies mainly involving backyard system of management, the prevalence of *A. galli* was very much higher, ranging between 47 to 67% (Asfaw, 1992; Teshome, 1993; Abebe et al., 1997; Bersabeh, 1999; Hagos, 2000) in different parts of Ethiopia. However, in Zimbabwe, the most prevalent nematode species in chickens was *Allodapa suctoria* (Permin et al., 2002) which was not found in the present study. The effects of *A. galli* on chickens include droppiness, emaciation, diarrhea and sometimes obstruction (Figure 1). In heavily parasitized chicken, it can also easily cause obstruction of intestine (Kaufman, 1996; Urquhart et al., 1996). Other pathological lesions such as pericarditis and pneumonia were also observed during necropsy (Figure 2).Among the three species of cestodes recovered, the most prevalent cestodes was *R. tetragona* (8.49%), followed by *R. echinobothrida* (7.07%) while *R. cesticillus* (0.47%) was the least encountered. There was no statistically significant difference ($p > 0.05$) in the prevalence of cestodes infection between sex (males 16.25% and females 13.65%) as well as age groups [(6 ± 12 months (12%) and 12 ± 24^{+} months (19.64%)]. The findings of the present study in terms prevalence of cestodes infection in general agreed with the previous studies in backyard chickens in Ethiopia (Bersabeh, 1999; Hagos, 2000) and in agreement with reports in Zimbanwe by Permin et al. (2002), as well as globally with reports of Permin and Bisgaard (1999). *R. echinobothrida* induces the formation of nodules in the intestinal wall, which can lead to confusion with the lesion of avian tuberculosis (Calnek, 1991; Urquhart et al., 1996). In the present study, similar nodular swellings were observed in the intestinal wall associated with *R. echinobothrida* and *R. tetragona* infection. The control of intermediate host (beetles, snails, slugs and flies) is one of the major tasks in the control of tape worm infection (Yacob et al., 2009).

The present study clearly indicated that Fayoumi chickens kept under semi-intensive management system in DZARC poultry farm (Figures 3; A and B) were exposed to a very low range of ectoparasites and relatively high

Table 1. Prevalence of ectoparasites in DZARC poultry farm based on sex and age of the examined birds

Lice species	No. examined	Positive Prevalence (%)	Prevalence in sex (%)		Prevalence in age; months (%)	
			M	F	6±12	12±24^{+}
M. stramineus	212	3 (1.41)	2/80 (2.5)	1/132 (0.75)	2/100 (2)	1 /112 (0.47)
M. gallinae	212	2 (0.94)	1/80 (1.25)	1/132 (0.75)	1/100 (1)	1 /112 (0.47)
Total	414	5 (2.35)	3/80 (3.75)	2/132 (1.52)	3/100 (3)	2/112 (1.79)

Table 2. Prevalence of nematode (*A. galli*) infection in DZARC poultry farm based on sex and age.

Nematode species	No. examined	Prevalence in sex (%)		Prevalence in age; months (%)	
		M	F	6±12	12±24^{+}
A. galli	212	19/80 (23.75)	33/132 (25)	37 /100 (37)	15/112 (13.39)

Table 3. Prevalence of cestodes infection in DAZRC poultry farm based on age.

Cestodes species	No. examined	Prevalence (%)	Prevalence in sex (%)		Prevalence in age; months (%)	
			M	F	6±12	12±24^{+}
R. tetragona	212	18 (8.49)	4/80 (5)	11/ 132 (8.33)	8/100 (8)	10/112 (8.93)
R. echinobothrida	212	15 (7.07)	9/80 (11.25)	6/132 (4.54)	4/100 (4)	11/112 (9.82)
R. cesticillus	212	1 (0.47)	0/80 (0)	1/132 (0.76)	0/100 (0)	1 /112 (0.89)
Total	212	34 (16.04)	13/80 (23.75)	18/132 (25)	12/100 (12)	22/112 (19.64)

Figure 1. Intestinal obstruction due to *A. galli*.

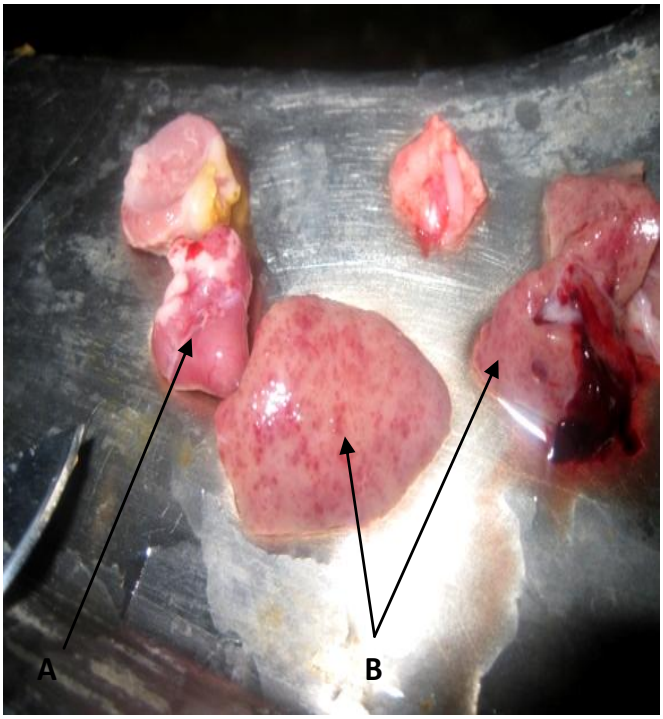

Figure 2. (A) Pericarditis and; (B) Pneumonia.

Figure 3. (A) Male Fayoumi; (B) Female Fayoumi.

Figure 4. Housing for semi-intensive Fayoumi breed management.

high number of GI-helminthes. Fayoumi chickens on the other hand were seen to have resistance to diseases and adapted to different environmental conditions.

Poultry production system in Ethiopia shows a clear distinction between the traditional low input system, on one hand and the modern production system, using relatively advanced technology, on the other hand. Traditional poultry production system operates indigenous chickens using a small sized flock without input and with poor or no housing facilities (Tadelle, 1997). Given the management conditions and the magnitude of input provision, the connotation that “the productivity of local chickens in Ethiopia is poor” is virtually unfounded (Yilma et al., 1998).

Due to the high mortality rate and lack of adaptation to the rural environment in exotic breeds, professionals are arguing that the crossbreeding scheme underway in the country is not based on justified ground. Also, the merits

and demerits of both the existing and imported germplasms have not been studied thoroughly. The low prevalence of both ectoparasites and endoparasites in this study is probably related to less frequent exposure of chicken under this semi-intensive management system (Figure 5). Chickens are usually exposed to multiple parasitic infections under extensive management system as indicated in the results obtained by Yacob et al. (2009) conducted on scavenging chickens kept under traditional management practices.

Management has a great role in controlling both ectoparasites and GI-helminthes. In addition to good management, periodical deworming can also reduce the effect of parasitic diseases (Tadelle, 1997). The present study clearly indicated that Fayoumi chickens kept under semi-intensive management system in DZARC poultry farm were exposed to ectoparasites and GI-helminthes parasites; thus causing major constraints to poultry production. Since the Fayoumi chickens are exotic and recently introduced to Ethiopia, detail studies should be conducted to assess the impact of these ectoparasites and GI-helminthes on productivity of this chicken species.

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Full Length Research Paper

Major reproductive disorders of dairy cows in and around Asella town, Central Ethiopia

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Reproductive disorders are one of the most important problems that affect the production and productivity of dairy cows. A cross sectional study was conducted to determine the prevalence of major reproductive disorders of dairy cows in and around Asella town, Central Ethiopia. For this purpose, a total of 82 owners and/or attendants of dairy cow herds were interviewed using structured questionnaire, and in addition, a total of 300 blood samples were randomly collected and analyzed from dairy cows in the study areas. Accordingly, the overall prevalence of reproductive disorders in the study area was 18.3%, with bovine brucellosis (32.9%), repeated breeding (26.8%) and abortion (14.6%) as the major reproductive disorders of dairy cows. It was also revealed that abortion is mostly common in exotic breeds (48.8%) and relatively less in local cows (9.8%) varying among parity and stage of pregnancy. Thus, it is recommended that strict animal husbandry especially animal feed and health management should be in place to minimize the risk of reproductive disorder occurrence in order to increase the production and productivity of dairy cows in the study area.

Key words: Reproductive disorder, bovine brucellosis, repeated breeding, dairy cows.

INTRODUCTION

Cattle production has been considered as the main component of agricultural development in most parts of sub-Saharan Africa. The overall cost of keeping cattle in terms of costs associated with the health care, nutrition and reproduction management, however, has not matched to their contribution to the livelihood and the economy of the people in the region. As in many countries, livestock, particularly cattle play multiple roles in Ethiopia being a source of milk, meat, hide, etc (Mekonnen et al., 1989). However, their productivity is low due to various constraints such as disease, feed, poor management and poor reproductive performance of indigenous zebu breeds (ILCA, 1988). Hence, income derived from livestock sector could not impart a significant role in the economic development of the country (Mukasa-Mugerwa, 1989).

It has been reported that among infectious diseases, brucellosis is a common genital disease which induces abortion in animals and humans, continues to cause heavy economic losses and public health concern throughout the world (Arthur et al., 1996). It is transmitted to people by direct or indirect contact with infected animals or their products (FAO, 2010). Abortion in cattle is an important fertility problem causing a serious economic setback due to direct losses of concepts and consequent impairment of fertility. Moreover, the incidence of retained fetal membrane after birth is often high in *Brucella* infected herds (Mukasa-Mugerwa, 1989);

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however, varieties of factors are responsible and it causes considerable losses at farm level, especially when the incidence exceeds the average rate of 5 to 10% (Arthur et al., 1996). In Ethiopia, even though dairy cattle are maintained under different production systems (ILCA, 1994; Shiferaw et al., 2003), the differences in management (production) systems and environmental conditions under which cattle are maintained could greatly affect the occurrence of reproductive disorders. In spite of this fact, little has been known about the magnitude of the major reproductive disorders in dairy cows in and around Asella town. Therefore, this study was designed to determine the prevalence of the major reproductive disorders of dairy cows in and around Asella town, Central Ethiopia.

MATERIALS AND METHODS

Study area

The present study was conducted in and around Asella town located in Oromia region, Central Ethiopia. Asella town, the capital of Arsi zone, is located at about 175 km Southeast of Addis Ababa at 7°57'N and 39°7'E with an altitude of 2430 m above sea level. Agricultural production system of the study area is of mixed crop and livestock production. Dairy farming using improved breeds is a common practice in urban and peri-urban areas (KARC, 2008).

Study population and their management

A total of 300 dairy cows and 82 households rearing dairy cows were included in this study. Classification of management systems was done based on the criteria adopted by Richard (1993). Accordingly, by taking the history of animals from owners as: semi-intensive management system for those animals that were kept indoor and fed and watered in their house/shade by cut and carry system while extensive management system for all animals that were kept outdoor during the day time and allowed to graze on a communal or private owned pasture land.

Study design and method of data collection

Questionnaire survey

A total of 82 households (owners and/or attendants of dairy cows) rearing dairy cattle were randomly selected and interviewed using structured questionnaire. In the survey, information on reproductive disorders as well as management system and particulars related to individual cow such as parity, breed, age, and history of abortion were collected and documented.

Blood sample collection and serological tests

A total of 300 dairy cows more than 6 months old were randomly selected, included for the study purpose where animals greater than 2 years represents adult animals (used for breeding purpose) while less or equal to 2 years of age represents young animals. Accordingly, about 10 ml of blood was collected from the jugular vein of each selected animal using plain vacutainer tubes and allowed to clot overnight at room temperature. The serum samples were separated and transported in iceboxes to National Veterinary Institute (NVI), Debre Zeit, Ethiopia, and were stored at -20°C until testing. Serum samples were screened for antibodies for *Brucella* species using the Rose Bengal Plate test (RBPT). In brief, 30 µl of serum was mixed with an equal volume of antigen suspension on a glass plate and was agitated. After 4 min of rocking, any visible agglutination was considered as positive (OIE, 2008). Agglutinations were recorded as 0, +, ++ and +++, according to the degree of agglutination (Millan, 1990). A score of 0 indicates the absence of agglutination; + indicates barely visible agglutination; ++ indicates fine agglutination; and +++ indicates coarse clumping. Samples with no agglutination (0) were recorded as negative while those with +, ++ and +++ were recorded as positive. RBPT *Brucella* antigen (Institute Pourquier, France), positive control and negative control sera (National Veterinary Institute, Debre Zeit, Ethiopia) were used for the RBPT.

Data analysis

The data collected from the field were entered into a computer on a Microsoft Excel spreadsheet and analyzed using Statistical Package for Social Sciences Inc. (SPSS, 2010). Descriptive statistics such as mean, range, frequency and percentage were used to summarize and present the results. Categorical variables (breed, age, parity number, stage of pregnancy and others) were expressed in percentages. The seroprevalence of *Brucella brucellosis* was calculated as the number of animals testing positive by the RBPT, divided by the total number of animals tested. Prevalence of reproductive disorders of dairy cows was determined based on the proportion of the respondents involved in the interview. The degree of association between or among each risk factor was assessed using Chi-square (χ^2) test (http://www.fourmilab.ch/rpkp/experiments/analysis). For all analyses, a p-value of less than 0.05 was taken as significant.

RESULTS

Socioeconomic characteristics of the respondents and animal husbandry

The educational level of the respondents involved in dairy cattle rearing in the study area were diverse from literate to illiterate people in that they do have degree (2.4%), diploma (7.3%), grade 9 to 12 (29.3%), grade 1 to 8 (40.2%) and no education level (20.7%). There are no female respondents having higher educational level (diploma and degree) and majority of the illiterates were female (70.6%) when compared with male respondents (29.4%) engaged in dairy cattle rearing. Accordingly, 75.6% of the respondent's dairy cattle are reared for both home consumption (23.2%) and market (1.2%) purposes. Individuals involved in milk selling sell a litter of milk on the average by 4.5 Ethiopian Birr (ETB) which is very cheap when compared with a litter of high land water in terms of its nutritional value.

The farm type in majority of the respondent is dairy (87.8%) and some of them mixed (dairy and beef) type of farms categorized under small scale dairy farm in different kind of management practices such as extensive (20.7%), semi-intensive (46.3%) and intensive (33.0%) system. Most of the farmers in the study area use cross breed (64.6%), local breed (22.0%) and both local and

cross breed cows (9.8%), where some of them use pure exotic breeds (3.7%) for their dairy purpose. The types of the cross bred dairy cows in the study area were Holstein Friesian with Arsi (75.6%) and Borena (24.4%) breeds. In majority of the cases, women and husbands were the ones involved in dairy cattle husbandry such as feeding, milking, herding and others (Table 1).

This study showed that 4.9% of the respondents do not have house or fenced enclosure (barn) for their animals. The most common housing systems for dairy cattle in the study area were indoor (separate house) and outdoor (tether) system, 69.5 and 25.6%, respectively where their sanitary condition vary from poor, medium to excellent accounting 39.0, 56.1 and 4.9%, respectively. Most (81.7%) of the respondents on the average posses 1 to 5 dairy cattle, whereas 15.9% of them have 6 to 10 cattle.

Reproductive disorders of dairy cows in the study area

The three top major constraints of dairy cattle in the study area were animal feeds, animal diseases and reproductive disorders figuring 40.2, 30.5 and 18.3%, respectively, where bovine brucellosis (32.9%) and repeated breeding (26.8%) were the major reproductive disorders (Figure 1).

Participants were asked the type of breeding system they were accustomed to breed their dairy cattle. Accordingly, most of the respondents (62.2%) used artificial insemination whereas others were using natural mating (29.3%) and both type (8.5%) of breeding system. From their practice and experience, most of the respondents prefer the utilization of artificial insemination services (65.9%) than natural mating (bull services) (34.1%) to breed their dairy cattle even though it is only 37.8% of the respondents who knows, as there is a risk of disease transmission in using bull or artificial insemination services.

In the study area, it was reported by 14.6% of the respondent's that they encountered abortion in their dairy cattle. It was also revealed that abortion is mostly common in exotic breeds (48.8%) and relatively less in crossbred, local cows and all breed types figuring 28.0, 9.8 and 13.4%, respectively where it also vary among parity and stage of pregnancy (Table 2). According to 53.7% of the respondents, by the time they encounter abortion cases in their herd they do not take any measure, whereas some of them take them to veterinary clinic and traditional healers, 25.6 and 20.7%, respectively.A relatively high proportion (73.2%) of the interviewed household members in the study population reported consumption habit of milk and milk product in their family by boiling, whereas 26.8% of them as raw. About three fourth (75.6%) of the respondents do not have awareness on bovine brucellosis as it can be transmitted to human through raw milk and milk products consumption from infected cows. Similarly, 79.3% of the respondents do not have awareness on bovine brucellosis as it can also be transmitted to human through handling aborted fetus and other reproductive organ discharges from infected cows.

Blood sample collection and serological test results

The overall individual animal level sero-prevalence, 5.3% (16/300), of bovine brucellosis was recorded from the study area on the basis of RBPT. The higher prevalence was detected in Pluriparous females than primiparous animals.

DISCUSSION

This study showed that 18.3% of dairy cattle in the study areas were affected by either one or more reproductive disorders based on questionnaires to the owners. This is in close agreement with the questionnaire survey result report by Gizaw et al. (2007) and Molalegne and Shiv (2011) from Adama and Bedelle accounting for 25.81 and 24.8%, respectively.

Repeated breeding can be caused by a number of factors, including sub-fertile bulls, endocrine imbalance, malnutrition, reproductive tract infections and poor management practices such as wrong time of insemination or faulty heat detection, inappropriate semen handling and insemination techniques (Arthur et al., 1996). The higher proportion of repeated breeding (26.8%) found in the present study is closer to the 21.8% prevalence rate reported by Mekonnen (2000) from Ada'a district of Central Ethiopia, but higher than the 8.9 and 4.6% prevalence rate reported by Gizaw et al. (2007) and Tigre (2004) from Adama and Holetta, respectively.

The overall individual animal level seroprevalence, 5.3% (16/300), of bovine brucellosis was recorded from the study area on the basis of RBPT. This is in agreement with previous report, 8.26%, by Bayleyegn (1989) from the same area using RBPT.

In the study area, abortion in dairy cattle was found to be more common in pluriparous cows. Pluriparous, primiparous and at any other parity status accounted for 67.1, 18.3 and 14.6%, respectively. It was also reported by 14.6% of the respondents' as they encountered abortion mostly at third trimester and any stage of pregnancy, 45.1 and 36.6%, respectively, in their dairy cattle. This is inconsistent with the previous findings (Tadesse, 1999; Oumer, 2003; Mamo, 2004) which is possibly due to the repeated exposure of the genital tract of pluriparous cows as their age increases to environmental risk factors that can impart uterine infection. The prevalence, 14.6%, of abortion recorded by questionnaire survey in this study is similar to the 13.0% reported by Molalegne and Shiv (2011) in and around

Table 1. Family members responsible for dairy animal husbandry.

Type of activity	Family member responsible for dairy animal husbandry					
	Husband (%)	Wife (%)	Son (%)	Daughter (%)	Daily laborer (%)	All family (%)
Feeding	9 (11)	33 (40.2)	9 (11)	6 (7.3)	7 (8.5)	18 (22.0)
Milking	8 (9.8)	64 (78.0)	-	2 (2.4)	8 (9.8)	-
Breeding	47 (57.3)	25 (30.5)	7 (8.5)	-	3 (3.7)	-
Herding	16 (19.5)	28 (34.1)	24 (29.3)	4 (4.9)	10 (12.2)	-
Health care	49 (59.8)	27 (32.9)	6 (7.3)	-	-	
Housing	45 (54.9)	25 (30.5)	2 (2.4)	6 (7.3)	4 (4.9)	-

Table 2. Abortion at different stage of pregnancy and parity in and around Asella town.

Risk factor	Group (Categories)	No. of cases of abortion	Prevalence (%)
Stage of pregnancy	First trimester stage	8	9.8
	Second trimester stage	7	8.5
	Third trimester stage	37	45.1
	At any stage	30	36.6
Parity status	Primiparous	15	18.3
	Pluriparous	55	67.1
	At any stage	12	14.6

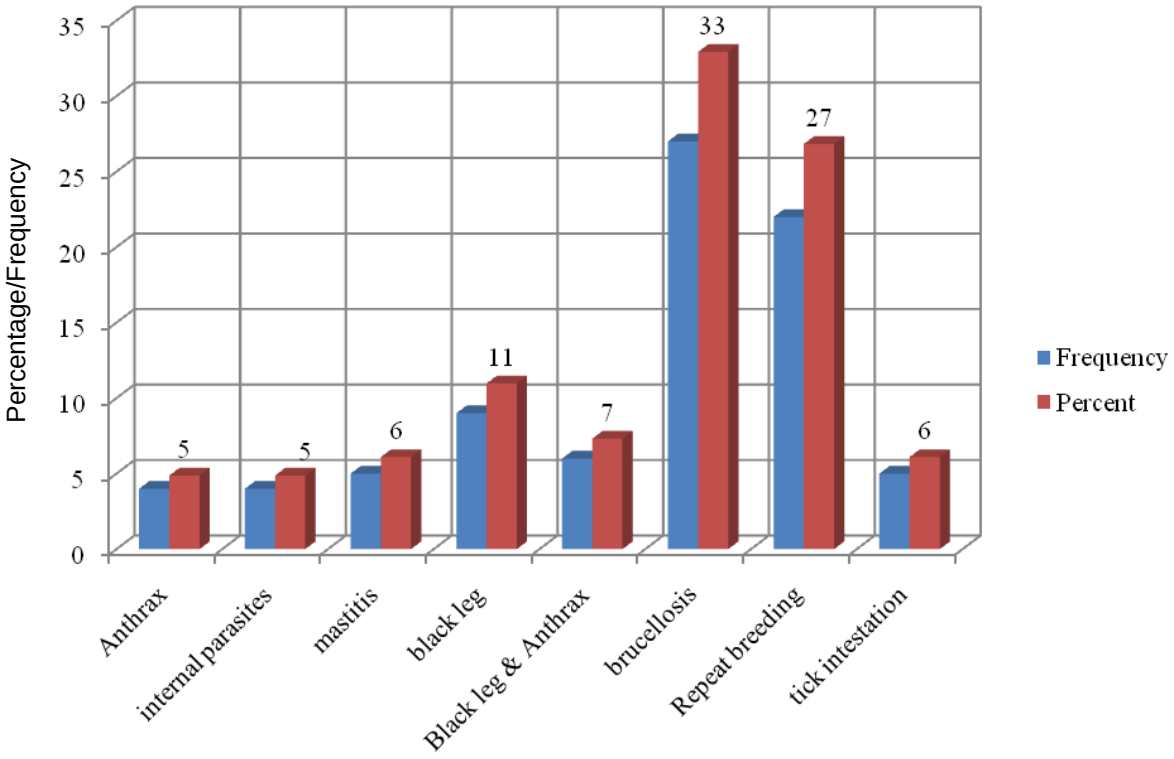

Figure 1. Major reproductive disorders and animal diseases in the study area.

Bedelle and significantly higher than the 2.23, 3.19, 5.33, and 6.30% reported earlier (Gizaw et al., 2007; Oumer, 2003; Shiferaw, 1999; Kassahun, 2003) from Adama, Kombolcha, Holetta and Hawassa, respectively. Higher percentage of abortion may be attributed, because Arsi zone is one of the districts in Ethiopia where dairy production using high yielding breeds of cattle has been started earlier, due to its agro-ecology that fairly favors the introduction and development of crossbred dairy cattle, where the problem is more common in exotics than local animals. Abortion is a frequent complication of brucellosis in animals, where placental localization is believed to be associated with erythritol, a growth stimulant for *Brucella abortus* (WHO, 2006).

Conclusively, reproductive disorder problems were prevalent in smallholder dairy cows in the study area calling for formulation of strategic control measures, including health education about the disease transmission, in order to reduce associated reproductive wastage and their risks factors.

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Major prepartum and postpartum reproductive problems of dairy cattle in Central Ethiopia

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A survey to determine major prepartum and postpartum reproductive problems of dairy cattle was carried out in three selected dairy farms in Debre Zeit town. Retrospective data analysis together with survey on major prepartum and postpartum reproductive problems were carried out through regular clinical follow up. A retrospective analysis of clinical data collected from a total of 711 cows in three farms was done and 44.3% of the cows were found with major prepartum and postpartum reproductive problems. Postpartum anestrus (12.9%) was the major reproductive problem followed by repeat breeding (11.4%) in the retrospective study. A total of 104 cows were used in the regular follow up and 33.6% (n =35) were found to be affected either with one or more of clinical reproductive problems. Postpartum anestrus (12.5%) was found to be the leading reproductive problem followed by repeat breeding (10.6%), metritis (8.7%), abortion (6.7%), retained placenta (3.8%), dystocia (2.9%) and prolapse (1.9%). Statistical analysis was performed to evaluate the difference in prevalence of the major reproductive problems on the basis of parity and body condition score. Hence, the impact of increasing parity on the occurrence of reproductive problems was examined and there is a direct relationship between increasing parity and prevalence of the reproductive problems with a highly significant variation (P<0.01) between primiparous and pluriparous cows. There is an inverse relationship of body condition and prevalence of reproductive problems with a highly significant variation (P<0.01). This study demonstrated that anestrus, repeat breeding, metritis and abortion were noticed as the most common major reproductive problems in the study farms in Debre Zeit town, Central Ethiopia.

Key words: Anestrus, postpartum, prepartum, repeat breeder, reproductive problems, survey.

INTRODUCTION

Livestock are vital sources of economic and social support for millions of poor people throughout Africa. Although Ethiopia is known to have a large livestock population, the contribution of livestock does not commensurate with the number of animals or the extent of land resources used. The major contributing factors that have impeded the full exploitation of cattle potential in Ethiopia are seasonality and quality of nutrition, low genetic potential for production in indigenous breeds, occurrence of disease and parasites, poor livestock management systems, and large socio-economic factors (Tegegne, 1989; International Livestock Centre for Africa (ILCA), 1998). Majority of Ethiopian cattle have been reported to be indigenous breeds and small non-descript Zebu types (Mukasa-Mugerwa, 1989) that are poor in major economically important traits but they have got adaptation to tropical climates (Tegegne, 1989).

Cross breeding with improved exotic dairy breeds in

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wide scale has been introduced as an option some 36 years back (Brannang et al., 1980) for upgrading the genetic potential of the indigenous Zebu cattle and subsequently to improve the dairy sector in Ethiopia. Moreover, the development and use of artificial insemination (AI) techniques have also revolutionized cattle production and genetic improvement, particularly in the dairy sectors (Tegegne et al., 1995). The performances of animals depend not only on their genetic merit, but also on other factors like nutrition, management, health and environment. On the other hand, reproduction is a vital factor in determining the efficiency of animal production (Peters and Ball, 1995). Thus, the success of cross breeding programme needs to be monitored regularly, by assessing the performances of crossbred cows under the existing management system.

Ethiopia is a country which is known for its highest livestock population from Africa. However, its level of productivity is low due to the constraints of disease, nutrition, poor management and poor performance of indigenous breeds. These constraints also result in poor reproductive performance of dairy cattle. Among the major problems that have a direct impact on reproductive performance of dairy cows are: abortion, retention of the fetal membrane (RFM) and metritis. These results in considerable economic losses to the dairy industry due to slower uterine involution, reduced reproductive rate, prolonged inter conception and calving interval, cost of medication, drop in milk production, reduced calf drop, and early depreciation of potentially useful cows (Thomas, 1989; Barnouin and Chacoranc, 1992; Merga, 1992).

Although, the major reproductive problems are greatly responsible for high economic loss in the dairy industry, there is scarcity of reliable information regarding the reproductive performances of dairy cows in subsistence dairy farms in the tropics, particularly in Ethiopia. Information pertaining to reproductive performance and interacting factors is of paramount importance primarily to the livestock owners and also to the extension agents, veterinarians and researchers. Moreover, it can assist in the development of strategies and prioritization of possible intervention options for performance improvement. Accordingly, the objective of this study was to identify major prepartum and postpartum reproductive problems of dairy cattle in the selected farms and to compare their relative occurrences.

MATERIALS AND METHODS

Study area

This study was conducted in Debre Zeit town which is located 47 km South East of Addis Ababa, Ethiopia. The town lies between 9°N latitude and 40°E longitudes and has an altitude of 1950 m above sea level. The rainfall is bimodal with an annual rainfall of 1151.6 mm of which 84% is during the long rainy season covering June to September and the remaining in the short rainy season extending from March to May. The dry season extended from October to February. The mean maximum and minimum temperature of the area are 34.7 and 8.5°C, respectively, and mean relative humidity is 61.3%. Mixed farming system followed in the area, crop and livestock production are an intensive type of production (NMSA, 2004).

Study animals

In this study, all animals which gave birth between first November, 2009 and end of February, 2010 were used. The cows used in this study were Holstein Friesian of different parity, but kept under similar production system (intensive type of management) in selected farms in Debre Zeit town.

Study design

The study had two parts; the first study was a retrospective data collection and analysis to establish the prevalence and to identify the major reproductive disorders in the selected farms. The second part of the study was a regular clinical follow up on purposively selected dairy cows kept by the selected dairy farms.

Data collection

Secondary data

Secondary data was collected from the record books kept by the farms in the last three years; from January, 2006 to December, 2008 to identify the major prepartum and postpartum reproductive problems and to assess their prevalence in the selected farms.

Regular follow up

A regular visit of the farms was carried out to collect data on the major prepartum and postpartum reproductive problems of dairy cows. The study animals were identified by their tag number/ID, breed, parity and followed up their gestation time until delivery and 85 days post delivery by referring their individual case book for time of delivery. During gestation, at and following calving, cows were closely observed; their body temperature measured and feed consumption monitored. After delivery, cows were examined for the presence of retained fetal membrane left hanging in the vulva in the first 24 h and if any abnormal vaginal discharge occurred. Cows with abnormal vaginal discharge were subjected to vaginoscopy to determine the type and nature of the vaginal discharge according to Bekena et al. (1994a).

Finally, parturient cows were grouped in to those giving birth without any problem and those giving birth with problems according to the following definitions.

Abortion: Is the expulsion of a dead fetus of recognizable size at any stage of gestation (Robert, 1986; Bekana et al., 1994a, b; Noakes, 1986).

Dystocia: A condition in which the first or especially the second stage of parturition was prolonged markedly for more than 6 h and the cow required assistance (Robert, 1986; Stevenson and Call, 1988; Noakes, 1986).

RFM: A lack of expulsion of the placenta with in the first 24 h after calving (Robert, 1986; Arthur et al., 1989; Bekana et al., 1994a, b).

Metritis: An inflammation of the uterine wall characterized by reddish brown, white or whitish to yellow mucopurulent, with fetid vaginal discharge along with thickness of uterine wall at transrectal palpation (Morrow, 1980; Robert, 1986; Bekana et al., 1994a, b).

Repeat breeders: A cow or a heifer that failed to conceive for three or more consecutive services time (Morrow, 1980; Allenstein, 1981; Robert, 1986; Noakes, 1986; Shiferaw et al., 2003).

Anestrus: Cows which failed to show clinical heat signs for 90 days or more after parturition (Hafez, 1993; Bekana et al., 1994a, b).

Uterine prolapse: The coming out of uterus through the vulva commonly shortly after parturition and hanged out with the inner surface outer most (Morrow, 1980; Noakes, 1986).

Vaginal prolapse: The protrusion of the vagina and sometimes with the cervix through the vulva (Noakes, 1986; Robert, 1986).

Body condition scoring

For all of the animals under study, body condition was scored in order to assess the nutritional status of the animal and the prevalence of post parturient reproductive problems. Therefore, animals were grouped in to 0, 1, 2, 3, 4 and 5 body condition scores according to Richard (1993).

Data management and statistical analysis

Both the retrospective data and the regular clinical follow up result was entered to a Microsoft Excel sheet 2007 and analyzed using a software SPSS® version 16. The different parameters (that is, parity, management and body condition score) that were considered during the study period were analyzed using the Chi-square technique. The possible association of metritis with abortion, dystocia and RFM was also tested using these techniques.

RESULTS

In the retrospective study, from a total of 711 assessed recorded data on pregnant and parturient cows during the last three years, 44.3% (n=315) were found with major prepartum and postpartum reproductive problems. Postpartum anestrus accounted for the highest prevalence of 12.9% followed by repeat breeding, abortion, dystocia, RFM, metritis and vaginal/uterine prolapse that accounted for 11.4, 6.6, 4.2, 3.5, 3.5 and 0.7%, respectively in the three farms.

During the survey using regular follow up, a total of 35 (33.6%) cows were diagnosed to be affected at least by one of the major prepartum and postpartum reproductive problems. The average prevalence of abortion, dystocia, RFM, prolapse, metritis, anestrus and repeat breeding in the three farms during the regular follow up was found to be 6.7, 2.9, 3.8, 1.9, 8.7, 12.5 and 10.6%, respectively. Out of the total 35 affected cows, some 16 (45.7%) of them were diagnosed to have more than one reproductive problems.

The relative occurrence of abortion on the base of stage of gestation was assessed and the corresponding results in the first, second and third trimester was 1 (14.3%), 2 (28.6%) and 4 (57.1%), respectively. This result indicates that more than half of the abortion cases occur in the third trimester.

The dairy cows under this study were all exotic (HF) breeds kept under the same management (intensive) system. But other risk factors such as parity of a cow and body condition score at calving were considered to assess its association with the occurrence of the reproductive problems. Accordingly, the possible impact of increasing parity on the occurrence of the post parturient reproductive problems was examined and there is a direct relationship of increasing parity and the prevalence of the post parturient reproductive problems with a highly significant ($P<0.01$) result (Table 1).The influence of body condition score at calving on the occurrence of the major post parturient reproductive problems was assessed (Table 1) and the result showed that there is an inverse relationship of body condition and prevalence of the post parturient reproductive problems. The variation in prevalence among the different body condition score was found statistically highly significant ($P<0.01$). Although an attempt was also made to see sanitation of farms as a possible cause of reproductive problems; this was not statistically significant ($P>0.05$).

Analysis of the result was also made to observe the relationship of clinical metritis cases with the other predisposing factors. The result showed that 7 (77.8%) of the clinical metritis were developed as a sequel to abortion, dystocia and RFM accounting for 3 (33.3%), 1 (11.1%) and 3 (33.3%) respectively. Only 2 (22.2%) of the total cases were developed after normal parturition.

The association of metritis with abortion, dystocia and RFM was tested statistically and it was found out that metritis was not significantly associated ($P>0.05$) with these predisposing factors (Table 2).

DISCUSSION

The prevalence of anestrus observed in this study is in line with the results of Zewdu (1992) and Amene (2006) who reported a prevalence of 0.7 to 20.4%, and 10.2% in ILCA herds in Debre Zeit and at Alage dairy farm, respectively. This prevalence is lower than the range indicated by Befekadu (2007) and Darwo and Zerbini (1998) as 16.4 and 24% in cross bred dairy cows in Debre Zeit and in central high lands of Ethiopia, respectively. This might be due to the breed and management system differences. The 10.6% prevalence of repeat breeding found in this study agrees with the 5 to 15% reported by Puntam (1986), 9.6% reported by Amene (2006) and 13% reported by Micheal (2003). The higher repeat breeding cases recorded in pluriparous cows (63.6%) than those of primiparous (36.4%) is in agreement with the finding of Wolf (1993) who described the decline in fertility of cows with an increase in parity.

Table 1. Prevalence of prepartum and postpartum reproductive problems and its association with risk factors.

Risk factor	No. of cows examined	No. of cows with RP	No. of cows with normal parturition	Percent of affected cows	χ^2 Value	DF	P-value
Parity							
Primiparous	31	17	14	54.8	8.88	1	0.003
Pluriparous	73	18	55	24.7			
Total	104	35	69	33.6			
Sanitation							
Good	70	20	50	28.6	2.48	1	0.116
Poor	34	15	19	44.1			
Total	104	35	69	33.6			
BCS							
0	3	2	1	66.7			
1	12	8	4	66.7			
2	28	14	14	50.0	18.72	5	.002
3	34	8	26	23.5			
4	22	3	19	13.6			
5	5	0	5	0			
Total	104	35	69	33.6			

Table 2. Association of clinical metritis with the other predisposing factors of cows in the selected farms.

Predisposing factors	Cases with clinical metritis	Cases without clinical metritis	Total
Abortion	3 (42.9)	4 (57.1)	7
Dystocia	1 (33.3)	2 (66.7)	3
RFM	3 (75.0)	1 (25.0)	4
Total	7 (50.0)	7 (50.0)	14

χ^2=1.48, DF=2, P>0.05.

The problem of repeat breeding could be possibly due to wrong time of heat detection and insemination as well as problems related to semen handling and insemination techniques (Robert, 1986).

Prevalence of metritis recorded in this study (8.7%) agrees with that of Micheal (2003) who reported a prevalence of 11.3%. However, a lower prevalence of metritis was obtained in this work compared to the results of Gebremariam (1996), Ebrahim (2003) and Amene (2006) who have reported 19.6, 18.7 and 28.5%, respectively. This could be possibly due to differences in breed and management systems. Another important clinical reproductive problem in this study was abortion, which accounted for 6.7%. This figure is similar to the results of Tekelye et al. (1991, 1992a), Zewdu (1992) and Gebremariam (1996) who have reported a prevalence of 1.7 to 20.2%, 1.0 to 8.4%, 1.5 to 7.8% and 6.1% in three state dairy farms in central high lands of Ethiopia, in indigenous cattle at Abornessa farm, in Debre Zeit ILCA herds and in extreme Northern Ethiopia, respectively. This finding was also similar with the report of Awad et al. (1977) who obtained an abortion rate of 6.52% in Egypt. At Ghibe farm, Tekelye et al. (1992b) reported a prevalence of 2.6 and 2.0% in 1988 and 1989, respectively. This low abortion rate may be the effect of vaccination against brucellosis as it is indicated by the author.

The other results of reproductive problems considered in this study were 2.9% dystocia, 3.8% RFM and 1.9% prolapse which are lower when compared with most of the earlier reports. 2.9% prevalence of dystocia is in agreement with Zewdu (1992), Amene (2006) and Gebremariam (1996) who reported a prevalence of 2.2 to 4.4, 3.1 and 3.7%, respectively. But it is lower than the 9.7% reported by Micheal (2003) and 5.8% by Tadelech (2004) in small holder dairy cows in and around Awassa and Debre Zeit, respectively. This wide variation in the prevalence of dystocia is due to the fact that it is influenced by factors such as, the age and parity of the dam as well as breed of the sire as has been reported

earlier (Morrow, 1980; Noakes, 1986). The 3.8% prevalence of RFM is in line with the reports of Morrow (1980) and Paisley et al. (1986) who reported a morbidity range of 3 to 12% and 1.96 to 55%, respectively. But the prevalence of RFM in this study was lower than the reports of Tadelech (2004), Gebremariam (1996), Tekelye et al. (1992a), Amene (2006) and Ebrahim (2003) who reported 14.3, 16.8, 15.5, 26.6 and 7.1 to 28.9%, respectively. The lower prevalence of RFM in this study could be due to lower rate of dystocia and management difference especially feeding and sanitation.

Similar to the previous findings (Gebremariam, 1996; Melkamu, 1999; Desalegne, 2000; Micheal, 2003; Ebrahim, 2003; Tadelech, 2004), the present study confirms that pluriparous cows are more affected by postpartum reproductive problems than the primiparous. This might be possibly due to repeated exposure of pluriparous genital tract to environmental risk factors, which indeed, caused uterine infection. Moreover, in this study, the prevalence of post parturient reproductive problems had highly significant inverse relationship ($P<0.01$) with the body condition of the animals. The higher prevalence of reproductive problems found in animals of poor body condition may be attributed to the fact that such animals do have weak expulsive force to drop their after birth or to give birth without assistance which is followed by secondary complication. However, animals in good body condition have been reported to have better ability to meet the energy requirement of parturition, lactation and involution of uterus than a cow in poor body condition and hence, are better resistant to the possible infections that may be the result than a cow in poor body condition (Hafez, 1993).

Finally the association of metritis with the other reproductive problems (that is, abortion, dystocia and RFM) as a predisposing factors was not statistically significant ($P>0.05$). In fact, various authors (Bretzlaff et al., 1982; Markusfeld, 1984; Noakes, 1986; Paisley et al., 1986; Robert, 1986; Arthur et al., 1989; Barnouin and Chacornac, 1992; El-Din et al., 1995) all reported the presence of high degree of association between metritis and the aforementioned predisposing factors. In this study, 3 (75.0%) of the RFM cases develop metritis and additionally 7 (77.8%) of the metritis cases develop postpartum anestrus which is in agreement with that of Allenstein (1981) who indicated that RFM of long course is the main cause of metritis and metritis in turn has long been known to create anestrus condition in dairy cows.

CONCLUSION AND RECOMMENDATIONS

The result of the present study indicated that postpartum anestrus and repeat breeding are the leading reproductive problems followed by metritis and abortion, respectively. In general, the prevalence of the major reproductive problems in the study farms is high beyond the economically tolerable limit. This might be accounted to the poor management at calving, poor farm hygienic practices and lack of regular clinical follow up by animal health professional in the farms. Hence, improvement of hygienic condition of the farms and proper care, management and handling of cows at the time of parturition should be practiced. Routine and periodical examination of cows during postpartum period is essential, since most cows acquire uterine infection during this period.

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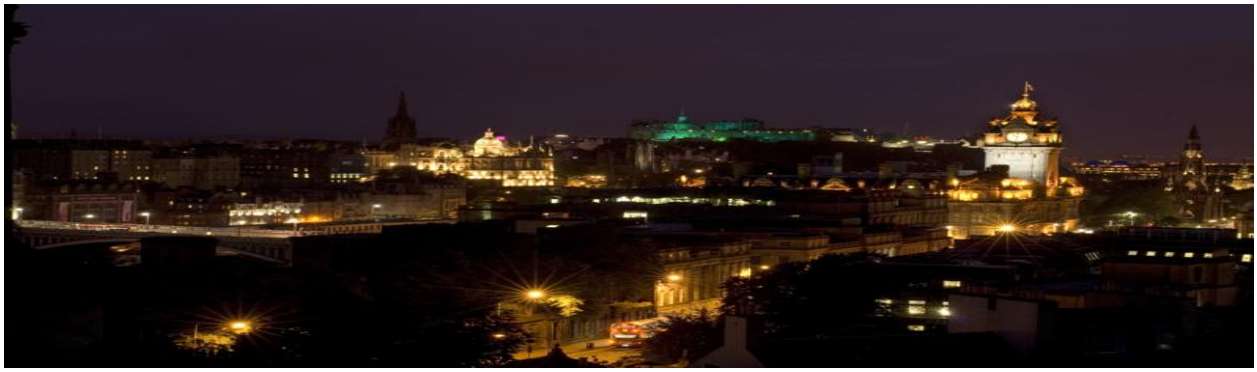

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