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Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Animal health constraints in dairy goats kept under smallholder farming systems in Kongwa and Mvomero Districts, Tanzania

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This study was conducted to determine animal health constraints for dairy goats kept by small-scale farmers in Kongwa and Mvomero districts, Tanzania. A total of 129 dairy goats belonging to 108 farmers were screened for gastrointestinal nematode (GIN) infection, coccidiosis, haemoparasites, brucellosis and contagious caprine pleuropneumonia (CCPP) over a period of 11 months. Other clinical diseases and mortalities were recorded. The goats used were Norwegian crosses and Toggenburg crosses. The mean prevalence of GIN infection and coccidiosis in all goats were 54.8 and 57.4%, respectively. Prevalence of GIN infection was higher ($P \le 0.05$) during the rainy months than in the dry months, but the prevalence of coccidiosis did not differ (P > 0.05) between the dry and rainy seasons. The EPG in goats did not differ (P > 0.05) between Kongwa (169.79 \pm 0.03 EPG) and Mvomero (171.51 \pm 0.04 EPG) districts, but the OPG differed significantly ($P \le 0.05$) with values of 793.15 ± 0.04 (Kongwa) and 364.02 ± 0.05 (Mvomero). The prevalence of CCPP in the goats was 26.4%. Other clinical diseases included respiratory diseases, infectious keratoconjunctivitis and orf (scabby lesions around mouth and nostrils). Both tests for haemoparasites and brucellosis indicated negative results for all goats tested. Mortality rate during the study period was 15.5% and the major causes of deaths were respiratory diseases, bloat and food poisoning. In conclusion, gastrointestinal nematodes are prevalent in both districts, but the burdens are relatively low to justify mass treatment. The Norwegian goats are more susceptible to GIN infection and coccidiosis compared to Toggenburg goats.

Key words: Coccidiosis, diseases, gastrointestinal nematodes, mortality, Norwegian goats, Toggenburg goats.

INTRODUCTION

In Tanzania, promotion of small-scale dairy goat production for poverty alleviation and combating malnutrition malnutrition started in early 1980s. Since then the number of dairy goats in the country has increased mainly

*Corresponding author. E-mail: chenyasw@yahoo.com. Tel: +255 784 754574. Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> through the goat-in-trust schemes promoted by nongovernmental organisations (NGOs), church organisations and research institutions/Universities. At the moment, the number of dairy goats in the country is estimated at 419,533 (United Republic of Tanzania (URT), 2012) and are predominantly kept by smallholder farmers in rural areas, especially women. The common goat breeds include Toggenburg, Saanen, dairy Norwegian, Anglo-Nubian and Alpine (Ministry of Livestock Development (MLD), 2006). The introduction of dairy goats in rural areas has provided an alternative source of milk to poor households, which cannot afford keeping dairy cattle.

Dairy goat production under smallholder production system is constrained by many factors, including poor husbandry practices, inadequate nutrition and disease challenges. The most common diseases affecting small ruminants different parts of Tanzania in are parasitism, gastrointestinal respiratory infections (especially pneumonic pasteurellosis and contagious caprine pleuropneumonia (CCPP)) and contagious ecthyma (Mtenga and Kusiluka, 1997; Kusiluka, 2002; Magona and Musisi, 2002). Some vector-borne diseases such as Rift valley fever (RVF) and bluetongue are associated with epidemic episodes (Sindato et al., 2011; Swai and Schoonman, 2009).

Gastrointestinal nematode (GIN) infection in small ruminants is of considerable significance in a wide range of agro-climatic zones and affects production through losses resulting from mortalities, reduced weight gain, milk yield and reproduction efficiency (Fabiyi, 1987; Bekele et al., 1992; Singla, 1995). The effects of GIN on production depend mostly upon the age of the animals, breed, parasite species involved and the worm burden in the affected animal (Wadhwa et al., 2011). Gastrointestinal nematode infection is the most serious health challenge that limits goat production, especially in rural areas of developing countries (Kusiluka and Kambarage, 1996; Githiori et al., 2006). This is mainly because most developing countries located in the warm tropical zone, which has favourable climate for the survival and development of GINs, have poor management practices and inadequate animal health control programmes (Akhtar et al., 2000). The impact of GINs is manifested by morbidity, mortality, cost of treatment and control measures against the syndrome (Mahusoon et al., 2004; Nwosu et al., 2007). However, most of the economic losses caused by GINs are mainly due to production losses rather than mortality (Waller, 2004) because the more prevalent subclinical infections cause sub-optimal productivity and insidious losses with negative impact on long-term animal productivity. Most cases of GINs infection rarely get veterinary attention due to their chronic and insidious nature (Sanval, 1998: Dimander et al., 2000) and clinical signs may be evident only during terminal stages (Valentine et al., 2007).

Another important disease is coccidiosis, caused by different species of Eimeria. This is the most common form of coccidial infections in small ruminants, often occurring concurrently with GINs (Kusiluka, 1995; Kambarage et al., 1996; Kusiluka et al., 1998). Clinical coccidiosis is less common under traditional smallholder production system, except where poor hygiene leads to gross contamination of the environment that favours the build up of heavy oocyst burdens. This precipitates a clinical disease in young and animals with concurrent infections (Assoku, 1981; Barger et al., 1994). Most species of Eimeria affecting small ruminants have limited pathogenicity, however, in the presence of pathogenic GINs, their additive parasitic effect may lead to a clinical disease. The environmental factors that favour the establishment, survival and development of GINs and coccidiosis are very similar and guite often animals have mixed infections (Kusiluka, 1995), making it difficult to quantify the pathogenic effects of the individual parasite. Because of this, it is often advisable to study the epidemiology of both groups of parasites concurrently.

Contagious Caprine pleuropneumonia is another important disease affecting goats in Tanzania. The disease has been suspected to be in the country since early 1980s and was confirmed by isolation of Mycoplasma capri pneumoniae by Msami et al. (1998) and then Kusiluka et al. (2007). Hence, the disease has been in the country for over three decades and probably a longer time. Symptoms such as high fever, anorexia, laboured breathing, productive coughing, purulent nasal mucous emanating from the nose and reluctance to walk are considered as being indicative of CCPP and the predisposing factors include animal contact when sharing water and pasture (Swai and Neselle, 2010). Brucellosis is a disease with important effects on both public health and animal health. In goats, it is mainly caused by Brucella melitensis. The organisms persist in the genital system of the males and the disease is transmitted to the females at the time of service. The disease is of economic importance because it causes loss of milk production and abortion. The disease is characterized by abortion after third month of gestation and birth of weak kids.

In order to develop sustainable strategies for control of small ruminant diseases, there is a need to determine the most important diseases affecting the animals in different areas. This can be achieved by undertaking longitudinal studies to determine the spatial and temporal patterns of diseases of socio-economic importance with the aim of identifying appropriate intervention strategies. The present study was intended to establish the diseases of socio-economic importance in Toggenburg and Norwegian crossbred dairy goats kept under smallholder farming systems in Kongwa and Mvomero districts of

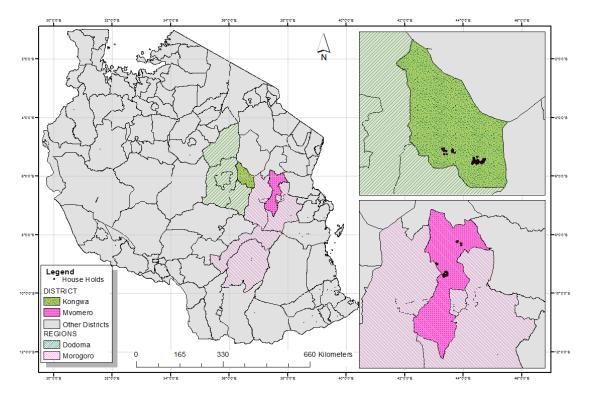


Figure 1. Map of Tanzania showing the study areas in Kongwa and Mvomero districts.

Tanzania. Toggenburg and Norwegian goats are the predominant dairy breeds in Tanzania. The animals were introduced in the rural areas of the two districts in order to improve human food and nutritional security and income of the resource-poor families. More specifically, the study was undertaken to: (i) determine the causes of mortality of dairy goats in semi-arid and sub-humid environments; (ii) determine seasonal patterns of nematode and eimeria infections and, (iii) compare the tolerance to common diseases between Toggenburg and Norwegian crossbred dairy goats introduced in the study areas. The epidemiological data gathered through the study could form the basis of designing disease control strategies in the study areas.

MATERIALS AND METHODS

Description of the study areas

The present study was conducted in Masinyeti and Ihanda villages of Kongwa district located in northeast of Dodoma region and Kunke and Wami-Luhindo villages of Mvomero district located in the north-eastern part of Morogoro region (Figure 1). Kongwa district is located in the semi-arid area and has an altitude ranging from 900 to 1000 m above sea level (asl), mean annual temperature of 26.5°C and rainfall of 400 to 800 mm per annum. Mvomero is located in the sub-humid tropical zone at an altitude of 600 to 2000 m asl, has temperatures that range from 18 to 30°C and receives annual rainfall of 600 to 2000 mm. Crosses of Toggenburg with the Small East African (SEA) goats were obtained from Babati district, Manyara region in northern Tanzania, which has a drier climate while the crosses of Norwegian goats with the SEA goats were obtained from Mgeta division, which is located on the slopes of the Uluguru Mountains in Morogoro region and has a cool mountain climate. The two types of crossbred goats were introduced in the study areas in order to improve food and nutritional security status of the communities.

Experimental animals

A total of 72 Norwegian (65 females; 7 males) and 57 Toggenburg (52 female; 5 males) crossbred goats were distributed between March and April, 2012 to 108 small-scale farmers willing to participate in the project in the four project villages (Table 1). In each village, half of the farmers received Norwegian crosses and the remaining farmers received Toggenburg crosses. For both breeds, the proportion of dairy goat blood was 75% while that of SEA goats was 25%. Before distribution to the project farmers, all animals were ear-tagged for identification and screened to know their health status with regard to GINs and coccidia burdens. Before the beginning of data collection, all goats were treated with an anthelmintic drug (Ivomec[®]) to control endoparasites and sprayed with acaricides to control ectoparasites. In both breeds, the animals were classified as young if they were below one year and adult if they were above one year of age.

District	Village	Breed	Does	Bucks	Total
	Ihanda	Norwegian	19	3	22
	manua	Toggenburg	16	1	17
Kongwa	Maginyati	Norwegian	15	1	16
	Masinyeti	Toggenburg	15	2	17
	Kunke	Norwegian	16	2	18
	Kulike	Toggenburg	16	1	17
Mvomero	10/	Norwegian	15	1	16
	Wami	Toggenburg	5	1	6
Total			117	12	129

 Table 1. Number of dairy crossbred goats distributed to farmers in the study areas.

Screening for gastrointestinal nematode eggs and coccidia oocysts

Screening of goats for GINs, coccidiosis and haemoparasites and general health monitoring of the animals was done from June, 2012 to April, 2013. During this period, field visits were made every month to the study areas and faecal samples were collected from rectum of each animal. Each faecal sample was placed in a separate polythene bag and then all samples were packed and stored in a cool box and transported within 24 h to the laboratory at Sokoine University of Agriculture (SUA) where they were stored at 4°C until when analysis was done. The presence of gastrointestinal nematode eggs and coccidia occysts in faeces were determined using the McMaster counting technique using saturated salt solution with specific gravity of 1.200 as the floating medium (Hansen and Perry, 1994). The number of eggs and oocysts counted in the McMaster slide was multiplied by 100 and expressed as nematode eggs per gram of faeces (EPG) and oocysts per gram of faeces (OPG), respectively. The nematode egg load of each animal was graded as low (< 500 EPG), medium (500 to 1,000 EPG) and high (> 1,000 EPG). Similarly, the oocyst burdens were graded as low (≤ 50,000 OPG), medium (50,000 to 100,000 OPG) and high (> 100,000 OPG) according to Soulsby (1982). Animals with medium to high rate of infections were treated. For each village, faecal samples from all positive samples were pooled and cultured. Nematode larvae were harvested using sedimentation technique and species of the nematodes were identified using standard keys (Soulsby, 1982; Uhlinger, 1991; Foreyt, 2001). For identification of Eimeria species, faecal samples were allowed to sporulate and species identification was performed according to Duszynski and Wilber (1997).

Other clinical diseases, conditions and symptoms observed in dairy goats kept by small-scale farmers

Blood sample for each animal was collected from jugular vein using 10 ml vacutainer tubes containing Ethylenediaminetetraacetic acid (EDTA). The blood samples were stored in a cool box containing ice during the field work and were transported to the laboratory for analysis within 24 h. A thin blood smear was prepared for each sample, air dried, fixed in methanol and stained with Giemsa. The smears were examined for the presence of parasites (Quinn et al., 1994). The parasites of interest were Trypanosoma, Babesia and Anaplasma. Also packed cell volume (PCV) and haemoglobin concentration (HB) were determined as complementary tests for parasitism. Collection of blood samples was done every month concurrently with the collection of faecal samples. Blood samples for serological screening were collected in vacutainer tubes containing heparin as anticoagulant. Serum samples were screened for brucellosis using the Rose Bengal test with the Brucella abortus as an antigen (Alton et al., 1988; Robinson, 2003). Presence of antibodies against *Mycoplasma capricolum* subspecies capri pneumoniae (M. capri pneumoniae), the causative agent for CCPP in serum samples was screened using the M. capri pneumoniae Antibody Test Kit (c-ELISA kit) developed based on the method by Thiaucourt et al. (1994). Farmers reported any clinical sign of diseases in the study animals to the village extension officers who then visited the respective farmers, verified and recorded the symptoms such as diarrhoea, rough hair coat, coughing, nasal and ocular discharges and skin lesions. Deaths and their causes (if established) were also recorded as they occur by the village extension workers.

Statistical analyses

Data on EPG, OPG, PCV and HB were analysed using the General Linear Model procedures of SAS (2009) to determine the effect of location (village), season, breed, sex, age of the goats and their interactions on EPG, OPG, PCV and HB. Before conducting statistical analysis, the EPG and OPG values were logarithmically transformed using log_{10} (EPG + 100) and log_{10} (OPG + 100) to normalize the distribution. No transformation was done for HB and PCV. Data on prevalence of various diseases and mortality rate were analysed using the chi-square test to test the significance of the differences between the breeds and among the villages.

RESULTS

Gastrointestinal nematode and coccidia infections

Goats purchased from Babati and Mgeta were screened for nematode and *Eimeria* infections before being

Factor	Parameter	Variable					
Factor	Parameter	EPG	OPG	HB (g/dl)	PCV (%)		
	Toggenburg	$465.41^{b} \pm 0.07$	5561.78 ^a ± 0.1057	$9.22^{a} \pm 0.38$	27.87 ^a ± 1.15		
Breed	Norwegian	$792.35^{a} \pm 0.06$	$722.62^{b} \pm 0.089$	7.91 ^b ± 0.36	23.67 ^b ± 1.08		
	P-value	0.0008	0.0480	0.0008	0.0004		
	Adult	501.50 ± 0.09	1172.47 ^b ± 0.13	8.54 ± 0.36	25.80 ± 1.07		
Age group	Young	756.26 ± 0.11	5111.93 ^a ± 0.15	8.59 ± 0.42	25.74 ± 1.27		
	P-value	0.6822	0.0361	0.9120	0.9640		
	Female	281.61 ± 0.06	2985.62 ± 0.08	8.76 ± 0.23	26.48 ± 0.70		
Sex	Male	976.16 ± 0.16	3298.79 ± 0.23	8.38 ± 0.63	25.07 ± 1.91		
	P-value	0.0820	0.2690	0.5928	0.5133		

Table 2. Least squares means \pm SE for EPG, OPG, HB and PCV of Toggenburg and Norwegian crosses before being transported to the study villages.

^{a,b}The means with different letters in the same column within a factor differ significantly ($P \le 0.05$). PCV = packed cell volume; HB = haemoglobin concentration; EPG = nematode eggs per gram of faeces; OPG = *Eimeria* oocysts per gram of faeces; n = Number of animals observed; P = Probability value.

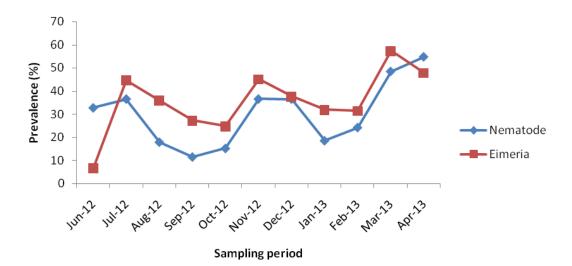


Figure 2. Prevalence of nematode and *Eimeria* infections in dairy goats during the experimental period in the study areas.

transported to the study sites. The prevalence of nematode and *Eimeria* infections in Toggenburg crossbred goats (n = 57) was 32.6 and 63.0%, respectively. In Norwegian crosses (n = 72), the prevalence was 54.7% for nematodes and 62.5% for *Eimeria*. Table 2 shows the effects of breed, age and sex of the animals on the nematode egg and coccidia oocyst burdens. Toggenburg crossbred goats from Babati had lower EPG (P ≤ 0.001) and higher OPG (P ≤ 0.05) than the Norwegian crossbreds from Mgeta. The Toggenburg crosses had higher HB (P ≤ 0.001) and PCV (P ≤ 0.001)

values than the Norwegian crosses. The difference between young and adult goats was not significant (P > 0.05) for EPG, HB and PCV, but it was significant (P \leq 0.05) for OPG. The values of EPG, OPG, HB and PCV did not differ significantly (P > 0.05) between male and female goats. However, males had slightly higher EPG and OPG and lower HB and PCV values compared to females.

The overall trend for the prevalence of GIN and *Eimeria* infections in Norwegian and Toggenburg crosses after being taken to the study areas is presented in Figure 2.

Villege	District	Dread	Variable					
Village	District	Breed	EPG	OPG	HB (g/dl)	PCV (%)		
		Norwegian	280.55 ± 0.03	707.91 ± 0.04	7.23 ± 0.48	24.73 ± 1.33		
Ihanda	Kongwa	Toggenburg	87.73 ± 0.03	655.82 ± 0.04	7.31 ± 0.48	26.31 ± 1.33		
		P-value	0.1437	0.8661	0.8901	0.3278		
		Norwegian	175.26 ± 0.04	878.33 ± 0.05	6.67 ± 0.59	22.14 ± 1.66		
Masinyeti	Kongwa	Toggenburg	135.59 ± 0.03	930.52 ± 0.05	7.56 ± 0.49	27.45 ± 1.38		
		P-value	0.8067	0.8907	0.2052	0.2711		
		Norwegian	86.94 ± 0.03	279.22 ± 0.04	6.68 ± 0.48	24.73 ± 1.28		
Kunke	Mvomero	Toggenburg	106.15 ± 0.03	276.60 ± 0.04	6.49 ± 0.48	24.05 ± 1.31		
		P-value	0.8831	0.9932	0.7449	0.6729		
		Norwegian	304.37 ± 0.05	791.47 ± 0.06	7.06 ± 0.69	24.12 ^b ± 1.94		
Wami-Luhindo	Mvomero	Toggenburg	188.55 ± 0.05	108.79 ± 0.07	6.98 ± 0.80	29.04 ^a ± 2.25		
		P-value	0.5332	0.1172	0.9193	0.0363		

 Table 3. Comparison of EPG, OPG, HB, and PCV of Norwegian and Toggenburg crossbred goats during the study period in the project villages.

^{a,b}The means with different letters in the same column within a factor differ significantly ($P \le 0.05$). PCV = packed cell volume; HB = haemoglobin concentration; EPG = nematode eggs per gram of faeces; OPG = *Eimeria* oocysts per gram of faeces; n = Number of animals observed; P = Probability value.

The prevalence of GIN infection in all goats was below 40% while that of *Eimeria* was below 50% for the most part of the study period. At the start of the experiment in June 2012 less than 30 and 10% of the animals were infected with nematodes and *Eimeria*, respectively. During the study period peak infections occurred in March (57% of the animals were infected) for *Eimeria* and April (55% of the animals were infected) for GINs. The levels of infection for both parasites were lower during the main dry season (July - October) and short dry spell (January - February). There was a slight increase in the levels of infections during November - December and March - April, during which the study areas experienced short rains and the main rain season, respectively.

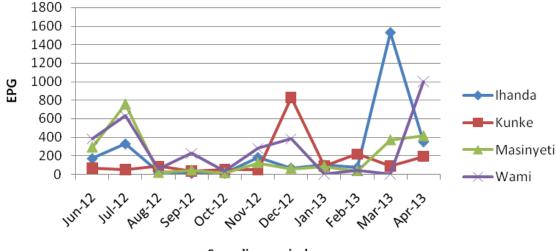
Table 3 shows the least squares means for EPG, OPG, HB and PCV of each breed in each village during the study period. In all the villages there were no significant (P > 0.05) differences between the breeds for all parameters observed. However, the Norwegian crosses had slightly higher values for EPG and OPG compared to Toggenburg crosses in all villages, except Kunke for EPG and Masinyeti for OPG. On the other hand, the Norwegian crosses had slightly lower HB and PCV values in Ihanda and Masinyeti and higher values in Kunke and Wami-Luhindo villages. The effects of location (village) and breed on EPG, OPG, HB and PCV observed in goats during the study period is shown in Table 4. Location did not significantly (P > 0.05) influence EPG, HB and PCV, but had significant ($P \le 0.05$) influence on OPG. Goats in Ihanda (681.86 ± 0.04) and Masinyeti (904.43 ± 0.04) had higher OPG values than those in Kunke (277.91 ± 0.03) and Wami-Luhindo (450.13 ± 0.06). With regard to EPG, goats in Wami-Luhindo (246.46 ± 0.06) had the highest value while those in Kunke had the lowest value (96.55 \pm 0.02), though the differences among the villages were not statistically significant. Goats in Ihanda $(7.27 \pm 0.38 \text{ g/dl})$ had the highest HB value while those in Kunke (6.59 \pm 0.36 g/dl) had the lowest. For PCV, goats in Wami-Luhindo (26.58 ± 1.75%) had the highest value while those in Kunke (24.39 ± 1.01%) had the lowest. Breed had no significant effects (P > 0.05) on EPG, OPG and HB, but significantly influenced (P \leq 0.05) PCV. The Norwegian crosses had slightly higher values for EPG (211.78 ± 0.02) and OPG (664.23 ± 0.03) than the Toggenburg crosses (129.51 ± 1.02) 0.02 EPG and 492.93 ± 0.03 OPG), while the Toggenburg crosses had higher HB (7.09 ± 0.35 g/dl) and PCV (26.71 \pm 0.99%) values than the Norwegian crosses. The monthly values of EPG observed in goats in the study areas are presented in Figure 3.

In all villages, the initial EPG values in June, 2012 were low but raised tremendously in July in Masinyeti and lhanda villages. For the most part of the study period, the mean EPG was below 400. The highest mean EPG was recorded in March, 2013 in Ihanda village and April in Wami-Luhindo village. This corresponded to the rainy

		Variable					
Factor -		EPG	OPG	HB (g/dl)	PCV (%)		
	Ihanda	184.14 ± 0.03	$681.86^{a} \pm 0.04$	7.27 ± 0.38	25.52 ± 1.06		
	Kunke	96.55 ± 0.02	$277.91^{b} \pm 0.03$	6.59 ± 0.36	24.39 ± 1.01		
Village	Masinyeti	155.43 ± 0.03	$904.43^{a} \pm 0.04$	7.12 ± 0.41	24.79 ± 1.16		
	Wami-Luhindo	246.46 ± 0.06	$450.13^{ab} \pm 0.06$	7.02 ± 0.62	26.58 ± 1.75		
	P - value	0.6776	0.0401	0.3630	0.5706		
	Norwegian	211.78 ± 0.02	664.23 ± 0.03	6.91 ± 0.34	$23.93^{b} \pm 0.96$		
Breed	Toggenburg	129.51 ± 0.02	492.93 ± 0.03	7.09 ± 0.35	26.71 ^a ± 0.99		
	P - value	0.2638	0.3209	0.5856	0.0023		

Table 4. Effects of location and breed on EPG, OPG, HB and PCV of goats during the study period.

^{a,b}The means with different letters in the same column of the same factor differ significantly ($P \le 0.05$). PCV = packed cell volume; HB = haemoglobin concentration; EPG = nematode eggs per gram of faeces; OPG = *Eimeria* oocysts per gram of faeces; n = Number of animals observed; P = Probability value.



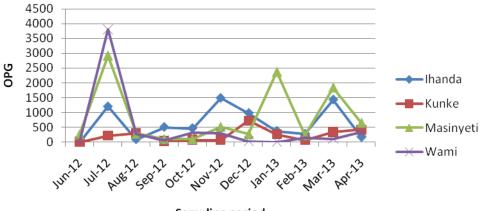
Sampling period

Figure 3. Monthly burdens of EPG for goats from Ihanda, Kunke, Masinyeti and Wami-Luhindo villages

season in all the study villages (March to May). The lowest EPG values in all the villages were recorded during the driest period of the year (August to November). A slight rise in EPG values was recorded in December, 2012, a month, which is within the short rainy period. The mean monthly OPG values observed in goats in the four study villages are presented in Figure 4. The OPG values were below 500 for the most part of the study period. The peak OPG values were recorded in July, 2012 in Wami-Luhindo and Masinyeti villages. For the rest of the period, the OPG values remained below 2000, even during the rain season, except in Masinyeti village in which the OPG was about 2,500 in January,

2013.

The distribution of the GIN genera in the study villages did not differ significantly (P > 0.05), though there were slight variations of the dominant species in each village. In Masinyeti village the most predominant genus was Haemonchus (47.83%), followed by Bunostomum (34.78%) and Strongyloides (17.39%). In Ihanda village main genera were Bunostomum (69.23%). the Haemonchus (15.38%) and Strongyloides (15.38%) while in Wami-Luhindo village Bunostomum, Haemonchus and Strongyloides accounted for 66.67, 16.67 and 16.67%, respectively. In Kunke village Bunostomum (60%) was the most predominant genus, followed by Haemonchus



Sampling period

Figure 4. Monthly burdens of OPG in goats from Ihanda, Kunke, Masinyeti, and Wami-Luhindo villages

(20%) and *Strongyloides* (20%). Moreover, at Wami-Luhindo village *Monieza expansa* and *Monieza ben* were also observed in four and two animals, respectively. *M. expansa* was also observed in two animals at Masinyeti villages.

The *Eimeria* species observed in the goat faecal samples from the study villages were *Eimeria arloingi*, *Eimeria parva*, and *Eimeria ninakohlyakimovae*. At Masinyeti village *Eimeria arloingi* (66.67%) was the most abundant species, followed by *E. parva* (20%) and *E. ninakohlyakimovae* (13.33%). At Ihanda village the predominant species was *E. arloingi* (63.64%), followed by *E. parva* (27.27%) and *E. ninakohlyakimovae* (9.01%). At Kunke village the most predominant species was *E. arloingi* (62.5%), followed by *E. parva* (25%) and *E. ninakohlyakimovae* (12.5%). No *Eimeria* species were observed after faecal culture of samples collected from goats of Wami-Luhindo village.

Other clinical diseases, conditions and symptoms observed in dairy goats kept by small-scale farmers

Of the 129 dairy goats screened for CCPP in all project villages, 26.4% of the goats were seropositive. Screening for brucellosis and haemoparasites (*Trypanosoma, Babesia* and *Anaplasma*) revealed negative results for all goats. A total of 284 cases of clinical diseases, conditions and symptoms were observed and treated in the study goats and these included diarrhoea (27.46%), coughing (13.73%), worm infection (39.08%), orf (16.55%), heartwater (2.82%) and abdominal hernia (0.35%). A total of 20 goats died during the study period due to various reasons and the overall mortality rate was 15.5%.

The causes of mortality are shown in Table 5. The causes for most (55%) of the deaths could not be established because of lack of veterinary services in the study villages. The lack of timely provision of veterinary services and poor communication system in the study villages contributed to the observed high mortality.

DISCUSSION

The present study has demonstrated that dairy goats in the study areas are infected with gastrointestinal nematodes throughout the year, despite the fact that they are kept indoors. This is contributed by the fact that some farmers allow their goats to graze in natural pastures around the homesteads and the majority of the farmers collect forages for feeding goats from communal lands in which local goats and other ruminant animals graze. This goats to infection predisposes the dairy with gastrointestinal parasites. The observation that most nematode egg and coccidia oocyst counts were highest during the rainy and humid months indicates that rainfall, humidity and temperature play a significant role in the epidemiology of gastrointestinal parasites as it has been reported by others (Regassa et al., 2006; Mbuh et al., 2008). Climatic conditions during the rainy season are favourable development, for the survival and translocation of pre-parasitic stages of gastrointestinal nematodes. In this study, nematode burdens declined during the dry season with the lowest values being observed in the driest months of August, September and October. This concurs with Nwosu et al. (2007) who reported that the climatic conditions in the dry period are not favourable for the survival of the free-living stages of

Table 5. Causes of death of dairy goats in the study villages.

	B		
Cause of death	Norwegian crosses	Toggenburg crosses	Total
Pneumonia	-	1	1
Plastic bag impaction	-	1	1
Starvation	-	1	1
Excessive consumption of cassava leaves	-	1	1
Consumption of insecticide treated maize grain	-	1	1
Abscess	-	1	1
Cowdriosis	2	-	2
Bloat of unknown origin	1	-	1
Cause of death not well established	10	1	11
Total	13	7	20

the parasites. The nematode genera observed in the present study have been reported in previous studies in Tanzania (Kusiluka, 1995; Kusiluka et al., 1996, 1999) and in neighbouring countries. Ng'ang'a et al. (2004) and Odoi et al. (2007) reported that in Kenya, the widely reported nematode genera of small ruminants include *Haemonchus, Trichostrongylus, Cooperia* and *Oesophagostomum*.

The prevalence of coccidiosis in dairy goats from the four villages observed in this study is similar to what has been reported in other sub-Saharan African countries (Kanyari et al., 2009; Kanyari et al., 2010). In this study, the dairy goats were infected with Eimeria throughout the year in all four villages, suggesting that the environmental conditions were conducive for the survival and development of the Eimeria species throughout the year. Most of the dairy goats in the study villages are kept indoors under the cut-and-carry system. This system increases the risk of goats for being infected with coccidiosis. The finding that both prevalence and loads of coccidia in this study were higher in young goats compared to adult goats agrees with the findings reported by several authors (Abo-Shehada and Abo-Ferieha, 2003; Regassa et al., 2006; Mbuh et al., 2008). This is due to the fact that coccidiosis is mainly a disease of young goats that have not yet developed immunity against coccidia (Matjila and Penzhorn, 2003). It is possible that the mixed infection of nematodes and Eimeria increased the susceptibility of young goats to coccidiosis.

The proportion of animals infected with nematodes and *Eimeria* and the respective EPG and OPG values were very low at the beginning of the study in June, 2012, simply because the animals were given treatment against gastrointestinal parasites before being distributed to the farmers. The high burdens of both EPG and OPG

recorded in July, 2012 indicates that the animals got new infections after being introduced in the research villages, implying that the project villages are infested with nematodes and Eimeria. The higher OPG values observed during the month of July compared to EPG reflect the resistance of coccidia oocysts to desiccation. Kanvari (1993) observed that sporulated oocvsts are more resistant to desiccation compared to helminth eggs or larvae and consequently oocyst counts in goats during the dry season are higher than the nematode egg counts. The overall low prevalence and burdens of nematodes and Eimeria in goats during the entire study period in all villages and both breeds may be due to the low stocking rate of goats which did not favour a build up of high nematode egg and oocyst burdens because on average, each household had one or two goats. Moreover, presence of few animals per household coupled with zero grazing minimized the risk of goats for being infected with the nematodes.

The persistently higher levels of EPG and OPG among the Norwegian crossbred goats compared to Toggenburg crosses may suggest that the Norwegian goats are more susceptible to gastrointestinal nematodes than the Toggenburg goats. Breed differences with respect to nematode infection in dairy goats have been reported by other studies (Richard et al., 1990; Costa et al., 2000). The Toggenburg goats have been in the country for longer time (since early 1960s) compared to the Norwegian goats, which were introduced in the late 1980s. Hence, the Toggenburg goats may have adapted better to the local conditions and developed traits for tolerance to endemic diseases compared to the Norwegian goats.

Screening for *M. capripneumoniae* revealed that a significant proportion of goats in the project villages were infected with CCPP. The observation in the present study supports the findings of previous studies which have

reported the existence of CCPP in Tanzania (Noah et al., 2011). CCPP infection in goats was confirmed for the first time in late 1990s (Msami et al., 1998). The disease is one of the major limiting factors for improved goat production in Eastern Africa (Eshetu et al., 2007). The disease causes direct economic loss due to high mortality, reduced milk and meat production, costs of diagnosis, treatment, vaccination and surveillance. Also it causes indirect loss resulting from trade restrictions. Therefore, there is a need to develop sustainable disease control strategy for CCPP in order to promote commercial goat production. Apart from CCPP, a peste des petits ruminants (PPR) has emerged in recent years as the major threat to the small ruminant industry in the country (Karimuribo et al., 2011, 2012). However, it was not encountered in this study. Similarly, trypanosomosis and tick-borne diseases, which are the major haemoparasitic diseases of small ruminant in sub-Saharan Africa (Kusiluka and Kambarage, 1996), were not observed in the current study.

The main cause of animal death in the present study was poor management practices. Some of the causes of deaths encountered in this study such as excessive eating of cassava leaves and insecticide-treated maize, plastic bag impaction and starvation could have been prevented if the farmers had the relevant skills required for provision of adequate care of dairy goats. This underlines the need for educating the farmers on improved animal husbandry practices, in addition to animal health control programmes. The fact that the causes of death for most of the animals were not determined reflects the unavailability of veterinary services and underscores the need for improvement of extension service delivery system in rural areas. It is possible that even the deaths reported to be caused by cowdriosis were erroneous because of the difficulty in field diagnosis of this disease. The introduction and sustainability of dairy goat keeping in the study villages will be successful if the farmers acquire basic animal husbandry and health management skills so that appropriate interventions can be made before losses are encountered. The situation can be improved by introducing community animal health workers in the study villages who will be responsible for early diagnosis and treatment of diseases in the villages.

Conclusion

It can be concluded that gastrointestinal parasites are widespread among dairy goats in the study areas and that the rainy season is the major factor, which influences the epidemiology of these parasites. The most predominant nematode genera in the study villages are *Bunostomum* (57.67%), *Haemonchus* (24.97%) and *Strongyloides* (17.36%) while for *Eimeria* the most

common species are *E. arloingi, E. parva* and *E. ninakohlyakimovae*. Although nematodes and coccidia were found in the study sites throughout the year, their burdens were relatively small to justify mass treatment. Therefore, treatment of individual clinically sick animals that are suspected to be highly affected by GINs or coccidiosis may be a rational option. Control of other diseases such as CCPP by vaccination is also recommended in view of its potential impact on goat mortality. Moreover, the study has revealed that the Toggenburg goats are relatively tolerant to helminthosis and coccidiosis compared to the Norwegian goats.

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Conflict of interest

The authors have no conflict of interest.

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Full Length Research Paper

Prevalence, cyst viability, organ distributions and financial losses due to hydatidosis in cattle slaughtered at Nekemte municipal abattoir, Western Ethiopia

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A cross-sectional study was conducted from October, 2013 to March, 2014 to assess the prevalence, cyst viability, organs distribution and direct financial losses of hydatidosis in cattle slaughtered at Nekemte municipal abattoir. Out of 473 inspected cattle at postmortem inspection, 82 (17.34%) were harboring a single or multiple hydatid cysts. Significantly (P < 0.05) higher infection rate was observed in different age groups and body condition scores. Anatomical organ distributions of cysts showed 64.2, 32.4, 0.93, 2.16 and 0.308% in lung, liver, kidneys, spleen and heart, respectively. Of 324 total cysts collected, 74 (22.84%) were calcified while the rest 250 (77.16%) were non-calcified cysts. From those non-calcified cysts, 62 (24.8%) were fertile while 188 (75.2%) sterile. Furthermore, viability analysis of fertile cysts showed 34/62 (54.84%) viable cysts. The rate of cyst calcification was higher in liver (60%) than other organs whilst the fertility was higher in lungs (23.6%). Size assessment revealed 87/250 (34.8%) small, 94/250 (37.6%) medium and 69/250 (27.6%) large sized cysts. In this study, annual economic loss from organs condemnation was estimated to be 8561.61 Ethiopian Birr (ETB) (450.6 USD) per annum based on the local market prices in the study period. This showed that hydatidosis is an economically important disease of cattle which necessitates appropriate strategic control.

Key words: Bovine, financial loss, hydatidosis/Echinococcosis, Nekemte, prevalence.

INTRODUCTION

Hydatidosis is a term used to describe the infection of animals and humans with metacestode stage of Echinococcus species (Parija, 2004). The metacestode larva stage of the dog tapeworm, *Echinococcus granulosus* is the causative agent of Cystic echinococcosis. Cystic echinococcosis is recognized as one of the major helminth zoonoses affecting humans and various animals' species in different parts of the world (Cringlo et al., 2007). It is a cosmopolitan zoonotic infection with dogs and other canids and domestic and wild ungulates

*Corresponding author. E-mail: nebmoje@yahoo.com. Tel: +251-910-248878. Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> being involved in the life cycle as definitive and intermediate host, respectively (McManus, 2006).Cystic echinococcosis is associated with severe morbidity and disability, and is one of the world's most geographically widespread zoonotic diseases as it affects humans (Craig et al., 2007; Cringoli et al., 2007). Human hydatidosis is often associated with clinical signs and the function of the affected organ is often impaired. This is especially true if the heart or the brain are involved (Kaufman, 1996). Unlike that of animals, when a human is involved as an intermediate host, the hydatid in its pulmonary or hepatic site is often of pathogenic significance. One or both lungs may be affected, causing respiratory symptoms, and if several hydatids are present in the liver there may be gross abdominal distension (Urguhart et al., 1996). Moreover, the parasite causes considerable economic losses and public health problems in many countries (Eckert and Deplazes, 2004; Karimuribo, 2009). The disease is endemic in a number of countries and has greater public health importance and economic impact in countries where livestock industry is an important segment of agricultural sector and when livestock production is based mainly on extensive grazing system (Berhe, 2009). Its distribution is higher in developing countries including Ethiopia, especially in rural communities where there is close contact between dogs (definitive host) and various domestic animal intermediate hosts (Eckert and Deplazes, 2004).

Ethiopia has been noted for a high prevalence of hydatid disease since the 1970s (Schaller and Kulus, 1972). Moreover, reports of findings from abattoirs in various locations revealed that hydatidosis is widespread in Ethiopia with great economic and public health significance (Jobre et al., 1996; Sisay et al., 2008; Berhe, 2009). Knowledge about the prevalence of the diseases together with associated risk factors as part of the epidemiology of the disease is crucial for any attempt of prevention and control of the disease in question. Moreover, determination of the economic significance of the disease is important for decision making, planning, development and implementation of local control strategies. Therefore, the objectives of this study were to determine the prevalence, proportion in relation to characteristics and organ distributions of E. granulosus cysts and estimation of direct annual economic losses due to involved organs condemnation in cattle slaughtered at the Nekemte slaughterhouse.

MATERIALS AND METHODS

Study area

The study was conducted from October, 2013 to March, 2014 at Nekemte municipal abattoir, Oromia regional state, West Ethiopia. Nekemte manucipal abattoir is situated in the capital city of East Wollega Oromia regional state which is situated at the West part of Ethiopia at a distance of about 328 km away from the capital (Addis Ababa). It is located in latitude and longitude of 9° 5'N 36° 33'E/9.083°N 36.55°E and has an elevation of 2088 meter above sea level. The area gets 1500 to 2200 mm Hg rainfall annually. The mean monthly minimum and maximum temperatures were 10.5 and 31°C, respectively. The livestock population of the area comprises of 74574 cattle, 11110 sheep, 1007 goats, 5074 equines and 36186 heads of chickens (Community Supported Agriculture (CSA), 2011).

Study animals

The study animals were local zebu cattle (Bos indicus) brought to the abattoir for slaughter from districts around the town (Arjo, Arjo Guddatu, Getema, Diga, Bandira, Uke, Sasiga, Wayu Tuka, Nekemt). Slaughtered animals were both male and female.

Study design

A cross sectional study was conducted to determine prevalence and associated risk factors of hydatidosis and to assess burden and size of cysts in cattle slaughtered at Nekemte municipal abattoir.

Sample size and sampling methods

The sample size was calculated according to Thrusfield (1995) by considering 23.17% prevalence (Abunna et al., 2011) and 95% confidence level with a 5% desired absolute precision. The calculated sample size was 273 and additional 200 samples were included to increase the precision and a total of 473 animals were included in the study.

Study methodology

Cattles brought to abattoir were selected by systematic random sampling. The first animals was selected randomly and the rest with equal intervals and subjected for both antemortem and detail postmortem inspection.

Antemortem examination

Antemortem inspection recommended by Gracey (1986) was utilized. The age, sex and body condition of each individual animal was identified and recorded. Based on the body condition, animals were grouped as poor, medium and good (Nicolson and Butterworth, 1986). The age of the animal was estimated on the basis of the dentitions (De Lahunta and Habel, 1986) and conventionally grouped into three; young (4 to 6 years), adult (7 to 9 years) and old (>10 years).

Post-mortem examination

Postmortem inspection procedures recommended by Food and Agricultural organization (FAO) (1994) were used during study. Visceral organs, particularly the lung, kidney, liver, spleen and heart were inspected with visual, palpation and systemic incision of each organ. The infected organs from each positive animal were collected; the total number of hydatid cysts were counted per infected organ and recorded on the sheet prepared for it.

Cyst characterization

Individual cyst was grossly examined for any evidence of degeneration and calcification. Cysts size measurement, cyst counting, cyst fertility and viability determination was also conducted. The size of the diameter of hydatid cyst was measured and classified as large (diameter >10 cm), medium (5 to 10 cm) and small (diameter < 5 cm) (Oostburg et al., 2000). The volume of hydatid fluid was measured and classified as high (volume >20 ml), medium (volume between 6 to 20 ml) and low (volume <6 ml). The collected cysts were carefully incised and examined for protoscolices, which looks like white dots on the germinal epithelium in hydatid fluid via microscope, so as to classify cysts as fertile or infertile. The infertile cysts were further classified as sterile (fluid filled cysts without any protoscolices) or calcified (Kebede et al., 2009a). Fertile cysts were further subjected to viability test. A segment containing protoscolices was placed on the microscope glass slide and covered with cover slip and observed for amoeboid like peristaltic movement with (40x) objective. For clear vision, a drop of 0.1% aqueous eosin solution was added to equal volume of protoscolices in hydatid fluid on microscope slide with the principle that viable protoscolices should completely or partially exclude the dye while the dead ones take it up (Dalimi et al., 2002). Direct financial analysis was made by measuring the kilograms of both partially and totally condemned organs. For simplicity to calculate annual abattoir loss due to hydatidosis measurement of kilograms were converted into equivalent organ numbers rejected due to hydatid cyst by taking organ average.

Direct financial loss

This is the loss resulted from organs condemnation at the abattoir and was assessed by modifying the formula set by Ogunrinade and Ogunrinade (1980) for fascilosis effect on liver but in this case considering the percentage of hydatidosis for organs condemnation (rejection rate of organs), the average number of animals slaughtered in the abattoir during a year from abattoir record and the average current market price of organs.

AELC = (ACS* PLi* ACLi) + (ACS* PLu* ACLu) + (ACS* PHe* ACHe) + (ACS* PKi* ACKi) + (ACS* PSpl* ACSpl)

Where AELC = Annual economic loss due to organ condemnation, ACS = Average number of cattle slaughtered per year at Nekemte municipal abattoir, ACLi = Average cost of liver in Nekemte town, PLi = percentage of hydatidosis in liver, ACLu = Average cost of lung in the town, PLu= percentage of hydatidosis in lung, ACHe = Average cost of heart in Nekemte town, PHe = percentage of hydatidosis in heart, ACKi = Average cost of kidney in Nekemte town, PKi = percentage of hydatidosis in kidney, ACSpl = Average cost of spleen in Nekemte town, PSpl= percentage of hydatidosis in spleen.

Average market price of lung, liver, spleen, kidney and heart were 10, 40, 3, 10, and 15 Ethiopian Birr (ETB), respectively. The mean annual numbers of cattle slaughtered was determined from the last five years abattoir records and found to be 5685.

Data management and analysis

The data was recorded on specially designed formats and preliminary analysis was done in Microsoft Excel. Descriptive statistics was carried out to summarize the prevalence and relative percentage of hydatid cyst in each organ. Univariate and multivariable logistic regression analysis was conducted to see the association between the risk factors and the occurrence of the disease.

Confidence interval and p-value were employed to see the presence of association. Additionally, odds ratios was used to assess the strength and direction of this association using STATA statistical soft ware version 9.

RESULTS

Hydatid cysts distribution

Cysts distribution among risk factors

Out of the total 473 cattle slaughtered and examined at Nekemte municipal abattoir, 82 (17.34%) were found to be infected with one or more hydatid cysts involving different visceral organs. Infection prevalence of hydatidosis was correlated with age group and body condition score of cattle. Rate of infection of hydatidosis with respect to age group showed that higher prevalence was in cattle 7 to 9 years and >10 years than in below 6 years (P<0.05) and with respect to body condition of cattle, highest prevalence (27.65%) was in poor body condition followed by medium and good body condition scores 23.53 and 13.35%, respectively (P<0.05). However, no significant variation was observed with related to sex of cattle (Table 1).

Proportions of animal and organs affected

Both single and multiple infected organs were recorded. Out of the total cattle (82) harbouring hydatid cyst; 60 (73.2%) animals involved a single infected organ whereas the remaining 22 (26.83%) infected animals had multiple organs involvement. From the total infected cattle (82), 45 (54.9%) of hydatid cyst were in their lungs, 15 (18.3%) in livers and 22 (26.83%) in multiple organs as mixed infection (Table 2).

Viability test

Out of 324 cysts counted and evaluated cysts; 62/324 (19.14%) were fertile and contained protoscolices whereas the remaining 188/324 (58%) and 74/324 (22.8%) were sterile and calcified cysts, respectively. Of the fertile cysts (62); 34/62 (54.84%) were viable while 28/62 (45.16%) were non-viable. More fertile (49/208 or 23.6%) and sterile (148/208 or 71.2%) cysts were observed in lungs. The rate of cyst calcification was higher in the liver (63/105 or 60%) than in the other organs (Table 3).

Risk factor	No. of examined animals	No. (%) of affected animals	Crude OR (95% CI)	Adjusted OR (95% Cl)	P value
Sex					
Male	434	77 (17.74)	1	1	-
Female	39	5 (12.82)	1.47 (0.56, 3.85)	1.01 (0.33, 3.13)	0.986
Age					
4-6 years	107	9 (8.41)	1	1	-
7-9 years	229	49 (21.4)	2.96 (1.397, 6.28)	2.72 (1.168, 6.312)	0.020
≥10	137	24 (17.5)	2.31 (1.026, 5.21)	1.56 (0.589, 4.133)	0.370
BCS					
Good	307	41 (13.35)	1	1	-
Medium	119	28 (23.53)	1.99 (1.17, 3.41)	2.05 (1.134, 3.707)	0.017
Poor	47	13 (27.65)	2.48 (1.208, 5.08)	2.80 (1.251, 6.291)	0.012
Origin					
Arjo	12	3 (25)	1	1	-
Arjo G	46	11 (23.9)	1.06 (0.24, 4.55)	1.01 (0.22, 4.74)	0.987
Bandira	159	25 (15.72)	1.8 (0.45, 7.14)	1.92 (0.45, 7.7)	0.369
Diga	135	19 (14.07)	2 (0.5, 8.33)	2.0 (0.47, 8.33)	0.348
Getema	31	8 (25.80)	1.04 (0.23,4.84)	1.25 (0.26, 6.25)	0.775
Nekemt	20	5 (25)	1.0 (0.2, 5.0)	1.2 (0.20,6.49)	0.869
Sasiga	11	1 (9.09)	3.33 (0.3, 33.3)	4.5 (0.37, 11.8)	0.236
Uke	40	7 (17.5)	1.56 (0.34, 7.14)	1.24 (0.25, 6.1)	0.795
W/tuka	19	3 (15.78)	1.8 (0.3, 11.1)	1.47 (0.23, 8.33)	0.679

Table 1. Logistic regression analysis of various risk factors association with the occurrence of cattle hydatidosis in Nekemte Municipal Abattoir.

Table 2. Distribution of Hydatid cysts in different visceral organs of infected cattle.

Infected organ	No. of infected animal	Proportions (%)
Lungs only	45	54.9
Liver only	15	18.3
Liver and lung	15	18.3
Lung and spleen	1	1.22
Liver and kidney	1	1.22
Lung, liver and spleen	2	2.45
Liver, spleen and kidney	1	1.22
Lung, liver, spleen and heart	1	1.22
Lung, liver, spleen and kidney	1	1.22
Total	82	100

Cyst size and volume

Out of the 324 recorded hydatid cysts, 74 were calcified cysts which reduce the total number of cysts to be

assessed for size and volume to 250. Accordingly, 87/250 (34.8%) were small, 94/250 (37.6%) medium and 69/250 (27.6%) large in size, while 75/250 (30%) were low, 103/250 (41.2%) medium and 72/250 (28.8%) large

0	No. of offeriad environments		I	Fertile cyst (%)			Unfertile cysts (%)	
Organ	No. of affected organs	No. of cysts (%)	Fertile	Viable	Non-viable	Sterile	Calcified	
Liver	37	105 (32.4)	12 (11.4)	5 (4.76)	7 (6.67)	30 (25.6)	63 (60)	
Lung	65	208 (64.2)	49 (23.6)	29 (13.94)	20 (9.62)	148 (71)	11 (5.3)	
Heart	1	1 (0.308)	0	0	0	1 (100)	0	
Kidney	3	3 (0.93)	0	0	0	3 (100)	0	
Spleen	6	7 (2.16)	1 (14.3)	0	1 (14.3)	6 (85.7)	0	
Total	112	324	62 (19.14)	34 (10.43)	28 (8.64)	188 (58)	74 (22.8)	

Table 3. Fertility and viability status of hydatid cyst in different organs.

Table 4. The Total non-calcified hydatid cyst counts with respect to size and volume in each infected organs of cattle slaughtered at Nekemte municipal abattoir.

0	Organ Total nam calcified syste		Cyst size (%)			Cyst volume (%)		
Organ	Total non-calcified cysts -	Small	Medium	Large	Low	Medium	High	
Liver	42	30 (71.4)	6 (14.3)	6 (14.3)	29 (69.1)	7 (16.7)	6 (14.3)	
Lung	197	51 (25.9)	83()	63 (42.1)	44 (22.3)	91 (46.2)	62 (31.5)	
Heart	1	1 (100)	0	0	0	1 (100)	0	
Kidney	3	2 (66.7)	1 (33.3)	0	2 (66.7)	1 (33.3)	0	
Spleen	7	3 (42.9)	4 (57.1)	0	0	3 (42.9)	4 (57.1)	
Total	250	87 (34.8)	94 (37.6)	69 (27.6)	75 (30)	103 (41.2)	72 (28.8)	

Table 5. Direct economic losses associated with Hydatidosis in infected cattle in Nekemte municipal abattoir.

Organ inspected	No. of organs condemned	Weight (No.) of organ condemned in kg	Price each organ (ETB)	Annually total price of organs
Liver	37*	18 (12)	40	5775.96
Lung	65 (62* + 3)	22 (22)	10	2643.53
Heart	1*	0.25 (0.5)	15	93.8
Kidney	3*	0.1 (0.2)	10	22.74
Spleen	6*	0.245 (0.7)	3	25.58
Total	112	40.6	78	8561.61 ETB

"'-organs partially condemned.

in volume (Table 4).

Direct financial loss

In direct financial analysis, both partially and totally discarded due to hydatidosis was taken into account. For simplicity in using a formula used by Ogurude and ogurude (1980), both partially and totally condemned organs were measured with kilograms which were converted into individual organs. Due to cattle hydatidosis, 22/kg or (22) lung, 18/kg (12) liver, 0.25/kg

(0.5) heart, 0.1/kg (0.2) kidney and 0.245/kg (0.7) spleen were condemned during the study period with an economic loss of 480, 220, 7.5, 2 and 2.1 ETB, respectively (Table 4). This was assessed from the mean retail market price of each organs and average weight of organs condemned during the study period. Annual economic loss on the other hand was estimated considering annual slaughter rate of cattle and prevalence of hydatidosis per organ and was calculated to be 8,561.61/ETB (450.6/USD) per annum (1 = 19/ETB) (Table 5).

DISCUSSION

Prevalence

Prevalence of hydatidosis varies from country to country or even within the country and has been reported by various researchers from developing countries under extensive production system (Gracey et al., 1999). In current study, 17.34% bovine hydatidosis prevalence was reported. This result agrees with the findings of Bizuwork et al. (2013) (17%), Assefa and Tesfay (2012) (18.6%), Bekele and Butako (2011) (16.85%), Kebede et al. (2009b) (16%) and Regassa et al. (2009) (15.4%) in Southern Wollo, Adigrat, both at Wolaita sodo, and in Hawassa, respectively. In general terms, throughout the world, there had been different magnitude records of hydatidosis in cattle with low, medium and high rates of occurrences. The present study was higher than the prevalence of 11.26% in Mizan Teppi by Jemere et al. (2013), in Mekelle Abergelle export abattoir (11.6%) by Yitbarek et al. (2012), 2.1% from Zambia by Fredrick et al. (2012), 6.99% from Iran by Ahmadi and Meshkehkar (2011) and 2.8% from Sudan by Sahar Adam and Atif Elamin (2011). On the contrary to the current findings, high prevalence rates were registered in other areas of the country such as 61% in Assela (Koskei, 1998), 52.69% in Hawassa (Regassa et al., 2010), 48.9% in Debre Markos (Kebede et al., 2009a), 46.5% in Debre Zeit (Jobre et al., 1996), 40.5% at Addis Abeba abattoir enterprise (Dechassa et al., 2012), 34.05% in Bahir Dar (Kebede et al., 2009c), 32.1% in Mekelle (Berhe, 2009), 23.17% in Nekemte (Abunna et al., 2011), 22.98% from Morocco by Azlaf and Dakkak (2006) and 22% in Tigray (Kebede et al., 2009d). A possible reason for the difference in the prevalence of hydatidosis might be due to the contact between large numbers of stray dogs with the herd of cattle. Dogs, which are the primary factor for the disease transmission are used as guards for herds and are routinely fed with uncooked offal which deemed unfit for human consumption (Getaw et al., 2010). The other possible reason for the variation in prevalence rate in different countries and regions may be attributed mainly to strain difference of E. granulosus that exists in different geographical situation (Arene, 1995). Moreover, other factors like difference in culture and social activities in different regions may contribute to these variations (Kebede et al., 2009abc).

Risk factors

This study showed that the infection rate increases as the age increases; it was found that there was statistical association between the age of cattle examined and infection rate (P < 0.05). This finding is in agreement with

the reports of Endrias et al. (2010) at Ambo Abattoir. This may be due to the fact that cattle are slaughtered at their medium or older age with which they have greater chance of being infected with E. granulosus (Assefa and Tesfay, 2012). Moreover, the growth of the hydatid is slow, maturity being reached in 6 to 12 months (Gemmell et al., 2001). Thus, the reason for the lower prevalence rate of hydatidosis in younger cattle may be early culling of the infected young cattle through selling or slaughtering before they reach old age. Sex and origin factors showed no significance association with the prevalence's of the disease (P > 0.05). This could be due to the similarity in the socio-economic status and animal husbandry practices of community in all areas from where animals were bought for slaughter. The other risk factors were body condition score and the result indicated that there was a significant difference (P < 0.05) in rate of infection among different body condition scores. Animals having poor body condition were found to have high cyst infection. This is similar with previous studies by Zelalem (2012), Miheret et al. (2013), Gebretsadik (2009) and Melaku et al. (2012). Battelli (1997) explained that in moderate to severe infection, the parasite may cause retarded performance and growth, reduced quality of meat and milk as well as live weight loss.

Organ distributions

In current organ distribution of hydatid cyst, lung and liver were the dominant organs affected with this cyst. This result is in agreement with the findings of Bekele and Butako (2011), Njoroge et al. (2002) and Eckert and Deplazes (2004). This could be justified by the fact that lungs and liver possess greater capillary fields which allow these organs to efficiently filter the ingested oncospheres from the blood liver, and lungs undergo sequential filtration of blood, liver undergoes primary filtration of blood from portal veins which is followed by pulmonary filtering actions before other organs are invaded. Only those oncospheres which transfer the blood will reach the systemic circulation and other tissues (Eckert and Deplazes, 2004). From liver even lungs (64.2%) were found to be infected with cysts. Similar findings were reported from different part of Ethiopia (Bizuwork et al., 2013; Dechassa et al., 2012; Gebretsadik, 2009) and from other countries (Anwar et al., 2000), from Pakistan (Islam et al., 2003), from Bangladesh and from Iran (Ahmadi and Meshkehkar, 2011). Similarly result had been obtained in the same abattoir by Bizuwork et al. (2013). Other similar reports from abroad also indicated that lungs were found to be the most infected organs in cattle, buffalo and sheep (Manandhor, 2005).

Fertility and viability tests

In examining the condition of cyst fertility and viability, the findings of 58% sterile, 19.4% fertile and 22.8% calcified were examined. The variation in fertility rate among different species could be due to the differences in the strain of *E. granulosus* (McManus, 2006). In comparison of the fertility rate among the organs, it was higher in lungs than liver. It has been stated that the relatively softer consistency of the lung tissue allows easier development of the cysts and the fertility rate of hydatid cysts may show a tendency to increase with advancing the age of the hosts (Himonas et al., 1987). This may be attributed to reduced immunological compatibility of animals at their older age of infection (Getaw et al., 2010).

The variation between tissue resistances of the infected organs may also influence the fertility rate of hydatid cysts. The fertility rates observed in this study are law; however, could serve as potential source to infection and perpetuate the cycle of hydatidosis when infected animals are slaughtered and raw offal fed to dogs and also leftovers during backyard slaughter are eaten by wild carnivores. It was observed that majority of the households had livestock, including cattle, sheep, goat and donkeys, which are the intermediate host of the parasite. Similarly, many households had dogs and cats, which were not dewormed regularly and were managed under free-range system. The percentage of calcified cysts is found to be higher in the liver than in the lungs. This may be associated with the relatively higher reticuloendothelial cells and abundant connective tissue reaction of the organ which encapsulates the cyst within a fibrous wall up to 13 mm thick (Shambesh et al., 1999).

Cyst volume and size

Higher numbers of medium (39.9%) and large (30.3%) sized cysts were found in lungs than in the liver while the liver harbored higher number of small sized (28.6%) and calcified (60%) cysts. The reason for higher percentage of medium and large sized cysts in lungs might be related to spongy consistency of the lung and allow easier development of the cyst (Anwar et al., 2000). The relatively higher proportion of small cysts in liver may be due to immunological response of the host that might preclude expansion of cyst size (Islam et al., 2003). In addition to that, it might be due to the case in which the infected cattle are slaughtered before the cysts become larger in size (Assefa and Tesfay, 2012).

Annual direct economic loss

In this study, financial loss due to organ condemnation by

hydatidosis was estimated to 8561.61 ETB (450.6 USD). Affected organs were condemned either partially or totally based on the degree of infestation. In this study, except 3 lungs (n = 65), all others are condemned partially through trimming. The weight of organs condemned as a result of hydatidosis was, 22 kg for lungs, 18 kg for liver, 0.25 kg for heart, 0.1 kg for kidney and 0.245 kg for spleen.

CONCLUSION AND RECOMMENDATIONS

The moderately high overall prevalence observed in the study indicated that hydatidosis is an important disease of economic and public health concern in Nekemte area. The high fertility and viability rates of hydatid cyst obtained from the study area together with the existing socio-economic situations of the community makes hydatidosis an important parasitic disease in the area. These warrant preservation and control of the parasite. As to recommendation of public education on means of transmission, prevention and control strategies of E. granulossus is crucial. At the same time disposal of affected offal freely for dogs and wild canids (the usual practice in the community) should be stopped and all the condemned organs should be either buried or incinerated. Moreover, backyard and roadside slaughtering practices should be prevented by putting the law and regulation of meat inspection into action.

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Full Length Research Paper

Seroprevalence of brucellosis in small ruminants in pastoral areas of Oromia and Somali regional states, Ethiopia

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A cross-sectional study was conducted from November, 2013 to April, 2014 in pastoral areas of Oromia and Somali regional states to determine the prevalence of brucellosis in small ruminants and assess associated risk factors. The multistage sampling technique was used on total population in the selected district during the study period. A total of 420 serum samples were collected from 129 sheep and 291 goats in extensive management system, with no previous vaccination history. Of 420 sera examined, 36 (8.5%) were positive to Rose Bengal plate test (RBPT). The sera screened positive by RBPT were retested using complement fixation test (CFT) and among 36 sera sample tested, 15 (3.6%) were positive for brucella antibodies. The prevalence of brucellosis among sheep and goats was found to be 2 (0.48%) and 13 (3.09%), respectively. The results of the present study showed that there was no significant difference in seroprevalence to *Brucella* antibodies and species, sex and age of the animals examined (p > 0.05). The occurrence of brucellosis among small ruminants in selected districts could pose productivity and reproductive problem in addition to public health risk. Thus, implementing control measures and raising public awareness on prevention methods of brucellosis should be suggested.

Key words: Brucellosis, complement fixation test, Ethiopia, Rose Bengal plate test, pastoral areas, small ruminant.

INTRODUCTION

Ethiopia is one of the developing countries with domestic

small ruminant population estimated to be 26.1 million

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> sheep and 21.7 million goats (Community-supported Agriculture (CSA), 2006). Small ruminants are the chief source of cash income to small holders (EPAIAT, 2003; Akbarmehr and Ghiyamirad, 2011). This is because sheep and goats provide rapid cash turn over (Corbel Center for food security and Public Health and OIE, 2009; Godfroid et al., 2011). Most of the sheep and goat populations in Ethiopia are raised under pastoral conditions. These small ruminants and their milk/meat products represent an important export commodity, which significantly contributes to the National economy. There is also a growing export market for sheep and goats meat in the Middle Eastern Gulf states and some African countries. At optimum off take rates, Ethiopia can export 700,000 sheep and 2 million goats annually, and at the same time supply 1,078,000 sheep and 1,128,000 goats for the domestic market (Alemu and Markel, 2008).

Even though the animals contribute much to the National economy, its development is hampered by different constraints. The most important constraints to small ruminant productions are poor management system, low genetic endowment and widespread endemic diseases including parasitic infestation, viral and bacterial diseases. Among many factors that limit economic return from small ruminants, reproductive diseases including brucellosis are the major disease constraints found in pastoral areas (International Livestock Research Institute (ILRI), 2006).

Brucellosis is a highly contagious and important zoonotic disease which particularly impedes international trade (Refai, 2002). It is caused by different species of the genus *Brucella*, a small, gram negative, non-motile, non spore forming, rod shaped (Coccobacilli) bacteria (Amenu et al., 2010; Kaoud et al., 2010) that are pathogenic for a wide variety of animals and also for humans (Mantur and Amarnath, 2008). In animals, it mainly affects reproduction and fertility, reduces the survival of newborns and diminishes milk yield. In human beings, the symptoms of disease are weakness, joint and muscle pain, headache and undulant fever (McDermott and Arimi, 2006).

It is an important disease of both livestock and people in sub-Saharan Africa (Radostits et al., 2008). The disease has much significance due to its transmission between animals and human through animal products and by products (OIE, 2004). *Brucella melitensis* (biovars 1, 2 or 3) is the main causative agent of caprine and ovine brucellosis and it is highly pathogenic for humans causing one of the most serious zoonoses in the world (OIE, 2008; Ragassa et al., 2009). All infected tissues, cultures and potentially contaminated materials should therefore be handled with great care (Alton et al., 1975).

Despite being endemic in many developing countries, brucellosis remains under diagnosed and under-reported. Furthermore, since brucellosis is an important cause of veterinary morbidity and mortality, the disease can also cause important economic losses in developing countries (Radostits et al., 2008). Even though the disease is endemic in the country, especially in pastoral areas, very limited researches have been done on small ruminant brucellosis. Hence, the study was designed to determine the seroprevalence of small ruminant brucellosis and assess potential risk factors in the selected districts of Oromia and Somali regional states of South Eastern Ethiopia.

MATERIALS AND METHODS

Study areas

The study areas were the pastoral areas located in Somali and Oromia National states, where most of the pastoralists of Ethiopia live. Borana Zone is found at 724 km South of Addis Ababa. It is one of the Oromia Regional States located at 3° 36' North 3° 43' East. The total human population of the district (Woredas) is estimated to be 43,837 (CSA, 2011) whereas small ruminant population was sheep (100, 261) and goat (99,201) (CSA, 2006). The average annual rainfall ranges from 400 to 700 mm. The mean daily temperature is 25 to 44°C. Livelihood of the people is largely dependent on livestock and livestock products, subsequent food and water shortage for the settled and mobile population of the area. Whereas, Somali districts are found around 820 km East of Addis Ababa, Somali regional states of Ethiopia, part of Liban Zone and located in coordinates 4° 25' North, 41° 25' East. The zone is bounded by the confluence of the Ganale Dorya with Dawa river by Afder Zone on the South West by Somalia and on the South by Kenya. The altitude of Woreda ranges from 200 to 1000 m above sea level, with an average daily temperature of 29 to 45°C. The Woreda has a total human population of 37,404, sheep (380,030) and goats (436,099). The rainfall pattern can be characterized as erratic, unpredictable and unreliable, with average rainfall of 200 mm. The livelihood is largely dependent on livestock and livestock products, with subsequent food and water shortage for the settled and mobile population of the area. Elevation of this Woreda ranges from 500 to 1500 m above sea level, with average daily temperature of 28 to 44°C. The total human population of these districts accounts for 66,495, with sheep (180,000) and goats (420,000).

Study animals

Animals (ovine and caprine) of both sexes, different age groups greater than six months and no history of vaccination against brucellosis, kept under the extensive whereas, small ruminants which were diseased and who have had history of vaccination against brucellosis were excluded.

Study design

A cross-sectional study was conducted from November, 2013 to April, 2014 in the study areas to determine the sero-prevalence of small ruminant brucellosis and assess potential risk factors for the transmission and spread of the disease. The cross sectional study design measures all variables on participants at same point in time

Sampling and sample size determination

Multistage sampling technique was used according to Dohoo et al. (2003) in the survey of small ruminant (sheep and goat) brucellosis. The peasant association (PA) was considered as primary unit, the herds as secondary units and individual animals as tertiary units. Sheep and goat herd in 8 PAs from four districts (2 kebeles per District) were sampled during the study based on the livestock population of each district. In order to determine the desired sample size, there were no previous reports of prevalence in the districts. The average expected prevalence rate was assumed to be 50% for the area within 95% confidence intervals (CI) at 5% desired accuracy as stated by Thrusfield (2007) formula:

$$n = \frac{1.96^2 (p) (1-p)}{d^2}$$

Where n = sample size; p = expected prevalence; d = desired level of precision (5%). However, the sample size was 420 to increase the representativeness of the samples to the wider population. Hence, n = 420 goats and sheep were sampled by considering 10% non respondent rate. Sampling was proportionally distributed based on the total small ruminant population in the study districts and accessibility to road for peasant association (PAS).

Sampling procedures

Blood samples were collected from a total of 420 study animals in the study areas during the study period, while laboratory analysis of specimens was made in National Animal Health Diagnostic and Investigation Center (NAHDIC). Essential materials that were used for sample collection and transportation were offered by the research institute. The blood samples were collected from the jugular vein of the animals aseptically. About 5 to 7 ml of blood was collected from sheep and goats through sterile vacutainer test tube and venoject needle. Immediately, each animal was tagged and the respective blood samples were labeled accordingly. This blood was let down to clot for about 2 to 3 h in room temperature then the clotted blood samples were stored at 4°C till serum extraction, usually within 24 h. Then, sera were extracted and dispensed into cryovials in NAHDIC and serum storage was made at -20°C. Then each serum samples were subjected to the laboratory test through the OIE (2004) recommended diagnostic tool.

Serological test procedures

Rose Bengal plate test (RBPT)

RBPT was performed in NAHDCI on all sera samples collected as per the procedure described by Alton et al. (1975) and OIE (2004). The antigen was obtained from Institute Pourquier, Montpellier, France. The test was conducted in National Animal diagnosis and Investigation center (NADIC) in Sebeta Veterinary laboratory. The interpretation of the results was done according to the degree of agglutination.

Complement fixation test (CFT)

Sera samples found positive by RBPT were further tested by CFT at NAHDCI, Sebeta, Ethiopia, according to the protocol described in

OIE Manual (2004). The CFT is the test approved by the World Organization for Animal Health (OIE) as the definitive test for further confirmation.

Data analysis

The data collected in the field were entered into a computer on a Microsoft Excel spreadsheet. Statistical analysis (multivariate logistic regression) was performed using 'Statistical package for the social sciences' (SPSS), version 20. Categorical variables (species, sex, age and area) were expressed in percentages. The prevalence proportion was calculated as the number of animals testing positive by the RBPT/CFT, divided by the total number of animals tested. The association between each risk factor and the outcome variable were assessed using the Chi-square test. For all analyses, a p-value of less than 0.05 was taken as significant.

Ethical consideration

Before any attempt to collect data, the protocol was approved by Institutional Review Board (IRB) of School of Veterinary Medicine, College of Medical and Health Sciences, Wollega University. Official permission was also obtained from animal owners and Agricultural Administration Office of the districts (Woredas). Moreover, the guideline was also used.

RESULTS

Out of 420 small ruminant sera tested, 36 (8.60%) sera were positive by RBPT. Among these, sheep (ovine) and goat (caprine) account for 7 (5.42%) and 29 (9.96%), respectively (Table 1). The results were further confirmed by CFT, where 15 (3.6%) were positive for small ruminant brucellosis during the study period (Tables 2 and 3). In the present study, statistical analysis of the data showed that there was no significant difference between the brucellosis and potential host risk factors (species, sex and age) of the examined animals (p > 0.05). However, significant difference was observed between the disease and origin of the animals (p < 0.05) (Table 3). The prevalence of small ruminant brucellosis in various origins was indicated (Figure 1).

DISCUSSION

The present study indicated the overall seroprevalence of small ruminant brucellosis in Oromia and Somali regional states to be 36 (8.60%) by the RBPT and 15 (3.6%) CFT. The result revealed a moderate prevalence and natural transmission of *Brucella* organisms in the study area. The finding was in line with the previous studies conducted by Omer et al. (2000) in Eritrea who reported, 3.8% in goat and in imported sheep by Refai (2002) in Iran who reported 3%. However, the result was higher than the result reported in Borena by Teshale et al. (2006), with

Table 1. Seroprevalence screened by RBPT of Small Ruminant brucellosis.

Host risk factor		No. of Sera tested	RBPT +ve results (%)	
Cracico	Caprine	291	29 (9.96)	
Species	Ovine	129	7 (5.42)	
Total		420	36 (8.60)	

Table 2. Sero prevalence of small ruminant brucellosis based on species, sex and age.

Host risk f	actor	No. of sera tested	CFT +ve result (%)	X ² (P-value)
Species	Caprine	291	13 (3.09)	X ² =2.2 (p>0.05)
	Ovine	129	2 (0.47)	x =2.2 (β>0.05)
Sex	Male	82	1 (0.24)	
	Female	346	14 (3.33)	<i>X</i> ² =1.59 (p>0.05
Age	Young	77	1 (0.24)	V^{2} 4 4 (D : 0.05)
	Adult	343	14 (3.33)	X ² =1.4 (P>0.05)

 Table 3. Sero prevalence of small ruminant brucellosis based on origin.

Origin	No. of Sera Tested	CFT +ve result (%)	X ² (P-value)
Oromia. M	110	5 (1.19)	X ² =5.41 (P<0.05)
Somali .M	107	4 (0.95)	
Dillo	109	4 (0.95)	
Dolo Ado	94	2 (0.47)	
Total	420	-	-

1.09% sheep in Yabello; Megersa et al. (2010), with 1.56% in Borena; Bekele et al. (2011), with 3.2% in Somali. On the other hand, the finding was lower than that reported by Wesinew et al. (2013), with 4.8% and Ashenafi et al. (2007), with 11.6% in Afar region, in the low lands of Ethiopia; Waghela (1976), with 6.01% in sheep, 6.01% in goat in Kenya; El-Ansary et al. (2001), with 14.2% in sheep, 16.2% in goat in Sudan. This difference could be due to various factors such as differences in diagnostic assay, sampling technique, study area and sample size used.

Higher prevalence was observed in goats (3.09%) than in sheep (0.47%). This finding is lower than the reports of PFE (2004), with 14.2% sheep, 16.72% in goat and Benkirane (2006), with 7.2% in sheep and 5.29% in goat. Goats are at higher risk of acquiring *Brucella* infection than sheep. This may be due to the greater susceptibility of goats to *Brucella* infection and also excreting the organism for a long period, unlike sheep; this reduces the potential for disease spread among sheep flocks.

Little difference was also recorded in the prevalence of brucellosis between adults and young animals. The prevalence in adult age is higher (3.3%) than young age (0.24%). It has been reported that brucellosis is essentially a disease of sexually mature animals (El-Ansary et al., 2001). Sexually mature and pregnant animals are more prone to *Brucella* infection and brucellosis than sexually immature animals of either sex (Walker, 1999). On the other hand, it is also true that younger animals tend to be more resistant to infection and frequently clear an established infection, although latent infections can occur. This might be due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity (Walker, 1999).

In this study, there was significant difference between

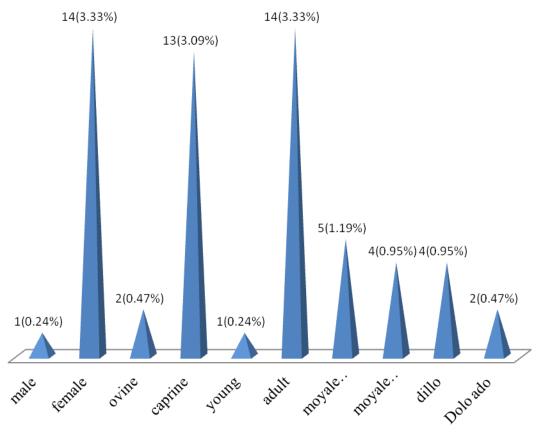


Figure 1. Seroprevalence of small Ruminant Brucellosis among different risk factors

the disease and origin of the animals (p < 0.05). Higher prevalence was found in Moyle Oromia (1.16%) and low prevalence was found in Dolo Ado (0.93%). This may be due to the difference in animal management system, sample size, presence of carrier animal in the origin of the region and population density of small ruminant in the area. Brucellosis is, therefore, well entrenched across the entire regions of Oromia and Somali. This might be attributable to the use of similar animal production and management systems throughout the districts of the region as well as similar agro-ecological conditions. Moreover, unrestricted animal movements may have enhanced the spread of infection, such as: Movements of animals in search of pasture and water/nomadic movement within the animal in search of feeding across the countries, trade within and between zones and districts, the mixing of animals at marketplaces and watering points. Accordingly, Quinn et al. (1999) found that the prevalence of small-ruminant brucellosis was higher, at points such as river and grassing land, especially during the dry season, in the regions, zones and district where there was frequent mixing of flocks.

The study indicated that RBPT which is based on *B. abortus* antigen was less sensitive in detecting antibodies against *Brucella melitensis* (Yibeltal, 2005). Almost half of the sera were found to be tested positive for anti-*Brucella* antibodies by RBPT and negative by CFT. This could be due to cross-reactions between *Brucella* and other bacteria which share similar epitopes. It might also be due to variations in animal management and production systems.

Conclusion

The study revealed that brucellosis is a widespread and well-established infection among goats and sheep in the study areas. The sero-prevalence of brucellosis was higher in goats than sheep, as well as female, adult aged and animals within dense and large herd size. It could be concluded that the positive animal can be a potential risk factors to the free disease animals in the areas, unless the management system is improved. Thus, the author recommend that the ongoing veterinary extension program for the community should be strengthened in order to effectively control the animal movement for successful prevention and control of the disease.

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Conflict of interest

Authors declare that there are no conflicts of interests.

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