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Case Report

Listeriosis in a 5 month old white Fulani-cross heifer

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A five month old calf was presented to the Large Animal Clinic of the Veterinary Teaching Hospital Ahmadu Bello University, Zaria, with a chief complaint of nervous dysfunction. The calf was hospitalized and a thorough physical examination was carried out. The condition was diagnosed to be listeriosis based on the clinical signs, though efforts were made to isolate the organism from urine without success. The calf was treated with procaine penicillin at 25,000 IU I/M bid for 2 weeks and multivitamin 5 ml I/M for 3 days, after which the calf recovered completely. Treatment of listeriosis can be rewarding if aggressive antibiotic therapy is instituted early in the disease.

Key words: Calf, listeriosis, antibiotics.

INTRODUCTION

Listeriosis is a sporadic infection caused by the bacteria *Listeria monocytogenes* that affects a wide range of animals including birds, and is of immense public health importance (Woo-Sem, 1999). Although three forms of the disease exist (meningoencephalitis, abortion and septicemia), only one clinical form usually manifests at a time in an animal or a group of animals and only one serovar can be isolated from clinically affected animals. However, an overlap of these clinical forms has been reported (Low; Renton 1985). The encephalitic form is the most common which mainly affects ruminants; it is clinically characterized by depression and fever. In addition, the affected animal is usually disoriented, indifferent to its surroundings and separates itself from the rest of the herd (Dennis, 1993). Microscopically, there is mixed non suppurative and suppurative inflammation centered in the pon and medulla oblongata. Additional characteristics are the formation of microabcesses, prominent perivascular cuffs that includes lymphocytes, monocyte, plasma cells and to a lesser extent neutrophils (Jubb et al., 2007). The septicemic form affects the viscera with or without meningoencephalitis and is common in the monogastrics and humans (Melinda et al., 1997; Lecuit, 2007).

L. monocytogenes can be found in soil, manure, water and vegetation which make transmission very easy between animals through dissemination in feces (Ramaswamy et al., 2007). Listeriosis in ruminants has also been associated with feeding large quantities of poor quality silage with a pH in excess of 5.5. Spoilage and high pH of silage, particularly the top and side layers of the silage, result in growth of the bacteria (Schneider, 1994). However, it has been reported that the pervasive nature of the bacterium can make source of the infection difficult to identify (Borucki et al., 2005). Provisional diagnosis can be made based on the clinical signs, while

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Figure 1. Showing scoliosis of cervical vertebrae (arrow).

confirmatory diagnosis can only be made by isolating the organism from the brain or internal organs, as well as faeces, urine, blood and milk of the affected animals. Rabies, otitis media, brain stem abscess, thromboembolic meningoencephalitis, lead poisoning and head trauma have been considered as differentials in the clinical diagnosis of Listeriosis.

Successful treatment of listeriosis depends on early detection and aggressive antibiotic therapy. However, as soon as signs of the affection of the central nervous system appear, the prognosis becomes unfavorable (Schneider, 1994; Coetze and Tustin, 2004). This paper reports a case of successful treatment of listeriosis in a 5 month old calf.

CASE PRESENTATION

A 5 month old orphaned white Fulani × Friesian cross weighing 50 kg from a herd of five was presented to the Large Animal Clinic of Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria with complaints of restlessness, inappetence and walking in circles. History further revealed that the calf was being fed with compounded milk and concentrates. Physical examination reveals an elevated rectal temperature of 42.1°C, rapid pulse, increase respiratory rate (50 cycles per minute) with markedly increased crackled and wheezily lung sound, excessive salivation with no obvious lesion in the buccal cavity. The ocular mucous membranes were congested and a lateral deviation (scoliosis) of the cervical vertebrae was evident (Figure 1).

Urine sample was taken and submitted to Department of Veterinary Microbiology for possible identification and isolation of the causative organism. The calf was put on procaine penicillin at 25,000 IU I/M bid ×2/52 and multivitamin 5 ml IM/ ×3/7. The animal was hospitalized for 2 weeks.

RESULTS AND DISCUSSION

Culture of the urine sample yielded no growth, this is however not surprising because Coetzer and Tustin (2004) reported that the pathogens are very irregularly distributed in the body, and for isolation, specimen must be collected from various organs of the body. The diagnosis of the disease was based on the prevailing clinical signs. The transmission of the pathogen has been associated with environment contaminated with lochia, faeces, urine nasal discharges and milk of infected animals. Immunosuppression (Schneider, 1994) has also been incriminated to be a major predisposing factor in this disease which is strongly suspected to be the cause of infection in this animal, thus this is supported by the fact that the calf in question lacked colostrum and had been bottle fed since birth. Since the causative organism can be a normal flora of the gastrointestinal tract, it could have gained entry through the intestinal epithelium and possibly ascended to the brain through the branches of the trigeminal nerve via lesions in the oral cavity, thus resulting in the observed nervous manifestations. The head and neck tilt resulting in concavity in the neck region was as a result of the dysfunction of cranial nerve VIII (vestibulocochlear) which is a nerve responsible for maintaining equilibrium (balance) and transmitting sound information from the inner ear to the brain (Sanders and Gilling, 2010). Excessive salivation could be responsible for the dyspnea due to aspiration pneumonia; this was due to the dysfunction of cranial nerve cnIX (Glossopharyngeal) that also helps in voluntary control of the stylopharyngeus muscle which aids in swallowing. The high values for all the vital parameters could be attributed to the presence of the infection and restlessness in this animal.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Age-related changes in haematologic parameters of cage-raised Japanese quails (*Cortunix japonica*)

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Blood samples were collected from clinically normal, cage-raised Japanese quails at regular intervals, over a six-week period. Full routine haematological analyses were carried out to establish reference values for growing individuals of this species and to assess age-related changes. The results of this study showed an initial significant reduction ($p<0.001$) in the values of the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) at the second week of age relative to the values at the first week of age. However, MCV values significantly increased ($p<0.001$) weekly, up to the sixth week of age. Similarly, significant decrease ($p<0.05$) was observed in the total white blood cell count at the second week of age, but subsequently remains unaltered throughout the period of the experiment. However, the heterophil:lymphocyte ratio increased significantly ($p<0.05$) with increase in the age of birds. The observations in this study compares favourably with published literature.

Key words: Quails, haematology, age.

INTRODUCTION

The Japanese quail, *Cortunix japonica*, belongs to the family Phasidae and the genus *Cortunix* (Mizutani, 2003). The bird originally domesticated around the 11th century as a pet song bird, is now highly valued as a food animal in several parts of the world, because it constitutes an important source of eggs and meat (Kayang et al., 2004). In addition, the relatively short life span of *C. japonica* (3 to 4 generations per year) is a useful experimental tool with which to study the effect of age, nutrition, disease and environment on haematological parameters of birds (Ottinger, 2001; Holmes et al., 2003).

Haematology offers a valuable contribution in the veterinary care of avian species in providing differential diagnosis of pathological conditions and in monitoring responses to disease (Campbell, 1994). Moreover, the evaluation of haematological parameters is important clinically for analyzing the health status of farmed animals animals, providing reliable information on metabolic disorders, nutrient deficiencies and chronic stress status (Bahmani et al., 2001). Consequently, measuring and comparing haematological values with known standards is among the methods which contribute to the detection of some changes in the health status of avian species (Gavett and Wakeley, 1986). Unfortunately, these haematological values are influenced by various factors including breed, sex, age, reproductive status, exercise, circadian rhythm, handling procedure, degree of excitement and nutritional status of animals (Kramer, 2000).

Unlike most mammalian species for which information for haematological parameters abound, the reports on the baseline haematological values in birds and the various factors affecting these values are few. Some of the

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available reports include the reports of Torgowski and Kontecka (1998) on the blood cell count of pheasants; that of Vahala and Kase (1993) on age-and sex-related differences in haematological values of captive white-tailed gnu (*Connochaetes gnou*); and that of Olayemi et al. (2006) on haematological and plasma biochemical parameters of the Nigerian laughing dove (*Streptopelia senegalensis*), and the Nigerian duck (*Anas platyrhynchos*). Similarly, Gildersleeve et al. (1985) reported the haematological response of the Japanese quail to haemorrhagic stress. McCue et al. (2013) reported that targeted 13C enrichment of lipid and protein pools in the body reveals circadian changes in oxidative fuel mixture during prolonged fasting, suggesting alteration in plasma metabolite of the Japanese quail following a 6-day fasting period. Overall, there is a dearth of information on the physiological variations of haematological parameters in birds. Specifically, apart from the work of Ayub et al. (2012), that reported the effect of age on the haematological and biochemical profile of Japanese quails raised under the litter system, data on age-related haematological variations of the Japanese quail, to our knowledge, are nonexistent. Therefore, it is prudent to evaluate the effect of age on the haematologic parameters of the Japanese quail under experimental conditions to provide baseline information about the physiological status of these birds.

The purpose of this study was to observe haematological values in growing Japanese quail chicks up to the age of 6 weeks in order to establish reference values, determine any significant age-related changes and present analysis of the dynamics of changes in haematological values with age.

MATERIALS AND METHODS

Experimental animals

Sixty unsexed Japanese quails were obtained at one day of age from a local hatchery. The birds were kept at the brooder for three weeks and for another three weeks in metal cages with wire mesh doors and floors, measuring 80 × 80 × 40 cm at the ratio of 15 birds to one cage. Feed containing approximately 23% crude protein, 12.9 MJ/kg metabolisable energy, with no medication and fresh clean water were provided *ad libitum*. Birds were kept in the dry season at room temperature (27 to 30°C) and relative humidity of about 70%.

Haematological analyses

Blood samples were collected from birds, in the mornings between 0800 and 0900 h, by wing venopuncture into sterile vials with 20 µl of 10% ethylenediaminetetraacetic acid (EDTA), weekly for six weeks. Blood samples were quickly refrigerated at 4°C and analysed within 24 h. Packed cell volume (PCV) and haemoglobin concentration (Hb) were determined by the microhematocrit and cyanmethaemoglobin methods, respectively, as described by Jain (1986). Erythrocyte count was determined by the haematocytometry method as described by Jain (1986). Total white blood cell (WBC) counts were made in a haemocytometer using the WBC diluting fluid and differential leucocytes counts were made by counting the different types of WBC from Giemsa stained slides viewed (Coles, 1989). Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined from the values obtained from red blood cells (RBC) count, haemoglobin concentration and PCV values (Duncan et al., 1994).

MCV expresses the average volume of the individual RBC:

$$\text{MCV (fl)} = \frac{\text{PCV (\%)} \times 10}{\text{RBC } (10^6\ \text{ul}^{-1})}$$

MCH expresses the average weight of hemoglobin (Hb) in the erythrocyte:

$$\text{MCH (Pg)} = \frac{\text{Hb (g/dl)} \times 10}{\text{RBC } (\times 10^6\ \text{ul}^{-1})}$$

MCHC gives the percentage of the MCV which the Hb occupies:

$$\text{MCHC (g/dl)} = \frac{\text{Hb (g/dl)} \times 10}{\text{PCV (\%)}}$$

Statistical analyses

Statistical analyses were carried out by using graph pad PRISM software package (version 5.0). Statistical significance was assessed by the student t-test and the repeated measures analysis of variance (ANOVA). Results are presented as mean ± standard error of mean, and value of probability less than 5% was considered statistically significant.

RESULTS AND DISCUSSION

The results for the age-related variations in haematological parameters of cage-raised Japanese quail are presented as shown in Table 1 and Figures 1 to 3. All recorded haematological values are considered normal, because the birds remain clinically normal throughout the period of the experiment. However, the values of the MCV and MCH significantly decreased ($p<0.001$) at the second week of age relative to the values at the first week of age (Figure 1). After the initial decrease, the value of the MCV significantly increased ($p<0.001$) weekly, up to the sixth week of age (Figure 1). MCV measures the average size of individual red blood cells (Dacie et al., 1995). In most instances, a reduction in the value of MCV occurs when iron deficiency becomes severe and is a fairly specific indicator of iron deficiency once thalassemia and the anaemia of chronic disease have been excluded (Garby, 2002). The initial significant reduction in the values of MCV, observed in this study may be due to accelerated erythropoiesis that significantly increases the demand for iron during haemoglobin formation. Accelerated erythopoiesis can also cause an increase in MCV values when iron is available in abundance, primarily due to the stimulatory effects of erythropoietin

Table 1. Weekly haematological paarameters of cage-raised Japanese quail.

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PCV (%)	30.01±3.13	31.60±2.62	31.62±1.14	32.41±1.14	31.82±2.58	39.8±1.50
Hb (g/dl)	9.91±1.08	10.51±0.85	10.52±0.41	10.74±0.36	10.54±0.87	13.18±0.45
Rbc ($\times10^{12}$ L^{-1})	2.28±0.64	4.51±0.70	4.80±1.60	4.39±0.98	3.62±0.81	3.75±0.74
Wbc ($\times10^{9}$ L^{-1})	8.01±1.38	3.36±2.21	14.21±3.41	14.80±5.94	10.88±0.81	16.38±0.74
Platelet ($\times10^{9}$ L^{-1})	8.87±0.81	9.20±1.09	9.72±0.38	9.92±0.18	9.62±0.89	11.25±1.09
MCV (fl)	131.24±23.41	71.42±12.50*	71.21±22.53**	77.12±21.87**	88.81±22.07**	108.63±19.91**
MCH pg	43.15±7.95	21.77±4.02*	23.21±7.31	25.21±7.15	29.21±7.33	35.81±6.26
MCHC (g/dl)	33.18±0.89	33.21±0.46	33.09±0.45	32.63±0.89	33.41±1.14	33.61±0.89
Lymphocyte ($\times10^{9}$ L^{-1})	70.15±6.35	62.41±4.35	60.15±3.81	58.41±3.78	55.81±3.89	54.21±3.49
Heterophil ($\times10^{9}$ L^{-1})	29.10±5.76	37.21±3.96	39.21±3.96	41.62±3.78	44.11±4.18	45.43±3.65

*Significant increase compared with week 1; **Significant increase compared with week 2.

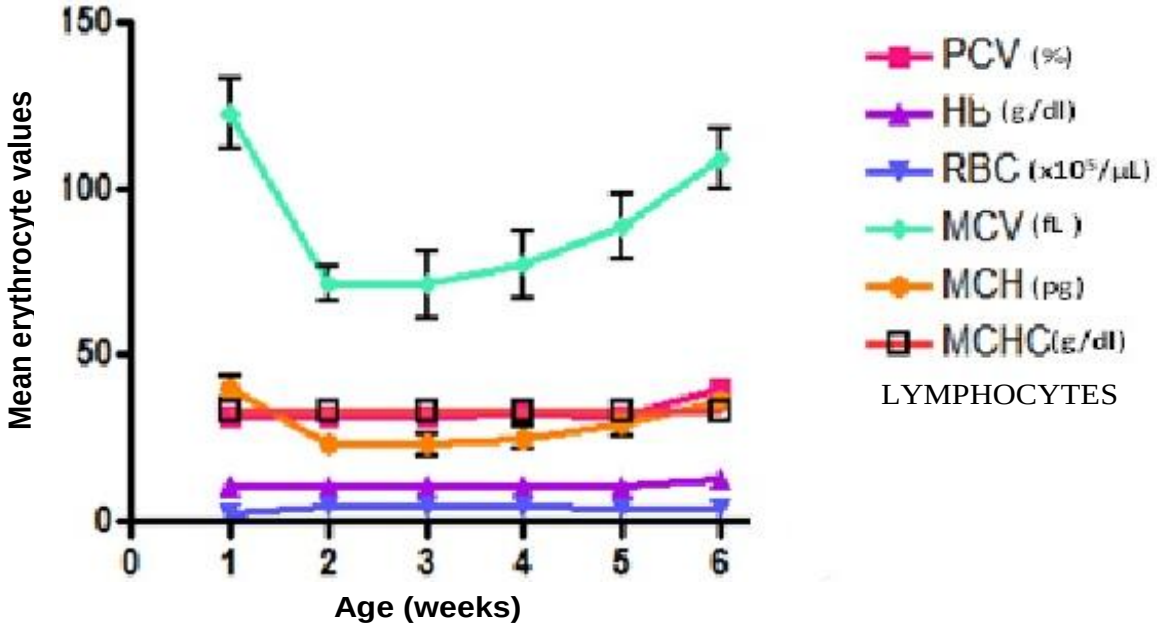

Figure 1. Mean weekly erythrocyte values of cage raised Japanese quail (*Cortunix cortunix japonica*). Asterisked values are calculated from the values of PCV, Hb and RBC.

on erythropoiesis. This is because erythropoietin causes the skipping of some non proliferative stages of erythopoiesis, leading to the release of large amount of reticulocytes into the circulation, and consequently increasing the MCV value. However, increase in MCV values due to accelerated erythropoiesis is usually preceded by acute and/or severe haemorrhagic or haemolyticanaemia, which is not the case in this study.

The decrease in MCV could have also resulted from changes in the relative proportions of erythrocyte type in bird embryos, compared to that of neonatal life. The larger primary erythrocytes which predominate in prenatal life decrease gradually during the second week, leaving predominantly mature erythrocytes, which may contribute to the gradual decrease in MCV. The subsequent gradual increase in the value of MCV is presumably due to the eventual predominance of mature erythrocytes in adulthood. In contrast to the age related variations in the values of MCV and MCH, variations observed in the values of the PCV, Hb, RBC and MCHC do not appear to be age related. This study agrees with the work of Ayub et al. (2012) who reported non-significant age-related variations in the values of PCV of Japanese quails raised under the litter system.

Also in this study, significant decrease ($p<0.05$) was observed in the total white blood cell count at the second

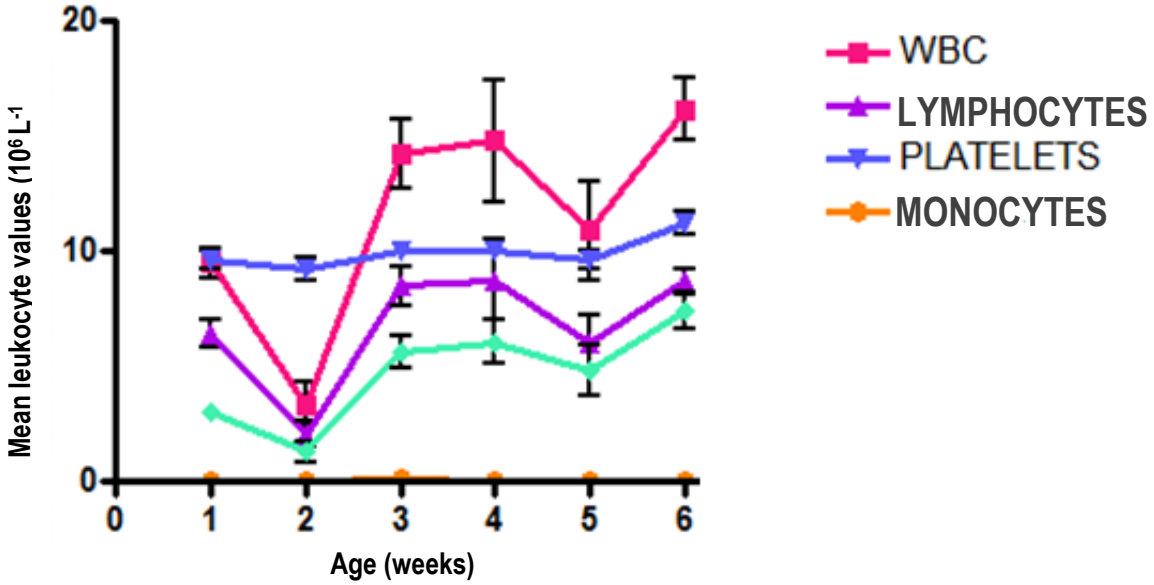

Figure 2. Mean weekly blood leukocyte values of cage raised Japanese quail (*Cortunix cortunix japonica*).

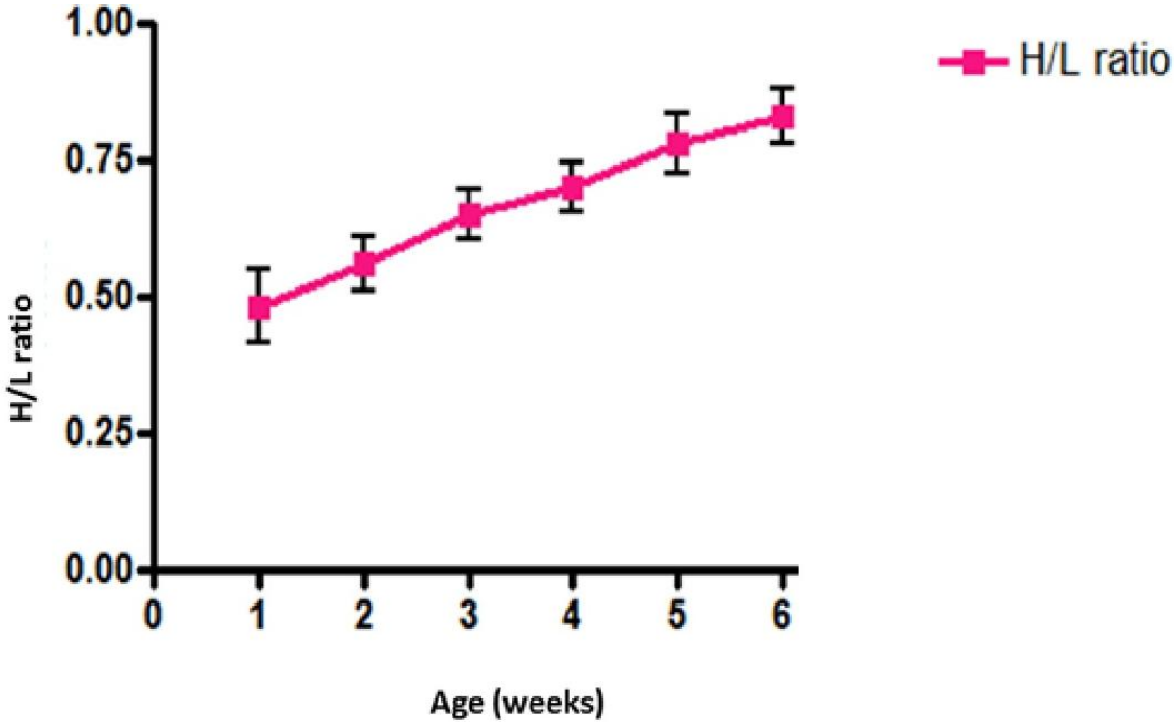

Figure 3. Mean weekly heterophil/lymphocyte ratio of cage raised Japanese quail (*Cortunix cortunix japonica*).

week of age, relative to the values recorded in the first week of age but subsequently remain unaltered throughout the period of the experiment (Figure 2). However, heterophil:lymphocyte ratio increased with increasing age of birds (Figure 3). This is similar to the results of Fouad et al. (2008) where a greater heterophil:lymphocyte ratios in caged chickens, compared to those reared in floor pens was observed.

The heterophil/lymphocyte ratio has been shown to be a reliable indicator of stress associated with injury,

reproductive cycles and seasonal changes in birds in both captive and field situations (Moreno et al., 2002). Consequently, changes in this ratio have been used as indicators of stress response in domestic animals (Gross and Siegel, 1985). Birds normally have high numbers of circulating lymphocytes and may develop leukopenia and lymphopenia in the initial stress response (Ritchie et al., 1994). High numbers of circulating lymphocytes beyond the normal range might be physiologic, reactive, or proliferative (Jain, 1993). Transient physiologic lymphocytosis is observed with a marked release of epinephrine, because of physical or emotional stress (Aengwanich et al., 2003). In fact, epinephrine level increases within seconds of exposure to stress and glucocorticoids rise within minutes of stress exposure (Le Maho et al., 1992). Therefore, the increase heterophil:lymphocyte ratio observed in this study may indicate a physiologic response of the Japanese quail to the stress presented by confinement in cages. A number of stress factors, including water deprivation, temperature extremes and exposure to novel social conditions have been suggested to elevate the number of heterophils and depress the number of lymphocytes in birds (Gross 1989).

In conclusion, the observations in this study may aid understanding of haematological responses of the Japanese quail to different disease conditions, especially under intensive management. However, further studies of haematologic responses of this species to specific pathological conditions are required to further aid the management of disease condition of cage-raised Japanese quails.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Prevalence of bovine tuberculosis in dromedary camels and awareness of pastoralists about its zoonotic importance in Eastern Ethiopia

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The aim of this study was to investigate bovine tuberculosis in camels which was conducted in Eastern part of Ethiopia using post mortem examination, *Mycobaterium* isolation, tuberculin skin test and questionnaire based interview. Tuberculosis (TB) is an important disease for pastoralist, particularly due to prevailing habit of consuming raw milk and sharing house during night time with their animals. This study has showed prevalence rate of 8.3% (33/398) based on the post mortem examination and 6.0% (29/480) at cut off >4 mm based on the tuberculin test. From 33 camels with suspected tuberculosis compatible lesions, 12% (4/33) showed mycobacterial growth on Lowenstein-Jensen (LJ) media supplemented with pyruvate and 18% (6/33) on LJ media supplemented with glycerol. 94.7% (36/38) of the interviewed pastoralists knew about TB while only 50% (19/38) were aware about its zoonotic importance. However, only 18.4% (7/38) of the interviewed camel owners usually boil camel milk before consumption. The result of this study highlights the potential public health risk posed by TB from camels. Hence, prompt measures are required to control the possible zoonotic transmission of the disease-prioritizing on educating the pastoralist to consume camel milk after boiling.

Key words: Dromedary camel, tuberculosis-compatible lesions, tuberculin test, zoonotic importance.

INTRODUCTION

Thirty-four percent of the land surface distributed world-wide is deserts and semi-deserts (Roger, 2006). The dromedary camel (*Camelus dromedarius*) is an important multipurpose livestock species uniquely adapted to these harsh environments that can be used for meat, milk, wool and hide production and transportation and as a source of entertainment, celebration and competition (Abdelhakim and Youcef, 2012). There are 25.89 million dromedary camels in the world with 80% of them in Africa. Ethiopia takes the 3rd place in Africa next to Somalia and Sudan in possessing 2.40 million dromedary camels (FAO, 2011). Ethiopia camels are kept in arid and semi-arid lowlands such as Borana, Somalia and Afar regions (Teshome et al., 2003). They serve as an important source

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of milk, meat, draught power and transportation for the pastoralists (Bekele, 1999). Zoonotic infections, transmissible between humans and animals, are closely associated with pastoralists, because of their close contacts with their domesticated animals (Macpherson, 1995; Schelling et al., 2003; Li et al., 2005) and habit of raw camel milk consumption (Farah et al., 1990; Radwanet al., 1992; Younan, 2004).

Animal tuberculosis (TB) is a zoonotic disease caused by members of the *Mycobacterium tuberculosis* complex, including *M. tuberculosis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium bovis*, *Mycobacterium caprae*, *Mycobacterium canettii* and *Mycobacterium pinnipedii* (Ayele et al., 2004). In dromedary camels, *M. tuberculosis* (Elmossalami et al., 1971; Zubairet al., 2004), *M. bovis* (Kinne et al., 2006), *M. capre* (Pate et al., 2006) and *M. microti* have been isolated from tissue lesions (Wernery and Kaaden, 2002; Kinne et al., 2006). *M. bovis* strains were also isolated by Donchenko et al. (1975) in Russia from bulked samples of raw dromedary milk. Recent studies in Ethiopian abattoirs also reported isolation of *M. bovis* (Mamo et al., 2011) and *M. tuberculosis* from tissue lesions (Gumi et al., 2012; Zerom et al., 2012). Five to twelve percent abattoir-based prevalence of tuberculosis compatible lesions (TCL) in dromedary camels slaughtered at Dire Dawa abattoir in Eastern Ethiopia and in Addis Ababa abattoir were recorded (Mamo et al., 2009; Mamoet al., 2011; Zerom et al., 2012).

The incidence of human TB in Ethiopia is high with an annual incidence of 261 cases/100,000 population (WHO, 2011). TB lymphadenitis in cervical lymph nodes (TBLN) accounts for ≈33% of all new cases in the country (WHO, 2011), raising the possibility of orally acquired infection. Thus the zoonotic risk arising from dromedary camel milk should be considered, because camel milk is mostly consumed in its raw state (Farah et al., 1990; Radwan et al., 1992; Younan, 2004).

This study was designed to estimate the prevalence of bovine TB among dromedary camels of Eastern Ethiopia based on post mortem examination and comparative tuberculin test; and to assess the awareness of camel holders about the zoonotic nature of dromedary TB.

MATERIALS AND METHODS

Study area

This study was carried out in Dire Dawa City Administrative Council (DDAC) and Somali pastoral region from June 2007 to June 2008. Dire Dawa is located between 9°49' North latitude and between 41°38' and 42°19' East longitude at 520 km East of Addis Ababa. It comprises of diversified topographic features with altitude varying from 950 m above sea level (m.a.s.l.) in the north-east to 2260 m.a.s.l in the southwest. The mean annual rainfall in the area varies from 550 to 850 mm. The monthly mean maximum temperature ranges from 28.1°C, which is recorded in the months of December and January, to 34.6°C recorded in the month of June (Agricultural Development Office of DDAC, 2001). The livelihood of the rural residents of DDAC is based on agriculture in which raising livestock (such as goats, camels and cattle) plays a great role.

The Somali pastoral regional state is situated in the Eastern part of Ethiopia at 4 to 11° North latitude and 40°48° East longitude. The altitude ranges between 500 and 1600 m.a.s.l. The average annual rainfall is about 560 mm. The annual daily minimum and maximum temperature is 13 and 27°C, respectively (National Meteorology Service, 2001). Eighty five percent of the population of the region makes up pastoral community. Two zones, namely Jijiga and Shinile, were selected for the purpose of this study.

Study design

This study was a cross sectional study using post mortem examination of camels slaughtered in Dire Dawa municipal abattoir and sampling of suspicious cases for bacterial isolation and laboratory tests; a Single Intradermal Comparative Tuberculin Test (SICTT) carried out in rural parts of Dire Dawa, Shinile and Jijiga; and interview with camel holders about the awareness of the zoonotic nature of bovine TB.

Abattoir survey

Age, sex, body condition and origin of each camel were recorded prior to slaughter. Age estimation and body condition scoring (BCS) were done according to Wilson (1984) and Faye et al. (2001), respectively.

Post mortem inspection was conducted according to procedures mentioned by Croner (1994). Thorough examination was conducted on the mandibular, parotid, retropharyngeal, bronchial, mediastinal and mesenteric lymph nodes, together with the thoracic and abdominal organs. The lobes of the left and right lungs were palpated and inspected externally then, each lobe was sectioned into about 2 cm-thick slices to facilitate the detection of lesions with sterile surgical blades. Similarly, lymph nodes were sliced into thin sections (about 2 mm-thick) and inspected for the presence of visible lesions. TB suspected tissue samples were collected in to sterile bottle in normal saline (0.9%), labelled and kept in a deep freeze (-20°C) in Dire Dawa Veterinary Diagnostic and Investigation Laboratory until being transported to Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University. The tissue samples were transported in cold chain using an ice box packed with ice.

Comparative tuberculin test

Peasant associations (PA's) were randomly selected from Dire Dawa, Shinile and Jijiga, and where security was in doubt and transport was not convenient, PA's with similar conditions were replaced. Proportional number of households was randomly selected from each PA. Cluster sampling was conducted for the SICTT, a household was considered as a cluster. Camels which were younger than six months, in late pregnancy and recently calved dams were excluded from the study. The tuberculin test was done using purified protein derivatives (PPD), which was produced by Central Veterinary Laboratories, Weybridge, UK. Volumes of 0.1 ml 30,000 iu/ml and 25,000 in/ml of bovine and avian PPD were administered to the middle of the cervical skin at 10 cm apart. Before the PPD was injected each site was shaved, cleaned with alcohol and marked with ink. The skin thickness was measured before injection and after 72 h using a digital calliper. The SCITT was considered positive if the reaction to the bovine PPD was >4.0 mm greater than that to the avian PPD, inconclusive if it was only from 1.0 to 4.0 mm greater, and negative result was recorded if the reaction to the bovine PPD was equal to or smaller than that obtained at the avian site of injection (OIE, 2004).

Questionnaire survey

Questions about the management of camels and public health importance of bovine TB were collected using a structured questionnaire. The questionnaire was administered by 'face to face' interviews with camel owners by local languages (Oromifa and Somaligna) side to side with the tuberculin test.

Laboratory test

Direct smears were made from the centrifuged sediments of 33 suspected tuberculous tissues for direct microscopic examination and stained using the Ziehl-Neelsen staining technique (Dekanter et al., 1987). Tissue samples were macerated in a sterile Petri-dish to get fine pieces by using a sterile blade and forceps and then each samples was homogenized using a sterile mortar and pestle for 10 min in 5 ml of normal saline. Two milliliters of the homogenate was transferred to centrifuge tube and decontaminated by adding an equal volume (2 ml) of 4% NaOH by centrifugation at 3,000 rpm for 15 min. The supernatant was discarded, while the sediment was neutralized with 1% (0.1 N) HCl with phenol red as indicator. Neutralization achieved when the color of the solution changed from purple to yellow (OIE, 2004). Therefore, 0.1 ml of suspension from each sample was spread on to a slant of Lowenstein-Jensen (LJ) medium. Duplicates of LJ media were used; one was with 4% sodium pyruvate while the other was enriched with glycerol. The cultures were then incubated aerobically at 37°C for up to 12 weeks with weekly observation; Ziehl-Neelsen staining was performed to confirm the presence of acid–fast bacilli (AFB) (Quinn et al., 1994; WHO, 1998). Initial identification of mycobacterial species were based on the rate of growth, pigment production and colony morphology. Species belonging to the *M. tuberculosis* complex show a slowed growth rate (Quinn et al., 1994).

Data management and statistical analysis

The data generated from the abattoir and the tuberculin test were analysed using STATA (Version 9) and Win Episcope epidemiologically software. The animal level prevalence was expressed as the number of camel positive to the post mortem examination and the SICTT per 100 separately. The chi-square (χ^2) test was carried out to study the association between bovine TB status of camels and host factors, and a test of agreement (Kappa) to compare the direct microscope and the bacterial growth (Thrusfield, 1997). The confidence level was set at 95% (α=0.05).

RESULTS

Post mortem examination

The detailed post mortem examination carried out at the abattoir revealed that 33 (8.3%) of the 398 examined carcasses showed TCL. Large proportions of the lesions (57.5%) were found in the thoracic lymph nodes and the lungs followed by the lymph nodes of the head (27.2%) and the complement in mesenteric lymph nodes. The average number of lesions per infected animal was 1.12, and about 87.8% of carcasses with TCL possessed only a single lesion. Out of 33 camels with TCL, 4/33 (12%) showed growth on LJ media supplemented with pyruvate and 6/33 (18%) on LJ media supplemented with glycerol. Ziehel-Neelsen staining of centrifuged sediments of the 33 lesions smears demonstrated only 11 acid fast bacteria (AFB) positive slides. Out of these 11 AFB positive smears, only six proved positive upon bacteriological examination culture (Table 1).

The association of risk factors like sex, age and body con-dition with the TCL status of camels was studied using χ^2 test (Table 2). The age category showed significant difference ($p<0.05$) as regard to the abattoir-based prevalence of bovine TB. The prevalence of bovine TB varies among the various origins of camels slaughtered in Dire Dawa (Figure 1). The highest was found in camels originated from PA's predominantly inhabited by Oromo ethnic group such PA's are Bishan Bahe, Mermersa and Dujuma.

Tuberculin test

Of the total 480 examined camels by the SICTT, 29 (6.0%) of them were positive to bovine TB (Table 3). The lowest prevalence was observed in Shinile zone; though the difference among the three study sites was not statistically significant ($p>0.05$). Likewise, the differences in prevalence between the sex and between different age groups were not significant ($p>0.05$).

Questionnaire survey

Thirty eight dromedary camel owners (households) were interviewed to gather information on public health risk factors of camel TB. Six (15.8%) of the interviewed respondents, house their camels during night along with the family, and only 18.4% consume milk usually after boiling (Table 4). Strikingly, none of the respondents consume raw meat. The awareness of the zoonotic nature of camel TB is 50%.

DISCUSSION

The prevalence of bovine TB in dromedary camels in Eastern Ethiopia was 8.3 and 6.0% based on the post mortem examination and the SICTT, respectively. The abattoir-based prevalence is a little higher than a similar work done in the same place one year earlier, which was 5.1% (Mammo et al., 2009). The camels that were slaughtered in the abattoir originated from the pastoral areas where the pastoralists depend on the milk of their camels for subsistence and where no treatment of milk is practiced before consumption, either it is consumed raw or when just soured. A previous study shows that the raw camel milk plays a role in transmission of TB into human (Younan, 2004). Therefore, this finding shows the possible public health risk of the disease on the pastoral community.

Based on the culture on the LJ media, only 4 camels

Table 1. Comparison of direct microscopic and bacteriological examinations of samples taken from camels with tuberculous compatible lesions slaughtered in Dire Dawa municipal abattoir, 2007-2008.

Direct microscopy	Culture		Total
	Positive	Negative	
Positive	6	5	11
Negative	4	18	22
Total	10	23	33

Sensitivity= 60% (95% CI: 30-90.4), Specificity= 78.3% (95% CI: 61.5-95.2), Kappa (κ) = 0. 37.

Table 2. Association of macroscopic tuberculous lesions with host factors in dromedary camels in Dire Dawa, 2007-2008.

Variable	Category	Number of examined camels (n)	Number of positive camel (%)	χ^2 (P-Value)
Sex	Male	301	24 (7.97)	4.5 (0.49)
	Female	97	9 (9.3)	
Age	≤ 5 years	11	0	6.9 (0.03*)
	6-10 years	200	17 (7.9)	
	>10 years	188	22(11.7)	
Body condition score	Poor	97	15(15.5)	8.5 (0.14)
	Medium	212	13(6.13)	
	Good	88	5(5.7)	

*Statically significant.

Table 3. The result of single intradermal comparative tuberculin test carried out in Eastern Ethiopia to study the status of bovine tuberculosis in dromedary camels, 2007-2008.

Variable		Tested camels [n (%)]	Reactors [n (%)]	χ^2 (P-value)
Site	Dire Dawa	118	8 (6.8)	1.9 (0.39)
	Shinile	208	6 (3.9)	
	Jijiga	154	15 (7.2)	
Sex	Male	121	4 (3.3)	2.2 (0.19)
	Female	359	25 (7.0)	
Age	< 4 years	120	4 (3.3)	2.3(0.31)
	4 - 10 years	259	19 (7.3)	
	> 10 years	101	6 (5.9)	
Total	-	480	29(6.0)	-

(12%) showed growth on the pyruvate supplemented media and 6 camels (18%) on the glycerol supplemented media. The small number of culture positive samples when compared with the number of macroscopic tuberculous lesions may arise from classification of non-tuberculous lesions as TCL due to similarity with parasitic

Table 4. The awareness of camel owners about zoonotic importance of bovine tuberculosis in camels in Eastern Ethiopia, 2007-2008.

Factor	Category	Number of respondents (%)
Housing of camels during night	Separate barn	32 (84.2)
	Along with families	6 (15.8)
Ways of milk consumption	Usually boiled	7 (18.4)
	Usually raw	21 (55.3)
	Occasionally boiled	9 (23.7)
	Occasionally raw	1 (2.6)
Ways of meat consumption	Usually cooked	38 (100)
	Usually raw	0 (0)
	Occasionally cooked	0 (0)
	Occasionally raw	0 (0)
Awareness about TB	Aware	36 (94.7)
	Non-aware	2 (5.3)
Awareness about zoonotic nature of TB	Aware	19 (50)
	Non-aware	19 (50)

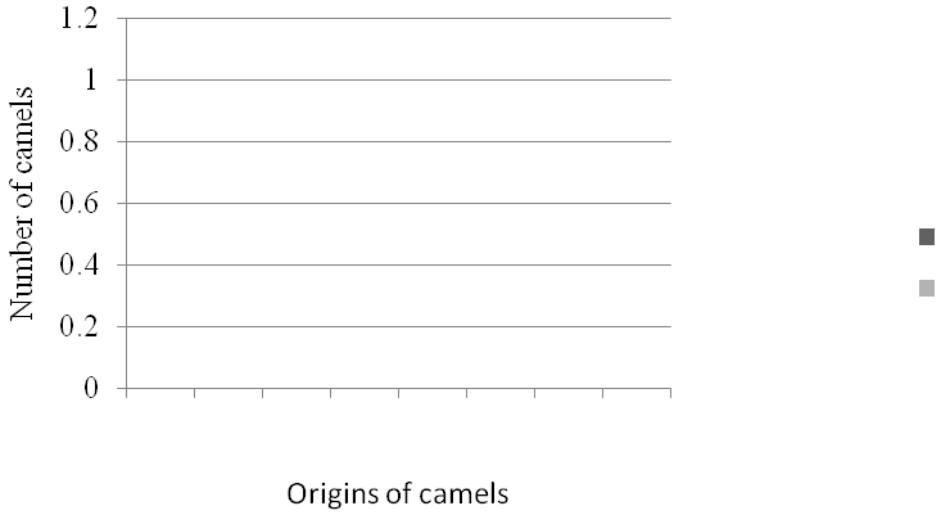

Figure 1. Origins of dromedary camels slaughtered in Dire Dawa municipal abattoir and their status of the post mortem examination for macroscopic tuberculous lesions, 2007-2008.

granulomas and abscess and decontamination process. Nevertheless, it could also be due to the absence of viable mycobacteria in calcified lesions. In completely calcified lesions tubercle bacilli are dead and no growth will be obtained up on culture (Gracey, 1986; Quinn et al., 1994; Tekluet al., 2004) or due to inappropriate handling of samples.

TCLs were found most frequently in the lungs and associated lymph nodes (57.7%) followed by lymph nodes of the head (27.2%), which is in consistence with the findings of Mammo et al. (2009) and Zerom et al. (2012) in camels. Croner (1990) reported that up to 95% of cattle with visible TB lesion could be identified by examination of lungs and associated lymph nodes. This finding suggests inhalation as the principal route of TB infection in cattle which might also be true in camels. The presence of lesions in the mesenteric lymph nodes (15.3%) indicates infection through ingestion (Radostitis et al., 1994). The average number of infected tissues per infected camel was 1.12, while 87.8% of the camels had only a single lesion. This finding complies with previous report on camel (Mammo et al., 2009).

The low agreement (κ=0.37) between direct microscopy and bacteriology (Table 1) complies with a previous report from Ethiopia (Teklu et al., 2004) and indicates that AFB other than *Mycobacteria* (e.g. *Nocardia*, *Actinomyces)* may have produced the spurious results. Irrespectively, direct microscopy is considered to be the least sensitive of all diagnostic methods for the detection of AFB (Baron et al., 1994).

The difference in TB prevalence between male and female based on macroscopic tuberculous lesions was not statistically significant, which is consistent with Mammo et al. (2009) in camels and Assegid et al. (2004) and Teklu et al. (2004) in cattle. In contrast to sex based study, the difference in TB prevalence among different age groups is statistically significant (χ^2=6.9, P=0.03). Animals older than ten years had the highest prevalence (11.6%) compared to other age groups. This is consistent with Abdurrahman and Bornstein (1992); they reported TB as chiefly disease of old camels. This may be the fact that, in spite of their susceptibility to infection, camels are very resistant to the effects of the disease and a long period elapses before they become cachectic and emaciated (Manson, 1917) and may also to develop tuberculous lesion.

Higher prevalence of the bovine TB was observed in camels that originated from PAs inhabited by Oromo society like Legaoda, BeshanBahe, Mermersa and Dujuma when compared to the camels that originated from areas inhabited by Somali society like Shinile (Table 3). The reason for this might be the difference in production system in the two camel rearing societies where mixing of camels with other livestock possibly contribute for the higher prevalence in the former ones. In Eastern Africa, the gradual spread of the camel to tribes not owning it in ancient times resulted from a pre-existing nomadic or semi nomadic way of life (Wilson, 1989) might cause change in production system that can have a great role in transmission of TB to camels. The Ethiopian Somalia camel rearing community in Shinile such as Issa is almost pure camel herders and keep their camels in open land just making a fence with thorny plants around it during night time, but in case of Oromia camel rearing areas where most of the camels are kept in close confinement with cattle, especially during night camels and cattle are kept together. Close confinement of camels and cattle together might be the source of cross infection (Mamo et al., 2011).

Based on tuberculin test, the difference in prevalence among the different age groups is insignificant, though the highest rate was observed in the middle age group (4 to 10 years). It is an established fact that TB is a chronic disease (Radostitis et al., 1994) and so the older an animal, the higher the probability of establishment of infection. But this finding does not favour this fact.

The questionnaire survey reveals that the camel rearing people are at high risk of contracting TB if their camels get infected by *Mycobacterium* species. Because, in the society which lack general awareness of TB and its zoonotic importance and family members share the same living house with their camels during the night time, there is high possibility of transmission of the infection between humans and camels. On top of that, only few of them have the habit of boiling milk before consumption. Raw camel milk consumer of the camel rearing society and their urban customers are in higher health risk. Personal communication with health workers at Dire Dawa and Jijjiga indicated that extra-pulmonary TB is one of the common problems of the regions.

Conclusion

The livelihood of camel rearing pastoralists is closely related with the health of their camels. Camels are kept mainly for milk production and transportation in camel rearing areas of Eastern Ethiopia. However, the finding of 8.3 and 6.0% prevalence of bovine TB in dromedary camels based on detail post mortem examination and SICTT in the present study is alarming in the population where only 50% have awareness of the zoonotic importance of TB and only 18% usually boil milk before consuming. The result of this study shows the presence of high public health risk posed by bovine TB in dromedaries up on Eastern Ethiopia camel rearing communities. Hence, prompt measures are required to control the possible zoonotic transmission of the disease; prioritizing on educating the pastoralist to consume camel milk after boiling and avoid sharing night accommodation with their camels.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Microbiological study on bacterial causes of bovine mastitis and its antibiotics suscebtibility patterns in East Showa Zone, Akaki District, Ethiopia

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The study was conducted during September, 2008 to 2009 in East Showa Zone, Akaki district to determine the prevalence of mastitis, identify risk factors, isolate and identify major bacterial causes and conduct *in-vitro* antimicrobial susceptibility test. Forty six (46) small holder dairy farms and 200 dairy cows were selected by one stage cluster sampling. Questionnaire survey, farm inspection and clinical examination of cows were used to collect data. Milk samples which were positive on california mastitis test (CMT) was collected aseptically using sampling bottles and transported to veterinary microbiology laboratory at Addis Ababa University, Faculty of veterinary medicine for bacteriological study. The prevalence of clinical mastitis at herd, cow and quarter level accounted 17.3, 3.0 and 1.2%, respectively whereas 60.8% herd, 25.0% cow and 12.7% at quarter level during subclinical mastitis. Major bacterial isolates in subclinical mastitis include *Staphylococcus aureus* (38.9%), coagulase negative *Staphylococcus* (23.4%), *Bacillus* spp. (10.4%), *Escherichia coli* (7.8%), *Streptococcus agalactiae* (6.5%), *Streptococcus dysgalactiae* (1.3%), *Staphylococcus uberis* (1.3%) and *Staphylococcus intermidius* (5.2%). Likewise during clinical mastitis, *S. agalactiae* and *S. aureus* accounted (33.3%) and (22.2%), respectively. Univariate logistic regression indicated that stage of lactation, parity number, teat lesion and milking mastitic cow at last had significant effect ($p < 0.05$) on prevalence of mastitis. The *in vivo* antimicrobial sensitivity tests showed that gentamicin, kanamycin, chloramphenicol and vancomycin were the most effective antibiotics, followed by streptomycin and penicillin; bacitracin, polymxin and amoxicillin were least effective drugs.

Key words: Antibiotics Sensitivity test, mastitis, prevalence, risk factors.

INTRODUCTION

Dairy production is a biologically efficient system that converts large quantities of roughage, which is the most abundant feed in the tropics into milk and meat (Bradley, 2002). Milk is a very nutritional food that is rich incarbohydrate, proteins, fats, vitamins and minerals. However, milk can be associated with health risk to consumers, which is linked to presence of zoonotic pathogens and anti-microbial drug residues. The quality

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of milk may be lowered by a number of factors such as milk adulteration, contamination during and after milking and presence of udder infection (Esron et al., 2005).

Milk serves as an excellent medium for certain microorganisms. Particularly, bacterial pathogens whose multiplication depends mainly on temperature compete with other microorganisms and their metabolic products with regards to their disease producing capacity. These pathogens depend upon the initial load of infection in the milk and on the subsequent dilution, processing, time lapse before the milk is consumed and other factors (Wellenberg et al., 2002). Pathogenic organisms in milk are derived from different sources such as the cow itself, human hands or the environment. These organisms can be excreted from the udder through the milk or may originate from the skin and mucous membranes of the animal or milker, resulting in the contamination of milk and utensils (Bradley, 2002).

Mastitis is an inflammation of mammary gland. It is a highly prevalent problem in dairy cattle and is one of the most important threats affecting the world's dairy industry. Although it may be caused by chemical or physical agents, the causes are almost entirely infectious and mostly are bacterial infections. At least 137 biological infectious agents causing bovine mastitis are known to date and in large animals the commonest pathogens are *Staphylococcus* species, *Streptococcus* species and *Coliforms.* It may be associated with many other microorganisms including *Actinomyces pyogens, Pseudomonas aeruginosa, Nocardia asteroids, Clostridium perfringens* and others like *Mycobacterium*, *Mycoplasma*, *Pastuerella* and *Prototheca* and *Yeasts* species (Radostits et al., 2000)

Bovine mastitis can be clinical and the clinical signs vary with the severity of the disease which includes pain, heat and swelling of the affected quarter. Half of the gland shows abnormality of milk either as clots or flakes and wateriness of the milk. Sub-clinical mastitis manifests with production losses and lowered milk quality. Both forms of mastitis produce significant economic losses due to rejection of milk for consumption, degraded milk quality and early culling of cows (Mifflin, 2004).

The economic impacts of bovine mastitis and intra-mammary infection have lead to the development of various therapeutic strategies to control them. Many drugs belonging to various therapeutic classes have been assessed (Koivula et al., 2005). The production of high quality milk is realistic and a meaningful goal for all aspects of dairy industry and the primary motivator for establishing a mastitis control program in dairy herd. Herds that have undergone successful comprehensive mastitis control program also need to develop strategies to control infection with environmental organism and need to use an effective monitoring system for new infection (Smith et al., 1985).

In Ethiopia, the disease is inadequately investigated and information related to its cause, magnitude of distribution and the risk factor is scanty. Such information is important to envisage when designing appropriate strategies that would help to reduce its prevalence and effect. The conventional drugs used for treatment of mastitis are of limited values in most of the woredas (districts), and due to this and other factors causative agents have showed variable degree of resistance. Some of the bacterias like *S. aureus*, *Streptococcus* species and some other pathogens have already developed resistance to many antibiotics (Kerro, 1997). The present study attempted to determine the prevalence of bovine mastitis, identify risk factors, isolate and identify the bacterial causes of bovine mastitis and conduct *in vitro* antimicrobial susceptibility test on those isolated pathogens.

MATERIALS AND METHODS

Study area

Akaki district is located 35km away from Addis Ababa at 9°-10°24' North latitude and 37°56'-40°35' East longitude with an altitude range of 1500-3100 meter above sea level. Its annual temperature ranges from 15°C-27°C. The mean annual rainfall of the district is 800-900 mm and the short rain occurs during February, March and April and the long rain extends from June up to August (Unpublished data of 2010/11). The report also shows that of all the domestic animals raised in the District, cattle population takes the first rank with 91,040, followed by 39,055 goats, 39,048 sheep, 22,676 donkeys, 6,136 horses, and 2,015 mules.

Study population and study design

Smallholder dairy farms in Akaki districts and dairy cattle owned by them represented the study population. The study was a cross-sectional type with some retrospective surveys and it was conducted from September, 2008 to May, 2009 on smallholder dairy farms. The data collection methods were based on structured questionnaire survey, California mastitis test (CMT), milk sample collection, bacteriological culture and *in vivo* antimicrobial susceptibility tests.

Sample size determination and sampling procedure

The sample size was determined using simple cluster type of sampling where farms were sampling units in which all dairy cattle in the farm were included in the study. The sample size was determined based on the formula recommended by (Thrusfield, 2005).

$$g = \frac{1.962}{nvc + Pexp\,(1-Pexp)\,nd^2}$$

Where g = number of clusters to be sampled, Pexp = expected prevalence, d = desired absolute precision, n = number of animals per cluster and vc = between cluster variance.

With 95% confidence level, 5% precision, an average herd size of 5

animals and 60% prevalence from previous studies (Workneh et al., 2002), forty six (46) clusters samples were selected randomly in Akaki Woreda. Thus, two hundred (200) dairy cattle were included in the study from Akaki districts.

Data collection

Structured questionnaire survey was conducted to collect data on potential risk factors for the occurrences of mastitis. The animals level factors considered in study were parity numbers, herd size, stage of lactation and presence of teat lesion. The farm level factors were housing, farm hygiene, and barn floor status, milking hygiene and milking sequence. The questionnaire survey format was structured and it was pre-tested and administered to owners of the animal.

Clinical examination of cows

The clinical inspection of the udder was done in the following way. The udder of selected animals was first examined visually and then by palpation to detect fibrosis, inflammatory swelling, visible injury, tick infestation, atrophy of tissue and swelling of supra mammary lymph nodes. The size and consistency of mammary gland were inspected for presence of any abnormalities such as disproportional symmetry, swelling, firmness and blindness.

California mastitis test

The CMT was carried out as screening test for sub-clinical and clinical mastitis and for selection of samples for culture. A squirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures, in horizontal plane for 5 s. The reaction was interpreted based on the thickness of the gel formed by CMT reagent and milk mixture, and the test result were scored as negative (0), trace (T), + (weak positive), ++ (distinctive positive) and +++ (strong positive) according to (Quinn et al., 2002). Quarters with CMT score of (+) or above were judged as positive. Cows were considered positive when at least one of the quarters becomes positive for CMT and a herd was considered positive, when at least one cow in the herd was tested positive with CMT.

Milk sample collection

Milk samples were collected according the procedure recommended by Quinn et al. (2002). Strict aseptic procedures were followed while collecting milk samples in order to prevent contamination with microorganisms present on the skin of cow's flasks, udder and teats, on the hands of the samplers and in the barn environment. The teats were washed with soap and water and dried with towel, 70% ethyl alcohol was also applied before sample collection. Sterile universal bottle with tighten fitting caps were used. The universal bottle was marked or labeled with permanent marker before sampling. First few streams of milk were removed and discarded to reduce the number of contamination bacteria in the teat canal. To reduce contamination of the teat ends during sample collection, the near teats were sampled first then (Wellenberg et al., 2002) followed by the far ones. Universal bottle was held as horizontal as possible to the teat and 15 ml of milk sample was collected into the universal bottle. After samples were collected, they were properly packed and stored in an icebox and transported to the Microbiology Laboratory of Faculty of Veterinary Medicine of Addis Ababa University (FVM-AAU).

Bacterial isolation and identification

Bacterial isolation and identification was conducted at the Microbiology Laboratory of FVM-AAU. Bacteriological examination of the milk was carried out using the standard procedure (Quinn et al., 2002). One loop (0.01 ml) of milk sample was streaked on 5% blood agar. Inoculated plates were incubated aerobically at 37°C and examined for growth at 24 to 48 h. The isolated micro-organisms were analyzed by their colony characteristics. From culture positive plates, typical colonies were subjected to Gram's stain to see the staining properties and cellular morphology of the bacteria. Pure cultures of a single colony from the blood agar were transferred into nutrient and blood agar plates for further bio-chemical examinations such as coagulase, catalase, DNase, triple sugar iron (TSI) and indole, methyl red, Voges-Proskauer, and citrate (IMViC) tests.

Antimicrobial sensitivity test

The antimicrobial susceptibility pattern of the bacterial isolates was determined using the Kirby-Bauer-disk diffusion method (Quinn et al., 1994). The disks were impregnated with the following antibiotics: kanamycin (K 30), streptomycin (S 10), penicillin 10 units (P 10), amoxicillin (Aml 2), gentamicin (CN 10), chloramphenicol (C 30), polymyxin (PB 300), bacitracin (B 10) and vancomycin (VA 30). Disks were stored under refrigeration to ensure maintenance of their potency. Well isolated bacterial colonies of the same morphologic type were inoculated into 5 ml of a Tryptophan soy broth and incubated at 37°C for 8 h until a visible turbidity was compared to the 0.5 McFarland standards. Mueller Hinton agar for less fastidious bacterial isolates and 5% sheep blood agar for *Streptococcus* species isolates were used as planting medium. Fifteen minutes after the plates were inoculated antibiotic impregnated disks were applied to the surface of the inoculated plates with sterile forceps. All disks were gently pressed down onto the agar with forceps to ensure complete contact with the agar surface. The plates were inverted and then aerobically incubated for 18 h at 37°C. The diameters of the zone inhibition were measured to the nearest whole millimeter using the transparent ruler. Zones of inhibition for individual antimicrobial agents were translated into susceptible, intermediate and resistant categories by referring the recommended interpretative standards (NCCLS, 2000).

Data storage and analysis

Data collected through questionnaire survey, farm inspection, animal examination, bacterial isolation and identification and antibiotic susceptibility test were entered into the data base management software, Microsoft-Excel computer program (Version 6.0., 2000). Descriptive statistics was estimated using SPSS for windows (release 15.0, 2006). Analyses of associations between the prevalence of subclinical mastitis at quarter, cow and herd level, with risk factors, were estimated by univariate and multivariate logistic regression of STATA (Version 10, 2007).

RESULTS

The current study revealed that out of 200 dairy cattle an

overall prevalence of sub clinical and clinical mastitis based on CMT and culture accounted 25% (n = 50) and 3% (n = 6), respectively.

Prevalence of clinical mastitis

On the bases of CMT and clinical observation 17.3% (n = 8) herds, 3.0% (n = 6) cows and 1.2% (n = 10) quarters had clinical mastitis. Most of the quarters were affected by acute mastitis which is characterized by swelling of udder and change of milk content. Milk samples were aseptically collected for CMT, positive dairy cattle were inoculated into culture media and enabled to isolate bacteria from 17.3% (n = 8) herds, 3.0% (n = 6) cows and 1.2% (n = 10) quarters (Table 1).

Prevalence and risk factors affecting sub clinical mastitis

The prevalence of sub clinical mastitis was determined by CMT and microbiological cultures as presented in Table 1. Of 200 cows examined, sub-clinical mastitis accounted 60.8% (n = 28) herd, 25% (n = 50) cow and 12.7% (n = 101) quarters level. Bacterial culture for CMT positive dairy cattle indicated prevalence of 50% (n = 23) at herd, 24% (n = 48) at cow and 9.1% (n = 91) at quarter level.

The prevalence of subclinical mastitis at cow level was significantly affected ($p < 0.05$) by stage of lactation, parity number and presence of teat lesion. The prevalence was significantly higher in cows at the end of lactation (86.1%), with high parity number (55.7%) and teat lesion (80%). When those factors with p-value less than 0.25 were fitted in the multivariate model, only stage of lactation had significant effect on cow level prevalence ($p < 0.05$). Cows at the end of lactation were more affected by subclinical mastitis than others (OR = 30.33) (Table 2).

Regarding herd level prevalence, only the practice of milking mastitic cows last had significant effect ($p < 0.05$). Almost seventy seven percent (77.6%) of dairy farms which were not practicing udder washing before milking were prone to mastitis, whereas only 68.1% of the dairy farms which were practicing udder washing were infected. Among selected dairy farms, only one farm which practice hand washing before milking was infected with mastitis (71.4%). Risk factors with p-value less than 0.25 were fitted in a multivariate model and only the practices of milking mastitic cow last had significant effect on herd level prevalence of subclinical mastitis (Table 2).

Bacterial isolates

From the total isolates, the major contagious pathogens were *S. aureus* and *S.agalactaie* and accounted for 37.2 and 9.3%, respectively. The most important pathogens isolated from clinical cases as indicated in Table 3 were *S. agalactaie* 3 (33.3%), *S. aureus* 2 (22.2%) and coagulase-negative *Staphylococci* (CNS) 2 (22.2%). In the case of subclinical mastitis, *S. aureus* 30 (38.9%), CNS 18 (23.4%), and *Bacillus* species 8 (10.4%) were the most frequently isolated pathogens. The major environmental pathogens isolated were *E. coli* 6 (7.8%), *S. uberis* (1.2%), *S. dysgalactaie* 1 (1.3%) and *A. pyogense* 2 (2.6%). Other minor isolated pathogens included *Bacillus* species, *S. intermidius*, *Micrococcus*, *Corynebacterium bovis and Coagulase negative staphylococcus* was the most important pathogen.

In vitro antimicrobial susceptibility test result

Antimicrobial sensitivity test was done for all of the bacterial isolates in which *S .aureus* showed high resistance to penicillin (75%), polymyxin (80%), bacitracin (82%) and amoxicillin (75%). But it was sensitivity to gentamicin (92%), kanamycin (95%), chloramphenicol (65%), streptomycin (80%), vancomycin (85%).

In the present study *S. intermidius* were found sensitive to all antimicrobial disks. CNS isolates were highly resistant to Penicillin (70%), Bacitracin (90%) and Polymyxin (75%) and highly sensitive to Chlramphenicol (100%) and Vancomycin (81%). The test also indicated that *S. agalactaie* was highly resistance to amoxicillin (50%), Polymyxcin (90%) and highly sensitivity to Gentamicin (100%), Vancomycin (100%), Penicillin (84.6%), Chloramphenicol (95%) and Streptomycin (72%).

S. dysgalctiae was highly sensitive to almost all antimicrobial disk applied except for amoxicillin, for which it was highly resistant. Isolate of *S. uberis* was resistant to Bacitracin (70%), Amoxicillin (65%) and highly sensitive to almost all antibiotic disks, whereas *E.coli* isolates were sensitive to all antimicrobial disks except Penicillin, Amoxicillin, Bactracidin and Polymixin. *Bacillus species* were highly sensitive to almost all antimicrobial disks applied except for Penicillin and Bacitracin.

DISCUSSION

Prevalence of bovine mastitis

The overall prevalence of clinical mastitis accounted 5.9% based on CMT and clinical examination at cow level, which is in consistent with 3.0% prevalence reported by Gizat et al. (2007). The variability in the prevalence of bovine mastitis is due to interaction of several factors mainly of management, environment and factors related to animal and causative organism. The present

Table 1. Prevalence of subclinical and clinical mastitis at herd, cow and quarter level.

Observation level	N	Prevalence subclinical mastitis in % (n)		Prevalence of clinical mastitis in % (n)	
		CMT (n)	Culture (n)	Clinical observation (n)	Culture (n)
Heard	46	60.8(28)	50(23)	17.3(8)	17.3(8)
Cow	200	25 (50)	24(48)	3.0(6)	3.0(6)
Quarter	793	12.7 (101)	9.1(91)	1.2(10)	1.2(10)

N: number examined; n: number positive.

Table 2. Risk factors affecting the prevalence of subclinical mastitis at cow and herd level.

Factor	Category	N	Number positive (%)	P value	OR	95% CI of OR
Herd size	1-5	134	39 (29.1)	0.568	0.84	0.47-1.51
	> 5	66	17 (25.8)			
Stage of lactation	Beginning	32	3 (9.4)	-	-	-
	Middle	125	16 (12.8)	0.643	1.41	0.32-6.23
	End	43	37(86.1)	0.000	59.61	11.58-306.80
Parity	1-3	121	12 (9.9)	0.000	11.42	4.89-26.65
	> 3	79	44 (55.7)			
Presence of teat lesion	Yes	5	4 (80.0)	0.032	11.00	1.23-98.69
	No	195	52 (26.7)			
Herd level						
Herd size	1-5	131	94 (71.76)	0.361	1.84	0.50-6.76
	>5	17	14 (82.35)			
Udder washing before milking	Yes	72	49 (68.06)	0.192	0.61	0.30-1.28
	No	76	59 (77.63)			
Milking mastitis cow last	Yes	35	9(25.7)	0.00	0.05	0.02-0.13
	No	113	99(87.6)			
Hand washing before milking	Yes	7	5(71.4)			
Drainage structure	Good	90	61 (67.78)	0.079	2.03	0.92-4.48
	Bad	58	47(81.0)			

the treatment of clinical cases, however, sub clinical form of mastitis was neglected which is responsible for high economic loss (Kerro, and Tareke, 2003). Though dairy farming is mostly a sideline business for the owners of small holder dairy farms in the study areas, they were not well informed about the invisible loss from sub clinical mastitis.

Bacterial isolation and identification

In the present study *S. aureus* was the predominant pathogen (38.9%) compared to all isolates in the area which is comparable with the findings of Workneh et al. (2002) and Barbuddahe (2001) who reported 39% and 38.8% isolates of *S. aureus* respectively. However, the current finding is in contrary to previous 9% research reported by Gizat et al (2007). Similar study conducted in Jamaica and India by Zingeser et al. (1991) and Barbuddahe et al. (2001) indicated lower *S. aurues* isolates which accounted 27% and 23.2% respectively than the current finding. The relative high prevalence of *S. aureus* in this study could be associated with lack of

Table 3. Bacterial isolates from quarters affected by clinical and subclinical mastitis.

Bacterial isolates	Clinical (%)	Subclinical (%)	Total (%)
S. aureus	2(22.2)	30(38.9)	32(37.2)
CNS	2(22.2)	18 (23.4)	20 (23.3)
S. intermidius	-	4(5.2)	4 (4.7)
S. agalactaie	3 (33.3)	5(6.5)	8 (9.3)
S. dysgalactaie	1 (11.1)	1(1.3)	2(2.3)
S. uberis	-	1(1.3)	1 (1.2)
E. fesalis	-	-	-
Bacillus species	1 (11.1)	8(10.4)	9 (10.5)
E. coli	-	6(7.8)	6 (7.0)
Enterobacter species	-	-	-
Klebssela species	-	-	-
Micrococcus	-	1(1.3)	1(1.2)
C. bovis	-	1(1.3)	1(1.2)
A. pyogens	-	2(2.6)	2 (2.3)
Total	9(100)	77 (100)	86(100)

effective udder washing, hand washing before milking, use of separate towel, post milking teat dipping and disinfection routine milking area. Kerro and Tareke (2003) indicated 40.5% *S. aureus* prevalence in southern Ethiopia and 44.4% in Sebeta Miline et al. (2002) which are higher than the present finding.

The result of coagulase negative staphylococcus (CNS) in present study accounted 23.2%, which is lower than 42% (Hussien, 1999) and 46% (Gizat et al., 2004) findings. However, it is higher than 10% prevalence reported (Miline, 2002). CNS is regarded as minor pathogen and normally considered as normal inhabitants of bovine udder (Gentilini et al., 2002).

The prevalence of *S. agalactaiae* in this study is lower than 13.1% reported by Kerro and Tareke (2003). Gizat et al. (2007) indicated a 1.5% prevalence of *S. uberis* which is in consistence with the current findings. Isolates of *S. dysgalactaie* were higher than 0.5 and 5.6% indicated by Gizat et al. (2007) and Kerro (1997), respectively. *E. coli* which was the predominant isolate in the current study might be associated with poor farm cleanness and stable areas. This study also showed that environmental pathogens were isolated in similar proportion. An increased herd size, poor manure disposal and sanitation problem leads to the building up of bacterial population such as coliform and environmental *Streptococcus* in the cows' immediate environment.

In-vitro antibiotics sensitivity test

S. aureus, the major cause of mastitis were found sensitive to gentamicin (92%), chloramphenicol (65%), kanamycin (95%), vancomycin (82%) and highly resistant to bacitracin (100%), polymyxin (80%), penicillin (89%) and amoxicillin (75%). This finding is in close agreement with the findings of Edward et al. (2002) who reported *S. aureus* was highly resistant to bacitracin and amoxicillin at 94 and 74%, respectively. Sanmartin et al. (2007) report *S. aureus* was found resistant to amoxicillin (60%) and penicillin which was in accordance with 68% current findings. Similar study in Argentina indicated that *S. aureus* was found highly susceptible to gentamicin (90%) and chloamphenicol (Gentilini et al., 2002). According to Kang et al. (2007), *S. aueus* and *Streptococcal* species including CNS were highly resistant to penicillin, which is similar to the current study that may be due to the long term use of beta-lactam antibiotics in intra-mammary infusion therapy.

Sanmartin et al. (2007) indicated that CNS strain were resistant to penicillin (56%) and amoxiciline (42%), this result is comparable to the present study in which CNS isolates were resistant to amoxicillin (35%). *S. agalactiea* in this study showed 100, 95, 85, 50 and 100% sensitivity to gentamicin, chloramphenicol, penicillin, kanamycin and vancomycin, respectively; however, they are resistance to amoxicillin (50%) and polymyxin (90%). *S. agalactaie* were 100% sensitive to gentamicin according to Shakuntala (2003), which is in agreement with the current findings. Shakuntala (2003) also indicated 75% sensitivity to chloaphenicol, which is comparable with the present finding.

The study also revealed that *E. coli* isolates was sensitive to chloraphenicol (100%), kanamycine (78%), gentamicin (80%), streptomycin (78%) and vancomycin (100%), whereas resistant to penicillin (65%) and bacitracine (92%). These results are close to the report in India (ICAR) in which *E. coli* was found to be 100%

sensitive to chloramphenicol and 50% to gentamicin (Shakuntala et al., 2003).

Conclusion

The current finding indicated an occurrence of low to moderate prevalence of clinical mastitis and moderate to high prevalence of subclinical mastitis at cow, herd and quarter level. Stage of lactation, parity number and presence of teat lesion were the most important risk factor affecting the prevalence of sub clinical mastitis in cow level and milking mastitic cow at least at herd level. *S. aureus* was the predominant pathogens in clinical and subclinical mastitis. *S. agalactaie* was the most frequently encountered bacteria during clinical mastitis. All the current Isolates were sensitive to gentamicin, kanamycin, chloamphenicol and vancomycin; moderately sensitive to streptomycin and penicillin. However, the isolates were resistant to bacitracin and polymixin which is similar to different researches made on similar isolates. Since mastitis is an economically important disease, hygienic milking practice, use of effective antibiotics, proper extension packages to dairy farm owners and strategic mastitis control programs should be of paramount importance.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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