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Full Length Research Paper

Prevalence and the associated risk factors of bovine trypanosomiasis in nyangatom pastoral woreda, Southern Nation and Nationalities People Region (SNNPR), Ethiopia

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A cross-sectional study was carried out in Nyangatom wereda of South Omo zone, Southern Nation and Nationalities People Region (SNNPR), Ethiopia with the general objectives to find out the prevalence of bovine trypanosomiasis and the risk factors associated with its prevalence from January to June 2015. To identify the protozoa blood samples (n =384) collected from the marginal ear vein of indigenous zebu cattle of more than one year age and both the sexes from three kebeles were examined by buffy coat technique, direct blood smear, thick blood smear and thin blood smear after staining. The overall prevalence of bovine trypanosomiasis was 26.3%. On peasant associations (PA'S) basis Lebere kebele has the highest prevalence 39(30.5%) followed by Shenkora kebele 34 (26.6%) and Ayipa kebele 28 (21.9%). *Trypanosoma congolense* is the most prevalent species (14.3%) followed by *Trypanosoma vivax* (5.70%) and *Trypanosoma brucei* (5.50%). A significant association was observed (P<0.05) between the disease positivity and age, sex and body condition score. The prevalence of trypanosomiasis was 16.20 and 31.50% in young and adult respectively. The prevalence 42.80 and 16.30 % in poor and good body condition score respectively. There was significant association between the risk factors and the species of trypanosomiasis (P<0.05). The result of the present study revealed that trypanosomiasis is the most important problem for animal production in the study area. Strategic control of bovine trypanosomiasis should be strengthened to improve livestock production and agricultural development in the area.

Key words: Bovine, buffy coat, Nyangatom, prevalence, trypanosomiasis.

INTRODUCTION

In Ethiopia, tsetse flies (the vector of different species of *Trypanosoma*) infest an area of approximately 240,000

km²; most of this area is located in the southern region of which; 25,000 km² is found in the southern rift valley of

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Ethiopia. About 10 to 14 million heads of cattle in Ethiopia, 4 to 6 million heads of cattle in southern region and 2 to 3 million heads of cattle in the southern rift valley are at risk from trypanosomosis.

Trypanosomosis is a complex immunosuppressive disease caused by unicellular, eukaryotic, hetetro specific haemo- parasites (trypanosomes) of blood and other tissues of vertebrates including cattle and man (Uilenberg, 1998; Singla et al., 2009). The disease by flagellated protozoa is transmitted by a number of different arthropod vectors but mainly by biting flies (Kumar et al., 2012; Sharma et al., 2012; Sharma et al., 2015). The disease is coincident with the distribution of the tsetse fly (*Glossina* spp.) and other flies which acts as a vector for the parasite (Sumbria et al., 2015) and infests an area of some 10 million square kilometer encompassing 36 countries in Africa (Black and Seed, 2002).

Trypanosomosis, a disease of major economic importance is caused by several species of trypanosomes, a major constraint to livestock animal production (Urquhart et al., 1996; Aulakh et al., 2005). Pathogenic species of salivarian trypanosomes are present throughout vast areas of Africa including Ethiopia (Jahnke et al., 1988). The disease in livestock creates great losses in terms of mortality, abortion, reduced fertility, milk and meat production, and working abilities of animals (Juyal et al., 2005). Depending on the species of *Trypanosoma* the organisms are transmitted cyclically by tsetse flies of the genus *Glossina* or mechanically by tsetse or other biting flies. The disease is the most economically damaging and widely distributed in most parts of Ethiopia (DACA, 2006).

Bovine *trypanosomosis*, which is one of the most important protozoa diseases of cattle in Ethiopia, affects the health status of animals. In general bovine are susceptible to trypanosome infection such as *T. vivax*, *T. congolense*, *T. brucei* and *T. evansi* (Leak, 1996). Therefore, the objectives of this study were to determine prevalence and risk factors of trypanosomosis in the study area and to identify and define the existing species of *Trypanosoma* in the study area.

MATERIALS AND METHODS

Study area

The study was conducted from January to June/2015 in the Nyangatom woreda, Ethiopia located in south omo zone of SNNPR, comprising of 20 (1 urban and 19 rural) kebele administrations. It is one of the eight woredas in south omo zone with an area of 2652 km and is located at 4.850 to 5.670N and 35.750 to 36.230E. It's bordering with Bench -maji zone and Selamagoworeda in north, Dassenech woreda in south, Hamar woreda in east and Kenya and South Sudan in west (CSA, 2013). The traditional agro ecology of the woreda is kola with an altitude that ranges between 300 and 450 m a.s.l. The mean annual temperature of the woreda ranges between 33 and 42°C. The woreda has a rainfall pattern of bimodal type (Belg from March to

May and Meher from August to October). The mean annual rainfall ranges from 350 to 500 mm. Livestock production is the dominant livelihood source in the woreda. It has an animal resource with an estimate of about 415,292 cattle, 132,604 goats, 109,217 sheep, 11,218 donkeys and 5,474 chicken. There are three ethnic groups in the woreda. Nyangatom is the dominant one followed by Murulle and Koygu (Muguji) (SOFEDD, 2012).

Study population

The study population was indigenous zebu cattle of more than one year age group, with poor and good body condition and both sex which are found within three kebeles of the study area. Particularly Lebere, Shenkora and Ayipa. A total of 384 cattle were examined to estimate the existing prevalence rate of trypanosomosis.

Study design and sample size determination

A cross - sectional study was conducted in order to determine the prevalence of bovine trypanosomosis and associated risk factors from selected kebeles of the woreda. The size of sample was determined by the following formula (Thrusfield, 2005) with 95% confidence and an expected prevalence of 50 and at 5% absolute precision. Based on the formula the total sample size was 384.

Sampling procedures

The sampling site (marginal ear vein) of the cattle was prepared and disinfected with ethanol. Then the ear vein was punctured by lancet and the blood sample was collected by heparinized capillary tube. One end of the tube was sealed by crystal seal and finally, the blood samples were immediately transported to jinka, the town of south omo zone, regional laboratory in tightly closed ice box.

Sample processing and examination techniques

Thin blood smear

A small drop of blood from a micro-hematocrit capillary tube was applied to a clean slide and spread by using another clean slide at an angle of 45°. The smear was air dried and then fixed for 2 min in methyl alcohol. The thin smear was flooded with Giemsa stain (1:10 solution) for 30 min. Excess stain was drained and washed by using distilled water. Then it was allowed to dry by standing up right on the rack and examined under the microscope (x100) oil immersion objective lens (OIE, 2008).

Buffy coat technique

Heparinised micro haematocrit capillary tubes, containing blood samples were centrifuged for 5 min at 12,000 rpm. After the centrifugation, trypanosomes were usually found in or just above the buffy coat layer. The capillary tube was cut using a diamond tipped pen 1 mm below the buffy coat to include the upper most layers of the red blood cells and 3 mm above to include the plasma. The content of the capillary tube was expressed onto a glass slide, and covered with cover slip. The slide was examined under x40 objective and x10 eye piece for movement of parasite (Paris et al., 1982). *Trypanosoma* species were identified according to their morphological descriptions on Giemsa stained blood film as well as movement in wet film preparations provided by (Radostitis et al., 2007).

Table 1. Prevalence of trypanosomiasis in Nyangatom on basis of peasant associations.

Kebele	No. of positive (%)	No. of negative (%)	P- value
Lebere	39 (30.5)	89 (69.5)	0.119
Shenkora	34 (26.6)	94 (73.4)	
Ayipa	28 (21.9)	100 (78.1)	

Table 2. Species of *Trypanosoma* involved with different risk factors.

Variable		<i>T. congolense</i>	<i>T. brucei</i>	<i>T. vivax</i>	p-value
Age	Adult	46 (18.1%)	13(5.1%)	16 (6.3%)	0.005
	Young	9 (6.9%)	8(6.2%)	6 (4.6%)	
Sex	Male	10 (9.3%)	3(2.8%)	7(6.5%)	0.027
	Female	45 (16.3%)	18(6.6%)	15 (5.4%)	
Body condition	Good	20 (8.9%)	10 (4.2%)	7 (2.9%)	0.000
	Poor	35 (24.1%)	11(7.6%)	15 (10.3%)	
Kebele	Debre	19 (14.8%)	10 (7.8%)	10 (7.8%)	0.192
	Shenkora	20 (15.6%)	7 (5.5%)	4. (3.1%)	
	AyPA	16(12.5%)	4 (3.1%)	8 (6.2%)	
Total		55(14.3%)	20(5.5%)	22 (5.7%)	

Table 3. Prevalence of *Trypanosoma* infection in both sexes.

Variable	No. of positive (%)	No. of negative (%)	p-value
Male	20 (18.5%)	88 (81.5%)	0.030
Female	81(29.3%)	195(70.7%)	

Data management and analysis

The data on individual animal and parasitological examination was entered into Microsoft Excel (MS-Excel). These data was analyzed using Statistical Package for Social Science (SPSS version 20.0) software. The descriptive statistics called Pearson Chi-Square test was used to see the association of trypanosomiasis infection rates with different variables like age, sex and body condition score statistical significance for categorical data. Throughout the analysis, 95% confidence interval with 5% degrees of freedom ($P < 0.05$) was considered to say statistically significant difference.

RESULTS

Parasitological survey

The result of the survey shows an overall prevalence for trypanosomiasis 26.3% and there was no significant association between the prevalence for trypanosomiasis and kebeles.

On Peasant associations basis Lebere has the highest prevalence of 39(30.5%) followed by Shenkora 34 (26.6%) and Ayipa 28(21.9%) (Table 1).

Distribution of *Trypanosoma* species

The species of *Trypanosoma* identified by direct smear, buffy coat technique and thin smear showed that *T. congolense* is the most prevalent (55.45%) followed by *T. vivax* (23.76%) and *T. brucei* (20.8%). There was a significant association between the species of *Trypanosoma* with age, sex and body condition ($p < 0.05$) and there was no significant association between the species of trypanosome with in kebelles ($p > 0.05$) (Table 2).

Prevalence for *Trypanosoma* infection in both sexes

During the present survey, from a total of 384 cattle examined 276(71.87%) were females and 108 (28.12%) of them were male animals.

From the females examined, 29.3% were positive for trypanosoma infection while 18.5% of the male animals were found infected. There was significant association between sex and prevalence for *Trypanosomiasis* (Table 3).

Table 4. Prevalence for *Trypanosoma* infection in different age groups.

Parameter		No. positive (%)	No. negative (%)	p-value
Age	Adult > 3 years	80 (31.5%)	74 (68.5%)	0.001
	Young 1-3 years	21 (16.2%)	109 (83.8%)	

Table 5. Prevalence for trypanosoma infection in poor and good body condition in animals.

Parameter		No. positive (%)	No. negative (%)	p-value
Body condition	Good	39 (16.3%)	200 (83.7%)	0.000
	Poor	62(42.8%)	83 (57.2%)	

Prevalence for *Trypanosoma* infection in different age groups

The animals examined were categorized in different age groups as the young (1-3years) and adults (>3 years). The prevalence was 16.2% in young and 31.5% in adult animals. There was significant association between age and prevalence for trypanosomiasis ($p < 0.05$) (Table 4).

Prevalence for *Trypanosoma* infection in different body condition

The prevalence of trypanosomiasis on two body condition groups was seen and the result showed that 42.8% in poor and 16.3% in good body condition. Relatively it was seen that higher proportion of poor body condition cattle were positive than good body condition animals. There was significant association between body conditions and prevalence for trypanosomiasis (p value < 0.05) (Table 5).

DISCUSSION

The overall result of prevalence of the present study (26.3%) was higher than the result of the previous work (12.79%) by Wondwosen et al. (2012). The present finding was lower than that registered by Shimelise et al. (2005) in the Ghibe valley 40.3% in late rainy and higher than in similar area (19.01%) in dry season by Wondoson (1986) in Bunno and Abiy (2002) in Goro district (19.01%). This may be due to the difference in agro ecology of the study area, prophylactic measure and difference in season. The positivity of trypanosomiasis was not significantly different within kebelles of the woreda whereas the positivity of trypanosomiasis was significantly variable with age, sex and body condition score of cattle.

In the present study *T. congolense* was predominant species in the study area which may be due to the development of better immune response to *T. vivax* by

the infected animal (Leak et al., 1999). The dominance of *T. congolense* (55.45%) in the present study is comparable with the previous result of Getachew and Jobre (1996) for tsetse infested areas of Ethiopia (66.1%), Afework (2001) at Pawe North west Ethiopia (60.9%), Terzu (2004) in selected sites of southern region (63.4%) and Wodwosen et al. (2012) worked in selected villages of Arbaminch, Ethiopia.

The result of Tewelde et al. (2004) at Kone (75%) and village-1 (93%) settlement area of Ethiopia, Woldeyes and Woldeyes and Aboset (1997) at Arbaminch zuria districts (85.2%) and Rowland et al. (2001) in Ghibe valley, South West of Ethiopia (84%) had shown higher prevalence of *T. congolense* than the present finding. These high ratios of *T. congolense* suggest that the major cyclical vector or *Glossina* species are more efficient transmitters of *T. congolense* than *T. vivax* in East Africa (Langridge, 1976).

The distribution of *Trypanosoma* species was not significantly different within kebelles of the woreda whereas the distributions of species were significantly variable with age, sex and body condition score of cattle. The prevalence in adults was higher than the young ones. In the calf group the prevalence was lower which the result of low exposure to the vector was. Conversely in the adult and older age groups of animals the prevalence of *Trypanosoma* infection was higher due to the constant contact existing with the tsetse fly in the field.

The higher prevalence of poor body condition cattle than good body condition animals in the study was also comparable with the result of Wodwosen et al. (2012) in Arbaminch area and Abraham and Tesfaheyw et al. (2012).

Abbreviation: **ALC**, Annual loss from liver condemnation; **DACA**, Disease Administration and Control Authority; **FAO**, Food and Agricultural Organization; **HAT**, Human African Trypanosomiasis; **P**, Prevalence rate of the disease at the study area; **PA**,

Peasant Association; **SNNPR**, Southern Nation and Nationalities People Region; **SOFEDD**, South Omo zone Finance and Economy Development Department; **STEP**, Southern Tsetse Eradication Program.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Observations of oxytetracycline treatment effects in a contagious bovine pleuropneumonia naturally infected herd in Zambia

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An observational study on the effects of oxytetracycline treatment on contagious bovine pleuropneumonia in a naturally infected herd of 500 cattle was conducted. A total of 68 cattle that showed pneumonia-like clinical signs were treated. Treatment was effected the moment an animal showed signs of illness. A total of 429 cattle were slaughtered after diagnosis of contagious bovine pleuropneumonia and at slaughter, 40.8% (175) had lesions compared to 59.2% (254) that did not have lesions. Out of the total cattle that were treated with oxytetracycline, 57.4% (39) died from contagious bovine pleuropneumonia over a period of 9 weeks while 42.6% (29) survived. Of the treatment group that survived, 37.9% (11) had fibrous lesions indicative of healing, while 62.1% (18) had pathological lesions consistent of active contagious bovine pleuropneumonia (CBPP). Categorisation of carcasses with pathological lesions within the treatment group showed 66.7% (12) and 33.3% (6) of acute and chronic lesions, respectively. The CBPP causative agent was isolated through culture and confirmed using polymerase chain reaction (PCR). The results obtained suggest that oxytetracycline did not stop the spread or death of cattle in this particular herd with the treatment of a proportion of the herd. However, large scale field trials are needed in order to validate these findings. It is therefore recommended that any antibiotic that will be developed and advocated for use in the treatment of contagious bovine pleuropneumonia should be effective to contain spread within the herd by treating only a proportion showing signs of the disease.

Key words: Contagious bovine pleuropneumonia, antibiotics, lung, lesions, oxytetracycline, treatment, Zambia.

INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is a highly infectious disease of cattle caused by a mollicute bacteria *Mycoplasma mycoides* subspecies *mycoides* (*Mmm*) and

is characterised by severe fibrinous exudative pneumonia (OIE, 2010; Provost et al., 1987). The disease has been recognised as a major hindrance to increased livestock

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production in sub-Saharan Africa causing great economic losses due to cattle mortality and morbidity leading to weakness, emaciation, reduced work ability and reduced fertility in affected herds (Amanfu, 2009). Tambi et al. (2006) described the economic importance due to the high financial and economic losses the disease causes to cattle owners and nations, the associated socio-economic implications of these losses and the economic wide impacts (resulting from reduced export earnings and a decline in economic activity in those industries that depend on cattle and their products).

Although CBPP has been successfully controlled in most developed countries (Scacchia et al., 2011), it has continued to spread and affect new areas in sub-Saharan Africa. The disease in Africa is complicated by the uncontrolled movement of cattle and the availability of chronic carriers that have been implicated in the perpetuation of the disease (Masiga et al., 1986).

The main control method that has been adopted by most countries in sub-Saharan Africa is vaccination using an attenuated live T1/44 vaccine strain. The vaccine confers a short immunity requiring booster vaccinations annually and can also revert to pathogenicity at the injection site that can cause death if not treated with antibiotics (Thiaucourt et al., 2007).

Alternative control of CBPP using antibiotics has officially been discouraged although it has been shown that it is widely practised in many African countries (Mariner and Catley, 2004). Mitchell et al. (2012) have demonstrated the effectiveness of oxytetracycline, danofloxacin and tulathromycin in biological matrices as well as artificial media in inhibiting the growth of *Mmm*, the causative agent of CBPP and suggested the possible use of the three in the treatment of CBPP. Provost et al. (1987) reported that the use of antibiotics may alleviate the clinical signs but would not inhibit the spread of the disease. Studies by Yaya et al. (2004) showed that in an experimental infection, oxytetracycline was able to reduce the losses due to CBPP but could not prevent the persistence of viable *Mmm* in the treated animals suggesting that treated animals could still spread the disease to susceptible animals. However, in similar studies of naturally infected cattle, Huebschle et al. (2006) reported success in reducing spread of the disease in a trial using danofloxacin, but failed to reduce clinical effects. In an investigation by Niang et al (2010), it was reported that oxytetracycline failed to induce sequestra formation under experimental conditions demonstrating the probability of cure and not the evolution of the disease to chronicity to the treated group. Nicholas et al. (2012) reported success in treatment and prevention of CBPP spread using danofloxacin in naturally infected herds in Caprivi Strip of Northern Namibia.

In FAO meeting of 2002 in Ghana, the effectiveness of antibiotic therapy was put to scrutiny and it was advocated that further research should be carried out to arrive at

informed decisions concerning the use of antibiotics in the treatment of CBPP (FAO, 2003). This paper highlights the failure of long-acting oxytetracycline in reducing the clinical lesions in individual cattle and in halting the spread of CBPP within a herd of naturally infected cattle that were in quarantine.

MATERIALS AND METHODS

Study site

The study was limited to a commercial farm located in the Copper belt Province of Zambia. The farm is mainly a crop farming enterprise that was diversifying into cattle ranching. The farm imported a 500 herd of cattle from Tanzania as its start-up herd. The cattle under study were the only ones on the premises. The farm is triple fenced with a barbed wire, game fence and an electric fence all around its perimeter. It has additional fences within the farm to divide various areas for different activities.

Study cattle

The study was restricted to heifers that were imported from Tanzania and were in quarantine at the time of the study. The age range of the cattle was from 3 to 18 months.

History of illness and treatment

According to the available records, on the fourth day after arrival, 3 of the heifers died after showing difficulties in breathing. A post-mortem was conducted and lung samples showing pneumonic signs were collected, placed in the mobile car freezer with a temperature of -20°C and submitted to the laboratory. They were not processed as the preservation in transit was not good for bacteriological assessment. However, a tentative diagnosis of Pasteurellosis was made in view of the long distance (over 2,800 km) that the heifers covered. Consequently, medication with oxytetracycline was instituted for those heifers showing respiratory discomfort and thereafter those that develop these signs. This led to a total of 13.6% (68) of the herd being treated. A dosage of 20 mg/kg per day (1 ml/10 kg body mass) was given for five days to each of the sick heifers. The sick cattle were not separated from the herd after institution of treatment. Over a 3 month period, 39 of the treated heifers died. The diagnosis of CBPP was only made 112 days after the initial deaths and by then, 92 were showing overt clinical disease characterised by coughing, dyspnoea, polypnoea, and nasal discharge. On running, these signs were accentuated.

Slaughter and collection of samples from the herd

After confirmation of CBPP on serology using CFT (OIE, 2010) and c-ELISA (Le Goff and Thiaucourt, 1998), the herd was ear marked for slaughter.

Pathology/post-mortem

All the cattle that had remained (n=429) were subjected to post-mortem upon slaughter. On the slaughter line, all the animals were identified according to the mark on the ear tag and thus treated and untreated individuals were recognised. Tissue samples that included lungs, lymph nodes and pleural fluid were collected from

Table 1. Various stages of infection in oxytetracycline treated and untreated cattle.

Description	% (n)
Total cattle imported	500
Herd size on slaughter	429
Total mortality	14.0(60)
Untreated	86.4(432)
Treated	13.6(68)
Treated but died	57.4(39)
Treated survived	42.6(29)
Total with lesions at slaughter	40.8(175)
Lesions from treated	16.6(29)
Lesions untreated	83.4(146)
Total acute	52.0(91)
Acute from treated	6.6(6)
Acute from untreated	81.3(74)
Total chronic	52.0(91)
Chronic from treated	6.6(6)
Chronic from untreated	81.3(74)
Total fibrotic lesions	13.7(24)
Fibrotic lesions from treated	45.8(11)
Fibrotic lesions from untreated	52.2(13)
Total treated with active lesions	62.1(18)
Acute of active lesions	66.7(12)
Chronic of active lesions	33.3(6)
Fibrotic lesions treated	37.9(11)
Total untreated with active lesions	91.1(133)
Acute of untreated active lesions	44.4(59)
Chronic of untreated active lesions	55.6(74)
Fibrotic lesions untreated	8.9(13)
Total without lesions at slaughter	59.2(254)

both groups and stored at -20°C until analysis.

Culture and PCR of isolates

A total of 20 tissue samples from both the treated (10) and untreated groups (10) were cultured in PPLO broth and agar medium (Himedia® India) containing 20% Horse serum as described by Razinand Freundt (1984) for 10 days.

DNA was extracted from the broth cultures of *Mmm* and purified using the Maxwell® DNA purification kit following the manufacturer's instructions. They were subjected to PCR and then restriction enzyme digestion using *AsnI* as described by Bashiruddin et al. (1994), (Figure 1).

RESULTS

Of the total that were treated, 57.4% (n=39) died with CBPP symptoms before the decision to slaughter the

whole herd was made while 42.6% (n=29) survived until the whole herd was slaughtered (Table 1).

A total of 429 cattle were slaughtered and on post-mortem examination of all the carcasses, 40.8% (n=175) had lesions of various stages of disease progression while 59.2% (n=254) did not have any lesions. Of those with lesions, 16.6% (n=29) were those from the treated group while 83.4% (n=146) were of the untreated cattle. There was 13.7% (n=24) carcasses with fibrotic lesions with indications of healing. Of these 45.8% (n=11) were from the treated group while 52.2% (n=13) were from the untreated group. When the carcasses with lesions and those showing signs of recovery were compared in the treated group, it was found that 37.9% (n=11) had lesions in the lungs showing signs of recovery, while 62.1% (n=18) had classical lesions of clinical CBPP. Those from the untreated group showed 8.9% (n=13) with signs of lesions of CBPP. When the carcasses with classical

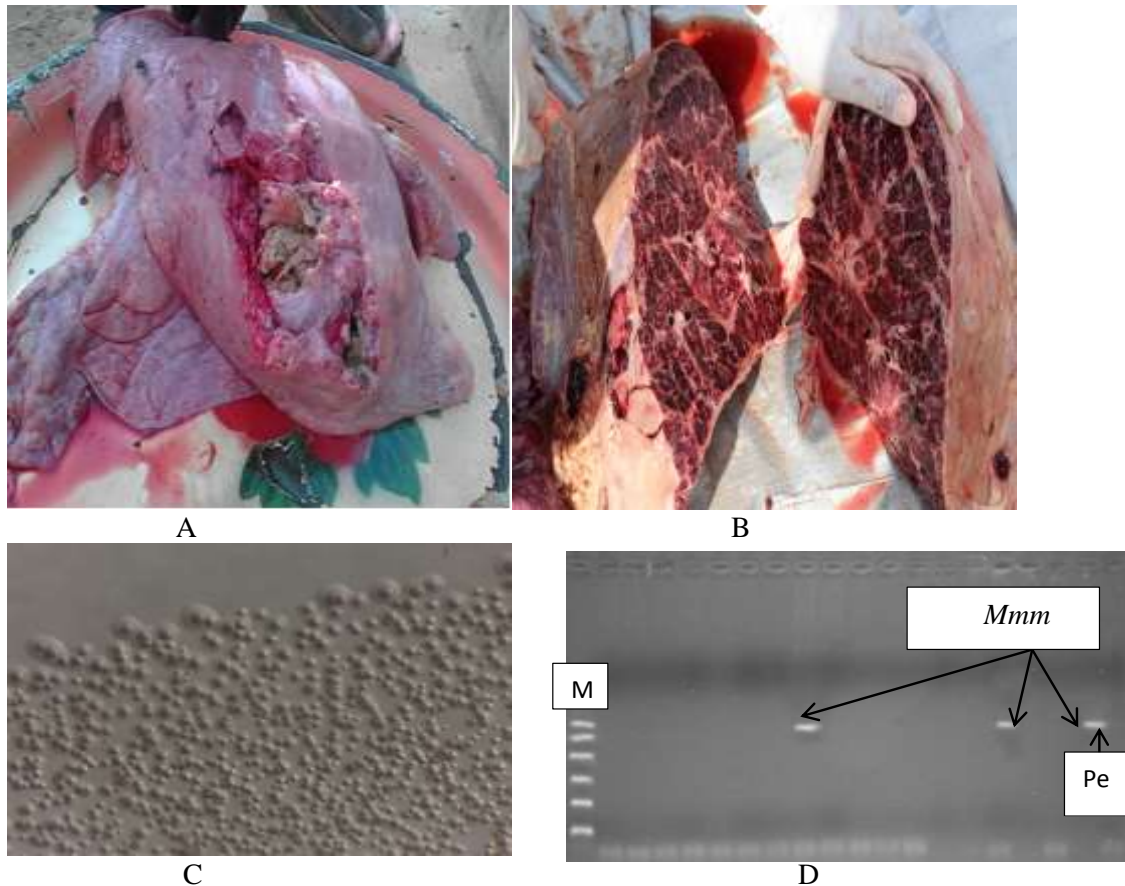


Figure 1. (A) Sequestra in one of the animals; (B) Lesions with typical marbling and consolidation characteristic of acute CBPP in one of the animals; (C) Colonies of *Mycoplasma mycoides* subsp. *Mycoides* as obtained on a plate X 3.2; (D) Gel electrophoresis results from PCR products of samples showing, the 574 bp amplicons of *Mmm* obtained using the Bashirrudin et al. (1994) method. M = Molecular weight markers, *Mmm* at 574 bp; and Pe = T1/44 positive control (*Mmm*) at 574 bp.

healing compared to 91.1% (n=133) that had classical lesions from the treated group were further examined, it was observed that 66.7% (n=12) had lesions in the acute phase of CBPP with typical marbled appearance of the lung, various degrees of adhesion to the pleural wall and copious pleural fluid, while 33.3% (n=6) had chronic lesions with sequestra of various sizes. Those from the untreated group showed 44.4% (n=59) in the acute phase of the disease while 55.6% (n=74) had lesions in the chronic phase of the disease.

DISCUSSION

Treatment of cattle infected with CBPP has long raised controversy due to the non-availability of adequate information on the repercussions of such interventions. This study has demonstrated the failure of oxytetracycline therapy in abetting clinical CBPP in a naturally infected herd where only cattle with clinical signs were treated. The proportion of cattle that was treated with the antibiotic

was 13.6% (68) of the whole herd. Considering the population (500) at risk when clinical signs were noticed, this is too low for an infectious disease as CBPP. This augments the point by OIE (2012) which states that in a CBPP infection, the whole herd will need to be treated with antibiotics in order to achieve recovery. Indeed the cost implication of treating the whole herd in such a situation would be very costly and prohibitive to the peasant farmers who are usually the victims of CBPP in sub-Saharan Africa.

The animals were in quarantine from the time they arrived in Zambia to the time of slaughter. They were not in contact with any other animals and as such, the disease was contracted at source in Tanzania. Thus the exact stages of the disease in these animals at commencement of treatment are not known and the time between exposure and transportation was not determined. However, this is the kind of situation faced in the field where treatment is initiated only after exhibition of clinical signs and the time of acquiring the disease is not known. Infected and non-infected cattle in herds usually share

pasture and watering points. In an experiment by Niang et al. (2010), it was noted that all the cattle that were subjected to oxytetracycline therapy recovered with the cicatrical lesions found on slaughter. In their study, all the cattle were treated early with the period of infection to therapy clearly outlined. In the current study however, the period between infection and therapy was not known.

It was observed that 57.4% (n=39) of the treated cattle succumbed to CBPP prior to mandatory slaughter of the herd. This is within the mortality rates expected in a CBPP outbreak where it has been shown that an outbreak usually causes mortality rates of 50 to 80% in a herd (Thomson, 2005). These results are also in agreement with studies of Huebschle et al. (2006) who showed that there was no difference between danofloxacin treated and untreated groups in terms of death due to CBPP. However, this is in contrast to reports made by Nicholas et al. (2012) where all the cattle that were treated using danofloxacin survived except for three that died in the first three months.

The lesions seen in the treated group at slaughter showed that 65.5% (n=19) were exhibiting signs of recovery indicated by fibrotic scar lesions while 34.5% (n=10) had lesions typical of active disease at various stages. This study also showed that 34.5% of the treated cattle had lesions of active CBPP. Of these 70% (n=7) had acute CBPP and 30% (n=3) had chronic lesions with sequestra of various sizes. The presence of sequestra indicates the transition of the disease into chronic phase which is known to be the probable cause of perpetuation of the disease where it exists. This finding is in contrast with the findings of Niang et al. (2010) who showed that none of the cattle treated with oxytetracycline developed sequestra.

The demonstration of *Mmm* colonies in pathological lesions from the treated group and the eventual confirmation using PCR indicate the presence of viable pathogen. This shows that these animals could still transmit the disease to susceptible individuals in the herd. This is in agreement with Yaya et al. (2004) who stated that the presence of *Mmm* in oxytetracycline treated individuals could still pose a risk of disease spread in the herds.

The observation of effective minimum inhibitory concentrations (MIC) by Mitchell et al. (2012) of some antibiotics including oxytetracycline on the growth of *Mmm* in biological matrices demonstrates their chemical effect *in vivo*. *In vitro* however, the concentration of *Mmm* is in many body fluids and tissues and the mycoplastatic effect of oxytetracycline may possibly not affect all the available pathogens. This could explain the observations made in this particular study.

Conclusion

This observation study has shown that the effects of oxytetracycline treatment of naturally infected CBPP cattle

in a herd is inconclusive and still requires further study. It has however shown that healing in some animals is possible.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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Full Length Research Paper

Incidence and economic impact of fasciolosis in Wolkite town, Community Abattoir

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The study was conducted to explore the incidence and economic loss related with fasciolosis in cattle at Wolkite town, Community Abattoir, Wolkite, Ethiopia. A cross-sectional study was conducted from February, 2016 - May, 2016 on bovine fasciolosis in Wolkite town, Community Abattoir. From a total of 392 cattle inspected coprologically 41.8% (164) were found positive for fasciolosis. The occurrence of cattle fasciolosis in the study sites was considerable ($p < 0.05$) which is mainly determined based on body conditions. Post mortem assessment was conducted on a total of 392 bovine and 41.8% were found infected by *Fasciola*. *Fasciola hepatica* was the major *Fasciola* species causing bovine fasciolosis at the study areas. Analysis of the abattoir data indicated a total yearly liver disapproval was identified to result in 182582.4 Ethiopian birr. Likewise, the mean carcass weight loss was calculated to be 4984499.52 Ethiopian birr due to fasciolosis in cattle. The total yearly monetary loss due to fasciolosis in Wolkite town, Community Abattoir was calculated to be 5167081.92 birr. The results of the present study thus illustrated that the incidence and economic loss of fasciolosis in bovine slaughtered at Wolkite town, Community Abattoir was exceptionally elevated and necessitates urgent need for control and prevention of the parasite in the study area in specially and in Ethiopia as a whole.

Key words: Cattle, *Fasciolosis*, incidence, Wolkite town.

INTRODUCTION

Fasciolosis also named as distomitosis, or liver rot is an important helminth disease caused by trematode *Fasciola* commonly called "liver fluke". This disease belongs to plant born trematode (Mas-Coma et al., 2005). The definitive host range is very broad and includes many herbivores, mammals, including humans (Chhabra and Singla, 2009). The life cycle include fresh water snail as intermediate host of parasite (Torgerson and Claxton, 1999). The disease is a type of helminthosis and has

been classified as Neglected tropical disease.

In recent times, worldwide fatalities in animal production because of *Fasciola hepatica* where predictably expected above 3.2 billion per annum. The WHO estimated at 180 million are at possibility of infection and 2.4 billion peoples are infected with *F. hepatica* (Spithil et al., 1999). In Europe, the American and Asia only *F. hepatica* is concerns but the distribution of the two species overlies in many areas of Africa as well as Asia (Mas-Coma et al.,

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2005). The prevalence of fasciolosis in cattle in various parts of the world has been reviewed. In Africa, Ethiopia 30-90% (Magaji et al., 2014), Addis Ababa 20.3% (Kassaye et al., 2012), Nekemte 20% (Alula et al., 2013), Hossona 34.9% (Bekele et al., 2014). Ethiopia has enormous number of cattle having high role for meat expenditure and produces cash revenue from export of live cattle, carcass, organs and skin.

The monetary losses due to fasciolosis throughout the humanity are massive and these losses are coupled with death, illness, stunted growth condemnations of fluky liver, and greater than before vulnerability to secondary infection and outflow because of have power over a measure (Malone et al., 1998).

Despite the aforesaid existing condition and the incidence of a number of troubles due to fasciolosis, there is scarcity of well-documented information on the incidence of fasciolosis among cattle in Ethiopia. For that reason, the present investigation was intended with the objectives of assessing the incidence of fasciolosis in cattle and the extent of direct economic loss due to liver disapproval and not direct carcass loss at Wolkite community abattoir, Ethiopia.

MATERIALS AND METHODS

Study period and area

The investigation was carried out from March, 2016 to May and it was carried out at Wolkite municipal Abattoir. Wolkite is located at south western part of Ethiopia 152 km away from Addis Ababa.

Study design and population

The study population comprised of 392 adult cattle from different parts intended for slaughter at Wolkite municipal abattoir.

Study design and sampling procedure

The study was cross-sectional study whereby the study animals were prevalence of fasciolosis in cattle brought from selected from the slaughter line using systematic random sampling in such a way that 14 animals were examined per a day from a group of varying number of cattle slaughtered in one day. Information regarding sex, age, breed and body condition of the study animals was recorded during ante-mortem assessment. Body condition was scored following the guidelines set by Nicholson and Butterworth (1986). Accordingly, animals were classified into lean (score 2 and 3), medium (score 4, 5 and 6) and fat (score 7, 8 and 9) categories. There was no animal with score 1.

Liver examination

The liver of each study animal was carefully examined for presence of lesions suggestive of *Fasciola* infection externally and sliced for confirmation. Liver flukes were recovered for differential count by cutting the infected liver into fine, approximately 1 cm, slices with a sharp knife according to Hansen and Perry (1994). Each mature fluke was identified to species level according to its shape and size

(Soulsby, 1982). All intact immature and mature flukes and only fluke heads -when a portion of fluke was found- were counted.

Sample size determination and sampling methods

Simple random sampling method was used for selection of sampling units at equal intervals. The sampled cattle were screened for the presence of trematodes of interest by coprological and post-mortem examinations. The body condition score was estimated using techniques suggested by Nicholson and Bufferworth (1986), and accordingly, animals were classified into lean (score 2 and 3), medium (score 4, 5 and 6) and fat (score 7, 8 and 9) categories. Sample size for this study was determined using the formula described by Thrusfield (1995). Since no study has been carried out so far on the prevalence of *Fasciola* in cattle at this abattoir, the expected prevalence was taken as 50%. Thus, using the following formula the sample size for the study was calculated as 384. However, to increase the precision of the study, we decided to include 392 cattle in our investigation.

Abattoir survey

Examination of livers for *Fasciola* was done immediately after removal of internal organs. The livers were examined by inspection, palpation and systematic incision to recover immature and adult *Fasciola* flukes. Those livers condemned as unhealthy for human consumption due to fasciolosis during post mortem examination were registered.

Data analysis

The data was record on particularly intended types. The occurrence of fasciolosis was calculated as the number of cattle found to be infected by *Fasciola* expressed as a proportion of the total number of cattle slaughtered. Difference between the results by body condition score examination and by post-mortem examination was estimated. A 95% CI and 5% significance level was used to agree on whether there was considerable difference in the parameters calculated between different groups.

RESULTS

Postmortem examination

Three hundred and ninety-two liver sections were scrutinized in the present study. More than one or only one *Fasciola* species were identified by regular postmortem inspection of the liver. The identification results proved a prevalence of 41.8% (182/392) (Figures 1 and 2) fasciolosis. All the parasites identified as *Fasciola* were tested for species assignment using customary guiding principles. The explicit incidence of *Fasciola* species were known to be 32.6% (128/392) *F. hepatica*, 0.51% (20/392) *F. gigantica*, 0.3% (12/392) mixed (both *F. hepatica* and *F. gigantica* species) and 10.01% (4/392) unidentified immature flukes (Table 1).

Body state score was taken as possible threat reason for the incidence of fasciolosis in the present study cattle. Maximum contagion rate of fasciolosis was existed in low

Table 1. Prevalence of fasciolosis by species.

Species	Positive	Prevalence (%)
<i>F. hepatica</i>	128	32.6
<i>F. gigantica</i>	20	0.51
Mixed infection	12	0.3
Unidentified (immature flukes)	4	0.01
Total	164	41.8

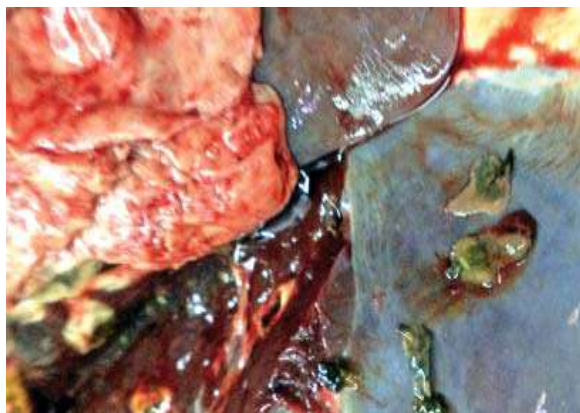


Figure 1. *Fasciola* in the liver of cattle.



Figure 2. Bovine liver with heavy infection due to *Fasciola*.

body state cattle (68.2%) go after by average body state cattle (44.1%). The least incidence of fasciolosis was identified to happen in better body state cattle (21.5%). Arithmetical scrutiny of the figures indicated the existence of arithmetical considerable disparity ($P < 0.05$) on the contagion rate of cattle with fasciolosis amongst the three dissimilar body stated examined cattle (Table 2).

Monetary loss

All the affected livers were condemned totally. Partial

condemnation is not practiced. The data was collected from the abattoir to estimate the economic losses by considering annually condemned livers. Annual data of the last three years regarding animals slaughtered and livers condemned were collected from retrospective abattoir record. Current retail market price of single liver and 1 kg meat in Wolkite, at the study time was known to be around 60 and 130 Ethiopian birr, correspondingly was determined from interviews with local butchers in Wolkite town according to the formula set by Ogunrinade and Ogunrinade (1980).

Based on this information, the total yearly liver disapproval (ALD) was identified to cause in 36,516 Ethiopian birr (1826 United States Dollar) loss ($ALD = 7280 \times 60 \times 0.418$) = 182582.4.

Likewise, the common carcass weight loss was known to be 99,690 Ethiopian birr (4984 United States Dollar) because of fasciolosis in livestock.

$$(IACW = 7280 \times (10\% \times 126) \times 130 \times 0.418) = 4984499.52$$

The total yearly economic loss due to fasciolosis at Wolkite town, Community Abattoir was known to be 5167081.92 Ethiopian birr (240329 United States Dollar).

$$ALC = CSR \times LC \times P$$

DISCUSSION

The ecological and climatic condition of the countries such as temperature, altitude, rainfall though variations in livestock managing method and the capability to notice infection can play a part. On the other hand, when we compare prevalence of Fasciolosis with Africa country the highest prevalence (53.9%) was recorded from Zambia (Phiri et al., 2005).

The world losses of animals due to fasciolosis agricultural communities and commercial producers in urbanized countries as well as the occurrence of *F. hepatica* can be up to 77% in hot countries. Fasciolosis is taken as the solitary and largest part important helminthes interaction of cattle with shown prevalence of 30-90 (neglected tropic disease).

The aggregate incidence of cattle fasciolosis (41.8%) calculated in the present study is in contrary with the

Table 2. Proportion of Fasciolosis among dissimilar body stated animals.

Body state	Inspected	Positive	Proportion (%)	X ² value	P-value
Better	140	32	21.5	8.48	0.001
Moderate	184	72	44.1		
Low	88	60	68.2		
Total	392	164	41.8		

work of Berhe et al. (2009) from northern part of the country who notified 24.3% proportion. Still, it is greatly less than that of many other reports of analogous studies from diverse abattoirs in the country and somewhere else in Africa. Yilma and Mesfin (2000) indicated a 90.7% prevalence of fasciolosis in cattle slaughtered at Gondar, whereas Tolosa and Tigre (2007) reported a prevalence of 46.2% at Jimma which was in concord with this study. The resemblance may be owing to the fact that two study places are so nearby and have more or less the same climatic conditions. Phiri et al. (2005) from Zambia and Pfukenyi and Mukaratirwa (2004) from Zimbabwe indicated 53.9 and 31.7% proportion respectively. On the other hand, a less occurrence of fasciolosis (14.0%) has been pragmatic in slaughter cattle at Wolaita Abattoir (Abunna et al., 2009). Dissimilarity in incidence amongst geographical locations is accredited primarily to the difference in the climate and ecology of the area. *Fasciolosis* commonness has been taught to be varying over the years largely due to deviation in quantity and pattern of rainfall (Mungube et al., 2006).

Similar to the present study's outcomes, numerous abattoir investigations in diverse localities of Ethiopia reported the high prevalence of *F. hepatica* to *Fasciola gigantica* (Tolosa and Tigre, 2007; Ibrahim et al., 2010; Berhe et al., 2009). Abunna et al. (2009), still, recorded privileged incidence of *F. gigantica* than *F. hepatica* in livestock butchered at Wolaita Abattoir in Ethiopia. The result of mixed infection with thus two species of *Fasciola* shows that there are places in the country where the climato-ecological conditions favor the existence of the intermediate snail hosts for both species. Disparity amongst the virtual incidence of the two species of *Fasciola* in cattle slaughtered in abattoirs situated in diverse regions of the country may possibly be clarified by the deviation in the climate-ecological conditions of the areas feeding the abattoirs. Quite a lot of studies in other African countries, however, indicated that *F. gigantica* is the leading if not only species ubiquitous (Phiri et al., 2005; Pfukenyi and Mukaratirwa, 2004; Kithuka et al., 2002; Opara, 2005).

This study showed there was a numerically considerable relationship ($P < 0.001$) among *Fasciola* incidence and body states of the cattle. In an analogous investigation, Bekele et al. (2010) identified elevated incidence of fasciolosis in livestock with low body state contrasted to cattle in moderate and better body state.

Chronic fasciolosis is distinguished by continuous loss of state (Urquhart et al., 1996). Though, it should be noteworthy that livestock originated from feedlots, which are anticipated to be in better body state, and are nearly all probably to be de-wormed than livestock originating straightforwardly from grazing.

As cattle butchered at Wolkite Abattoir came from roughly all place of the zone it could be said that Fasciolosis is still ubiquitous in cattle in the surrounding area. The climates as well as ecological situations are sympathetic for continued existence and expansion of the intermediate snail hosts for the two species of *Fasciola* are also common in the study area.

Conclusion

The present study confirmed that fasciolosis is a significant disease cause substantial loss of income at Wolkite town Abattoir. The country was known to suffer loss of 5167081.92 Ethiopian Birr (240329 United States Dollar) annually due to liver disapproval and corpse weight loss that occurred from fasciolosis. For this reason, from the present study one can wind up that fasciolosis is among the key livestock parasitic disease of cattle which has a blow on the country's wealth further than its crash on the farmers. Consequently, stress should be given to management of its distribution while fasciolosis is one of the prominent parasitic diseases that have gigantic indirect and direct losses in domestic animals population.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Prevalence and economic significance of bovine hydatidosis at Adama Municipal Abattoir, Adama, Ethiopia

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A cross sectional study was conducted to assess the prevalence and economic significance of bovine hydatidosis at Adama municipal abattoir. A total of 500 cattle were examined and 191 (38.2%) of them were found infected. Also, 253 visceral organs were found harboring one or more hydatid cyst. Prevalence of lung and liver cyst accounted for 94.5% and the involvement of other organs as many as 20 cysts were recovered from a single lung. Proportionally 35.7, 34.6, 20.8 and 8.82% of the cysts were calcified, small, medium and large sized, respectively. In addition, sterile and fertile cysts represent 46.2 and 18.1%, respectively. The rate of calcification is higher in liver than lungs, while that of most fertile cysts were recovered from lungs. The annual economic loss due to bovine hydatidosis at Adama abattoir is estimated to be about 171,436.36 Eth Birr (ETB). This information shows the risk of hydatid cyst distribution and economic significance in the study area. Therefore, appropriate control measures should be undertaken which include public awareness education program and a more aggressive effort that should include a reduction of stray dog population.

Key words: Adama abattoir, economic significance, hydatid cyst, fertility, prevalence.

INTRODUCTION

Hydatidosis (cystic echinococcosis) is a cosmopolitan food borne parasitic zoonoses caused by the larval stages of cestodes belonging to the genus *Echinococcus* (Family Taeniidae) (Chhabra and Singla, 2009). Larval infection (hydatidosis) is characterized by long term growth of metacestode (hydatid cysts) in the intermediate host. The two major species of veterinary importance are *Echinococcus granulosus* and *Echinococcus*

multilocularis which cause cystic Echinococcosis (CE) and alveolar Echinococcosis (AE), respectively. Both CE and AE are serious diseases, the latter especially so, with a high fatality rate and poor prognosis if not managed properly. Hydatid cysts of *E. granulosus* develop in internal organs (mainly the liver and lungs) of humans and other herbivores intermediate hosts (sheep, horses, cattle, pigs, goats and camels) as unilocular fluid filled

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bladders. These consists of two parasites derived layers, inner nucleated germinal layer and an outer acellular laminated layer surrounded by a host-produced fibrous capsule and protoscoleces bud off from the germinal membrane (Thompson and McManus, 2001).

E. granulosus is the smallest of all tape worms with only three proglottids (Eckert and Deplazes, 2004; OIE, 2004). The body or strobila has a number of reproductive units (proglottids), the mature penultimate proglottid and the terminal proglottid (Soulsby, 1982; Adem, 2006). The latter is gravid and is usually more than half the length of the worm. This gravid uterus/proglottid has 12-15 short lateral diverticuli and is usually filled with 100-1500 thick shelled eggs. The gravid proglottids and or eggs are shed in the faeces (McManus et al., 2003). The eggs are brown in color and morphologically indistinguishable from those of other tape worms of the genus *Taenia*. The egg has a single hexacanth embryo, the oncosphere, which has three pairs of hooks (Thompson and McManus, 2001).

Hydatidosis caused by *E. granulosus* is a serious concern in public health which is much more common on the rural areas of Ethiopia where dogs and domestic animals live in a very close association usually sharing the same accommodation with human. Man becomes infected by an accidental injection of oncospheres from contaminated food, water and environments, where as the dog is the commonest final host (FH), which becomes infected by ingestion of infected offals (Urquhart et al., 1996).

The disease has also an advert effect on the productivity of animal with huge economic losses. The level of which has not until now been precisely determined (Polydorou, 1992). In addition to its direct effect on livelihood of domestic animals and man, *Echinococcus* causes great economic losses. The losses due to this parasite is considerable when one considers its effect on the productivity of animals, the condemnation of infected viscera or even the whole carcass and costs incurred for its control (Hubbert et al., 1975).

Several researchers from different parts of the country (Abebe and Yilma, 2011; Kebede et al., 2010, 2011; Jobre et al., 1996) have reported a prevalence range of 13.7 to 72.4% in cattle slaughtered at Dire Dawa, Gonder, Adama and Asella, respectively by indicating its highly importance and existence of the disease. To establish the prevalence and estimated economic loss of hydatid disease in animals depend on mainly collection of data in slaughter houses. Prevalence of the disease in domestic food animals' show that sheep are the most commonly infected domestic intermediated host (IH), though cattle and various other types of live stock are also affected. Infection does not usually result in any sign in livestock (Soulsby, 1986).

Echinococcus parasites are difficult to detect in faeces of definitive host, due to their small size. Diagnosis has been performed by examination of purge contents for the

presence of *E. granulosus* even though the techniques have some disadvantage such as poor sensitivity, incomplete purgation, adverse reactions to the drugs in some dogs and infectivity of the purge material for the personal involved. The more recently developed serological tests, for the diagnosis of hydatid disease are enzyme linked Immuno sorbent assay (ELISA), radio immuno assay (RIA), immune electro phoresis (IEP) and indirect haem agglutination (IHA) (Sewell and Brockles, 2002).

In general, different organs lung, liver, spleen, heart and kidney (rarely) involved in hydatidosis of which lung and livers are the primary (most commonly) condemned organs due to hydatidosis, among cattle slaughtered. Nevertheless, study on prevalence and proper evaluations of economic losses due to this disease in different species of animals in the nation is lacking to which other wise is of great relevance where economic realities often determine the type and scope of the control measures to be envisaged. The principal objectives of this study were therefore to determine the prevalence of hydatidosis in cattle at Adama municipal abattoir and to estimate the magnitude of economic losses incurred due to hydatidosis.

MATERIALS AND METHODS

Study area

The study was conducted from November, 2008-May 2009, in Adama town, East Shoa zone of Oromo Regional State, Central Ethiopia. The town is located at 99 km South East of Addis Ababa at 39.1 N and 8.31 E, at an elevation of 1770 m above sea level and receives the annual rainfall raining from 400 to 800 mm; the temperature range is 13.9 to 27.7°C (NMSA, 2016).

Adama town is one of the most populous town ships in the country with important multi directional trade route. The town ship has one municipality Abattoir that supplies the inspected meat to more than 150,000 inhabitants and 61 legally registered butcheries. Backyard slaughter is also significant in spite of some pressure from the government authorities to ban this activity.

Study animal and design

The study was conducted on local breed cattle that originated from neighboring provinces such as Bale, Arsi, Harar, areas around Adama and Borena zone of Ethiopia. Almost all cattle presented for slaughter were male and adult.

A cross-sectional study type was carried out from November, 2008 to May 2009 by collecting data on events associated with hydatidosis in cattle slaughtered at Adama Municipality Abattoir.

Sample size determination

The sample size determined according to Thrustfield (2007) by using expected prevalence of hydatidosis 50% then the sample size required was 384 cattle at 95% confidence level and 5% expected error. But, in order to increase accuracy of the study, the sample sizes were increased to 500 cattle.

Study methodology

Post-mortem examination

During post mortem examination organs of the abdominal and thoracic cavities namely liver, lungs, heart, spleen and kidneys were systematically inspected for presence of hydatid cyst by applying the routine meat inspection procedures. The inspection procedure used during the post mortem examination consisted primary examination followed by a secondary examination if evidence of hydatid cyst were found. The primary examination involved, visualization and palpations of organs and muscles, whereas secondary examination involves further incision into each organ in case where a single or more hydatid cysts were found. Whenever and wherever hydatid cysts were apparent the number and the size of the cysts as well as calcified cysts per organ and per animal were recorded. The size of the cyst was categorized into three groups small (1-5<5 cm) in diameter, medium 5-8 cm in diameter and large (>8 cm in the diameter).

In organs with hydatid cysts, the cysts were carefully removed using a knife, collected in a clean container (ice box) and brought to Asella Regional Veterinary Laboratory then fertility tests of the Hydatid cysts were carried out and the result were registered.

Laboratory examination

Laboratory examinations were carried out on all collected specimens to determine the fertility and sterility of the cysts. The contents of the cyst was aspirated with a syringe to decrease its pressure and collected in a graduated beaker and the rest of the fluid was then added to it and measure its volume and it was allowed to stay on incubator for 30 min at 36°C to settle the content and then about 10 ml of these sediment was poured to the test tube and centrifuged at 1000 rpm for 3 min to separate the contents clearly from the liquid part and the supernatant was discarded, but the sediment with some fluid was left in test tube examination was done under objectives of 40X magnification for the presence or absence of protoscolex (Gupta and Singla, 2012). The protoscolex which preset as white dots on the germinal epithelium or brood capsules for hydatid sands with in the suspension cysts was categorized as fertile and where its absence categorize the cysts as sterile or non fertile (McPherson, 1985).

Economic loss assessment

The total economic loss due to hydatidosis in cattle slaughtered at Adama municipality abattoir was estimated from the summation of annual organ condemnation cost (direct loss) and cost due to carcass weight reduction (indirect loss).

Direct loss

All organs namely liver, lung, heart, spleen and kidney which are positive for hydatidosis were totally condemned and conditions leading to partial condemnations were poorly recorded. The economic losses due to total/partial condemnation of organs due to bovine hydatidosis was then assessed using the following formula set by Ogunrinade and Ogunrinade (1980).

$$ACL_1L_2HKC = P (CSR \times PL_1C \times L_1C) + P (CSR \times PL_2C \times L_2C) + P (CSR \times PHC \times HC) + P (CSR \times PKC \times KC) P (CSR \times PSC \times SC)$$

Where ACL_1L_2HKSC = Annual cost of live, lung, heart, kidney and spleen condemned; CSR – average number of cattle slaughtered per year at a abattoir; P – prevalence of hydatidosis at Adama

municipal abattoir; PL_1C – percentage of lungs condemned; L_1C – mean cost of one lung in Adama town; PL_2C – percentage of liver condemned; L_2C – mean cost of one lung in Adama town; PHC – percentage of heart condemned; HC – mean cost of one heart in Adama town; PKC – percentage of kidney condemned; KC – mean cost of one kidney in Adama town; PSC – percentage of spleen condemned; SC – mean cost of spleen in Adama town.

Indirect loss

A 5% carcass weight loss, due to hydatidosis in cattle, has been described by Polydrous (1992). So, the annual economic loss due to carcass weight reduction as a result of bovine hydatidosis was calculated as.

$$ACW = CSR \times P \times BC \times CL \times 126 \text{ kg}$$

Where, ACW = annual loss from carcass weight loss due to hydatidosis; CSR = average number of cattle slaughtered per annum in Adama; CL = carcass weight loss in individual cattle due to hydatidosis; BC = average market price of 1 kg beef in Adama town; P = prevalence rate of hydatidosis at Adama abattoir.

Data analysis

Basic data entry and handling was done using MS-Excel. From row data collected, total number of cases showing hydatid cyst were determined.

Prevalence of hydatidosis was calculated as the number of cattle found to be infected with hydatid cysts expressed as the percentage of the total number slaughtered (Thrusfield, 2007), economic loss assessed by formula set by Ogunrinade and Ogunrinade (1980) and variation between origin were evaluated by Pearson's Chi-square (χ^2) and differences were regarded statistically significant if $P < 0.05$ using STATA 7.0

RESULTS

Prevalence

Regular visit to Adama slaughter house during study period (November 2008 to May 2009) allowed examination of 500 cattle, of these 191 (38.9%) were found infected with hydatid cyst.

Observation during this period revealed that 166 (65.6%) lungs, 73 (28.9%) liver, 3 (1.16%) spleen, 9 (3.56%) kidney and 2 (0.79%) hearts were harbored with hydrated cyst representing a total 253 organs all together (Table 1).

Out of the total infected organs, the involvement of lung and liver accounted for 94.5%. In addition, pulmonary infection out weighted involvements of liver and other organs (Table 1). The frequency of distribution (infection rate) can also vary among different origin of animals in superiority of Arsi followed by Bale, Borena and Harar, respectively, even though the result was statistically insignificant (Table 2).

The total number of cysts found on each organ was in the order of 322 (72.9 %) in lungs, 105 (23.8%) in liver, 4 (0.9%) in heart in total cyst count being 442 (Table 1).

Table 1. Prevalence of bovine hydatidosis and organ involvement rate.

Organs involved	No. examined	No. Involved	Relative Prevalence (%)	Cyst count	
				Max. no of cyst/organ	Total
Lung	500	166	65.61	20	322
Liver	500	73	28.85	6	105
Spleen	500	3	1.16	2	4
Kidney	500	9	3.56	1	9
Heart	500	9	0.79	1	2
Total	500	253	100	20	442

Table 2. Prevalence of hydatidosis based on origin in the study area.

Results	Origin									
	Arsi		Bale		Borana		Harar		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Positive animals	65	34.03	49	25.65	39	20.42	38	19.90	191	100.00
Negative animals	112	36.25	78	25.24	67	21.68	52	16.83	309	100.00
Total	117	35.40	127	25.40	106	21.20	90	18.00	500	100.00

Pearson χ^2 (χ^2) =0.8771, P=0.831.

Cyst characterization

Cyst size

An exceptionally large cyst was found measuring 15 cm in diameter and containing about 1.5 L of fluid. A considerable number of hydatid sands were recovered from it, which up on microscopic examination was classified as fertile and viable cyst. The remaining cysts were by far smaller than the above described one and were classified as: 153 (34.62%) small, 92 (20.81%) medium, 39 (8.82%) large and 158 (35.75%) calcified cyst (Table 3). The result revealed that small cysts represent the highest proportion, while large cysts are the least in terms of their prevalence (Table 3).

Fertility and sterility of cyst

Out of the total hydatid cysts recovered in this survey, 204 (46.15%) were found sterile, 80 (18.10%) fertile and 158 (35.74%) are calcified (Table 4).

Economic losses assessment

Assessment of the retail market prices of organs from averaged sized zebu in the study area revealed that the cost for lungs, liver, kidney, heart and spleen were indicated below (Table 5). The price of a kilo of beef is

about 55 Ethiopian Birr in average. Therefore, the calculated annual economic loss due to bovine hydatidosis at Adama slaughter house from organ condemnation are 3,069.71 Ethiopian Birr and from carcass weight loss is 168365.74 Birr mounting to a total loss of 171,436.36 Birr.

DISCUSSION

It was noted that *E. granulosus* recovered from geographic regions have shown considerable variation which may have important epidemiological implication. In addition, other factors such as difference in culture, social activities and attitudes to dogs in different regions may contribute to the variations in its prevalence (Arene, 1986).

In this study, a prevalence of 38.2% was seen which is very close (37.7%) to a finding by Yamane (1990) and slightly lower (48.7%) than that of Ahmed et al. (2016) in East Shoa. Barsisa (1994) in Nekemte reported a prevalence of 36.66%, Abduljewad (1988) reported 36.66% in Jima, Tamene (1986) 33.78% and Belina et al. (2012) in Bahir Dar. Still high prevalence values were also registered in other places: 63% of prevalence in Robe (Wubet, 1988), 54.8% in Arsi (Alemayehu, 1990), 55.71% in Debrezeit (Abera, 2007), 54.9% in BahirDar (Nebiyu, 1990) and 46.5% in Debrezeit (Yilma, 1984).

The high prevalence may be related to the presence of favorable factors for the propagation and maintenance of

Table 3. Cyst size and organ involvement frequency distribution.

Organs involved	Small		Medium		Large		Calcified		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Lung	128	39.75	78	24.22	27	8.39	89	27.64	322	72.85
Liver	22	20.95	12	11.43	11	10.48	60	57.14	105	23.76
Spleen	0	-	2	50.00	1	25.00	1	25.00	4	0.9
Kidney	3	33.33	0	-	0	-	6	66.67	9	2.04
Heart	0	-	0	-	0	-	2	100.00	2	0.45
Total	153	34.62	92	20.81	39	8.82	158	35.75	442	100

Table 4. Distribution of cyst condition versus predilection site in affected animals.

Organs involved	Sterile		Fertile		Calcified		Total	
	No.	%	No.	%	No.	%	No.	%
Lung	163	50.62	70	21.74	89	27.64	322	72.85
Liver	35	33.33	10	9.52	60	57.14	105	23.76
Spleen	3	75.00	0	-	1	25.00	4	0.9
Kidney	3	33.3	0	-	6	66.7	9	2.04
Heart	0	-	0	-	2	100.00	2	0.45
Total	204	46.15	80	18.10	158	35.75	442	100

Table 5. Number of organs affected, % involvement and their current value in Adama market of cattle organs.

Type of organs	No. of condemned organs	Percentage	Price of each organ (Birr)	Total cost (Birr)
Lung	166	33.2	5.00	830
Liver	73	14.6	11.00	803
Spleen	3	0.6	1.00	3
Kidney	9	1.8	2.00	18
Heart	2	0.4	3.00	6

high level infection in the area. Moreover, the age of slaughtered animals is also anticipated as one of the reasons contributing to the high prevalence of the disease in the area most of the slaughtered animals were old probably culled from productivity and, hence they were exposed over a longer period of time with an increased possibility of acquiring the infection. Studies conducted in New Zealand (1958) also strongly suggested that prevalence is heavily influenced by age. The result from this study later indicated that 9% of affected cattle were 1-2 years old. While 74% were 5yrs older. Another study reported a prevalence in calves as 7%, in cows 52.2%, in fattening bullocks 69.7%, in bulls and bullocks as 81.2% (Abebe and Yilma, 2011; Gracy, 1994).

A maximum of 20 cysts were recovered from a single lung in this study. This finding is nearly close to the findings of Fikre (1994) and Nebiyu (1990). A much higher and lower result was also found previously by

Tamene (1986), Wubet (1988), Feyissa (1987) and Barsisa (1994) who found 132, 5, 99 and 63 cysts per organ, respectively. Such variation in cyst abundances in an organ is explained as probably to the special distribution and infectivity of *Echinococcus* eggs (Gemmel, 1987).

In the present study, it was found that about 94% of the case hydatid disease involves the lungs and liver, although lung infection was relatively higher. This finding concord with the observations of other workers: Barsisa (1994), Nebiyu (1990), Abera (2007), Tamene (1986) Wubet (1988), Fufa and Debele (2013) and Lati et al (2015) for this reason, the explanation shows that these organs are the first capillary sites encountered by the migrating *Echinococcus* oncosphere.

In addition as to why lungs are organs much more affected than the liver is related to the slaughtered subject, as most animals were slaughtered with the age

of above 5 years liver capillaries are dilated at older age that most oncospheres are easily pass directly to the lungs. This also facilitate the condition, then to the thoracic ducts and heart and finally to be trapped in the lungs.

In the present trial, higher number of medium and large sized cyst was found in lungs than in liver and most calcified cyst in liver. Similar observations were also described by other workers: Fikre (1994), Tamene (1986), Alemayehu (1990), Abera (2007) and Hagos (2007). The reason given for the occurrence of higher percentage of calcified cysts in the liver is associated with the relatively higher reticulo-endothelial cells and abundant connective tissue reaction of the organ (Gemmel, 1987); hence larger numbers of oncosphere are killed in this organs.

The study carried out to evaluate the condition of hydatid cyst revealed that rates of sterility and fertility vary among different organs. The finding of 46.25% sterile, 18.09% fertile and 35.74 calcified cysts may generally imply that most of the cysts in cattle are infertile. This finding is consistent with the observation of Fikre (1994), Barsisa (1994), Nebiyu (1990) and Wubet (1988). Conversely in Britain, up to 90% of the total cysts from cattle are said to be sterile. On the other hand, in some countries like South Africa, Belgium and Rhodesia, 96.9, 94.2 and 86.5%, respectively, of the uncalcified cysts were fertile (Arene, 1986). The variation in fertility rates in different geographic zone of the globe could be allocated to strain differences of *E. granulosus* (Arene, 1986) also strain of parasites and host can modify infectivity of parasite (Gemmel, 1987).

The economic loss due to bovine hydatidosis at Adama slaughter house from offal condemnations and carcass weight loss was estimated to be about 171, 436.36 Ethiopian birr per annum. These figures correspond to loss of 134.77 birr per head of slaughtered cattle. Interpretation of this result must be made with a very serious precaution particularly in light of the fact that in the study area, only few animals were brought to slaughter house for prevailing tradition in back-yard slaughtered. The calculated loss present is generally considered as by far lower than the real losses brought about by bovine hydatidosis.

Conclusions

Cystic echinococcosis/ hydatidosis is a disease of considerable importance both from public health and economic point of view. The prevalence of the disease and estimated corresponding economic losses in Adama municipal abattoir from offal condemnation and carcass weight loss were 38.2% and 171,436.36 ETB, respectively. Therefore, it is concluded that owing to the presence of socio- economic conditions that favors the propagation and maintenances of high level infection, and considering the incalculable indirect loss, hydatidosis

is one of the most economically important disease in Adama and its surrounding, warranting serious attention for its control and prevention.

Based on the results obtained and socio-economic realities in Adama town and its surrounding, the authors forwarded the following recommendations; public education of zoonotic importance, life cycle and economic importance of the disease through teaching at school for students, extension workers for farmer and other possible mass media (Radio, TV, etc); construction of abattoirs and provision of facilities such as well educated meat inspectors, construction of dog proof fence and construction of ideal disposal pits; imposing legislative measures that will put an end to back yard and road side slaughter activities and create favorable conditions for people to bring their animals to slaughter houses; control of dog population through killing of stray dogs in collaboration with rabies control campaign and detailed investigations for basic epidemiological factors governing the dissemination of hydatidosis/echinococcosis must be carried out.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Study on prevalence of ovine lungworm in Guna District, Arsi Zone, South East Ethiopia

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A cross-sectional study was conducted in Guna district, Arsi zone, South East Ethiopia, from November, 2013 to March, 2014 to determine the prevalence, associated risk factors and identification of species of ovine lungworm by using coproscopic examination and questionnaire survey. A total 384 faecal samples from randomly selected sheep of different age groups, body conditions, sexes and PAs with various altitudes. The finding indicated that 217 (56.5%) were infected with different species of lungworm, namely, *Dictyocaulus filaria* (28.4%), *Muellerius capillaries* (10.7%), *Protostrongylus rufescens* (7.6%), and mixed infection (9.9%). There were statistically significant difference ($p < 0.05$) in the prevalence of lungworm infection with regard to age (≤ 1 year 62.0% and > 1 year 51.0%) and PAs (Cire Anole 78.1%, Nano Hecho 52.1% and Re'e Amba 39.1%); however, sexes (female 59.9% and male 53.1%) and body conditions (poor 60.9%, medium 57.0%, and good 51.6%) were insignificant ($p > 0.05$). Parallely, questionnaire surveys on history of antihelmintic usage, manifestation of respiratory signs, and place where animal kept were undertaken on the same animals that were sampled for coproscopic examination. Accordingly, the prevalence of lungworm infection with antihelmintic usage (none dewormed 67.5% and dewormed 44.6%), manifestation of respiratory sign (No 44.1% and yes 68.2%), and place where animal kept (forest area 38.1% and swampy 67.9%) and statistically all considered factors for questionnaire survey are highly significant ($p = 0.000$). As conclusion, our work revealed that lungworm belongs to the major respiratory helminthes that affect the health and productivity of sheep in the study area; therefore, attention should be given for the control and prevention to reduce the current high prevalence.

Key words: Arsi, Ethiopia, Guna, lungworm, ovine, prevalence.

INTRODUCTION

Ethiopia lies within the tropical latitudes of Africa, and has an extremely diverse topography, a wide range of climatic features and a multitude of agro-ecological zones, which

makes the country suitable for different agricultural production systems. This in turn has contributed to the existence of a large diversity of farm animal genetic

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resources in the country (Anon, 1998a, 2004b). Ethiopia with its estimated 24.2 million sheep together with its variation in agro climatic zones represents a good reservoir of small ruminant genotypes (CSA, 2013).

Unlike the large potential of small ruminants in the country, their productivity is low. The major problems that greatly affect the economy of sheep and goat production in Ethiopia were diseases (Bekele et al., 1992). Disease alone accounts for 30% mortality in young's and 20% in adults. A loss of US\$81.8 million is reported annually due to parasite infection. In a country confronted with such enormous losses caused by parasites, it is great loss to the country (Biffa et al., 1999).

Helminth parasite of ovine is ubiquitous, with many tropical and sub-tropical environment of the world providing nearly perfect conditions for their survival and development (Hansen and Perry, 1994; Singla, 1995). Helminthosis is one of the considerable significance in a wide range of agro-climatic zones in sub-Saharan Africa and constitute one of the most important constraints to small ruminant production (ILCA, 1990). The production loss is a direct result of clinical and subclinical helminthes infections resulting in low productivity due to stunted growth, insufficient weight gain, poor feed utilization and mortality and indirect losses associated with treatment and control costs (Ayalew et al., 2011; Singh et al., 2016).

In the highland areas, infection with lungworm parasites is the common cause of high mortality and morbidity in sheep population (FAO, 2002). Lungworms are parasitic nematodes known for infection of the lower respiratory tract, characterized by respiratory distress, tracheitis, bronchitis, and pneumonia (Kimberling, 1988). Common lungworms in sheep are *Dictyocaulus filaria*, *Muellerius capillaris*, and *Protostrongylus rufescens* (Radostits et al., 2007). These nematodes belong to two super families, Trichostrongyloidea (*D. filaria*) and Metastrongyloidea (*P. rufescens* and *M. capillaries*) (Urquhart et al., 1996; Radostits et al., 2007). Dictyocaulidae and certain Metastrongyloidea are known to exist in East Africa (Ethiopia, Kenya and Tanzania) and South Africa (Torncy, 1989). Endoparasites, including *D. filaria*, are major cause of death and morbidity in the Ethiopian highlands. Up to half of all sheep deaths and morbidity on farms in Ethiopia highlands are caused by pneumonia and endoparasites (ILCA, 1990).

The previous findings of lungworm infection in Ethiopia (Bekele et al., 1981; Netsanet, 1992; Wondwossen, 1992; Paulos, 2000; Mihreteab and Aman, 2011; Abeje et al., 2016) are in Arsi and Bale, Wollo, Debre Birhan, Asella, Chilalo, and Tiyo respectively showing prevalence ranging from 30.74 to 73.25% which indicated the high prevalence of the infection in certain parts of the country; however, there was no research done on ovine lungworm in Guna District, Arsi Zone, Oromia Regional State, South East Ethiopia. Therefore, the objectives of this research

were to determine the current prevalence of lungworm infection and identify the circulating species and associated risk factors for its occurrence.

MATERIALS AND METHODS

Study area

This study was conducted in Oromia Regional State, Arsi Zone, Guna District which is located 224 km south east of Addis Ababa at altitude of 1500 to 3300 m.a.s.l. The area covers 38,398 km² in range lands. Area is comprised of sixteen peasant association (PAs) such as, Nano hecho, Re'e amba, Re'e dharayo, Walargi, Negelle, Nano jawi, Cire anole, Amuma harago, Guna genete, Samo bixana, Samo badosa, Bobe, Waqantera, Sorbo'e ertero, Andale abajema, and Dima. The area is bordered by different district such as, Merti by north, Cole by south, Golocha by west, and Su'ude by east. Topographically, it has 84% high land, 11% "weynadega", 2% low land and 3% "wurch". It receives an annual range of rainfall from 700 to 1300 mm, and the annual range of temperature from 12 to 23°C. It receives bimodal rainfall occurring from March to April (a short rainy season) and from July to October (long rainy season). It has a total of 110,610 livestock of which 8,609 were sheep (GDRAD, 2013).

Study population

The study population comprises of indigenous Arsi-Bale sheep breed from three agro-ecological areas (highland, midland and lowland); kept under similar extensive management system; young or adult; all body conditions (poor, moderate, good); dewormed or non-dewormed by antihelmintic; kept in forest or swampy area; apparent health or not were included.

Study design and sample size determination

Out of 16 villages of Guna District, three namely, Re'e amba, Nano hecho, and Cire anole were purposively selected considering their representation of the highland, midland and lowland of the district, respectively.

Cire anole is located at an altitude of 2700 to 3000 m.a.s.l.; Nano hecho is situated at altitude of 2200 to 2500 m.a.s.l. and Re'e amba is located at altitude of 1500 to 1800 m.a.s.l. The households and individual animals were selected using simple random sampling technique. Accordingly, equal proportions of animals were selected for the study, that is, 128 each from each selected PAs. During sampling age, sex, body conditions, antihelmintic usage, appearance of symptoms, and grazing area of the animals were recorded.

The sample size of this study was determined based on internationally set standard formula in Thrusfield (2005). Therefore, the sample size for this study was determined using standard formula indicated as follows:

$$N = 1.96^2 \cdot P_{\text{exp}} (1 - p_{\text{exp}}) / d^2$$

where N = required sample size, P_{exp} = expected prevalence, and d = desired level of precision (5%).

There was no previously documented ovine lungworm infection in the study area.

As stated earlier, confidence level chosen is 95% so that d= 5% and expected prevalence is 50%. By substituting the value, the required sample size was 384.

Table 1. Prevalence of different species of lungworm in total examined sheep.

Species of lungworm	No. examined animal	No. positive	Prevalence (%)	Df	χ^2	p-value
<i>D. filaria</i>	384	109	28.4			
<i>M. capillaries</i>	384	41	10.7			
<i>P. rufescens</i>	384	29	7.6	3	3.84	0.00
Mixed infection	384	38	9.9			
Total	384	217	56.5			

*Number of examined animal = Number of examined animals; *Df=degree of freedom; * χ^2 =Chi-square.

Sample collection and laboratory diagnosis

Fresh fecal samples collected from the rectum of the animals were immediately transported to Guna Veterinary Clinic for processing. Five grams of faeces were weighed from each sample for extraction of L1 larvae using Modified Baerman techniques according to Anne and Gary (2006). The faeces were fully enclosed in cheesecloth fixed with metallic stick (agraph) rest on the edges of the funnel glass. The glass was filled with clean cold water until the sample became submerged making sure that the corners of the cheesecloth did not hang over the edge of the funnel. The sample was allowed to sit overnight. Larvae were collected and morphologically identified as described by (Urquhart et al., 1996; Anne and Gary, 2006).

Questionnaire survey

Semi structured questionnaire survey was carried out to interview individual owners of 384 sheep taken for coproscopic examination in order to obtain general information about anthelmintic usage, symptoms of respiratory signs, and grazing area.

Data management and statistical analysis

Raw data and the results of parasitological examination were entered in to a Microsoft Excel spread sheets program. Simultaneously, they were transferred and analyzed by SPSS version 16 software program. The prevalence of lungworm infection was calculated by dividing positive samples for the total number of samples examined. The association between different variables and outcome variables was evaluated using Chi-square (χ^2) test. For all analysis, a p-value less than 0.05 at 95% confidence level were taken as significant.

RESULTS

Over all prevalence of lungworm infection

Out of 384 sheep faecal examined, 217 (56.5%) (CI= 51.38 - 61.5%) were infected with different species of lungworm. Out of these, 28.4, 10.7, 7.6, and 9.9%, were due to *D. filaria*, *M. capillaries*, *P. rufescens*, and mixed infection, respectively. Thus, *D. filaria* was the dominant species followed by *M. capillaries*, then by *P. rufescens*, alone or in mixed infection. There was statistical

significance difference between *D. filaria* and other species of lungworm identified (p<0.05) (Table 1).

Risk factors and prevalence of lungworm infection

Prevalence of lungworm infection was determined based on altitude, age, sex, and body conditions of the studied animals.

Prevalence of lungworm infection according to PAs with various altitudes

Based on altitude and climatic condition, the prevalence were found to be to be 78.1, 52.3, and 39.1% in high land (Cire anole), mid-land (Nano hecho), and low land (Re'e amba), respectively. *D. filarial* and *M. capillaries* were most prevalent in high land, while *P. rufescens* was most prevalent in midland. Statistically, there was significant difference among different PAs with different altitudes (p<0.05) (Table 2).

Prevalence of lungworm infection according to sex of the study animals

Prevalence of lungworm infection according sex of animals was 53.1 and 59.9% in male and female, respectively. Prevalence was higher in female than male; however, statistically there was insignificant difference between sex (p>0.05) (Table 3).

Prevalence of lungworm infections in different age groups of animals

The prevalence of lungworm infection according to age of study animals was 62% in less than one year and 51% in greater than one year. *D. filaria* was higher in less than one year, while *P. rufescens* was slightly higher in greater than one year. The prevalence of lungworm infection between age of study animals was statistically significant (p< 0.05) (Table 4).

Table 2. Prevalence of lungworm infection among PAs with various altitudes.

PAs	No. examined	No. positive	Prevalence of different species of lungworm				Total P (%)
			Df (%)	Mc (%)	Pr (%)	Mi (%)	
Cire anole	128	100	53 (41.4)	20 (15.6)	8 (6.2)	19 (14.8)	78.1
Nano hecho	128	67	25 (12.5)	11 (8.6)	15 (11.7)	16 (12.5)	52.3
Re'e amba	128	50	31 (24.2)	10 (7.8)	6 (4.7)	3 (2.3)	39.1
Total	384	217	109 (28.4)	41 (10.7)	29 (7.6)	38 (9.9)	56.5

*P= prevalence *Df=*D. filaria*, Mc=*M. capillaries*, Pr=*P. rufescens*, Mi=Mixed infection. ($\chi^2=42.093$; df =2; p=0.000).

Table 3. Prevalence of lungworm infection according to sex of study animals.

Sex	No. Examined	No. Positive	Prevalence of different species of lungworm				Total P (%)
			Df (%)	Mc (%)	Pr (%)	Mi (%)	
Male	192	102	48 (25)	23 (12)	15 (7.8)	16 (8.3)	53.1
Female	192	115	61 (31.8)	18 (9.4)	14 (7.3)	22 (11.5)	59.9
Total	384	217	109 (28.4)	41 (10.7)	29 (7.6)	38 (9.9)	56.5

$\chi^2=1.791$; df=1; p =0.181.

Prevalence of lungworm infection in different body conditions of study animals

Prevalence of lungworm infection according to body condition of study animals was 60.9, 57.0, and 51.6% in poor, medium, and good, respectively. Thus, prevalence of lungworm was the highest in poor body condition than others. *D. filaria* was almost equal in all body condition; *M. capillaries* and *P. rufescens* were the highest in medium and poor body conditions, respectively. Prevalence of lungworm infection according to body condition conditions was statistically insignificant ($p>0.05$) (Table 5).

Questionnaire survey and prevalence of lungworm infection

Prevalence of lungworm infection during questionnaire survey was assessed based on antihelmintic usage, manifestation of respiratory signs, and grazing area.

Prevalence of lungworm infections in relation to antihelmintic usage in study animals

Prevalence of lungworm infection in study animals in relation to dewormed by antihelmintic or non-dewormed was 44.6 and 67.5%, respectively and it is statistically significant ($p<0.05$). Thus, almost prevalence of all lungworm species was higher in non-dewormed animals ($p<0.05$) (Table 6).

Association between prevalence of lungworm infection and manifestation of respiratory signs

Prevalence of lungworm infection in study animals according to manifestation of respiratory signs was 44.1 and 68.2% in apparently health and diseased, respectively and it is statistically significant ($p<0.05$). Prevalence of all species of lungworm was higher in those that manifest respiratory signs (Table 7).

Association between prevalence of lungworm infections and grazing area

The prevalence of lungworm infection in study animals according to grazing area was 67.9 and 38.1% in swampy and forest grazing animals, respectively. Thus, infection was higher in swampy grazing animals. Prevalence of all species of lungworm was higher in swampy area. The prevalence of lungworm infection according to grazing area was statistically significant ($p<0.05$) (Table 8).

DISCUSSION

The result of the present study conducted from November, 2013 to March, 2014 in three PAs of Guna District, Arsi Zone, south-east of Ethiopia indicated that lungworm infection was one of the most common respiratory diseases of sheep with an overall prevalence of 56.5%. This agrees with the research findings that

Table 4. Prevalence of lungworm infections in different age groups of animals.

Age	No. examined	No. positive	Prevalence of different species of lungworm				Total P (%)
			Df (%)	Mc (%)	Pr (%)	Mi (%)	
≤1 year	192	119	64 (33.3)	23 (12)	12 (6.2)	20 (10.4)	62.0
>1 year	192	98	45 (23.4)	18 (9.4)	17 (8.9)	18 (9.4)	51
Total	384	217	109 (28.4)	41 (10.7)	29 (7.6)	38 (9.9)	56.5

$\chi^2=4.673$; df=1; p= 0.31.

Table 5. Prevalence of lungworm infection in different body condition of study animals.

Body condition	No. examined	No. positive	P(%) of different species of lungworm				Total P (%)
			Df (%)	Mc (%)	Pr (%)	Mi (%)	
Poor	128	78	38 (29.7)	12 (9.4)	15 (11.7)	13 (10.2)	60.9
Medium	128	73	36 (28.1)	20 (15.6)	9 (7.0)	8 (6.2)	57.0
Good	128	66	35 (27.3)	9 (7.0)	5 (3.9)	17 (13.3)	51.6
Total	384	217	109 (28.4)	41 (10.7)	29 (7.6)	38 (9.9)	56.5

$\chi^2=2.310$; df=2; p=0.315.

Table 6. Prevalence of lungworm infection in relation antihelmintic usage in study animals with response of respondents.

Did you deworm your sheep?	No. examd with rr	No. positive	P (%) of different species of lungworm				Total P (%)	
			Df (%)	Mc (%)	Pr (%)	Mi (%)		
Responses	Yes	184	82	41 (22.3)	10 (5.4)	15 (8.2)	16 (8.7)	44.6
	No	200	135	68 (34.0)	31 (15.5)	14 (7.0)	22 (11)	67.5
	Total	384	217	109 (28.4)	41 (10.7)	29 (7.6)	38 (9.9)	56.5

$\chi^2=20.51$; df=1; p=0.00. *p= prevalence, *No. examined with rr = number of examined animals with response of respondents.

Table 7. Association between prevalence of lungworm infection and respiratory signs with response of respondents.

Did your sheep cough?	No.exmd with rr	No. Positive	P (%) of different species of lungworm				Total P (%)	
			Df (%)	Mc (%)	Pr (%)	Mi (%)		
Responses	Yes	198	135	67 (33.8)	25 (12.6)	22 (11.1)	21 (10.6)	68.2
	No	186	82	42 (22.6)	16 (8.6)	7 (3.8)	17 (9.1)	44.1
	Total	384	217	109 (28.4)	41 (10.7)	29 (7.6)	38 (9.9)	56.5

$\chi^2=22.658$; df=1; p=0.000. *No. examined with rr = number of examined animals with response of respondents

Table 8. Association between prevalence of lungworm infections and grazing area with response of respondents.

Where do you keep your sheep?	No.exmd with rr	No. positive	P(%) of different species of lungworm				Total P (%)	
			Df (%)	Mc (%)	Pr (%)	Mi (%)		
Responses	Swampy areas	237	161	79 (33.3)	32 (13.5)	19 (8.0)	31 (13.1)	67.9
	Forest	147	56	30 (20.4)	9 (6.1)	10 (6.8)	7 (4.8)	38.1
	Total	384	217	109 (28.4)	41 (10.7)	29 (7.6)	38 (9.9)	56.5

$\chi^2= 32.85$; df= 1; p=0.00. *No. examined with rr = number of examined animal with response of respondents.

were conducted in Asella (Bekele et al., 1981; Wondwossen, 1992; Paulos, 2000; Mihreteab and Aman, 2011; Hasen et al., 2013; Abeje et al., 2016) with prevalence of 59.4, 58.8, 52.54, 57.1, 55.10 and 57.6%, respectively. In Dessie and Kombolcha, 50% prevalence was reported (Teffer, 1993). However, the current finding was lower than the prevalence reported by Eyob and Mathios (2013) in Asella province (72.44%); by Yohannes (1989) in DebreTabor Awraja (70.7%); by Netsanet (1992) in Debre Birhan (73.75%) and by Sefinew (1999) in six district of Wollo (71.3%). The result of the current finding highly disagrees with the study conducted by Frewengel (1995) in and around Mekele (13.24%) and by Ibrahim and Degefa (2012) in Mekele town (13.4%). The possible explanation for such prevalence variation could be due to variation in altitude, rainfall, humidity, temperature difference, and season of examination on the respective study areas which favor or disfavor the survival of parasite larvae (Soulsby, 1982; Bradford, 2002).

In the current study, the prevalence of different species of lungworm was 28.4, 7.6, 10.7 and 9.9% due to *D. filaria*, *P. rufescens*, *M. capillaries*, and mixed infection with two or three species of lungworm, respectively. With regard to the species of lungworms, it was observed that *D. filaria* was the most predominant species in the area followed by *M. capillaries*, whereas *P. rufescens* was the least prevalent. This finding is supported by Alemu et al. (2006), Mihreteab and Aman (2011), Netsanet (1992) and Nemat and Moghadam (2010) who reported that *D. filaria* was the most prevalent in their study area. In contrast to these findings, Sisay (1996) in Bahirdar and Mezgebu, in Addis Ababa reported that *M. capillaries* was the most prevalent. The possible explanation for the predominance of *D. filaria* in the study area might be attributed to the difference in the life cycles of the parasites. Thus, *D. filaria* has a direct life cycle and requires shorter time to develop an infective stage, while *M. capillaries* has an indirect life cycle which needs an intermediate snail for completing its life cycle. Thus, require longer time to develop to infective stage. In the present study area, the environment may not be favorable to the intermediate host as that of Bahirdar and Addis Ababa that make *M. capillaries* and *P. rufescens* lower. According to Soulsby (1982) after ingestion the larvae *D. filaria* parasites can be shed with faeces within five weeks. Compared with *D. filaria*, the transmission of *P. rufescens* and *M. capillaries* is epidemiologically complex event involving host, parasite and intermediate host. Hence, *M. capillaries* and *P. rufescens* in sheep require slugs or snails as intermediate host which must be eaten for infection to occur; this might make them low prevalent in the present study area than *D. filaria* (Urquhart et al., 1996). Mixed infection was also observed in the current study as in previous studies (Wondwossen, 1992; Hansen and Perry, 1994; Paulo, 2000).

On attempt to know the influence of altitude on the study area, there was statistically significant difference on prevalence of lungworm infection with prevalence of 78.1, 52.3, and 39.1% at high altitude (Cire anole) (2400 to 3000 m.a.s.l), midium altitude (Nano hecho) (1800 to 2200 m.a.s.l) and low altitude (Re'e amba) (1500 to 1800 m.a.s.l), respectively. These results indicate that, prevalence of lungworm infection increase as altitude increase. This result agrees with study reported by Mihreteab and Aman (2011) who reported 66.4, 57.5, and 47.2% in high altitude (>2400 m.a.s.l), medium altitude (1800 to 2200 m.a.s.l), low altitude (1600 m.a.s.l), respectively in Tiyo district. It was also inline within Alemu et al. (2006) findings who had reported 70, 47 and 43% in high, medium, and low altitude, respectively, in north east of Ethiopia. This finding disagrees with the reports of Wondwossen (1992) who indicated absence of significant difference in different lungworm species distribution between high and mid altitude in Asella Awraja. These differences between researchers might be associated with variation in sample size, duration of study time and season of the study period. It may also be associated with climate changes every year which could help in the agro-ecological expansion of previously highlands adapted parasites to medium and low altitude. In this finding, prevalence was the highest in high altitude than others; this might be due to it has low temperature, higher moisture and humidity than other ecologies which is favorable for survival of larvae and intermediate hosts (Radostits et al., 2007).

With regard to the prevalence of lungworms in different age groups, young animals were found to be more infected than adult. The higher infection rate was observed in less than a year (62.0%) while lower infection rate was observed in greater than a year (51.0%). This shows that young were more susceptible than adult. In less than one year, *D. filaria* was higher (33.3%) than greater than one year (23.4%); however, in greater than one year *P. rufescens* (8.9%) was higher than less than one year (6.2%). This finding agrees with Mihreteab and Aman (2011), Wondwossen (1992), and Teferra (1993) who reported that young sheep were more affected by *D. filaria* than adult sheep. The reason behind this is either due to development of acquired immunity in adult animals from previous exposure or recovered animals have better immunity against re-infection. In the other way, young animals had poorly developed immunity against *D. filaria*. In this finding, *P. rufescens* was higher in adults than in young; this might be due to impaired development of acquired immunity in adult or due to young animals may not be exposed to intermediate host (Radostits et al., 2007). This may be also associated with the life cycle and infection route of the parasite which is through ingestion of infected snail (IH) which results to lower infection in young, but accumulate through long time in adults that make them more susceptible.

On attempt to know the influence of sex, on variation of prevalence of lungworm infection, the prevalence was higher in female (59.9%) than male (53.1%), but the difference was statistically insignificant. This agrees with research reported by Addis et al. (2011), Nibret et al. (2011), Eyob and Mathios (2013), Dawit and Abdu (2012) and Hasen et al. (2013), but disagree with report of Alemu et al. (2006) and Mihreteab and Aman (2011). These differences between researchers might be either due to improper distribution of sample selection between the two sexes that makes prevalence higher in female (Addis et al., 2011) or most of the sampled females are not in preparturient period during the study time that make both sexes equally susceptible to disease. In the current finding, prevalence was higher in female; this might due to certain sampled animal were lactating which suppress immunity of the animal (Urquhart et al., 1996).

With regard to assessing the influence of body condition on variation of prevalence of lungworm infection, 60.9, 57, and 51.6% in poor, medium, and good, respectively was found. Hence, prevalence was higher in poor body condition than other; however, variation among body condition was statistically insignificant. This finding agrees with study reported by Dawit and Abdu (2012) who said the difference between conditions are insignificant; however, disagrees with study reported by Mihreteab and Aman (2011) and Desta et al. (2013) who reported the variation among body condition was statistically significant. The reason why current finding insignificant among body condition, might be either due to loss of weight cannot only be attributed by the lungworm infection alone but also inappropriate management and other helminth infection (Mengestom, 2008).

In this finding, even though variation is insignificant, prevalence of lungworm infection was higher in poor body condition than other; this might be due to poorly nourished animals less competent not to be infected by lungworm than others (Kimberling, 1988).

With attempt to know influence of antihelmintic usage on prevalence of lungworm infection, questionnaire survey findings were tried to associate antihelmintic usage with the faecal examination results. Higher prevalence (67.5%) of the parasites was recorded in sheep with the respondents that said non-dewormed than dewormed (44.6%) and it is statistically significant. The observation noted in this study agreed with study reported by Eyob and Mathios (2013), Yohannes (1989), Netsanet (1992) and Sefinew (1999). In these mentioned authors, their findings indicate that the prevalence of the parasite was found high in animals that were non-dewormed than dewormed. In the current study, even though the dewormed sheep revealed low infection prevalence compared to non-dewormed groups, about 44.6% of them were infected with lungworm. The reason why dewormed sheep infected might be either due to the

antihelmintic used in the area for the treatment only temporarily suppress egg production of the adult worms or parasite may become resistance to antihelmintic used. It may also be related to the poor quality of antihelmintic used in the country. In contrast, 32.5% of none dewormed animals were not infected by lungworm; this might be due to development of acquired immunity from previous exposure (Radostitiset al., 2007; Urquhart et al., 1996) and it may also be due to none exposure to the infective stages of *D. filaria* or to intermediate host of the other species of the lungworms of sheep throughout their life.

With regard to know appearance of symptoms of respiratory sign with lungworm infection, questionnaire survey findings were tried to associate manifestation of respiratory sign with the faecal examination results. Higher prevalence (68.2%) of the parasites was recorded in sheep with the respondents that said yes (it shows clinical respiratory signs) than that said no (did not show signs) (44.1%).

Hence, the variation was statistically significant. This finding agreed with the study reported by Paulos (2000), Eyob and Mathios (2013) and Hasen et al. (2013). In these mentioned authors, their findings indicate that the prevalence of the parasite was found high in animals which showed symptoms of respiratory signs than apparently healthy.

In current finding, even though apparently healthy sheep show low infection compare to those showing clinical respiratory signs groups, about 44.1% of them were infected with lungworm.

The reason why apparently health sheep appeared with lungworm might be due to the parasites in pre-patent stage, due to small adult worm burden in sheep which could not produce eggs and hence larvae, or as a result of immunity developed due to exposure to a few lungworms which is not associated with clinical sign, but animal shed larvae (Soulsby, 1982). 21.8% of these animals manifesting respiratory signs appeared negative on coproscopic examination; this might be due to bacterial or viral diseases that causes occurrence of respiratory signs (Gelagay et al., 2004).

Lastly, in this study, questionnaire survey findings tried to relate the grazing area with the faecal examination results. Higher prevalence (67.9%) of the parasites was recorded in sheep with the respondents that said they were kept in swampy area than forest (38.1%) and it is statistically significant.

Thus, the variation of lungworm infection according to grazing area may be due to antihelmintic effects of some trees and shrubs browsed from the forest that caused prevalence of lungworm infection low in forest grazing sheep (Rahman and Seip, 2006); prevalence in swampy area was high which might be due to the presence of moisture which is favorable for survival of larvae and intermediate host (Urquhart et al., 1996).

Conclusions

The present study revealed that prevalence of ovine lungworm was high in Guna district, Arsi zone. The major lungworm species identified in the area were *D. filaria*, *M. capillaris*, and *P. rufescens*. *D. filaria* was identified as the most dominant lungworm species. Coproscopic examination and questionnaire survey revealed that young, none dewormed, clinically diseased, swampy grazing animals, and animals from high and medium altitude harbor more infection than their counter parts; however, body conditions and sexes does not have much influence on variation of lungworm infection. In view of these facts, the following recommendations are forwarded: regular deworming with effective antihelmintic should be routinely practiced, sheep should be forbidden to graze swampy areas, young age groups should be isolated during the season when pasture contamination is expected and emphasis should be given to the control and prevention in order to reduce the prevalence from the current high finding.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Full Length Research Paper

Serological prevalence of *Babesia caballi* and *Theileria equi* in camels and donkeys from Karamoja sub-region, North-eastern Uganda

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Equine piroplasmosis is a severe disease of horses caused by the intra-erythrocyte protozoan, *Theileria equi* and *Babesia caballi*. *T. equi* and *B. caballi* infections were assessed in serum from camels and donkeys using competitive-enzyme-linked immunosorbent assay (cELISA) assay. A total 110 animals were studied including 25 donkeys and 85 camels from two districts viz. Moroto and Amudat in Karamoja sub-region, North-eastern Uganda. All the (100%) donkeys tested were positive for *Babesia/T. equi* while none of the camels had been exposed to the infection. All animals were negative to *B. caballi* cELISA. Our findings indicated that all donkeys sampled in Karamoja sub-region have been exposed to *T. equi* and this could be prevalent in equine population in Uganda. No exposure status to *B. caballi* was reported. This study represents the first report on the status of *T. equi* and *B. caballi* infection in Uganda.

Key words: Donkey, Camel, *Theileria equi*, *Babesia caballi*, Seroprevalence, cELISA, Uganda.

INTRODUCTION

Equine piroplasmosis (EP) is a tick-borne disease of horses caused by apicomplexan hemoprotozoan parasites *Theileria equi* and *Babesia caballi* of the order Piroplasmida (Wise *et al.*, 2013; Scoles and Ueti, 2015; Sumbria *et al.*, 2017). The nomenclature was changed from *B. equi* to *T. equi* based on evolutionary, morphologic, biochemical, and genetic evidence (Singla and Sumbria, 2017). The disease is also called biliary fever and affects all equid species including Horses, donkeys, mules and zebras (Friedhoff *et al.*, 1990;

Schein, 1988). Several genera of tick species including *Hyalomma*, *Rhipicephalus* and *Dermacentor* transmit both these parasites (De Waal, 1992; Sumbria *et al.*, 2016a). Clinical signs include fever, anemia, icterus, hepatomegaly, edema, intra-vascular hemolysis, hemoglobinuria and even death (Schein, 1988; Uilenberg, 2006). The disease is distributed in tropical and sub-tropical areas including some temperate zones (Shkap *et al.*, 1998; Steinman *et al.*, 2012). The equine disease has a worldwide economic importance especially

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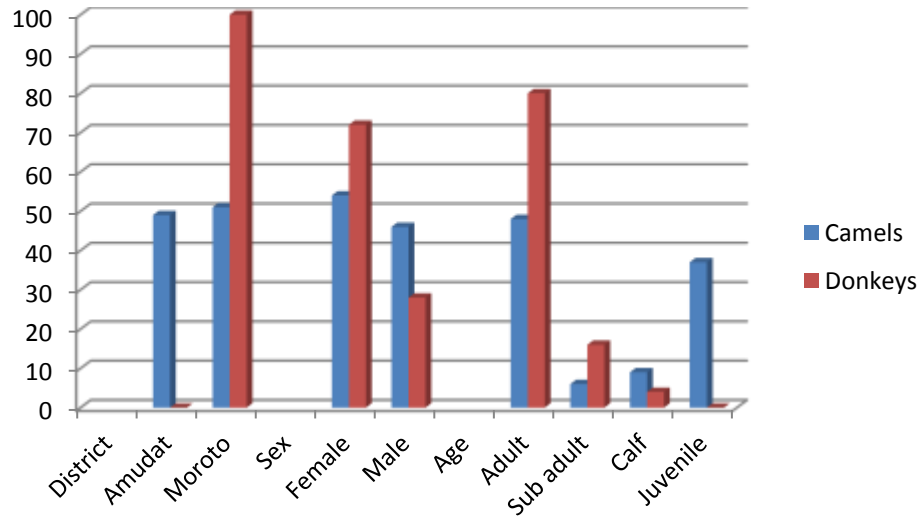


Figure 1. Demographic characteristics of sampled animals.

concerning international movement of horses because carrier horses and infected ticks can be introduced into disease-free countries (Friedhoff *et al.*, 1990; Sumbria *et al.*, 2016b). The vectors of *T. equi* and *B. caballi* are the same (Abedi *et al.*, 2014) although *T. equi* is more virulent than *B. caballi* (Friedhoff *et al.*, 1990; Mehlhorn and Schein, 1998; Posnett *et al.*, 1991). In endemic countries, mixed infections occur (Scoles and Ueti, 2015). Diagnosis depends on clinical observation especially in the acute phase of the disease and is confirmed by microscopic detection of intra-erythrocyte parasites in Giemsa-stained blood smears (Shkap *et al.*, 1998). However, the latent phase of the infection is characterized by low parasitemia (Kumar *et al.*, 2008; Sumbria *et al.*, 2015) hence the need for more sensitive diagnostics like enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) (Friedhoff and Soule, 1996; Moretti *et al.*, 2010; Alsaad *et al.*, 2012). Ponies, mules and donkeys act as natural reservoirs for disease transmission to the horses (Radostitis *et al.*, 2008).

In Uganda, camels and donkeys are kept in Karamoja and Sebei sub-regions, North-eastern Uganda. They are kept by peasant farmers, pastoralists in this semi-arid region. They are kept for meat, milk, dowry, prestige, and carriage. These animals receive little or no veterinary care. Nakayima *et al.*, (2017) detected helminth parasitosis in these animals in the absence of veterinary intervention. No studies have been undertaken on equine piroplasmiasis in Uganda. Therefore, the aims of this work were to determine the infection rate of EP in donkeys and possibly in camels given the fact that they occupy the same ecological setting and could therefore act as accidental hosts. Information from this study will help update current knowledge on the health of camels and donkeys in Uganda for their improved production and productivity.

MATERIALS AND METHODS

Serum samples were collected from Karamoja sub-region in two districts namely: Moroto: N 2° 31' 41.604", E 34° 39' 28.794" and Amudat: N 1° 47' 29.841", E 34° 54' 23.583" districts, Uganda. The study was conducted in March 2016. The camels and donkeys were classified as: Infant, juvenile, sub-adult and adult. Both sexes were sampled (Tables 1 and 2 and Figure 1). Blood was collected from the jugular vein of both camels and donkeys following restraint. 5 ml of blood was collected, 2.5 ml blood was put in anticoagulant ethylene diamine tetra acetic acid (EDTA) vacutainers for parasitological and DNA analysis while 2.5 ml blood was put in serum tubes. The serum was collected into plain vacutainer tubes without anticoagulant, serum was separated from blood cells by centrifugation at 2500 rpm for 15 min and stored at -20°C until use in a competitive-ELISA (cELISA) (Kouam *et al.*, 2010) for both *T. equi* and *B. caballi*. The total number of donkeys was 25, while camels were 85 giving a total of 110 animal samples collected. The sample size determination was based on purposive sampling based on the availability of the animals. The age and sex were recorded for each animal. The samples were transferred to the laboratory and stored at -20°C until use. The serum from clinically healthy animals was examined for *T. equi* and *B. caballi* antibodies by two separate cELISA.

ELISA

Commercial cELISA kits were used to analyze sera from donkeys and camels for the presence of antibodies to *T. equi* and *B. caballi* as described by the manufacturer (VMRD Inc., Pullman, WA, USA). The cut-off values for positive infections was 40% of inhibition for both tests, as indicated by the manufacturer (VMRD Inc., Pullman, WA, USA) (Shkap *et al.*, 1998; Kappmeyer *et al.*, 1999). Thus samples with %I above 40 are considered as positive, and below 40, considered as negative. The results were expressed as a value of the percent inhibition (%I) according to the following formula:

$$(\%I): \%I = 100 - \left\{ \frac{\text{sample O.D.} \times 100}{\text{mean negative control O.D.}} \right\}$$

Microscopic detection of hemoprotozoal parasites depends on morphological and biometrical parameters including the shape, site

Table 1. Infection of camels (Total = 85; all negative for both infections).

Category	Frequency	%
District		
Amudat	42	49
Moroto	43	51
Sex		
Female	46	54
Male	39	46
Age		
Adult	41	48
Sub adult	5	6
Calf	8	9
Juvenile	31	37

Table 2. Infection of donkeys (n=25) (All affected with *Theileria equi*, all negative for *Babesia caballi*).

Category	Frequency	%
District		
Amudat	0	0
Moroto	25	100
Sex		
Female	18	72
Male	7	28
Age		
Adult	20	80
Sub adult	4	16
Calf	1	4
Juvenile	0	0

location and size of parasite in an infected erythrocyte in Giemsa-stained blood smears (Sadeghi Dehkordi *et al.*, 2010). Morphological detection of the parasites could be described as single round, double round, single pyriform and double pyriform with obtuse or acute angle. Microscopically, *B. caballi* is a larger paired pyriform parasite, while *T. equi* is a smaller paired pyriform, rounded and tetrad or Maltese cross arrangement of merozoites (Kuttler, 1988; Levine, 1971).

Statistical analysis

Data were analyzed using SPSS (Version 16). A value of $p < 0.05$ was considered as statistically significant.

RESULTS

110 samples were analyzed with 25 donkeys and 85 camels. All donkeys were positive for *T. equi*. Donkey no. 22 was not done, the sample had dried out. All camels were negative for *Babesia/T. equi* ELISA. Corrected ODs were calculated from sample ODs and blank ODs (Table 3). Sample Id represents animal species, age, sex and

sample number.

All the serum samples from donkeys and camels were negative to *Babesia caballi* Competitive-ELISA. This suggests no history of exposure to this parasite. 110 samples were analyzed with 25 donkeys and 85 camels. Donkey no. 22 was not done, the sample had dried out. Corrected ODs were calculated from sample ODs and blank ODs (Table 4). Sample Id represents animal species, age, sex and sample number.

DISCUSSION

Diagnosis of piroplasmiasis can be achieved by clinical observation and confirmed by microscopy (Irwin, 2010). However, given the low sensitivity of parasitological diagnosis, there is need to combine parasitological diagnosis with molecular diagnostics (Abedi *et al.*, 2014; Salib *et al.*, 2013; Baptista *et al.*, 2013; Rosales *et al.*, 2013).

Camels were sampled alongside donkeys. Much as camels are not equines, they could serve as accidental hosts or reservoirs of the parasites since they are in the same ecological setting. However, all camels tested negative to *B. equi* cELISA (Table 3). The serological prevalence of *B. equi* cELISA was 100% in donkeys and 0% in camels (Table 3). Apparently, equine piroplasmids are enzootic in Uganda and their distribution pattern is likely affected by the presence and densities of suitable hosts rather than by ecological conditions. There is a significant correlation between host species and age with the distribution of EP. Horses are more susceptible to the infection than donkeys. Adult animals have a higher risk of infection. All the serum samples from camels and donkeys tested negative to *B. caballi* cELISA (Table 4). This suggests no history of exposure of these animals to this parasite or the fact that the infection is cleared in 1 to 4 years. All the 25 donkey serum samples (100%) were found positive for *T. equi* antibodies (Table 3), *B. caballi* was not detected in the study area (Table 4) while all the 85 camels tested negative to equine piroplasmiasis (Tables 3 and 4). A study of equine piroplasmiasis in horses in Sudan by Salim *et al.* (2008) recorded 100% prevalence of *T. equi* in Khartoum North (100%) and Atbara (100%); a low prevalence for *B. caballi* was reported and 0% prevalence was detected in Khartoum, Khartoum North, and Kosti areas. The infection rate of EP varies in different countries. Several factors account for this namely: disease management practices, tick vector abundance and climate. Climatic factors influence the habitat of tick vectors like rainfall, humidity and temperature (Oncel *et al.*, 2007). In endemic countries, equines adopt to infection possibly because of the phenomenon of endemic stability. However, stress and immune-suppression could revert otherwise sub-clinical infection to overt disease. *T. equi* infection results into life-long carrier status (Brüning, 1996) while *B. caballi* could persist in subclinical form for at least 1 to 4 years

Table 3. *Babesia / Theileria equi* competitive-ELISA of camel and donkey serum.

S/N	Animal species	Sample ID	Corrected Ods	% Inhibition (%)	Sample status
1	Donkey	D/A/M/01	0.262	75.40	Positive
2	Donkey	D/A/F/02	0.209	80.38	Positive
3	Donkey	D/SA/F/03	0.159	85.07	Positive
4	Donkey	D/A/F/04	0.218	79.53	Positive
5	Donkey	D/A/F/05	0.196	81.60	Positive
6	Donkey	D/A/F/06	0.395	62.91	Positive
7	Donkey	D/A/F/07	0.252	76.34	Positive
8	Donkey	D/A/F/08	0.572	46.29	Positive
9	Donkey	D/A/F/09	0.275	74.18	Positive
10	Donkey	D/A/M/10	0.173	83.76	Positive
11	Donkey	D/A/M/11	0.167	84.32	Positive
12	Donkey	D/A/F/12	0.291	72.68	Positive
13	Donkey	D/A/F/13	0.244	77.09	Positive
14	Donkey	D/A/F/14	0.232	78.22	Positive
15	Donkey	D/A/M/15	0.159	85.07	Positive
16	Donkey	D/A/M/16	0.147	86.20	Positive
17	Donkey	D/A/F/17	0.247	76.81	Positive
18	Donkey	D/A/F/18	0.241	77.37	Positive
19	Donkey	D/A/M/19	0.17	84.04	Positive
20	Donkey	D/SA/F/20	0.483	54.65	Positive
21	Donkey	D/SA/F/21	0.606	43.10	Positive
23	Donkey	D/A/F/54	0.48	54.93	Positive
24	Donkey	D/A/F/55	0.298	72.02	Positive
25	Donkey	D/SA/F/56	0.507	52.39	Positive
26	Donkey	D/C/M/57	0.328	69.20	Positive

110 samples were analyzed with 25 donkeys and 85 camels. All donkeys were positive for *T. equi*. Donkey no. 22 was not done, the sample had dried out. All camels were negative for *Babesia / T. equi* ELISA. Corrected ODs were calculated from sample ODs and blank ODs. Sample Id represents animal species, age, sex and sample number.

before being eliminated. This could be attributed to the fact that *T. equi* parasites are not completely eliminated from the blood of equines after treatment or natural recovery (de Waal and van Heerden 1994) as compared to *B. caballi*. Therefore, failure to detect *B. caballi* by ELISA is most probably due to the parasites clearance from the circulating blood by the host or reduction to a level beyond the detection of the host immune response or the diagnostic test. There was a significant difference between donkeys and camels with respect to the seroprevalence of *T. equi* and *B. caballi* ($P < 0.05$).

Treatment strategy for piroplasmosis in equines depends on the endemic status of the country. So it is either parasite elimination in disease free countries or resolution of clinical disease in endemic countries. It is not recommended to eliminate the parasite from equids in endemic countries because the animals need endemic stability due to constant exposure to the parasite at low levels (Donnellan and Marais, 2009). Several drugs can be used to treat EP. Generally, *B. equi* has been reported to be more refractory to babesiacidal drugs than *B. caballi*. More common drugs include imidocarb and

diminazene. However, donkeys are more susceptible to imidocarb toxicity than horses (Donnellan and Marais, 2009). Imidocarb causes a dose-dependent hepatotoxicity and nephrotoxicity (Donnellan and Marais, 2009). Other drugs could include artesunate, arteether, buparvaquone. Equine piroplasmosis is endemic in Karamoja sub-region and possibly Uganda at large due to the distribution of equine species and the tick vectors.

Conclusion

All donkeys tested positive to *Babesia / T. equi* cELISA while all camels were negative. This exposure status indicates that this piroplasm could be endemic in Karamoja sub-region and in the equine population in Uganda in the absence of veterinary intervention. No exposure status to *B. caballi* was reported. Camels are not accidental hosts or reservoirs of equine piroplasmosis. The results of this study will help inform policy on the improvement of the health and welfare of these animals through veterinary intervention. To our knowledge, this is the first report on epidemiology of

Table 4. *B. caballi* competitive-ELISA of camel and donkey serum.

No.	Animal species	Sample ID	Corrected Ods	% Inhibition (%)	Sample status
1	Donkey	D/A/M/01	1.115	-2.6	Negative
2	Donkey	D/A/F/02	1.257	-15.7	Negative
3	Donkey	D/SA/F/03	1.292	-18.9	Negative
4	Donkey	D/A/F/04	1.055	2.9	Negative
5	Donkey	D/A/F/05	1.168	-7.5	Negative
6	Donkey	D/A/F/06	1.175	-8.1	Negative
7	Donkey	D/A/F/07	1.138	-4.7	Negative
8	Donkey	D/A/F/08	1.08	0.6	Negative
9	Donkey	D/A/F/09	1.114	-2.5	Negative
10	Donkey	D/A/M/10	1.263	-16.2	Negative
11	Donkey	D/A/M/11	1.248	-14.8	Negative
12	Donkey	D/A/F/12	1.15	-5.8	Negative
13	Donkey	D/A/F/13	1.055	2.9	Negative
14	Donkey	D/A/F/14	1.127	-3.7	Negative
15	Donkey	D/A/M/15	1.181	-8.7	Negative
16	Donkey	D/A/M/16	1.151	-5.9	Negative
17	Donkey	D/A/F/17	1.044	3.9	Negative
18	Donkey	D/A/F/18	1.185	-9.0	Negative
19	Donkey	D/A/M/19	1.27	-16.9	Negative
20	Donkey	D/SA/F/20	1.174	-8.0	Negative
21	Donkey	D/SA/F/21	1.076	1.0	Negative
23	Donkey	D/A/F/54	1.098	-1.0	Negative
24	Donkey	D/A/F/55	1.141	-5.0	Negative
25	Donkey	D/SA/F/56	1.151	-5.9	Negative
26	Donkey	D/C/M/57	1.136	-4.5	Negative

All the serum samples from donkeys and camels were negative to *Babesia caballi* Competitive-ELISA. This suggests no history of exposure to this parasite. 110 samples were analyzed with 25 donkeys and 85 camels. Donkey no. 22 was not done, the sample had dried out. Corrected ODs were calculated from sample ODs and blank ODs. Sample Id represents animal species, age, sex and sample number.

equine piroplasms in Uganda. Molecular studies for accurate diagnosis of equine piroplasmosis are essential for providing baseline information about its epidemiology, distribution, and prevalence in the affected equine population and for effective control measures.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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