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Research Articles

Prevalence of Bovine Trypanosomosis in Abeshige District of Gurage Zone, South Western Ethiopia

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Abstract: A cross sectional study was conducted from November 2014 to May 2015 in Abeshige district of Gurage Zone in Southwestern Ethiopia with the objectives of estimating the prevalence and identifying the species of trypanosomes. A total of 498 blood samples were collected and tested using conventional thin smear and buffy coat techniques. The result revealed an overall prevalence rate of 12.4% trypanosomosis. There were no significant difference in prevalence between animals of different location, age, sex and breed ($p>0.05$). The mean PCV of parasitemic animals (24.5%) was significantly lower than that of aparasitemic animals (29%) ($p<0.05$). The most commonly encountered trypanosome species among parasitemic cattle was *T. congolense* (67.7%) followed by *T. vivax* (29%) and mixed (*T. congolense* and *T. vivax)* (2.3%) infections. In conclusion, the result indicated trypanosomosis to be a major livestock production challenge in the study area that warrant control strategies.

Keywords: *Bovine trypanosomosis; Buffy coat; Thin smear; PCV*

Introduction

Bovine trypanosomosis causes a significant loss in animal production and greatly hampers agricultural development in Africa (Uilenberg, 1998). The existing threat of Africa animal trypanosomosis ranked among the top priority cattle diseases on sustainable livestock production and mixed farming system which present a major constraint in the development of the African continent (Abenga *et al*., 2002; Samdi *et al*., 2010a). These constitute a major threat to achieving food security in several parts of Sub-Saharan Africa and Ethiopia (Samdi *et al*., 2010b). In Ethiopia, animal trypanosomosis is one of the most important diseases limiting livestock productivity and agricultural development due to its high prevalence in the most arable and fertile land of Southwestern and Northwestern part of the country following the greater river basins of Abay, Omo, Ghibe and Baro (Shimels *et al.,* 2005). Over 6 million heads of cattle and equivalent number of other livestock species are at risk of contracting the diseases. More than 20,000 heads die per annum and annual loss attributed to the diseases is estimated to be over US$236 million. Loss due to reduced meat and milk production and draft power is not included in this figure (OAU, 2002).

In Ethiopia, the most important trypanosome species affecting livestock are *T. congolense, T. vivax and T. brucei* in cattle, sheep and goats, *T. evansi* in camels and *T. equiperdem* in horses (Getachew, 2005). Although trypanosomosis is considered as an important disease of cattle in the region (Terzu, 2004) no study has yet been carried out on the epidemiology, prevalence and economic significance of bovine trypanosomosis in Abeshige district of Gurage Zone, Ethiopia. Therefore, the objective of this study was to estimate the prevalence of bovine trypanosomosis and identify species of trypanosomes affecting cattle in selected *Kebeles* of the study area.

Materials and Methods

Description of the Study Area

Abeshige district is located 165 kms South of Addis Ababa in Gurage Zone of Southern Nations Nationalities and Peoples Regional State, Ethiopia. The altitude of the study area ranges from 1001-2000 masl. It is located at latitude of 8° 20' 0" N, and longitude of 37° 40' 0"E longitude. It is characterized by minimum and maximum temperature ranging from 15.5-25^{o}C and the mean annual rainfall of 801-1400mm.The farming system is characterized by a mixed crop-livestock production system with the estimated population of 48,455 cattle, 2,615 sheep, 9,083 goats, 7,702 equine and 44,381 poultry (ADFDO, 2006). The district is divided into 26 *rural* k*ebeles*. Three *Kebeles* were selected for the purpose of the study by simple random sampling technique.

Study Animals

The study was conducted on cattle reared under extensive production system with consideration of different risk factors like age, sex and breed. The study

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animals were classified into different age groups according to the descriptions of Nicholson and Butterwarth (1986). Animals between one and three year of age were considered as young and those above three years as adults.

Study Design

A cross-sectional study was conducted from November 2014 to June 2015 to estimate the prevalence of bovine trypanosomosis in selected *rural kebeles* in the study areas.

Sampling Method and Sample Size Determination

Animals were sampled from the three *rural kebeles* based on proportional bases. At the *rural kebele* level, animals were selected by simple random sampling using lottery method. The number of cattle sampled from a particular herd in a given *rural kebele* depends on proportional weighting. The sample size was determined using the equations given by Thrusfield (2007) whereby:

$$n = \frac{Z^2 . PQ}{e^2}$$

Where, Q = 1-P
Z = 1.96
e = precision error (0.05); P = expected prevalence of about 50%.

Accordingly, a total of 384 animals were randomly selected. However, to avoid loss of sample units and to increase precision, additional 114 samples were collected. Thus, the total sample size was 498.

Parasitological Diagnosis

Blood samples were collected into two heparinized haematocrit capillary tubes from each animal from ear vein punctured by sterile lancet. The tubes were filled with blood to3/4 of their heights and sealed at one end with crystal sealant. The capillary tubes were then loaded on the microhematocrit centrifuge machine symmetrically and centrifuged at 1200 rpm for 5 minutes. Packed cell volume (PCV) was determined using hematocrit reader. Animals with PCV <24% were considered as anemic (Murray *et al.,* 1983). After the PCV was read, capillary tubes were broken 1mm below the buffy coat and the content were transferred on microscopic slides, mixed and covered with a 22x22 mm cover slip. Then it was examined using ground buffy coat technique to detect the presence of the parasites (Paris *et al*., 1982). For preparation of the thin smear, first the slide was polished with dry and clean cloth. The blood in microhematocrit capillary tube was expressed approximately 20 mm away from one end on the slide. The spreader (another slide) was placed on a head of the drop of the blood approximately at an angle of 45°. The spreader slide was drawn back to make contact with blood. Then, the blood was allowed to run to both ends of the spreader slide and spread the blood along the slide with steady motion. The slide was dried by waving it in the air and fixed for 5 minute with methyl alcohol. The smear was flooded with Giemsa staining solution for 45 minute. Excess stain was drained and washed off by using distilled water and allowed to dry for examination. Microscopic examination was made under oil emersion objective (Losos, 1986; Losos and Kede, 1972).

Data Processing and Analysis

The data collected were entered and managed in Microsoft excel. Coded data was transferred to Stata version 11.0 statistical software (STATA, 4905 Lakeway Drive, College Station, Texas, USA) program for analysis. The prevalence of the disease was determined by dividing the number of positive samples by the total number of samples tested for the disease. Chi-square test was used to assess if there was a statistically significant difference in infection among explanatory variables. The mean PCV of parasitemic and aparasitemic animals were compared using *t*-test to assess whether the means of two groups are statistically different from each other. P-value less than 0.05 was considered significant.

Results

Trypanosome Prevalence and Species

The overall prevalence recorded was 12.4% [CI: 10.7-14.1] (n=62). No statistically significant difference was observed between the three *rural kebeles* (p>0.05) (Table 1).

Table 1. The prevalence of trypanosomosis in the study area

Rural kebele's	No. of cattle examined	Total Positive	Trypanosome species			Prevalence (%)	X^2	p-value
			T. c[1]	T. v[2]	Mixed (T.c/T.v)[3]			
Nachakulit	166	24	15 (62.5)	7 (29)	0	14.4		
Kulit-2	166	24	14 (58.3)	5 (20.8)	2 (8.3)	14.4	2.183	0.336
Hudade-4	166	19	13 (68.4)	6 (13.6)	0	11.4		
Total	**498**	**62**	**42 (67.7)**	**18 (29)**	**2 (3)**	**12.4**		

T.c[1]= Trypanosoma congolense, T.v[2]=Trypanosoma vivax, T.c and T.v[3] = mixed.

Most of the infections in trypanosome positive animals were due to *Trypanosoma congolense* followed by *Trypanosoma vivax* and the rest were mixed infections of the two (Table 1). Relatively numerically higher prevalence was recorded among male animals than that of females, but the difference between sex groups was not statistically significant (p>0.05) (Table 2). The infection rate in adult cattle was slightly higher than the young but it was not significant (p>0.05).

Table 2. Prevalence of trypanosome infection among age, sex and breed

Risk factor	Number of cattle Examined	No. of Positive samples	Prevalence (%)	χ^2	p-value
Sex					
Female	264	25	9.4	5.35	0.461
Male	234	37	15.8		
Age					
Young (1-3years)	245	27	11		
Adult (>3 years)	247	35	14.2	2.183	0.336
Breed					
Local	423	51	12	1.241	0.987
Crossbreed	75	11	14.6		

Hematological Findings

Among a total of 498 animals examined, 12.2% of the animals were anemic (Table 3). There was strong statistical difference between the mean PCV of parasitemic and aparasitaemic animal (p<0.01).

Table 3. Mean PCV of parasitemic and aparasitemic animals

Conditions	No. Examined	No. examined PCV (%) ≥24	No. examined PCV (%) <24	Mean PCV (%)	t- test	p- value
Parasitemic	62	34 (54.8)	28 (45.2)	24.5	13.342	0.003
Aparasitemic	436	403 (92.4)	33 (7.5)	29		
Total	**498**	**437 (87.7)**	**61 (12.2)**	**26.25**		

Discussion

The overall prevalence indicated the disease to be an important constraint in livestock production in the study area. The prevalence value of the present study is in agreement with the 12.4% prevalence in Metekel and Awi Zones in northwestern Ethiopia (Solomon and Fitta, 2010) and 12.41% in Hawa Gelan in Oromia Region (Tewodros *et al*., 2012). However, the present prevalence is higher as compared to the studies conducted previously in different part of Ethiopia *(*Basaznew *et al.,* 2012; Teka *et al*., 2012; Zelalem *et al*., 2014; Amanuel *et al*., 2015; Gamechu *et al*., 2015; Reta *et al*., 2015). Prevalence of 9.1% by Abenga *et al.* (2001) and 2.2% by Samdi *et al.* (2011) from Nigeria, Kaduna state central abattoir was also reported. In contrast to the present result, higher prevalence was previously reported in different parts of Ethiopia (Shimelis *et al*., 2001; Dawud and Molalegne, 2011; Abraham and Tesfaheywet, 2012; Thomas *et al*., 2006). Similarly, higher prevalence of 46.8% reported by Sam-Wobo1 *et al.* (2010) and 31.62% by Ayodele *et al.* (2013) also were reported from Ogun and Jos States of Nigeria, respectively. The differences in the prevalence of the disease reported from different regions might be due to the variability in agro-ecology of the study areas and difference in season during data collection (Thomas *et al*., 2006). The identified *Trypanosoma* spp. was *T. congolense, T. vivax* and mixed infections. This findings were similar with results reported from different areas in Ethiopia (Abraham and Tesfahiwot, 2012; Wagari *et al*., 2012; Gamechu *et al*., 2015). Prevalence rate of 33.33% for *T. congolense* (Tewodros *et al*., 2012), 28.89% for *T. vivax* (Gamechu *et al*., 2015) and 2.27% (Reta *et al*., 2015) for mixed (*T. congolense* and *T. vivax)* reported from other parts of the country also confirms the importance of this parasite in hampering animal productivity. The differences in prevalence rate among the studies might be due to the fact that *T.congolense* requires an absolute presence of the biological vector (*Glossina* spp.), whereas *T. vivax* is more readily transmitted mechanically by biting flies than tsetse flies (Langridge, 1976) and also *T. congolense* is mainly confined to the blood, while *T. vivax* and *T. brucei* can also invade the tissues (Hoare, 1972). *T. vivax* is highly susceptible to treatment while the problems of drug resistance are higher in *T. congolense* (Leak, 1999).

The hematological findings showed that mean value in parasitemic animals was much lower than the

aparasitemic animals ($p<0.05$). This finding is in agreement with the previous result reported by Cherinet *et al.* (2006), Abebayehu *et al.* (2011) and Abraham and Tesfaheywet (2012). The parasitemic cattle with mean PCV <24% in this study could be due to direct impact of the disease since trypanosomes destroy RBC membranes resulting in early removal of the defective cells by the reticulo-endothelial system of the animals and therefore result in anemia (Murray *et al.*, 1977; Afework *et al.*, 2000).

Absence of difference in prevalence of the infection between sex groups is in agreement with that reported previously in the country (Abebayehu *et al.*, 2011). This result is also similar with previous results of Terzu and Getachew (2008) and Teka *et al.* (2012) who obtained no significant difference in susceptibility between the two sexes. This might be due to similar exposure of both sexs to the flies in grazing areas (Muturi, 1999; Terzu, 2004; Nega *et al.*, 2004). Similarly, age wise prevalence difference observed was also insignificant indicating that both young and adult animals are equally exposed to the fly in the field. Tethered young animals were also infected by the infection showing the flies are also found around homestead, but with low density relative to the grazing area (Fimmen *et al.*, 1999). On the other hand, the prevalence of the disease in local breeds was slightly lower than the in crossbreeds, although not statistically not significant ($p>0.05$). This might be due to the fact that both local and cross breed cattle are grazing together and have probability of equal exposure (Quadeer *et al.*, 2008).

Conclusion and Recommendations

In the present study, two species of trypanosomes were identified. *T. congolense* was the predominant species in the area followed by *T. vivax* and mixed infection (*T. congolense* and *T. vivax).* The 12.4% prevalence of bovine trypanosomosis suggests that the disease remains to be the major threat to livestock production. Therefore, appropriate control through different chemotherapeutic and chemoprophylactic drugs as well as tsetse control programs should be designed in order to reduce the impact of trypanosomosis in the study area. An active and continuous surveillance is also needed for better understanding of the epidemiology of the trypanosomosis. Further study should also be conducted in order to identify potential tsetse fly species in the study area.

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Conflict of Interests

The authors declare that they have no competing interests.

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Isolation and Identification of *Streptococcus Uberis* in Lactating Cows of Haramaya University Dairy Farm

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Abstract*:* A cross-sectional study was carried out in 40 lactating dairy cows of Haramaya University dairy farm from November 2014 to April 2015 to isolate *Streptococcus uberis* and assess risk factors. A checklist, farm inspection, and clinical examination of cattle were employed to collect data before laboratory examination of milk samples. Lactating animals were examined for the presence of clinical signs of mastitis. Physical examination of milk samples and California Mastitis Test (CMT) were conducted. Milk samples collected from clinically mastitic cows and CMT positive samples were subjected to microbiological examinations. Isolation and identification of *Streptococcus uberis* were carried out according to standard microbiological procedure. From 40 cows udders examined, 17 (42.5%) and 3 (7.5%) were sub-clinically and clinically affected, respectively. *Streptococcus uberis* was isolated from 2 (5%) of these cows. Out of 160 quarters examined, 11 (6.88%), 20 (12.5%) and 50 (31.25%) of the quarters were blind, clinically mastitic and sub-clinically mastitic, respectively. *Streptococcus uberis* was isolated from hind quarters of two sub-clinically mastitic dairy cattle. In conclusion, the present study revealed low isolation rate, however, it's potential to spread and negative impact on quality milk production should not be neglected. Therefore, emphasis should be given to the control of mastitis due to this pathogen by improving hygienic and sanitation management measures.

Keywords: *CMT, Isolation, Mastitis, Microbiological examination*, *Streptococcus uberis*

Introduction

Ethiopia is endowed with the largest livestock population in Africa with an estimated total cattle population of 53.99 million (CSA, 2013). However, this population size is not commensurate with its potential benefit to the country due to different constraints, among which animal diseases takes the top rank. The livestock production sector, particularly, dairy production, has not been fully exploited and promoted in the country (MOA, 2012).

Mastitis is one of the most important threat and highly prevalent problem in dairy cattle affecting the world's dairy industry (Viguier *et al*., 2009). Mastitis is an inflammation of the parenchyma of the mammary gland characterized by changes in the milk appearance and pathological alterations in the glandular tissue in clinical cases (Radiostits *et al*., 2007). However, in subclinical mastitis, there is no visible change in the milk or udder which makes it difficult to detect, even though milk production decreases and composition is altered due to bacteria. Subclinical mastitis is 3 to 4 times more common than the clinical mastitis (Mungube *et al*., 2005) and it results in severe economic losses from reduced milk production, treatment cost, increased labor, milk being withheld following treatment and premature culling (Viguier *et al*., 2009; Abureema, 2013).

Environmental mastitis is associated with bacteria that are transferred from the environment to the cow rather than from other infected quarters. The most common environmental mastitis causing bacteria are coliforms and environmental streptococci (Garcia, 2004; Radiostits *et al*., 2007). Among environmental streptococci, *Streptococcus uberis* is one of the most common mastitis pathogens found in dairy herds throughout the world and responsible for a significant proportion of clinical and subclinical mammary gland infections (Rambeaud, 2002; Tillman, 2006).

Streptococcus uberis is a gram-positive, facultative anaerobic and catalase negative bacteria which hydrolyzes esculin. It has complex and variable nutrition requirements, which reflect its adaptation as a commensal or pathogen and explain its high percentage as environmental mastitis causing pathogen in dairy cattle (Hossain *et al*., 2015). It is also ubiquitous in the cow's environment and found in manure and other organic matter, including bedding. Although its main source is the environment, a contagious cow-to-cow transmission may also occur (Celia *et al*., 2008).

The high infection rates of *Streptococcus uberis* in the dry period and the failure of post milking teat disinfection to control disease emphasize the independence of milking and transmission. Although the organism is sensitive *in vitro* to a range of antibiotics, intramammary therapy often is ineffective and chronic infections are common in some herds. Under these circumstances cow-to-cow transmission may become more important. More importantly, *Streptococcus uberis* can sometimes be

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associated with somatic cell count problems at low bacterial count (Andrews, 2004).

Previously, environmental mastitis constituted less than ten percent of total mastitis cases, but more recently there has been an increase in the incidence of environmental mastitis, particularly associated with *S. uberis* infection (Tiwari *et al*., 2013). Isolation of *S. uberis*, as a cause of bovine mastitis has come under increased scrutiny in dairy cattle, which were previously considered as minor pathogens associated with a mild inflammatory reaction but they are now known to cause bovine mastitis (Hussein, 2012). In fact, a high incidence of *S. uberis* as significant agents of mastitis in New Zealand and USA draw huge attention to this micro-organism as cause of clinical and subclinical mastitis (Rossitto *et al*., 2002; McDougall *et al*., 2004).

Although an increasing isolation of *S. uberis* mastitis has been reported throughout the world including Ethiopia, it still is relevant and important to study the recent status of environmental mastitis pathogen like *S. uberis*. Therefore, the present study was conducted with the objectives of isolating and identifying *S. uberis* in Haramaya University Dairy Farm.

Materials and Methods

Study Area

The present study was conducted in Haramaya University Dairy Farm where there was no regular and systematic detection of mastitis pathogens. Haramaya University is located at 09^{o} N and 42^{o}E at an altitude of 1950 meters above sea level. The area receives a bimodal rainfall; long rainy season (July to September) and short rainy season (March to June). The average rainfall is about 790mm. The mean maximum and minimum temperature are 23.6^{o}C and 10.1^{o}C, respectively (HADB, 2014).

Study Population

During the study period a total of 40 lactating cross bred (Holstein Friesian X Zebu) cows were present in the farm and all the cows were included in the study. The cows were kept under intensive husbandry practice and milked twice daily using a milking machine.

Study Design

A cross-sectional study was employed from November 2014 to April 2015. Clinical examination and laboratory test were conducted to isolate and identify *S. uberis* in lactating dairy cows of Haramaya University. Checklist, personal observation and farm records were used to collect data including husbandry system, age, parity, hygienic condition, lactation stage, production level, milking practices, barn drainage and milking personnel hygiene.

Study Methodology

Physical examination of udder and milk: The udder was examined visually and thorough palpation for detection of injury, blindness, presence of cardinal signs of inflammation, tick infestation and swelling. Viscosity and appearance of milk secreted from each quarter was examined for abnormalities in color, consistency, presence of clot, blood, flakes, and any other visible abnormalities. Depending on the clinical inspection findings, cases were categorized as clinical mastitis positive or negative**.**

After physical examination of the udder, milk samples were screened by California Mastitis Test (CMT) according to Quinn *et al.* (2002). A squirt of milk sample from each quarter of the udder was placed in a separate cup on the CMT paddle and an equal amount of CMT reagent was added and mixed well. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane for few seconds. The CMT results were read immediately and scored based on the amount and thickness of gel formed. Milk samples from animals with CMT positive were used for microbiological analysis.

Milk samples collection and transportation for bacteriological examination was conducted as follows: Udder washing was performed only when it was found with paste of dung. Teats were thoroughly cleaned with soap and water and dried with clean towel before milk collection. The teats were disinfected with cotton wool moistened with 70% ethanol and air dried before sampling. From each quarter an approximately 10ml of milk sample was collected into sterile universal bottle. All samples were labeled using the cow's identification number and quarter using permanent marker, the samples were placed in icebox and transported (Quinn *et al*., 2002) to Haramaya University College of Veterinary Medicine Microbiology Laboratory for bacteriological examination.

The milk samples were cultured to isolate *S. uberis* according to procedures recommended by Quinn *et al.* (2002). A loop full of milk was taken after mixing by swirling and inoculated onto blood agar enriched with 5% sheep blood. The inoculated plates were labeled and given numbers corresponding to the milk sample. Plates were incubated at 37^{o}C and reading was made initially after 24 hours then repeated after 48 hours of incubation. Identification of the bacteria on primary culture was done on the basis of colony morphology, hemolytic characteristics, and Gram stain reaction including shape and arrangements of the bacteria.

The small-medium sized colonies that were hemolytic or non-hemolytic on 5% sheep blood agar and yielding gram positive cocci were sub-cultured onto nutrient agar to obtain a pure isolate for further identification and subjected to catalase test. The catalase test was performed by transferring a bacterial colony with a sterile wire loop onto a cover slip and a drop of 3% H_2O_2 was added. Any colony that showed a positive reaction was discarded. Bacterial isolates that were Gram positive and negative for catalase production were set up for aesculin hydrolysis incorporated into the primary isolation media (Edward's medium). Esculin hydrolysis

positive cocci were transferred to Mac-Conkey agar to detect growth. Bacteria which did not grow on Mac-Conkey agar were considered as *S. uberis*. Bacteria which grew on Mac-Conkey agar were considered as *Enterococcus faecalis* (Quinn *et al*., 2002).

Statistical Analysis

The data was collected and recorded on specifically designed formats for this purpose and entered on Microsoft excel spreadsheet and analyzed with STATA version 12 statistical software. Descriptive statistics including frequency and percentage were used to summarize the data generated from the study.

Results

Among the 40 lactating cows examined, three (7.5%) and 17(42.5%) were affected by clinical and subclinical mastitis, respectively and two (5%) were identified positive for *S. uberis* (Table 1).

Table 1. Isolation of *Streptococcus uberis* in relation to cow level mastitis forms

Mastitis form	Number of animal affected	Percentile	*S. uberis* positive (%)
Clinical	3	7.5%	0 (0%)
Subclinical	17	42.5%	2 (11.76%)
Overall	20	50%	2 (5%)

Isolation of *S. uberis* on the bases of animals' age groups revealed prevalence of 5.88% for young and 4.34% for adult ages. *S. uberis* isolation was observed only in multiparous cows (5.56%). On the other hand, cows in late lactation were affected at 6.7% rate while cows in early lactation had 8.3% prevalence. In addition, isolation of *S. uberis* was observed in animals with high production and low production with isolation rates of 11.1% and 5%, respectively (Table 2).

Table 2. Isolation of *Streptococcus uberis* with respect to host related factors

Factors	Categories	Number examined	Positive (%)
Age	Young adult (3-5 years)	17	1 (5.88%)
	Adult (>5years)	23	1 (4.34%)
Parity	Primiparous	4	0
	Multiparous	36	2 (5.56%)
Lactation stage	Early (≤ 4 months)	12	1 (8.3%)
	Mid (5-7 months)	13	0 (0%)
	Late (>7 months)	15	1 (6.7%)
Milk yield	Low (≤ 5 lt)	20	1 (5%)
	Medium (6-10 lt)	11	0 (0%)
	High (>10 lt)	9	1 (11.1%)

Eleven (6.88%) of the 160 quarters were blind whereas 20 (12.5%) and 50 (31.25) were positive for clinical and sub clinical mastitis, respectively. On quarters' level, S. uberis was isolated from hind quarters of two (1.25%) cows with sub clinical mastitis (Table 3).

Table 3. Quarter level mastitis form and isolation of *S. uberis*

Mastitis form	**No Mastitis + (%)**	***Streptococcus uberis* positive (%)**	
Clinical	20 (12.5%)	0 (0%)	
Subclinical	50 (31.25)	2 (4%)	
Quarter	**No of quarter examined**	***Streptococcus uberis* positive (%)**	**Blind (%)**
RF	40	0 (0%)	2 (5%)
LF	40	0 (0%)	1 (2.5%)
RR	40	1 (2.5%)	3 (7.5%)
LR	40	1 (2.5%)	5 (12.5)
Total	160	2 (1.25%)	11 (6.88%)

RF=Right Front; LF=Left Front; RR=Right Rear; LR=Left Rear.

Discussion

The overall isolation of *S. uberis* is 5% which is slightly greater than noted by Belayneh *et al.* (2014), Bitew *et al.* (2010) and Bedada and Hiko (2011) who reported prevalence of 1.2%, 2.5% and 0.9% *S. uberis*. However, the current finding is in line with the findings of Girma *et al.* (2012), G/Michael *et al.* (2013) and Kerro and Tareke (2003) who reported 5.8%, 5.2% and 5.1% prevalence of *S. uberis*, respectively. This discrepancy between different studies is probably due to the fact that environmental *Streptococcus* (*S. uberis*) infection is strongly influenced by hygienic status, poor housing conditions and sanitation problem (Radiostits *et al.*, 2007). Despite the observed poor drainage and inadequate hygienic state of the farm, the prevalence of *S. uberis* remains low. This could be due to the fact that udder infection with *S. uberis* is highly established in dry cows managed to stay in deep straw beddings, which is reported as major risk factor as it favours bacterial multiplication (Andrews, 2004).

Streptococcus uberis was isolated only from cows with subclinical mastitis. Belayneh *et al.* (2014) and Bitew *et al.* (2010) also isolated *S. uberis* only from animals with subclinical mastitis with prevalence of 1.3% and 2.63%, respectively. The present finding is also in agreement with Zadoks (2002) finding who reported *S. uberis* as a major cause of subclinical mastitis in dairy herds. On the other hands Girma *et al.* (2012), Bedada and Heko (2011) and Sori *et al.* (2005) reported higher isolation of *S. uberis* from clinical cases rather than subclinical cases. This difference could be attributed to variation in sample size and study setting among various studies.

In this study, isolation of *S. uberis* is observed in 2 (5.56%) cows that has two previous births. This finding is supported by Zadoks *et al.* (2001) who reported lower incidence of *S. uberis* in lower parity cows than higher-parity cows. Kerro and Tareke (2003) and Getahun *et al.* (2008) also showed direct relationship between parity and prevalence of mastitis. The high isolation rate in aged multiparous animals might be due to increase in teat patency and frequency of previous exposure (Ayano *et al.*, 2013). *S. uberis* isolation both in young adult 1(5.88%) and adult cows 1(4.34%) is in accordance with that noted by Pryor (2008) who reported age to have no influence on *S. uberis* isolations.

Isolation of *S. uberis* only from hind quarters concur with Pryor (2008) and Zadoks (2002) findings who reported the incidence of mastitis caused by any pathogen to vary between the quarters of the udder with the rear two quarters more likely to be infected than the front two quarters, which could be related to greater production capacity of hind quarter, likelihood of fecal accumulation, environmental contamination and difficulty of cleaning of the hind quarter (Sori *et al.*, 2005).

Higher prevalence at early lactation stage than late in the present study is in agreement with the findings of Abureema (2013) who indicated *S. uberis* to be the most common isolate at early lactation. However, Chairman *et al.* (2012) noted *S. uberis* to be dominant pathogen at all stages of lactation since *S. uberis* mastitis is mainly the result of heavy contamination of the teats and udder with water, mud and faecal matter at any stage during lactation (Radiostits *et al.*, 2007).

Isolation of *S. uberis* in both high 1(11.1%) and low 1(5%) milk production group agrees with the finding of Charles (2014) and Moges *et al.* (2012) who reported higher mastitis in cows with high milk yield. This could be due to ease with which injuries are sustained in large udders, so that foci for the entrance of microorganisms are created and stress associated with a high milk yield may weaken the defense system of the cow (Charles, 2014).

Conclusion and Recommendations

Even though occurrence of S. *uberis* can be considered low in the current study, as high as 50% of mastitis prevalence level is worrisome. It is necessary to take appropriate measures to minimize the overall mastitis problem and to prevent potentially harmful effect of *S. uberis* and its spread to other farms in the surrounding. Therefore, husbandry and sanitation management, screening of animals for subclinical mastitis and appropriate dry cow management should be employed to reduce the possible risk of *S. uberis*.

Conflict of Interests

The authors declare that they have no competing interests.

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Detection of Anthelmintic Resistance in Gastrointestinal Nematodes of Small Ruminants in Haramaya University Farms

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Abstract: The present study evaluated the status of anthelmintic resistance of gastrointestinal (GI) parasites of small ruminants. The study was conducted from December 2014 to January 2015 in Haramaya University sheep and goat farms. A fecal egg count reduction test (FECRT) was performed in naturally infected sheep and goats. A total of 30 black head Ogaden sheep and 30 Hararghie highland goats of age form 6-18 months not treated in the previous 8 weeks and with a fecal egg counts (FECs) greater than 150 eggs per gram of faeces were selected for the test. Both sheep and goats were grouped into two treatments and one control group (albendazole, ivermectin, and the control). In sheep, the percentage reductions in FECs and the 95% (lower and upper) confidence limit (CL) for albendazole was 82% (95%, CL 60-92), and for ivermectin 68% (95%, CL 0-90). In goats, the percentage reductions in FECs for albendazole was 63% (95%, CL 28-81), and for ivermectin 41% (95%, CL 0-72). The result show that albendazole and ivermectin resistance was detected in nematode parasites of sheep and goats. To overcome the problem, the farm should use anthelmintics only when necessary, employ rotation of anthelmintic every two or three years, use the correct dose of anthelmintics, reduce dependence on anthelmintics and use other management options such as rotational grazing, and adopt strategies to preserve susceptible worms.

Keywords: *Anthelmintic resistance; Sheep and goat; Albendazole; ivermectin, Fecal egg count reduction*

Introduction

Ethiopia has 25,489,204 sheep and 24,060,792 goats' populations (CSA, 2015). The proportion of total annual meat production in Ethiopia from cattle, sheep, and goats was 63%, 25%, and 12%, respectively. At the national level, sheep and goats account for about 90% of the live animal and 92% of skin and hide export trade value (FAO, 2004). In Ethiopia, small ruminant production and productivity is affected by diseases, inadequate nutrition, and poor management system (Addis, 2015). Several studies in different parts of Ethiopia revealed that gastro intestinal (GI) parasites are one of the major problems causing morbidity, production loss, and mortality (Shimelis *et al*., 2011; Sabkeber *et al.,* 2014; Sisay *et al*., 2007). The treatment and control of parasitic helminthes largely depends on the use of few classes of anthelmintics drugs. Consequently, anthelmintic resistance is becoming a serious problem worldwide, especially in developing countries where there is no rational use and variety of anthelmintic drugs (Urquhart *et al*., 1996).

Anthelmintic resistance is a heritable change in the ability of individual parasites to survive the recommended therapeutic dose of anthelmintics (Taylor *et al*., 2002). According to World Association for the Advancement of Veterinary Parasitology (WAAVP), resistance is present if the percentage reduction in egg count is less than 95% and the lower limits of 95% confidence level is less than 90%. If only one of the two criteria is met, resistance is suspected (Coles *et al*., 1992; Coles *et al*., 2006). Anthelmintic resistance in GI nematodes of small ruminant has been reported in different parts of the world, which made it a seriously increasing problem (Wolstenholme *et al*., 2004). Resistance to the major classes of anthelmintics has been recorded in Europe (Coles *et al*., 2004), Asia (Gills, 1993), North America (Uhlinger *et al*., 1992) and Latin America (Echevarria *et al*., 1996). In Ethiopia, anthelmentic resistance in small ruminants has been reported by many researchers from different part of the country (Ayalew *et al.,* 2014; Desie *et al*., 2013; Getachew *et al*., 2013, Sisay *et al*., 2006a; Sisay *et al*., 2006b).

A study conducted by Sissay (2007) on small ruminant helminth parasites in eastern Ethiopia identified more than nine genera of nematode parasites and the use of anthelmintics has been practiced for a long time and constitutes a considerable share of the costs spent by the country in the control of helminthosis (Demelash *et al*., 2006). However, the control of GI nematode parasites of livestock in smallholder farmer and pastoralist communities is done with limited anthelmintic drug and is performed mainly during the rainy seasons (Sisay *et al*., 2007). Drugs are relatively expensive and are often not easily accessible to smallholder farmers and stock owners in pastoralist communities, while frequent and indiscriminate use of different classes of anthelmintics

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has been reported in institutional and large commercial farms (Sissay *et al*., 2006a). Despite the frequent use of anthelmintic drugs in Haramaya University sheep and goat farms, the prevalence of GI nematodes is extremely high (Sabkeber *et al*., 2014). The higher prevalence might be due to anthelmintics drug resistance or problem in the quality of the drugs. Therefore, the objective of this study was to assess if there is anthelmintics drug resistance in the GI nematodes of sheep and goats in Haramaya University farm.

Materials and Methods

Study Area and Duration

The study was conducted in Haramaya University sheep and goat farms from Decmber 2014 to January 2015. The farm is situated at an altitude of 1600 to 2100 meter above sea level, with the mean annual temperature and relative humidity of 18°C and 65%, respectively (Sisay *et al*., 2006a). Geographically, it is located at latitude 09º 24' 10"N and longitude 041º 59' 58" E. There are four seasons in the area; a short rain season (from March to mid-May), a short dry season (from end of May to end of June), a long wet season (early July to mid-October) and a long dry season (end of October to end of February). Haramaya area receives an average annual rain fall of 900 mm, with a bimodal distribution pattern, picking in mid April and mid-August. The vegetation that constitutes the available pasture lands of the university is predominantly native grasses and legumes (HADB, 2009).

Study Animals

The sheep farm consist more than 134 (26 male and 108 female) sheep of different breeds. The flock consists of indigenous black head Ogaden breed (69), Hararghie highland (46), Washera (12), and exotic Dorper pure breed (7). The sheep breeds, except the Dorper, are kept in semi-intensive management system that is they graze the same field with Borena cattle breed and supplemented with concentrate at night. In addition to these, the farm has small paddocks where sheep graze for some time and are housed in separate housing units that accommodate 30-40 sheep per unit. The goat farm has 226 goats (32 male and 194 female) of different breeds and their crosses. Hararghie highland (56), Somali (52), Abargelle (45), Boer (10), Anglo-Nubian (1), and crosses of different breed (30) forms the goat population of the farm at the begging of the study. Hararghie highland, Somali and Abargelle goats are managed in semi-intensive system. All the remaining goat breeds are managed under intensive management. All the different breeds and cross breeds have separate pen. The separate grazing land for goat is also occasionally grazed by cattle, but sheep and goats are almost kept on separate grazing areas with rare cases of grazing the same area one after the other.

The farms have been using anthelmintics to control GI nematodes infection and the most frequently used anthelmintics were albendazole and ivermectin. The treatment frequency is at least twice per year and changed based on the condition of the animals. The conditions used as a justification for deworming were poor appetite, coughing and sneezing, diarrhea and loss of body condition. Sheep and goats that show these clinical signs are treated with the available anthelmintic without confirmatory diagnosis.

Experimental Design

Animals in both farms have identification number and based on their ear tag the animals used for the experiment were selected using simple random sampling technique. Before the actual experiment, equal number of black head Ogaden sheep (40) and Hararghie highland goat (40) were randomly selected considering the dropout during the experiment. Only animals that did not taken anthelmintics for the past 2 months were included in the study. Three gram of fecal materials were collected directly from the rectum by inserting two fingers and placed in universal bottle and labeled with necessary information. The fecal samples were screened in the Veterinary Parasitology laboratory of the University for the presence of nematode eggs within 2 hours after collection. Simple floatation and modified McMaster egg counting technique were used. Only sheep and goats with more than 150 egg per gram (EPG) of faeces were included in the study. The FECRT compares the treatment groups with untreated groups. The efficacies of albendazole and ivermectin were tested and interpreted according to the guideline provided by WAAVP recommendation (Coles *et al*., 1992).

Finally, 60 animals (30 for each species) were selected for the experiment based on the criteria set by WAAVP and assigned randomly to albendazole (10), ivermectin (10) and control (10) groups. Different age groups (6-18 months) and female sheep and goats were used for the study since there were no sufficient male goats in the farms. Individual animals were weighed and the two groups treated according to manufacturers recommended dose orally or subcutaneously depending on the type of drug, but the control groups were not. Albendazole 300mg bolus manufactured by Chengdu Qiankun veterinary pharmaceuticals Co. Ltd, China was used at a dose of 7.5mg/kg, orally. Ivermectin 1% (50ml injection) manufactured by Laboratorios Microsules, Uruguay, was used at a dose of 0.2mg/kg, subcutaneously.

The second rectal sample was taken after 10 days post treatment for albendazole and 14 days for ivermectin with their corresponding control group. The FECs were performed using Modified McMaster counting technique and the changes in the EPG count were recorded with a minimum detection limit of 50 EPG (Cole *et al*., 2006). Furthermore, the EPG was classified as light, moderate and heavy infection for a count of 50 to 799, 800 to 1200 and over 1200, respectively (Urquhart *et al.,* 1996).

Table 1. The degree of infestation of sheep and goats

Degree of Infestation	Number of Goat (%)	Number of Sheep (%)	Total (%)
Light	3 (10%)	10 (33.3%)	13 (21.7)
Moderate	3 (10%)	2 (10%)	5 (8.3)
Heavy	24 (80%)	18 (60%)	42 (70)

Analysis and Interpretation of Data

The effectiveness of albendazole and ivermectin was evaluated on the basis of the reduction in faecal egg count. Calculation of the arithmetic mean, percentage reduction and 95% upper and lower confidence limits was according to Coles *et al.* (1992). A computer program, RESO, Version 2 (Anonymous, 1990) was used for this calculation. Resistance is considered to be present if the percentage reduction in egg count is less than 95% and the 95% confidence level is less than 90%. If only one of the two criteria is met resistance is suspected (Coles *et al.*, 1992).

Results

The percentage reduction of faecal egg counts after treatment with albendazole and Ivermectin was 82% and 68%, respectively (Table 2). The lower confidence limit was within range of resistant parasites for both anthelmintics. The result revealed the development of resistance against albendazole and ivermectin by the GI nematodes of sheep in Haramaya University farm.

Table 2. Mean faecal egg counts and percentage reductions after treatment of sheep with Albendazole and Ivermectin

FECRT summary results	Treatment groups		
	Albendazole	Ivermectin	control
Number of animals (n)	10	10	10
Pre-treatment mean EPG	1640	1645	1370
Post-treatment mean EPG	180	465	1460
Reduction (%)	82	68	-
Upper 95% CI (%)	92	90	-
Lower 95% CI (%)	60	0	-
Interpretation	resistant	resistant	-

EPG= egg per gram; Mean EPG = arithmetic mean of faecal nematode egg counts; Control = Untreated group of animals, CI= confidence interval.

The percentage reduction of faecal egg counts after treatment with albendazole and Ivermectin was 63% and 41%, respectively (Table 3). The lower confidence limit was within range of resistant parasites for both anthelmintics. The result revealed the development of resistance against albendazole and ivermectin by the gastrointestinal nematodes of goats in Haramaya University farm.

Table 3. Mean faecal egg counts and percentage reductions after treatment of goats with Albendazole and Ivermectin

FECRT summary results	Treatment groups		
	Albendazole	Ivermectin	Control
Number of animals (n)	10	10	10
Pre-treatment mean EPG	3100	3455	1685
Post-treatment mean EPG	595	1220	2065
Reduction (%)	63	41	-
Upper 95% CI (%)	81	72	-
Lower 95% CI (%)	29	0	-
Interpretation	resistant	resistant	-

Mean EPG = arithmetic mean of faecal nematode egg counts; Control = Untreated group of animals, CI= confidence interval.

Discussion

The results obtained from the FECRT and 95% confidence limits indicated the presence of resistance to albendazole by the GI nematodes in Haramaya University sheep farm. The current finding was comparable with reports from Western Oromia for albendazole with 98% percentage reduction, 95% upper confidence limit (UCL) 100% and lower confidence limit (LCL) 86% (Aga *et al.,* 2013). Similar result was reported for albendazole against GI nematodes of sheep from Southern Ethiopia by Desie *et al.* (2013) with percentage reduction of 95%, 95%UCL (98.2%) and LCL (86.5%). However, the current finding of GI nematode resistance test against albendazole was contradictory with the reports from Wolaita Soddo by Desie and Amenu *et al.* (2010) with percentage reduction

of 100%, Bersissa and Ajebu (2008) from Hawassa with percent reduction of 100%, Getachew *et al.* (2013) from Bedelle with percentage reduction of 95.6%, 95% UCL (97.6%) and LCL (93.6%). Similarly, Kassahun *et al.* (2005) from Southern Ethiopia reported with percentage reduction of 100% using albendazole against GI nematode in sheep, and Sissay *et al.* (2006b) in experiment done in eastern Ethiopia done in different peasant association with percentage reduction 95% and above, and 95% UCL (≥96%) and LCL (≥91%).

The result obtained from the FECRT and the 95% confidence limits showed the presence of resistance to Ivermectin by the GI nematodes in Haramaya University sheep farm. Ivermectin resistance is not common in GI nematodes of sheep in Ethiopia. However, in other countries, resistance againest GI nematodes of sheep were reported. A study conducted on 25 lambs in New Zealand showed a reduction of the FECs only by 18% which was an indication for the emergence of Ivermectin resistance by the GI nematodes of sheep (Leathwick *et al.*, 2001). However, many reports from Ethiopia showed the effectiveness of ivermectin against GI nematode of sheep. Sissay *et al.* (2006a) from Eastern Ethiopia reported a percentage reduction of (98%), 95% UCL (99%) and LCL (95%), Desie *et al.* (2013) from Southern Ethiopia, with percentage reduction of (96.7%), 95% UCL (100%) and LCL (91%), and Getachew *et al.* (2013) from western Oromia, with percentage reduction of (96.7%), 95% UCL (98.8%) and LCL (94.5%) confirmed the effectiveness of this drug.

The result obtained from the FECRT and the 95% confidence limits showed the presence of resistance to Albendazole by the GI nematodes in Haramaya University goat farm. Similar findings were reported by Sissay *et al.* (2006a) from the same farm (Haramaya University goat farm) before ten years with percentage reduction of (57%), 95% UCL (63%) and LCL (49%) which confirmed the persistence and heritability of resistant genes in nematodes both in the host and in the refugia population for more than a decade. Desie *et al.* (2013) from Southern Ethiopia reported suspected resistance to albendazole against GI nematodes of goats with percentage reduction of (99.6%), 95% UCL (99%) and LCL (88.3%). On the other hand, Sissay *et al.* (2006b) from Eastern Ethiopia (Haramaya district small holder's goat flock) reported the effectiveness of albendazole with percentage reduction of (95%), 95% UCL (97%) and LCL (94%). This might be due to difference in the study area (different district), both the parasite population in the host and the refugia are different.

The present finding obtained from the FECRT and 95% confidence limits indicated the presence of resistance to Ivermectin by the GIT nematodes in Haramaya University goat farm. Similar result was reported by Sissay *et al.* (2006a) from the same farm before a decade with percentage reduction of 67%, 95% UCL (73%) and 95% LCL (60%). This might indicate the transfer of resistance genes vertically within generations for several years and its persistence after development in an area. On the other hand, many research findings were contradictory to the current finding of GI nematode resistance against ivermectin in Haramaya University goat farm. A study conducted by Sissay *et al.* (2006b) on goats in Eastern Ethiopia against GI nematodes indicated a percentage reduction of 96%, 95% UCL (97%) and 95% LCL (93%). Similarly, Desie *et al.* (2013) from southern Ethiopia reported a percentage reduction of 97.1%, 95% UCL (99.1%) and 95% LCL (99.0%).

The present finding confirmed the development of resistance for albendazole and ivermectin by GI nematodes of sheep and goats in Haramaya University farms. Different factors might contribute for the development of anthelmintics resistance in the farm such as the treatment frequency, inaccurate dosage determination, and indiscriminate use of anthelmintic, treatment without confirmatory diagnosis and management system (Suarez and Cristel, 2014). Besides these, the substandard drug quality might be the other probable reason for the ineffectiveness of the drug which needs to be investigated in the future in order to be sure about the cause for the development of resistance in both species.

In the majority of cases treatment frequencies in Haramaya University farms were influenced by clinical conditions of animals such as poor body conditions, coughing, sneezing, diarrhoea, emaciation and poor appetite. Indiscriminate use of anthelmintic will favor the selection pressure in which the susceptible population will extinct in the area whereas the resistant population will dominate both in the host and refugia.
The other contributing factor for the development of resistance in the farms were animal weight estimation by guessing to calculate the dosage which might accounted for error such as under or overdosing. Under dosing has been repeatedly blamed for the buildup of resistance which permits the survival of resistant heterozygous individuals and increases their chances of producing highly resistant parasites (Vadlejch *et al.*, 2014). Another factor that could explain the high levels of resistance in the farms were the use of the same drugs for many years in the same area. Frequent and longstanding use of the same anthelmintic is among the means to efficiently select one sub population of worms that presents the capability to survive for a particular type of drug. On the contrary, alternate use of the anthelmintic family reduces the effect of the selection pressure exerted by each type of drug and resistance development (Vadlejch *et al.*, 2014).

The management system in the farms is generally poor, the same grazing land was used before and after anthelmintic treatment which predisposed animals for the re-infection and contamination of the environment with surviving resistant strains. The gaps of the current study were inability to perform copro-culture before and after treatment, and lack to identify which species of nematodes developed resistance.

Moreover, the efficacy of the starting materials (drugs) is not known, hence it is not possible to directly declare that there is resistance; in previous reports, there was an evidence that dosage regimen for goats should be higher than sheep and manufacturers recommended doses usually are said not to be working. Hence different dose regimens should have been used in goats.

Conclusion and Recommendations

The growing resistance of GI nematodes of sheep and goats, to available veterinary anthelmintics, is a serious problem and a global issue. Our study findings indicated that GI nematodes of sheep and goats, in Haramaya University farm, might be resistant to albendazole and ivermectin. Based on literature and observations during the study period, it can be recommended that the farm should refrain from using frequent and unnecessary treatments, implement alternative use of anthelmintics such as a change of drugs every two/three years, reduce dependence on anthelmintics by employing management practices such as rotational grazing, improve the management of the farms and implement correct dosing of anthelmintics. Future researches should focus on the identification of resistant species by copro-culture before and after treatment, and perform *in vitro* egg hatchability assay using known standard drugs.

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Conflict of Interests

The authors declare that they have no competing interests.

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Protective Efficacy of *Lepidium sativum*, *Capsicum frutescens* and their Mixtures against Experimentally Induced *Eimeria tenella* Infection in Broiler Chickens

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Abstract: This study was aimed at evaluating the anticoccidial efficacy of *Lepidium sativum* (Garden cress (GC)), *Capsicum frutescens* (Hot red pepper (Hrp)) and their mixtures powder in broiler chickens. A total of 144 Cobb-500 broiler chickens were randomly allocated into six treatment groups with three replications. The experiment lasted for 42 days. Rations were fortified with 0.75% GC, 0.75% Hrp, 0.38% GC+0.38% Hrp and 0.0125% amprolium in groups 3, 4, 5, and 6, respectively and fed to the chicks starting on day two of age. Ration fed to chicks in group 1 and 2 were without any additives. Chicks in groups 2, 3, 4, 5 and 6 were infected with ~10^5 sporulated *Eimeria tenella* oocyst per chick at the age of 15 days. Animal performance, oocysts output, cecal lesion score, carcass and serum parameters were recorded during the experiment. Uninfected-unfortified and amprolium ration groups in the starter period and group that received ration fortified with a mixture of GC+Hrp and uninfected-unfortified ration in the finisher phase resulted in a higher body weight gain (BWG). Across the entire experimental period, BWG was higher in the uninfected-unfortified ration group. The average feed intake in the entire period was higher in uninfected-unfortified ration, GC and amprolium groups. Broiler chicks fed a diet fortified with GC 0.75% or amprolium (0.0125%) as additive were equally effective to reduce *E. tenella* oocyst shed at day 6, 7, 8 and average total count post inoculation. Infected chickens fed diet fortified with GC, Hrp and their mixtures showed cecal lesion similar to those fed with infected-unfortified diet group at day 7 post inoculation. Highest intestinal length at 27 days post inoculation was observed in the uninfected-unfortified ration group and the shortest length was noticed in infected-unfortified ration group. In conclusion, broilers fed diet fortified with GC 0.75%, Hrp 0.75%, GC 0.38%+Hrp 0.38% mixture and amprolium 0.0125% showed better BWG at the end of the production phase than infected-unfortified ration group. Garden cress and amprolium lowered oocyst shed indicating better protection against *E. tenella* infection.

Keywords: *Cecal lesion; Eimeria tenella; Feed additive; Oocyst shed; Phytogenic*

Introduction

Coccidiosis is a protozoan disease caused by *Eimeria* species that affect the intestinal tract of poultry and cause considerable economic loss to the poultry industry (Dalloul and Lillehoj, 2005). This disease causes moderate to severe damage to the intestinal epithelium, resulting in reduced growth rate, impaired feed conversion and often overt morbidity and mortality in chicken (Chandrakesan *et al*., 2009). Among the nine different *Eimeria* species known to infect chickens, *E. tenella* infection causes a severe disease characterized by hemorrhagic lesion development in the cecum leading to high morbidity and mortality, and reduced weight gain (McDougald and Fitz-Coy, 2008). In Ethiopia, the incidence of poultry coccidiosis has been reported to exist in many parts of the country. Lobago *et al.* (2005) reported 38% mortality in Kombolcha Poultry Multiplication Center and Dinka and Yacob (2012) noted 72% mortality in Debre zeit Agricultural Research Center poultry farm due to coccidiosis outbreak.

Currently, coccidiosis control programs in the poultry industry across the world are largely relying on chemotherapy and immunoprophylaxis measures. While these methods are effective for the control of avian coccidiosis, the continuous uses of anticoccidial drugs have led to the emergence of drug-resistant strains (Abbas *et al*., 2008). Furthermore, drug residue in poultry products has been a potential deterrent to the use of poultry products by consumers. Producers also incur high costs for medication, which results to increased cost of poultry products. Hence, searching for natural, cheap and safe alternative means of treatment against the control of coccidiosis infection has been an area of research in the recent past (Williams, 2006). Accordingly, anticoccidial properties of various natural products were reported (Abbas, 2012; Meskerem and Bookaewan, 2013). Herbs and spices rich in alkaloids, antioxidants (Naidoo *et al.,* 2008) and diets high in n-3 fatty acids (Allen *et al.,* 1996, 1997) are reported to have value for treating coccidiosis in chickens.

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Diwakar *et al.* (2010) noted that the essential oil derived from *Lepidium sativum* (garden cress (GC)) seed contains tocopherol, carotenoid, oleic acid and α-linolenic acid. Garden cress seed possesses varied medicinal properties and is known as "versatile medicine". It has been used to treat various kinds of human and animal ailments such as diarrhea, dysentery, unidentified gastrointestinal disorder, stomach-ache, indigestion, febrile disease and skin disorders (Teklehaymanot *et al.*, 2007). It also has antihypertensive, diuretic and hypoglycaemic effects (Maghrani *et al.*, 2005), known to improve asthmatic attacks (Paranjape and Mehta, 2006), and possesses aperients, alterative, tonic and carminative activities (Sumangala *et al.,* 2004; Patel *et al.,* 2009). Similarly, *Capsicum frutescens* (hot red pepper (Hrp) is said to have a pungent taste because of capsaicin and it is used as spice, feed additive and drug (Nwaopara *et al.*, 2007). Other biologically active phytochemical constituents in this plant include alkaloids, mucilages, reducing compounds, sterols and polyterpenes (Dougnon *et al.*, 2014). These components gives Hrp several pharmacological properties especially against obesity, hyperglycemia, hypercholesterolaemia, hyperlipidaemia (Takashi *et al.,* 2004), pain, gastric ulcer (Suk-Hyun *et al.*, 2006), pneumonia (Newall *et al.,* 1996), diarrhea and inflammation. Moreover, Hrp has been reported to have antioxidative (Sun *et al.,* 2007), immunomodulatory (Takano *et al.,* 2007) and anti-carcinogenic (Suk-Hyun *et al.*, 2006) effects. Furthermore, there is preclinical and clinical evidence that Hrp may have beneficial actions in protecting against lesion formation in the gastric and intestinal mucosa in part by alleviating oxidative stress (Holzer, 2004). Despite the aforementioned manifold health benefits of GC and Hrp, their natural anticoccidial effects against broiler coccidiosis was not sufficiently investigated. Therefore, the present study aimed to investigate the effects of Garden cress, Hot red pepper and their mixtures powder against *E. tenella* in experimentally infected Cobb-500 broiler chickens.

Materials and Methods

Experimental Site, Feed Ingredients and Ration Formulation

The experiment was conducted at Haramaya University poultry farm, Ethiopia, east of the capital Addis Ababa. The feed ingredients used to formulate the experimental rations are presented in Table 1. After contaminant materials were removed, the GC and Hrp were hammer milled to powder. Corn grain, noug seed meal and limestone were milled through a sieve size of 5 mm. Six standard isocaloric and isonitrogenous starter and finisher treatment rations were formulated (Table 1). The rations were formulated to meet the nutrient requirement of starter and finisher broilers as described by Leeson and Summers (2005).

Table 1. Ingredients used to formulate experimental rations and their chemical composition

	Treatment groups											
	G1		G2		G3		G4		G5		G6	
Ingredients%	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher
Corn grain	50	57	50	57	50	57	50	57	50	57	50	57
Noug Seed Meal	17	17.3	17	17.3	16	17.3	16	16.6	16.5	17.3	17	17.3
Soybean Meal	30	23	30	23	30	23	30	23	30	23	30	23
Garden Cress	-	-	-	-	0.75	0.75	-	-	0.38	0.38	-	-
Hot red pepper	-	-	-	-	-	-	0.75	0.75	0.38	0.38	-	-
Amprolium	-	-	-	-	-	-	-	-	-	-	0.0125	0.0125
Premix*	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.4
Limestone	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DL-meth.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
L-lysine HCL	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Dry matter (%) and nutrient contents (%DM) of the ration												
Dry Matter	93	93	93	93	92.7	93	92.7	93	92.7	93	93	93
Crude Protein	22.6	20.3	22.6	20.3	22.5	20.3	22.55	20.3	22.55	20.3	22.6	20.3
Ether extract	4.75	4.62	4.75	4.62	4.66	4.62	4.66	4.62	4.66	4.62	4.75	4.62
Crude fiber	7.17	7.0	7.17	7.0	7.2	7.0	7.4	7.0	7.3	7.0	7.17	7.0
ME (kcal/kg DM)	3171	3218	3171	3220	3178	3221	3172	3218	3174	3221	3189	3218
Calcium	0.93	0.91	0.93	0.91	0.92	0.91	0.92	0.91	0.92	0.91	0.93	0.91
Phosphorus	0.58	0.3	0.58	0.3	0.58	0.3	0.58	0.3	0.58	0.3	0.58	0.3

**Vitamin and mineral premix =25 kg contains: Vitamins: Vit. A (E672), 75,000,000 IU; Vit. D3 (E671), 25,000,000 IU; Vit. E (all-rac-alpha tocopherylacetate) (3a700), 20,000 mg; Vit. K3, 2,000 mg; Vit. B1, 1,500 mg; Vit. B2 (riboflavin), 5,000 mg; Vit. B3 (calcium-D-pantothenate), 9,001 mg; Vit. B6 (3a831), 5,000 mg; Vit. B12 (cyanocobalamin), 25,000 mcg; Vit. pp (nicotinic acid), 30,003 mg; Folic Acid, 1,000 mg; Biotin, 100,000 mcg, Choline, 648,750 mg; Minerals: Iron, 45,000; Copper (Cu, E4), 15,000 mg; Manganese (Mn, E5), 75,001 mg; Zinc oxide-Zinc (Zn, E6), 70,001 mg; Iodine (I, E2), 2,000 mg; Selenium (Se, E8), 400,050 mcg; Calcium, 1,231,662 mg; Magnesium, 12,687 mg; Sodium, 952 mg; Chloride, 185,313 mg; BHT, 500 mg. DM: Dry matter; ME: Metabolizable energy; G1: Uninfected-unfortified ration; G2: Infected-unfortified ration; G3: Infected-0.75% Garden cress; G4: Infected-0.75% Hot red pepper; G5: Infected-0.38% Garden cress+0.38% Hot red pepper; G6: Infected-0.0125% Amprolium.*

Managements of Experimental Broilers

The experimental house and pens, watering and feeding troughs were thoroughly cleaned, disinfected and sprayed against external parasites before placing the birds. The chicks were vaccinated with HB1 at day 7 as eye drop as a preventive treatment against Newcastle disease. The chicks were brooded using 250 watt infrared electric bulbs as sources of heat and light. *Teff* straw was used as a litter material at a depth of approximately 7 cm. Feed and clean tap water were offered *ad libitum* throughout the experiment.

Experimental Design and Treatments

The experiment was carried out using a completely randomized design (CRD). One hundred forty four unsexed day old Cobb-500 broiler chickens with initial body weight of 38.5 ± 0.82 g (mean ± SD) were randomly allotted into six dietary groups each consisting three replicates of 8 chicks (Table 2). At the age of 15 days, all chickens except those in group 1 were orally inoculated with ~10^5 sporulated *E. tenella* oocysts per 1 mL of inoculums (Long *et al.,* 1976) using a calibrated syringe. Chicken in group 3, 4, 5 and 6 were fed with ration fortified with 0.75% GC, 0.75% Hrp, 0.38% GC+0.38% Hrp mixture and 0.0125% amprolium, respectively from 2 to 42 days of age. The birds were monitored daily for the presence of clinical signs.

Table 2. Layout of the experiment

Groups		Chicks/ treatment	Oocysts challenge
G1	Uninfected-unfortified ration	24	–
G2	Infected-unfortified ration)	24	+
G3	0.75% *Lepidium sativum* seed powder	24	+
G4	0.75% *Capsicum frutescens* powder	24	+
G5	0.38% *L. sativum* + 0.38% *C. frutescens* mixture	24	+
G6	0.0125% amprolium	24	+

Oocysts challenge = Challenge with ~100,000 sporulated E. tenella oocysts; (-) = No infection; (+) = Infection with sporulated E. tenella oocysts.

Isolation and Propagation of Eimeria tenella Oocysts

Eimeria tenella oocysts were identified by a combination of oocysts size, location in the gut and appearance of the lesions (McDougald and Fitz-Coy, 2008). Following evisceration at post-mortem of coccidia suspected birds, the cecal contents were washed into a beaker using tap water and the oocysts were isolated using a flotation procedure (Permin and Hansen, 1998). Oocysts were sporulated by incubating concentrated suspension of oocysts in distilled water with 2.5% potassium dichromate solution ($K_2Cr_2O_7$) and with forced aeration at room temperature for 72 hours (Bowman, 2009). The sporulated *E. tenella* oocysts were suspended in 2.5% $K_2Cr_2O_7$ solution and refrigerated at 4 ºC until oral administration. The $K_2Cr_2O_7$ solution was removed through 5 times centrifugation by distilled water and the sporulated *E. tenella* oocysts were suspended in distilled water at the time of oral administration. The sporulated *E. tenella* oocysts were orally inoculated in three chickens for oocyst multiplication. Chickens were monitored daily for the development of clinical coccidiosis and the presence of *Eimeria* oocysts in their feces. Then the reproduced oocysts were sporulated, stored and washed as described earlier for the actual experiment.

Data Collection

Intake and body weight gain: The amount of feed offered and refused was recorded daily and the amount consumed was determined as the difference between the feed offered and refused. Birds were weighed weekly in a group per pen and pen average was calculated by dividing the total pen weight by the broilers alive on that day. Body weight (BW) change was calculated as the difference between the final and initial BW. Average daily gain (ADG) was calculated as BW change divided by the number of experimental days. Feed conversion ratio was computed as the ratio of daily DM consumption per ADG. The general health of the birds daily and mortality as occurred were also recorded.

Estimation of oocyst: Fresh fecal droppings from the ground of each pen were collected in sterile universal plastic bottles from all experimental groups on day 14 and oocysts counted were recorded as pre-inoculation measure. Oocyst count per gram of feces (OPG) was determined by McMaster egg counting technique and calculated using the technique described by Permin and Hansen (1998). During the post-inoculation period, fecal samples in each pen were collected from randomly selected sites on days 4, 5, 6, 7, 8, 9, 14, 20 and 27, mixed well, and the oocysts were counted and recorded.

Pathological study: On day 7 and 27 post inoculation, three randomly selected chickens from each replicate were euthanized by cervical dislocation for cecal lesion scoring according to the method of Johnson and Reid (1970). The scoring scales ranged from 0 to +4, where: 0 = no lesion (the wall is thin and presents characteristic longitudinal groves and mucosal folds; the contents are homogenous and creamy and do not contain any particles; the color is chestnut), +1= mild lesion (with few scattered petechia, the cecal contents are normal; the petechiae are more visible on the serous membrane than on the mucosal side), +2= moderate lesion (with numerous petechia, bleeding and the cecal wall is slightly thickening; visible bloody contents at the proximal end), +3= severe lesion (with severe bleeding and clotting, the caecal walls are greatly thickened; the feacal contents have practically disappeared) and +4= extremely severe lesion (with severe bleeding, a much thickened or

rupture of caecal wall, feacal debris is no longer visible; it is enclosed in the caseous plugs; gangrene or death, assigned to dead fowl). Cecal and intestinal length (gizzard to cloaca) was also measured.

Carcass measures: At the end of the experiment, three randomly selected broilers' used for cecal lesion scoring at 27 days post-mortum were selected randomly from each replicate, starved for 12 hours, weighed and slaughtered. Birds were eviscerated and carcass cuts and non-edible offal components were determined according to the procedure described by Kekeocha (1985). Dressed weight was measured after the removal of blood and feather and the dressing percentage was calculated as the proportion of dressed carcass weight to slaughter weight. Eviscerated carcass weight was determined after removing blood, feather, shank, head, kidney, lungs, pancreas, crop, proventriculus, small intestine, large intestine, caeca and urogenital tracts. The eviscerated percentage was determined as the proportion of slaughter weight. Drumstick, thigh, breast meat, heart, gizzard and liver were separated, weighed and calculated as a percentage of slaughter weight. Fat around the proventriculus, gizzard, against the abdominal wall and the cloacae were separated, weighed and expressed as a percentage of slaughter weight.

Serum biochemical parameters: Blood for serum biochemical analysis were collected from the same birds slaughtered for caecal lesion analysis. Each blood sample was collected without anticoagulant for serum biochemical analysis. Serum was separated after centrifugation at 3,000 rpm x g for 15 min and stored at -20 ^{0}C until analyzed. Total serum protein was determined by refractometer (George, 2001). Total serum immunoglobulin concentration was determined by serum zinc sulfate turbidity test by reading the optical density of the test and the control separately at 545 nm by using spectrophotometer (Mondesire, 2004).

Data Processing and Analysis

Data were analyzed using GLM model in a completely randomized design (CRD). Data analysis was done using SAS (SAS Institute, 2008). Duncan's multiple range test was used to detect the differences ($p<0.05$) among group means. Prior to statistical testing, mortality values were logarithmically transformed [$\log10^{(X+1)}$] to create a normal distribution. The difference between treatment groups were considered significant at $p < 0.05$ level.

Results

Feed Intake, Body Weight Gain, Feed Conversion Ratio, and Mortality

Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) during the first 15 days (pre-inoculation period) were not different ($p>0.05$) among treatment groups (Table 3). However, BWG and FCR were better during the third week (first week after inoculation) in uninfected-unfortified group followed by GC and amprolium received groups. At the end of week 4 higher BWG was recorded for uninfected-unfortified group. Overall BWG at the end of starter phase (1–21 days) was higher ($P<0.05$) in uninfected-unfortified and amprolium ration groups (Table 3). In the finisher phase (22–42 days) BWG in groups uninfected-unfortified and group consumed ration fortified with GC+Hrp mixture were not different and higher ($p<0.05$) than GC, Hrp and amprolium ration groups. When considered for the entire period of broiler production (1-42 days) BWG was highest for the uninfected-unfortified ration group, lowest for the infected-unfortified ration group, and intermediate for the other groups ($p<0.05$). However, FCR for the entire period was unaffected by treatment groups ($p>0.05$). Feed intake was lower for infected-unfortified treatment as compared to the uninfected-unfortified, 0.75% GC and amprolium groups.

Mortality was higher in the starter (1–21 days) than the finisher phase of the experiment with no deaths recorded in the group consumed ration fortified with 0.75% GC and 0.38% GC+0.38% Hrp mixture. In the finisher phase (22–42 days), mortality was recorded only in uninfected-unfortified and amprolium ration groups. However, mortality rate was not significant among the groups over the entire production period.

Table 3. Performance and mortality rate of broilers fed ration fortified with garden cress, hot red pepper, their mixture or amprolium and inoculated with *Eimeria tenella* oocysts

		Treatment groups						
Age	Parameters	G1	G2	G3	G4	G5	G6	SEM
Week 1	Initial weight (g/bird)	39.3	38.7	38.6	39.4	37.6	37.6	0.8
	BWG (g/bird)	104	107	109	102	91.0	108	6.12
	FI (g/bird/day)	23.6	23.1	22.5	23.5	21.7	23.7	0.96
	FCR (feed:gain)	1.54	1.52	1.48	1.62	1.70	1.55	0.08
Week 2	BWG (g/bird)	149	143	146	144	141	154	6.23
	FI (g/bird/day)	50.4	48.6	48.5	46.3	46.4	50.1	1.39
	FCR (feed: gain)	2.37	2.39	2.35	2.27	2.32	2.28	0.10
Week 3	BWG (g/bird)	286^{a}	204^{c}	237^{b}	219^{bc}	207^{c}	241^{b}	9.93
	FI (g/bird/day)	80.5	76.4	79.0	76.4	71.2	76.4	3.48
	FCR (feed:gain)	1.97^{d}	2.62^{a}	2.34^{bc}	2.44^{b}	2.44^{b}	2.24^{c}	0.04
Week 4	BWG (g/bird)	301^{a}	231^{b}	233^{b}	237^{b}	246^{b}	248^{b}	13.7
	FI (g/bird/day)	107	94.1	90.9	91.5	94.2	100	4.98
	FCR (feed:gain)	2.50^{bc}	2.85^{a}	2.51^{bc}	2.72^{ab}	2.44^{c}	2.83^{a}	0.07
Week 5	BWG (g/bird)	377^{a}	289^{c}	329^{bc}	345^{ab}	387^{a}	354^{ab}	13.9
	FI (g/bird/day)	138	114	132	125	127	134	6.43
	FCR (feed:gain)	2.56^{ab}	2.76^{a}	2.81^{a}	2.55^{ab}	2.30^{b}	2.64^{ab}	0.11
Week 6	BWG (g/bird)	320	349	340	358	380	325	17.0
	FI (g/bird/day)	137	120	130	135	135	134	6.08
	FCR (feed:gain)	2.97a	2.41^{c}	2.68^{b}	2.63^{b}	2.49^{c}	2.88^{a}	0.04
Week 1-3	BWG (g/bird)	543^{a}	453^{bc}	485^{bc}	465^{bc}	437^{c}	502^{ab}	16.3
	FI (g/bird/day)	51.5	49.4	49.7	48.8	46.4	50.3	1.41
	FCR (feed:gain)	1.96^{b}	2.18^{a}	2.05^{ab}	2.11^{ab}	2.15^{a}	2.02^{ab}	0.05
	Mortality (%)	0.00^{b}	0.46^{ab}	0.00^{b}	0.83^{a}	0.00^{b}	0.23^{ab}	0.14
Week 4-6	BWG (g/bird)	998^{a}	869^{c}	922^{b}	939^{b}	1019^{a}	927^{b}	17.2
	FI (g/bird/day)	127^{a}	110^{c}	120^{ab}	117^{bc}	115^{bc}	123^{ab}	2.94
	FCR (feed:gain)	2.68^{a}	2.69^{a}	2.65^{a}	2.63^{a}	2.41^{b}	2.78^{a}	0.11
	Mortality (%)	0.23	0.00	0.00	0.00	0.00	0.23	0.06
Week 1-6	BWG (g/bird)	1541^{a}	1322^{c}	1407^{b}	1404^{b}	1456^{b}	1429^{b}	21.4
	FI (g/bird/day)	89.2^{a}	79.4^{c}	84.7^{ab}	82.9^{bc}	81.6^{bc}	86.5^{ab}	1.77
	FCR (feed:gain)	2.32	2.43	2.36	2.37	2.28	2.40	0.04
	Mortality (%)	0.23	0.46	0.00	0.83	0.00	0.37	0.21

[a-c]Means within a row and under treatment groups with different superscripts differ ($p<0.05$); SEM: Standard error of the mean; BWG: Body weight gain; FI: Feed intake; FCR: Feed conversion ratio

Oocysts Shed, Lesion Score and Intestinal Length

Peak oocysts shed was observed on day 6 and 7 post inoculation and then after reduced until day 9 post inoculation. However, amprolium supplemented groups showed fast reduction of oocysts shed on day 8 post inoculation. Diet consisting 0.75% GC or 0.0125% amprolium significantly reduced oocyst counts compared to the group infected and not fortified with the feed additives, feed fortified with Hrp or a mixture of GC+Hrp groups (Table 4). The total oocyst shed for the entire count was not significantly different between amprolium and GC fortified groups. The highest average oocysts shed across the post inoculation period were detected in infected-unfortified, Hrp and GC+Hrp fortified groups.

Table 4. *Eimeria tenella* oocyst excretions of broilers fed diet fortified with garden cress, hot red pepper, their mixtures or amprolium inoculated with *Eimeria tenella* oocysts

	Treatments (Oocyst per gram of feces * 1000)						
Days	G1	G2	G3	G4	G5	G6	SEM
Pre-inoculation	0.00	0.00	0.12	0.02	0.02	0.03	0.05
Day 4 PI	0.03	0.25	0.22	0.10	0.12	0.07	0.06
Day 5 PI	0.52^{b}	8.80^{a}	6.37^{ab}	12.3^{a}	5.85^{ab}	11.2^{a}	2.42
Day 6 PI	0.10^{c}	99.6^{a}	23.7^{bc}	101^{a}	71.0^{ab}	54.4^{abc}	20.9
Day 7 PI	0.00^{b}	176^{a}	77.7^{b}	86.7^{ab}	174^{a}	60.0^{b}	28.5
Day 8 PI	0.50^{d}	47.0^{bc}	42.9^{bc}	75.4^{b}	136^{a}	15.2^{cd}	12.5
Day 9 PI	0.07	9.67	18.4	9.73	11.1	7.03	4.8
Day 14 PI	1.70	19.7	15.6	32.9	19.9	15.0	14.2
Day 20 PI	26.4	11.2	9.87	17.4	3.00	0.73	9.37
Day 27 PI	5.85	0.90	0.60	0.58	7.90	0.38	3.54
Total count	35.2^{c}	373^{a}	196^{b}	337^{a}	429^{a}	164^{b}	31.8

$^{a-d}$ Means within a row with different superscripts differ (p<0.05); SEM: Standard error of the mean; PI: Post-inoculation; G1: Uninfected-unfortified ration; G2: Infected- unfortified ration; G3: Infected-0.75% Garden cress; G4: Infected-0.75% Hot red pepper; G5: Infected-0.38% Garden cress+0.38% Hot red pepper; G6: Infected-0.0125% Amprolium.

Cecal lesion was significantly different (p<0.05) among treatment groups at 7 days post inoculation (Table 5; Fig. 1). Infected broilers fed a diet for tified with 0.75% GC, 0.75% Hrp and their mixtures showed higher cecal lesion similar to those in infected-unfortified ration group. As expected, no lesions were observed in the cecum of uninfected-unfortified and amprolium fortified groups at 7 days post-inoculation. Significantly higher cecal lesion score was observed in the uninfected-unfortified ration group followed by 0.75% GC fortified ration groups at 27 days post-inoculation. Shortest intestinal length (from gizzard to cloaca) at 7 days post-inoculation was recorded in uninfected-unfortified ration group followed by infected 0.38% GC+0.38% Hrp fortified ration groups. Longest length at 27 days post-inoculation was observed in the uninfected-unfortified ration group followed by infected 0.75% GC ration group and the shortest length was noticed in infected-unfortified ration group.

Table 5. Intestinal length and cecal lesion score of broilers fed with diet fortified with garden cress, hot red pepper, their mixtures, or amprolium and inoculated with *Eimeria tenella* oocysts

	Treatment groups						
Parameters	G1	G2	G3	G4	G5	G6	SEM
22 days of age (7 days post inoculation)							
Slaughter weight (g)	529	510	506	519	458	535	34.0
Cecal length (cm)	11.5	12.7	11.3	12.2	10.6	12.1	0.71
Intestinal length (cm)	121^{c}	137^{ab}	140^{ab}	147^{a}	130^{bc}	147^{a}	4.94
Cecal lesion score	0.14^{b}	1.83^{a}	2.33^{a}	2.19^{a}	2.17^{a}	0.00^{b}	0.47
42 days of age (27 days post inoculation)							
Slaughter weight (g)	1574	1398	1568	1524	1541	1523	72.8
Intestinal length (cm)	195^{a}	158^{c}	187^{ab}	179^{abc}	180^{abc}	170^{bc}	7.61
Cecal length (cm)	17.2	17.2	18.5	18.6	17.8	17.5	0.83
Cecal lesion score	0.67^{a}	0.00^{b}	0.25^{ab}	0.00^{b}	0.00^{b}	0.00^{b}	0.15

$^{a-c}$ Means within a row and under treatment groups with different superscripts differ (p<0.05); SEM: Standard error of the mean; G1: Uninfected-unfortified ration; G2: Infected-unfortified ration; G3: Infected-0.75% Garden cress; G4: Infected-0.75% Hot red pepper; G5: Infected-0.38% Garden cress+0.38% Hot red pepper; G6: Infected-0.0125% Amprolium.

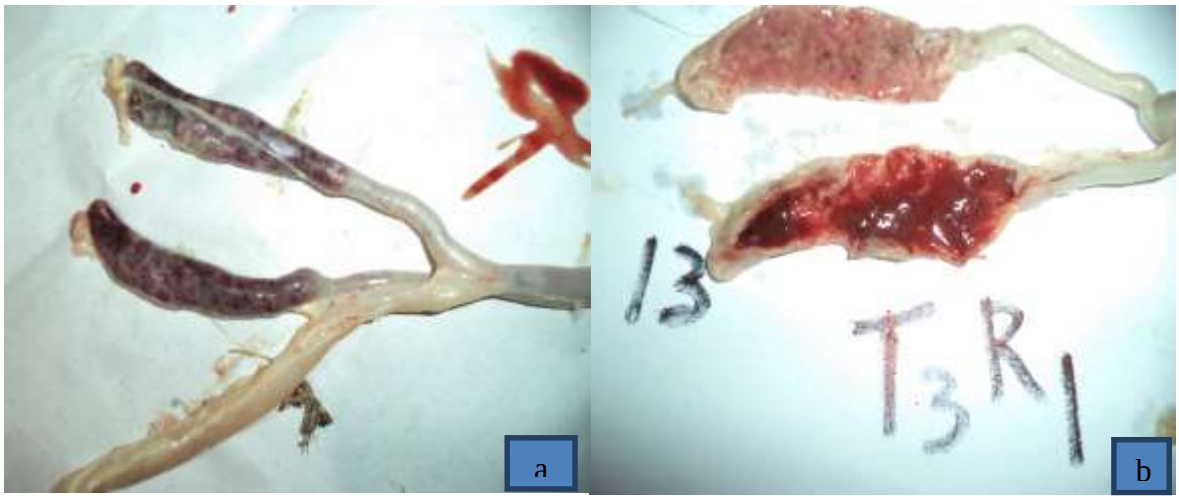

Figure 1. Gross lesions of *Eimeria tenella* oocysts infected broiler chicken caeca. a: Unopened ceca severely affected and distended with blood; b: Opened filled with blood.

Carcass Measures

Slaughter weight, dressed weight, eviscerated percentage, carcass, breast, drumstick, thigh, heart, liver and abdominal fat percentage were not significantly different ($p>0.05$) among the treatments. However, lower percentage of gizzard was observed in amprolium followed by GC+Hrp mixture and infected-unfortified ration groups (Table 6).

Table 6. Carcass characteristics of *Eimeria tenella* oocysts inoculated broilers fed with diet fortified with garden cress, hot red pepper, their mixtures or amprolium

	Treatment groups						
Parameters	G1	G2	G3	G4	G5	G6	SEM
Slaughter weight (g)	1574	1398	1568	1524	1541	1523	72.8
Dressed weight (g)	1438	1284	1437	1391	1412	1393	70.6
Dressing percentage	91.3	91.8	91.6	91.2	91.6	91.4	0.47
Eviscerated weight (g)	1143	1017	1117	1107	1097	1098	60.2
Eviscerated percentage	72.6	72.6	71.3	72.6	71.1	71.8	1.24
Carcass weight (g)	1065	952	1042	1032	1027	1030	58.2
Carcass percentage	67.7	67.9	66.5	67.7	66.6	67.4	1.32
Breast percentage	24.1	22.7	24.3	23.0	23.5	23.6	1.04
Drumstick percentage	8.68	8.51	8.88	8.70	8.92	8.71	0.27
Thigh percentage	10.2	10.4	10.2	10.5	10.4	10.0	0.33
Thigh + drumstick (%)	18.9	18.9	19.0	19.2	19.3	18.7	0.52
Heart percentage	0.58	0.67	0.64	0.68	0.61	0.64	0.06
Liver percentage	2.14	2.08	2.25	2.24	2.21	2.26	0.10
Gizzard percentage	1.93^{a}	1.86^{ab}	1.91^{a}	2.11^{a}	1.82^{ab}	1.55^{b}	0.11
Abdominal fat (%)	1.68	1.93	1.42	2.06	2.07	2.13	0.37

[a,b] Means within a row and under treatment with different superscripts differ ($p<0.05$); SEM: Standard error of the mean; G1: Uninfected-unfortified ration; G2: Infected- unfortified ration; G3: Infected-0.75% Garden cress; G4: Infected-0.75% Hot red pepper; G5: Infected-0.38% Garden cress+0.38% Hot red pepper; G6: Infected-0.0125% Amprolium.

Serum Biochemical Parameters

The effect of experimental diets on some serum biochemical parameters of broiler chickens at 22 days of age (7 days post-inoculation) is given in Table 7.

There was no significant differences ($p<0.05$) among groups in serum total protein and total serum immunoglobulin concentration.

Table 7. Serum parameters of broilers fed diet fortified with garden cress, hot red pepper, their mixtures or amprolium and inoculated with *E. tenella* oocysts at 7 days post inoculation

	Treatment groups						
Parameters	G1	G2	G3	G4	G5	G6	SEM
Total protein (g/dl)	3.10	2.80	2.70	3.03	2.33	3.20	0.23
TIG (mg/dl)	2.03	1.61	1.91	1.84	1.24	1.73	0.24

SEM: Standard error of the mean; TIG: Total immunoglobulin; G1: Uninfected-unfortified ration; G2: Infected-unfortified ration; G3: Infected-0.75% Garden cress; G4: Infected-0.75% Hot red pepper; G5: Infected-0.38% Garden cress+0.38% Hot red pepper; G6: Infected-0.0125% Amprolium.

Discussion

Improved growth performance and feed intake of *E. tenella* infected broilers fed ration fortified with GC, Hrp, their mixture or amprolium than the infected-unfortified ration group in the finisher and entire production period indicate that such feed additives play a role in the prevention of *E. tenella* in broilers. This could be an attribute of the immuno-stimulant or the appetite and digestive enzymes stimulating properties of GC and Hrp (Jang *et al.,* 2007) due to the different bioactive ingredients in these additives (Frankic *et al.,* 2009). Feed supplements with herbs and spices increase stability of feed and beneficially influence the gastrointestinal ecosystem mostly through growth inhibition of pathogenic microorganisms, which consequently reduce exposure of the digestive system to the toxins of microbiological origin (Windisch *et al*., 2008). Subsequently, herbs and spices help to increase the resistance of the animals exposed to different stress situations and increase the absorption of essential nutrients, and improve the growth of animals (Windisch *et al*., 2008). In his review, Rosen (1995) noted that supplementation with ionophores or phenolic compounds improve performance of fast-growing broilers. Meskerem and Bookaewan (2014) also reported significantly higher BWG for chickens challenged with *E. tenella* and fed diet containing *L. sativum* than those fed the control diet. Significantly lower BWG in infected-unfortified diet group following the infection was a consequence of reduced feed intake and the probably disrupted intestinal integrity, which might have affected the absorption of nutrients and the efficiency of feed utilization, which is ascribed to be the common effects of coccidiosis (Walk *et al*., 2011).

No mortality was observed in all phases of production period in groups fed diet fortified with GC or GC+Hrp even though no significant difference was recorded among the treatments. The result of the work by Meskerem and Bookaewan (2014) also demonstrated that supplementation with *L. sativum* following *E. tenella* infection significantly reduced mortality rate than those fed the control diet. Although broilers in the infected-unfortified, Hrp and GC+Hrp mixture ration groups had larger number of excreted oocysts, mortality remain lower across the production period. This was in agreement with the study conducted by Almeida *et al.* (2014) who infected broilers with *E. acervulina* and *E. maxima* and found larger oocyst count in the positive group, but similar mortality compared with the group not infected. Lower mortality might be due to relatively lower cecal lesion scores across all the treatment groups. Sever infection that may lead to death should display cecal lesion score of +4 (Johnson and Reid, 1970), but in this experiment the average higher cecal score recorded was 2.33, which is not sever enough to cause high mortality.

The parasite was not completely suppressed in any of the infected treatment groups. However, the coccidial oocyst loads of GC and amprolium groups were lower than the infected-unfortified and GC+Hrp groups. Lower oocyst in the GC group might be linked with the effect of the phenolic compounds, antioxidants and high n-3 fatty acids. *L. sativum* seed oil is rich in tocopherol, carotenoid and fatty acids such as oleic and α-linolenic acids (Diwakar *et al.,* 2010). The hydrophobic essential oils possess the ability to intrude the bacterial cell membrane and disintegrate membrane structure and cause ion leakage (Windisch *et al*., 2009). It has been also reported that antioxidant-rich plant extracts and high n-3 fatty acids have a potential benefit in treating cecal coccidiosis infections in chickens (Allen *et al.,* 1996; 1997; Naidoo *et al.,* 2008).The result highlights the importance of commercial preparations of plant-based products to reduce the effect of coccidiosis infection in organic production systems (Abbas *et al*., 2012). Such products will improve intestinal health of chickens and thus reduce the effects caused by coccidiosis infection (Waldenstedt, 2003). Regarding anticoccidial property of the chemical amprolium, it acts as a thiamine analog that competitively inhibits the active transport of thiamine, negatively affecting *Eimeria* species without harming the broilers due to the comparatively greater sensitivity of the parasite than the host (Ruff and Chute, 1991).

Broilers in the amprolium ration group showed no lesion in the cecum caused by *E. tenella*. Uninfected-unfortified group showed oocyst excretion between days 14 to 27 post-inoculation, even though there was no significant differences among treatments at these ages. As a result, cecal lesions at 27 days post inoculation were observed. This suggests contamination of pen litter of *Eimeria* parasites, even with the strict hygiene measures applied during the study. Flies, ants, other insects, and also the feet and hands of technicians may have served as vectors for the dissemination of the *Eimeria* parasites (Henken *et al*., 1994). But, BWG in the finisher and entire period was higher in uninfected-unfortified group than other treatment groups, suggesting that the low infection loads that occurred as a result of contamination

did not influence performance attributes (Rosen, 1995). All infected chicken groups showed typical signs of coccidiosis including bloody diarrhea and weight loss compared to uninfected-unfortified group at the early age of infection.

The higher cecal lesion occurred in GC during 27 days post-inoculation might be related to certain anti-nutritional factors present in GC. According to Agarwal and Sharma (2013), whole GC seed flour contains tannins, phytic acids, oxalic acid and cyanogens which might have an effect on lesion development in the cecum. Meskerem and Bookaewan (2014) noted that non-infected chickens fed a diet containing GC seed powder showed cecal lesions and mortality. The longer intestinal tract (gizzard to cloaca) recorded for the uninfected-unfortified group at 42 days of age might have provided larger absorptive capacity; hence the birds grew rapidly than infected-unfortified ration group. The shorter intestinal length observed in the infected-unfortified ration group might be due to the infection, thus, less surface area is available for absorption and consequently less tissue mass gain (Miles *et al*., 2006), which resulted to reduced performance of the birds.

Carcass characteristics of broilers relative to live body weight did not significantly differ among treatment groups, which were in agreement with Ocak *et al.* (2008) who found no significant effect on carcass traits in broiler chickens supplemented with thyme (*Thymus vulgaris*). Inclusion of GC and Hrp into the diet of broilers also did not affect the development of liver and heart differently. Al-kassie *et al.* (2012) also reported no difference between treatments consumed different levels of Hrp and black pepper mixture on liver and heart percentage.

Conclusion

Broilers fed ration fortified with GC 0.75%, Hrp 0.75%, GC 0.38%+Hrp 0.38% and amprolium 0.0125% in the entire production phase showed better BWG than infected-unfortified group. Garden cress (0.75%) and amprolium showed comparable reduction of *E. tenella* oocyst shed which is also an additional advantage over 0.75% Hrp and 0.38% GC+0.38% Hrp. However, 0.75% GC or 0.75% Hrp or 0.38% GC+0.38% Hrp can be used as an alternative feed additive to improve the body weight of broilers under areas where *E. tenella* infestation is common in all levels of production because of their availability, affordability and easy usage at farm level. However, the reason for the presence of slight cecal lesion in the GC group should be further investigated.

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Conflict of Interests

The authors declare that they have no competing interests.

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Husbandry Practices, Farmers' Perception and Constraints of Pig Farming in Bishoftu and Holeta Areas, Central Ethiopia

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Abstract: The study was conducted to describe and compare the current pig production practices in Bishoftu and Holeta towns and their surroundings, central Ethiopia. The areas were selected since they are the most important pig production areas in the country. A structured questionnaire was used to interview 20 and 23 pig farmers from Holeta and Bishoftu, respectively. The parameters studied in the survey included socio-economic characteristics, production and management, ownership, herd structure, purpose of keeping, feed resource, feeding and fattening practices, reproductive management, meat utilization and marketing, and pig production constraints. Results indicate that household characteristics of pig keepers did not differ significantly ($P > 0.05$) between the two study areas. Pig farmers keep adapted exotic pig breeds. Herd composition did not differ statistically ($P > 0.05$) between the two study areas. Mean pig herd size per household was 5.72. The majority (58%) practice both pig breeding and fattening. The two study areas were similar ($P > 0.05$) in the type of pig house. Pigs were permanently housed by 88.4% of the households. Major feed sources offered to pigs in both study areas include household wastes, market wastes and crop residues. Reproductive managements did not differ significantly ($P > 0.05$) between the two study areas. Similar results ($P > 0.05$) were obtained for origin of animal stocks in the two study areas. Most (83.7 %) of the farmers acquired their foundation stock from local markets. The farmers did not slaughter pigs for home consumption and pigs were kept as a source of income. Farmers in both study areas named high cost of feeds, followed by pig mortality due to diseases, marketing constraints and lack of capital as major constraints for pig production. Despite the existence of production constraints, most respondents had aspiration to continue rearing pigs and plan to expand pig farm. It can be concluded that an improvement of pig production in Central Ethiopia should consider an improvement in feeding practices, marketing system, prevention of diseases, and a reduction of inbreeding.

Keywords: *Feed sources, Households; Pig farming; Husbandry practices; Small scale*

Introduction

Pigs are considered as the only litter-bearing animal among meat producing livestock having the shortest generation interval and high feed conversion efficiency. Pig production forms an integral part of farmer's economy in many parts of the world, and plays enormous role in poverty reduction by creating employment opportunities for resource-poor farmers. Consequently, pig production is increasing from time to time in many parts of tropical countries (Serres, 2001). In the tropics pigs have been raised under various husbandry practices including free range feeding, tethering, and confinement (Kimbi *et al*., 2001). Traditionally kept pigs contributes about 80% of pigs kept in East Africa (Tanzania, Kenya and Uganda), 75% in Zimbabwe, 70% in Botswana (Setschewaelo, 1992), 65% in Sahel countries (Chad, Niger, Mali, Guinea Bissau, Senegal), and 80% in Namibia (FAO, 1998).

Ethiopia's pig production is concentrated in limited areas and the population is estimated at about 33,000 (FAOSTAT, 2013). To date, the pig production in the country has received very little attention and no systematic documentation of farming practices has been done (Seid and Abebaw, 2010; Theodros *et al*., 2013; Yeshambel and Bimrew, 2014). To formulate policies, improve pig production and to increase the income of the pig farmers, farming practices should be evaluated. Grass root-level surveys are needed in order to obtain farmers' perceptions on the pig production and feed utilization. Such approach will certainly help to generate appropriate technologies within the prevailing conditions of the different pig farming areas. There is, therefore, an increasing need to technically assess the problem at the ground level and identify ways of overcoming constraints to improve pig production in the country. The main objective of the current study was therefore, to generate and avail information on purpose

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of keeping, management practices, meat utilization, marketing, and production constraints of pigs in two study areas of central Ethiopia.

Materials and Methods

Description of the Study Areas

A survey was conducted within and around Bishoftu and Holeta towns. These were selected since they are the most important pig production areas. Bishoftu is located in the central highlands of Ethiopia at about 45 km southeast of Addis Ababa and its geographic location is at 8°45'00" N latitude and 38°59'00" E longitude. The area has an altitude of about 1900 meters above sea level, with average annual rainfall of 849 mm. The average minimum and maximum temperature ranges from 10.5 to 26.1°C with a mean value of 18.7°C. The average relative humidity is 58.6% (DZARC, 2001). Holeta is located at 40 km distance west of Addis Ababa and its geographic location is at 9°04'00" N latitude and 38°30'00"E longitude. It lies at an altitude of 2400 meter above sea level. The average minimum and maximum temperatures are around 6.1 and 24°C, respectively. The annual rainfall of the area is 1250 mm.

Data Collection

A rapid field visit was conducted before the data collection to get information about the study area and help to select pig farmers. Based on the assessment of the rapid field visit and in consultation with regional Ministry of Agriculture offices, pig farms within the towns and small scale farms around the towns were purposively selected based on their pig production potential, experience in pig farming and access to market. Households that have at least two pigs or landless farmers who have a minimum of one year experience in pig production were included for the study. Structured questionnaire was used to collect information from all households who own pigs. Interview was made for each respondent with the help of trained enumerators. Secondary information was also collected from development agents and experts of livestock working in the towns/districts. Based on information on pig husbandry, management practices and farmers' perception about piggery in the region, prospects and constraints were assessed to draw recommendations.

In general, the aspects covered in the farm questionnaires included farm-management practices, type of feeds and feeding practices, housing, reproduction, marketing and utilization of pig's meat, and production constraints encountered in pig production. General information on the socio-economic characteristics of the respondents such as family size, age, education, sex, occupation, farm size, household size, etc. were also collected. Production and management practices such as housing, herd structure, purpose of keeping, feed resources, feeding practice and fattening practices were also included in the study. Reproductive parameters included in the survey were breeding/mating system, methods of mating and weaning. Furthermore, information on meat consumption, buying and selling of animals, and on main constraints as they are perceived by farmers were collected.

Data Processing and Analysis

Data from the questionnaire was entered into SPSS (2007) database and validated for analysis. After validation, the data were analyzed using SAS (2004) statistical package. Descriptive statistics was employed for data involving frequencies and Pearson chi-square was used to compare variables between the two areas, whereas quantitative variables were analyzed using analysis of variance procedure. Indices were calculated to provide overall ranking of a particular trait according to the formula: Index = sum of [4 for rank 1 +3 for rank 2 +2 for rank 3 +1 for rank 4] given for an individual trait divided by the sum of [4 for rank 1 +3 for rank 2 +2 for rank 1 +1 for rank 4] summed over all traits.

Results and Discussions

Characteristics of Pig Keepers

Characteristics of pig farmers did not differ between the two study areas (Table 1). Male headed households were the majority involved in pig husbandry. This is similar to that has been reported earlier in other traditional production systems (Nsoso *et al*., 2006; Nwanta *et al.,* 2011; Riedel *et al*., 2012; Nath *et al.,* 2013; Ikwap *et al*., 2014). But, it is in contrast with the report in Kenya (Mutua, 2010), Zimbabwe, and South Africa (Halimani *et al.,* 2013; Chiduwa *et al.,* 2008) where pigs are traditionally owned by women. In Southern Africa also, females played a bigger role in pig farming (Madzimure *et al.,* 2013). The present study revealed that a high proportion of middle aged and small proportion of young household heads were involved in pig production. This is in agreement with the findings of Ajala *et al.* (2007). About 46.5% of the respondents had finished secondary school and higher education (Table 1). This is in agreement with the findings of Yeshambel and Bimrew (2014) and Theodros *et al.* (2013). The highest percentage of the respondents having formal education also agrees with the observations of Adesehinwa *et al.* (2003) who reported that a higher percentage of pig farmers in Oyo State of Western Nigeria had formal education. In the current study, the high level of education amongst the pig farmers could help them to implement good husbandry and health-management practices to enhance pig productivity. On average respondent farmers had 9.73 years of experiences in pig husbandry indicating the presence of better know how in pig keeping in the present study area. This is in contrast with the findings of Yeshambel and Bimrew (2014) who reported pig keeping to be a recent introduction in Northwestern Ethiopia. More than half (53.5%) of the respondents' in the study areas are full-time government employees who engaged in business and pig farming to earn additional income.

Such findings have also been reported in other countries in Africa and Asia (Kagira *et al*; 2010, Costales *et al*., 2007; Lemke *et al.,* 2006).

In the study areas farmers keep adapted exotic pig breeds. This is similar to that reported in a mountainous area of Northeast India (Kumaresan *et al*., 2009). But, this is in contrast to other previous reports where the majority of the farmers who kept or prefer local or crossbred pigs (Kagira *et al.,* 2010; Patr *et al.,* 2014; Madzimure *et al*., 2013; Nath *et al.,* 2013; Fualefac *et al.,* 2014). This is also in contrast to reports that indicated most of the pigs raised in developing countries are crosses or local breeds raised under traditional production systems (Permin *et al.,* 1999; Hide, 2003; Wabacha *et al.,* 2004). Keeping adapted exotic breeds in the present study area may not be a matter of preference, but lack of choice since there are no indigenous breeds or cross breeds in the country.

Livestock Holding

Farmers predominantly own pigs (26.77%) and poultry (30.25%), they also rear other animals such as cattle (17.19%), small ruminants comprising sheep (16.54%) and goats (9.25%). Ownership patterns of other livestock species were similar for the two study areas (Table 2). The majority of the livestock and poultry in the present study are indigenous breeds or their crosses that are managed using traditional practices. Generally, few external inputs are purchased for livestock. The farmers in the present study are smallholder livestock keepers which are similar to report from North Vietnam (Lemke *et al*., 2007).

Table 1. Characteristic of pig owners in the study areas

	Bishoftu (N=20)		Holeta (N=23)			Total (N=43)
Farmer's characteristics	Number	%	Number	%	P-value	Overall %
Sex					0.756	
Male	18	90	20	87.00		88.40
Female	2	10	3	13.00		11.60
Age (years)*	41.85±10.93		39.48 ± 10.45		0.472	40.60 ± 10.62
25-29	3	15	5	21.74		18.60
30-34	4	20	3	13.04		16.28
35-39	2	10	4	17.39		13.95
40-44	3	15	4	17.39		16.28
45-49	2	10	2	8.70		9.30
50-54	3	15	2	8.70		11.63
55-59	2	10	3	13.04		11.63
≥60	1	5	0	0		2.33
Family Size (*n*)*	4.90 ± 2.06		5.1 ± 2.33		0.728	4.98± 2.19
1-5	15	75	14	60.90		67.44
6-10	5	25	9	39.10		32.56
Educational level					0.928	
Never been to school	5	25	4	17.40		20.90
Primary education	6	30	8	34.80		32.60
Secondary education	7	35	8	34.80		34.90
Higher education	2	10	3	13.00		11.60
Primary occupation					0.960	
Full-time employee	5	25	6	26.10		25.60
Farmer	6	30	6	26.10		27.90
business	9	45	11	47.80		46.50
Experience in pig rearing*	8.28±5.39		11.00±5.87		0.122	9.73±5.75
1-5 years	10	50	7	30.43		39.53
6-10 years	4	20	5	21.74		20.93
11-15 years	3	15	3	13.04		13.95
16-20 years	3	15	8	34.78		25.58
Breed (%)					0.00	
Local	0	0	0	0.00		0.00
Large White (Yorkshire)	20	100	23	100		100

*P-value refers to the level of the difference between the proportions from the two study areas; *Mean ± Standard Deviation; N = number of respondents.*

Table 2. Number of livestock owned by pig farmers (mean ± SE) in the study areas

Livestock type	Bishoftu (N=20)	Holeta (N=23)	Total (N=43)	P-value
Cattle	3.50± 0.489	3.83±0.501	3.67±0.361	0.658
Sheep	3.30±0.442	3.74±0.549	3.53±0.356	0.545
Goat	2.20±0.268	1.78±0.301	1.98±0.204	0.313
Poultry	6.00±0.548	6.87±0.556	6.47±0.393	0.275
Pigs	5.25±0.459	6.13±0.551	5.72±0.283	0.501

*N= number of respondents, *Mean ± Standard Error.*

Origin of Pig Stocks

Similar results (P > 0.05) were obtained for origin of animal stocks in the two study areas (Table 3). Most of the farmers acquired their foundation stock from local markets while others got their foundation stock from home bred animals and neighbor herds and less than 10% of the respondents obtained foundation stock from family. Such observation coincide with results of Hossain *et al.* (2011) who reported that farmers in Bangladesh purchased pig from market or neighbors and started a family level farming. This is also similar to that reported in traditional pig farming in Nagaland, India where foundation piglets are mostly acquired from local market (Patr *et al.*, 2014).

Table 3. Acquisition Methods of the pig foundation stock

Origin of animal stocks*	Bishoftu (N =20)		Holeta (N =23)			Total (N =43)
	Number	%	Number	%	P-value	Overall %
Purchased	16	80	20	87.0	0.538	83.7
Homebred	6	30	9	39.1	0.531	34.9
Neighbours	5	25	7	30.4	0.692	27.9
Family	2	10	2	8.7	0.883	9.3

P-value refers to the level of the difference between the proportions from the two study areas; **Total observations >100% due to multiple responses; N= number of respondents.*

Herd Structure of Pig

Herd composition did not differ statistically (P > 0.05) between the two study areas (Table 4). The mean herd size was small (5.72) and consisted mostly of sows, gilts and piglets. Most households had less than three sows in their herd and a relatively small number of piglets and only four farmers owned boars. It was recorded that 32.56% of the farmers kept less than 5 pigs and 67.44 % of farmers kept 5-10 pigs in their house. The small herd size ownership is probably associated with availability of land (Katongole *et al.,* 2012). The low herd size observed in this study could also be due to the high cost of feeding. The pressure on land in the highlands of Ethiopia may impose a pressure on livestock feed resources forcing the pig farmers to keep an average herd size of no more than five pigs. This observation is consistent with the findings under small-scale farming system in other countries (Ajala *et al.*, 2007; Huynh *et al.,* 2007). The current average herd size was smaller than what has been reported in Democratic Republic of the Congo (Bienvenu *et al.,* 2014), India (Kumaresan *et al.*, 2009; De *et al.,* 2014) and higher than reported in Nigeria, northeast India, Zimbabwe (Ajala *et al.*, 2007; Patr *et al.,* 2014; Chiduwa *et al.,* 2008) and in western Kenya (Kagira *et al.,* 2010). But it is similar to that reported in North Vietnam (Lemke *et al.,* 2007).

Table 4. Herd structure of pig farms in the study areas

Study area	Piglet Male (0-2m)	Piglet Female (0-2m)	Sub-Adult Male (2-6m)	Sub-Adult Female (2-6m)	Adult Male (>6m)	Adult Female (>6m)	Total	No. of pigs per household
Bishoftu	11	10	15	21	22	26	105	5.25
Holeta	9	13	29	35	22	33	141	6.13
Total	20	23	44	56	44	59	246	5.72
Percentage	8.13	9.35	17.89	22.76	17.89	23.98	100	
SEM	0.126	0.122	0.108	0.171	0.071	0.145		
P-value	0.537	0.793	0.016	0.173	0.316	0.649		

m=months; SEM=standard error mean; p-value refers to the level of the difference between the proportions from the two study areas.

Purpose of Keeping Pigs

The two study areas were similar (P > 0.05) in purpose of keeping pigs (Table 5). Breeding, fattening, and mixed farming were considered as the purpose for keeping pigs in the study areas. Piglets for fattening were purchased from seller or from nearby farmers by 23% of the pig keepers who raised pigs primarily for fattening to sale. About 19% of the pig keepers are breeders keeping sows and are engaged in piglet production. The majority (58%) were mixed farmers that practice both breeding and fattening. Farmers fattened own farm produced offspring, but some farmers bought additional piglets for fattening. This is in agreement with that observed in other smallholder systems (Lanada *et al.,* 2005; Lemke *et al.,* 2007; Mutua *et al.,* 2011; Kagira *et al.,* 2010; Patr *et al.,* 2014).

Table 5. Purpose of keeping of pigs in the study areas

	Bishoftu (N=20)		Holeta (N=23)			Total (N =43)
	Number	%	Number	%	P-value	Overall %
Purpose of keeping					0.693	
Breeding	3	15	5	21.7		18.6
Fattening	4	20	6	26.1		23.3
Mixed/dual purpose	13	65	12	52.2		58.1

P-value refers to the level of difference between the proportions from the two study areas; N= number of respondents.

Housing Practices

In the present study, all the respondents in the study areas provide some form of housing to their pigs. The two study areas were similar (P > 0.05) in the type of pig house. Pigs were permanently housed by 88.4% of the households in a house constructed from mud walls either with thatched or zinc roof, while the rest of the households kept their pigs in temporary pig house in the backyard (Table 6). This practice is similar to that observed in North Vietnam where pigs were permanently penned (Lemke *et al.,* 2007). It is also consistent with pig production in Democratic Republic of Congo where the majority of the pigs were reared in pens without free roaming (Bienvenu *et al*., 2014). Moreover, the majority of the households in North East Indian State of Nagaland housed their pigs all the time (Njuki *et al*., 2010; Patr *et al*., 2014; Nath *et al*., 2013).

Table 6. Type of housing in the study areas

	Bishoftu (N=20)		Holeta (N=23)			Total (N=43)
	Number	%	Number	%	P-value	Overall %
Type of house					0.569	
Permanent house	18	90	20	87		88.4
Temporary house	2	10	3	13		11.6
Shelter					0.498	
Together	14	70	15	65.22		67.4
Separated pen	6	30	8	34.78		32.6

P-value refers to the level difference between the proportions from the two study areas; N= number of respondents.

In the present study very few households partially confined their pigs in temporary pig house/pen. In the temporary type of housing, pigs are housed in wooden or bamboo-made pens that are roofed with tin or locally available materials. This is consistent with the reports of low-input traditional free ranging pig farming system of other developing countries like Kenya (Kagira *et al.,* 2010), Zimbabwe (Chiduwa *et al.,* 2008) and other part of Africa (Madzimure *et al.,* 2013). In this type of housing pigs were allowed to scavenge/graze during day and confined during night time. In Northeast India (Kumaresan *et al.,* 2009) about 98% of the pig houses were of temporary type and made up of locally available materials. Kumaresan *et al.* (2009) also noted that permanent type of housing is more in urban areas where the exotic pig rearing is highly practiced. In other developing countries like Kenya and Nigeria, tethering of free-range pigs was undertaken during the rainy season since pigs were predisposed to damaging of crops (Mutua *et al.,* 2012; Ajala *et al*., 2007).

In the present study area, farmers use similar (P > 0.05) housing management. About thirty-three percent of the households had separated fattening and maternity pens, while the rest sheltered their pigs together. Thirty-three percent of the pig house had concrete floors, and are cleaned regularly, while the rest were earthen floors. Majority (92%) of the pig farms had mud walls either with thatched or zinc roof. The floor space per animal was found to be adequate in 92.98% of the farms. Recommended pig housing system was not found in the present study areas, and pigs were kept together regardless of their age, sex and reproduction status.

Feed Sources and Feeding System

The major feed sources that were offered to pigs by farmers in the present study area include household

wastes, market wastes and crop residues (Table 7). A large proportion of residues from cereal crops like maize, sorghum and millet which are available from households and unsuitable for marketing and family use are utilized as pig feeds. The market wastes varied from hotel wastes, potato peelings, fruits/vegetables and slaughter house wastes including blood and offal. Feeding household and market wastes and crop residues to pigs of all categories is a means of reducing feed cost. Generally utilization of commercial feeds for pigs was limited in the study areas. This is similar to what has been reported in other traditional production systems (Nsoso *et al.,* 2006; Lemke and Zarate, 2007; Kagira *et al,* 2010; De *et al,* 2014). The system of raising pigs on locally available resources has been reported in Northeast India (Kumaresan *et al*., 2009) and in North Vietnam (Lemke *et al*., 2006).

Table 7. Major pig feed sources in the study areas

Feed sources	Bishoftu (N =20)		Holeta (N =23)			Total (N =43)
	Number	%	Number	%	P-value	Overall %
Household wastes	15	75	16	69.6	0.692	72.1
Market wastes	13	65	15	65.2	0.988	65.1
Crop residues	13	65	14	60.9	0.780	62.8
Purchased feeds	2	15	2	8.7	0.520	11.6
Grazing	2	10	3	13	0.756	11.6

Percentages exceed 100% within a column due to multiple responses; P-value is a chi-square probability; N= number of respondents.

In contrast to the present study locally available indigenous plant materials (forages) serve as the main feed for pigs in other pig dominated states of India like the northeast part of Sikkim Himalayan region (Nath *et al*., 2013) and Nicobar group of islands (De *et al.,* 2014) as well as in other developing countries like Kenya (Mutua *et al*., 2012), Zimbabwe (Halimani *et al.,* 2013) and Democratic Republic of Congo (Bienvenu *et al.,* 2014). In this study, poor-quality feeds and inadequate feeding are mentioned to be the major factors limiting pig productivity, which needs to be addressed to enhance productivity and income from pig production.

Higher percentage of farmers (88%) practiced group-feeding inside the pen. Very few farmers (12%) allow their pigs for scavenging. Almost all farmers (98.1%) used local feeders, made up of wooden board or concrete for feeding pigs. Other materials used as feeder and waterer were cut tier of vehicles and aluminum plates.

Reproductive Management

Natural service is the only breeding method used by producers in the study areas (Table 8). This is in contrast to the practice in other tropical smallholder farms (Gatenby and Chemjong, 1992; Lanada *et al.,* 1999; Lemke *et al*., 2007) where sows are served by artificial insemination. The pig farmers recognized oestrus from the behavior of the sow based on standing reflex and by using boar. Heat detection techniques identified in this study coincided with results of Losada *et al.* (1997) who reported that majority of the farmers in east of Mexico city detected heat from the behavior and external changes in the reproductive organ of the sows.

Mean litter sizes in the present study was markedly low (6-7) which are in conformity with the report of Hossain *et al.* (2011) in Bangladesh and Chiduwa *et al.* (2008) in Zimbabwe. The average number of piglets per litter in commercial farms in Kenya was 8 (Wabacha *et al*., 2004). The small litter size reported in this study can be attributed to poor diets and inbreeding (Toro *et al.,* 1988). Inbreeding is a major issue in indigenous pig population for declining productivity (Patr *et al*., 2014). Majority of the sows in the current study farrowed twice a year, which is similar to the expected farrowing index of about 2.2 (Chiduwa *et al.,* 2008).

In the surveyed areas, the majority of farmers (81.4%) castrated their pigs at the age of 3-4 months using surgical method. This is similar to that reported in other traditional production systems (Nwanta *et al.,* 2011; Nath *et al.,* 2013). Farmers perceived that growth of the castrated pigs is better than the uncastrated ones. However, piglets with better vigor, body weight and health are kept uncastrated for breeding purpose.

Majority of the farmers (86%) practiced pig weaning at 60 days and above. Delayed weaning of pigs was observed on majority of the farms which is similar to that reported in Kenya (Mutua *et al.,* 2011; Kagira *et al.,* 2010), Zimbabwe (Chiduwa *et al.,* 2008) and Creole piglets in Guadeloupe (Gourdine *et al.,* 2006), in Nepal (Gatenby and Chemjong, 1992) and the Solomon Islands (de Fredrick and Osborne, 1977). Early weaning is, however, not ideal for smallholder farming areas as the practice should be supported by suitable and sustainable feeding regimes.

Consumption and Marketing

The pig farmers in the study areas did not slaughter pigs for domestic consumption. This is because the majority of the communities are Orthodox and Muslims religion followers who do not consume pork since it is forbidden by the religion. As a result, farmers reared pigs mainly as a source of income. Earlier studies in other parts of the country also indicated that all respondents keep pigs entirely as a means of income generation (Theodros *et al*., 2013; Yeshambel and Bimrew, 2014). This is also similar to what has been reported in Western Kenya

(Kagira *et al.,* 2010) where pigs are mainly kept as source of income. In other countries, low-input pig production has role of both income- generation and source of meat for home consumption (Ajala *et al.,* 2007; Lemke and Zarate, 2007; Patr *et al.,* 2014). In other African countries smallholder pig production is primarily for market (Ajala *et al.,* 2007; Kagira *et al.,* 2010; Bienvenu *et al.,* 2014) and consumption of animal products come only secondary. This lies in contrast with Asian areas where pigs are less market oriented but fulfill functions related to savings and household consumption (Kumaresan *et al.,* 2009; Lemke *et al.,* 2006).

According to respondents, there are no central markets for trading live pigs. Smallholder pig farmers use different marketing channels. From the farmers, the animals have to pass through several middle men before reaching direct consumers. Some pig farmers sell live pigs to agents or traders who come to collect them in villages. Pigs are also traded at the super market in Addis Ababa based on a negotiated price between the farmer and super market owner. The pig price paid to farmers is based on live weight, sex and age of the pig through negotiation implying that farmers have little influence on the price.

Table 8. Reproductive management of pigs in the study areas

Parameters	Bishoftu (N=20)		Holeta (N=23)			Total (N=43)
	Number	%	Number	%	P-value	Overall %
Heat detection method					0.265	
Boar	9	45	6	26.1		34.9
Standing reflex	6	30	6	26.09		27.9
Boar + standing reflex	5	25	11	47.8		37.2
Methods of mating					0.637	
Live boar	16	80	17	73.9		76.7
AI	0	0	0	0		0
Never use boar or AI	4	20	6	26.1		23.3
Farrowing frequency per year					0.775	
Twice	14	70	17	73.91		72.1
once	6	30	6	26.09		27.9
No of piglet per farrowing					0.971	
<6	2	10	2	8.70		9.3
6-10	15	75	17	73.91		74.4
>10	3	15	4	17.39		16.3
Castration					0.571	
Yes	17	85	18	78.3		81.4
No	3	15	5	21.7		18.6
Weaning age					0.597	
Two months	3	15	3	13		14
>two months	17	85	20	87		86

P-value refers to the level of the difference between the proportions from the two study areas; N= number of respondents

Constraints of Pig Production

Lack of feeds, disease risks, marketing problem and shortage of financial sources were mentioned as the main constraints to intensify pig production (Table 9). Feed ranked to be the first limiting bottleneck for pig production as perceived by farmers. The major feedstuffs available for pig production are of low quality, which do not meet their productive and reproductive performances. In both study areas, the price of concentrate feed is high and unaffordable to the pig farmers. Diseases were the second major constraints of pig production in the study area. The main disease constraints were diarrhea (39.5%), mange (37.2%), cough (20.9%), and worms (2.3%) which can lead to pig mortality. Marketing was equally reported by seventy percent of the farmers as a problem for pig production in the study areas. Quite a lot of farmers reported lack of collaterals to access bank loans as a factor that limits expansion of their enterprises.

Table 9. Ranking of the major problems of pig production in the study areas

Problems	Bishoftu (N =20)		Holeta (N =23)	
	HH	Index	HH	Index
High cost of feeds	17	0.19	22	0.43
Diseases	16	0.13	16	0.28
Marketing problem	14	0.08	16	0.22
Lack of capital	10	0.06	10	0.07

HH =number of household respondents ranking constraints; N= number of respondents.

Perception of Farmers toward Pig Production

Most respondents expressed the desire to increase their pig holdings. Majority of pig farmers showed interest in continuing pig production. Most respondents have also the plan to expand pig husbandry (Table 10). However, production constraints have been mentioned to have a drawback on their plans. Pigs often die from poor husbandry practices. Therefore, attention should be given to the sector to develop and make the sector better contribute to the livelihood of the smallholders.

Table 10. Tendency of households to continue pig rearing and expand pig farm

	Bishoftu (N=20)		Holeta (N =23)			Total (N=43)
	Number	%	Number	%	P-value	Overall %
Continuing rearing pig					0.747	
Yes	16	80	19	82.6		81.4
No	2	10	3	13		11.6
Not decided	2	10	1	4.3		7.0
Total	20	100	23	100		100
Expanding pig farm					0.765	
Yes	12	60	15	65.2		62.8
No	1	5	2	8.70		7.0
Not decided	7	35	6	26.1		30.2
Total	20	100	23	100		100

P-value refers to the level of the difference between the proportions from the two study areas; N= number of respondents.

Conclusion and Recommendations

This study revealed the emergence of small scale piggery in the central Ethiopia using family labor and locally available feedstuffs. The study identified pig production to have a great potential to enhance household income. However, for better income contribution of pig production, attention should be given to curb the prevailing constraints of feeds and feeding, health and marketing. Detail studies are required to understand accessibility of sufficient customer for pork and market out let.

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Conflict of Interests

The authors declare that they have no competing interests.

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Prevalence and Species of Ticks on Cattle in Borecha District, Southern Ethiopia

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Abstract: A cross sectional study was conducted from October 2014 to June 2015 to identify and estimate the abundance of bovine tick species. The ticks were collected from different attachment sites on cattle kept under extensive management system. Among the 384 animals examined, 63 percent (n=242) were infested by one or more tick species. A total of 4246 adult ticks were collected and five tick species belonging to two genera, namely *Amblyomma* and *Rhipicephalus* were identified. The prevalence of tick infestation in animals with poor body condition (73.95%) was significantly ($P<0.05$) higher compared to animals with good body condition (52%). The prevalence of tick infestation among the age groups was significant ($P<0.05$) and higher in old than young and adult. *Boophilus* tick species infested all body regions of animals. *Amblyomma s*pecies concentrated on the scrotum/udder regions whereas *Rhipicephalus* were restricted to the ear, neck, udder/scrotum, ano-genital and tail of the animals. The prevalence and abundance of tick in the present study is high and can reduce animal productivity. Therefore, appropriate and strategic tick control program should be formulated and implemented and this should be based on the distribution pattern of the tick species.

Keywords*: Bovine; Tick; Prevalence; Tick burden*

Introduction

Ethiopia has the largest livestock population in Africa and livestock contribute to the livelihoods of over 80% of the rural population by providing food such as milk and meat, and foreign currency earning to the country from export of live animals, hides and skins (Fufa *et al*., 2009). The utilization of hides and skins in Ethiopia is estimated at 48 for cattle, 75 for goats and 97% for sheep with off take rate of 7, 35, and 33%, respectively (Mahmud, 2000). Though hides and skin are very important source of export income, its contribution to the national economy is far below the expected potential mainly due to external parasite such as ticks (Kassa, 1998; Hagos *et al*., 2013) that cause significant economic losses through rejection and down grading of hides and skins.

Ticks and Tick Borne Diseases (TBDs) are widely distributed throughout the world, particularly in tropical and sub-tropical regions. It was estimated that 80% of the world's cattle population is exposed to ticks infestation (Fufa *et al*., 2009). Ticks have developed resistance to many classes of acaricide including organophosphates, formamidines (amitraz) and other acaricide group in different regions of the world. Target site mutations are the most common resistance mechanism observed, but there are examples of metabolic mechanism (Shearer and Wall, 2008). In Ethiopia, tick and tick borne diseases cause considerable losses to livestock industry and accounts for 75% of the animal exports loss. A conservative estimate of 1 million birr loss annually was incurred through rejection and down grading of hides and skin in the country (Hagos *et al*., 2013). Apart from direct effect on animal production and productivity, ticks are inviolably efficient vectors of pathogens to man and domestic animals (Pegram *et al*., 1981; Rahbari *et al*., 2009).

According to Walker *et al*. (2003) ticks that are considered to be most important to the health of domestic animals in Africa comprise about forty species. Among these, the most important tick species in cattle are *Amblyomma gema, A. varigatum*, A. cohaerens, *A. lepidum* (Sileshi *et al*., 2002; Ammanuel and Abdu, 2014), *Boophilus, decoloratus* (Sileshi, 2002), *B. annulatus* (Tamiru and Abebaw, 2010) *Rhipicephalus pulchellus, R. pravus, R. everts everties* (Alemayehu, 2000), and *Haemaphysalis aciculifer* (de castro, 1994; Mesele *et al*., 2010). The environmental condition and vegetation of Ethiopia are highly conducive for ticks and tick-borne disease perpetuation (Pegram *et al*., 1981).

The infestation of tick results in retardation of animal growth, loss of milk, and meat production by affecting the market desirability (Tsegaye *et al*., 2014). Even though various researches were carried out in different parts of the country, there were no studies conducted regarding tick infestation problem and tick species distribution in the present study area. Therefore, the present study was designed to estimate the prevalence, identify tick speciesand associated risk factors.

Materials and Methods

Study Area

The study was conducted in four selected Kebeles (Yirba dumancho, Bonoyachire, Konsore fulassa and Sadamo dikicha) of Borecha district, Sidama Zone, Southern Ethiopia. Borecha is located at about 304 km from Addis Ababa, the capital city of Ethiopia. It has a total land mass of 39, 504 hectare out of which about 17,934, 17,778, and 6,487 ha were covered by perennial crops, forest and grazing land, respectively. Administratively, there are 39 rural kebeles and 4 urban Kebeles. It has an altitudinal range of 1700-2000 above sea level and its agro-ecological condition is 22% highland and 78% lowland. The average annual rainfall ranges from 1232

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mm – 1242 mm and the annual average temperature is 16-29.8 ^{o}C.

Study Animals

The study population constituted local breeds that were available in the study area. Cattle in the rural areas are indigenous zebu breed kept in traditional management system. The animals depend on grazing throughout the year with little supplementation of crop residues.Cross breed cattle are increasing in number and they are primarily reared around urban and peri-urban areas.

Study Design

A cross sectional study was conducted from October 2014 to June 2015 to identify tick–species and their preferred site of attachment on the animal body. All animals investigated were categorized in to age, sex, season, and body condition score (BCS) (Table 1). The age of the cattle were grouped into young (1 to 2 years), adult (3 to 7 years) and old (>8 years) as described by Gatenby (1991). The BCS of the animals were categorized into poor and good based on the appearance of ribs and dorsal spines as applied for indigenous cattle (Nicholson and Butterworth, 1986). Samples were collected both in dry (December, January and February) and wet season of the year (October, November, March and April).

Sampling Method and Sample Size Determination

The study Kebele's were purposively selected based on the availability of transportation and logistics as well as their agro-ecological representativeness of all Kebele's of the district. From each selected Kebeles, the animals were selected by simple random sampling method. The sample size was determined by using the formula given by Thrustfield (1995). The expected prevalence of Ixodidae ticks of cattle in the district was assumed to be 50% since there was no known research conducted in the study area. The parameters used were 95% confidence interval and 5% desired level of precision. Accordingly, the sample was determined by employing the formula: $N = 1.962\ pexp\ (1\text{-}pexp)/d^2$, and by substituting these values in the formula, the sample size determined was n = 384. Where n = sample size; pexp = expected prevalence; d^2 = expected precision which is usually 5% (0.05).

Table 1. Study animals in terms of age, sex, season, and body condition score

Factors	Categories	Number of animals examined
Sex	Male	92
	Female	292
Age	Young	59
	Adult	296
	Old	29
Body condition	Good	192
	Poor	192
Season	Dry	139
	Wet	245
Kebele	Yirba dumancho	112
	Bonoyachire	100
	Konsore fulassa	81
	Sadamo dikicha	91

Tick Collection and Identification

First, the animals were properly restrained and checked for any tick infestation. Adult ticks were collected from half body regions such as ears, heads, dewlaps, belly/flunk, udder/scrotum, fore/hind legs, perineum and tails. Ticks were removed carefully and gently in a horizontal pull to the body surface. The collected ticks were preserved in universal bottles containing 70% ethanol and labeled with respect to predilection site, age, sex, BCS of the animal, and date of collection and transported to district veterinary laboratory. Ticks were counted and subsequently identified to genus and species level by using stereomicroscope according to standard identification keys given by Walker *et al.* (2003).

Data Processing and Analysis

The data collected were entered and managed in Microsoft excel. STATA (STATA Corporation, 2001) was employed for the data analysis. The overall prevalence of tick was determined by dividing the number of positive animals by total sample size and was expressed as percentage. Chi-square (x^2) test was used to assess the association in tick infestation between different variables.

Results

Overall Prevalence

Out of the 384 animals examined, the prevalence of ticks was 63.02% (n=242) and the animals harbored at least one tick species of varying number (Table 2). There was no difference among the study kebeles.

Table 2. Prevalence of tick infestation in cattle by the sample Kebeles

Kebele	No of animals examined	No of positive	Prevalence	χ^2	p-value
Yirba dumancho	112	66	58.9%	15.02	0.89
Bonoyachire	100	64	64%		
Konsore fulassa	81	62	76.5%		
Sadamo dikicha	91	50	54.9%		
Total	384	242	63.02%		

Relative Abundance of Tick Species

Boophilus decoloratus is the most commonly found tick species followed by *Ambylomma lepidium*, *Ambylomma varigatum,* R*hipiepcephalus eversi* and R*hipiepcephalus mushamae,* respectively (Table 3).

Table 3. Frequency of tick species identified in Borecha district

Tick species	Frequency	Percentage
B. decoloratus	2570	60.53
Lepidium	814	19.17
Varigatum	378	8.17
R. eversi	320	7.54
R. mushamae	164	3.86
Total	4246	100

Prevalence of Tick Infestation by Different Risk Factors

The overall prevalence of tick infestation was 63%. There was no significant difference in the occurrences of tick infestation among the study Kebeles (Table 2). Slightly numerically higher prevalence was recorded in Yirba duwancho Kebele (41.5%) and lower prevalence in Sadamo dikicha Kebele (31.5%). The occurrence of tick infestation were not significantly different ($p>0.05$) between sex. There was statistically significant association between age of the animals and level of tick infestation ($P<0.05$) (Table 4) in which older animals recorded the highest prevalence Animals with poor body condition had higher tick prevalence than animals with good body conditions (Table 4). There was no variation in tick infestation between seasons of the year ($p>0.05$) (Table 4).

Table 4. Prevalence of tick infestation in cattle of the study district by sex, body conditions, age and season

Factors	No of animals examined	No of positive Animals	Prevalence	χ^2	p-value
Sex					
Male	92	61	66.30%	9.36	0.69
Female	292	181	61.98%		
Age					
Young	59	33	55.93%	5.33	0.003
Adult	296	185	62.5%		
Old	29	24	82.75%		
Body condition					
Good	192	100	52%	6.8	0.0001
Poor	192	142	73.95%		
Season					
Dry	139	83	59.71%	3.55	0.36
Wet	245	159	64.89%		
Total	384	242	63.02%		

Distributions of Tick Species on Different Body Parts of the Animals

A total of 4246 ticks belonging to five tick species of two genera were identified. *Rhipicephalus (Boophilus) decoloratus* was the most abundant tick species followed by *A. lepidum, A. varigatum, R. evertsi and R. mushamae* in decreasing order (Table 5). *Boophilus* tick species infested all body regions of animals, *Amblyomma s*pecies are concentrated on the scrotum/udder regions whereas *Rhipicephalus* was restricted to the ear, neck, udder/scrotum, ano-genital and tail of the animals.

Table 5. Species of ticks and their distribution on body regions of cattle in the study area

Body region	*A. varigatum*	*A. lepidum*	*B. decoloratus*	*R. evertsi*	Total
Ear	0	0	6 (40)*	10 (92)	16 (132)
Face	0	0	41 (222)	0	41 (222)
Neck	1 (6)	1 (2)	126 (1308)	2 (182)	130 (1494)
Brisket	8 (30)	13 (46)	38 (136)	0	59 (212)
Abdomen	2 (14)	2 (10)	76 (498)	0	80 (526)
Limbs Scrotum	14 (42)	14 (72)	36 (222)	0	54 (336)
Udder	54 (262)	85 (652)	14 (78)	1 (4)	154 (996)
Back	1 (6)	0	13 (38)	0	14 (44)
Ano-gential	5 (18)	11 (32)	1 (2)	49 (202)	96 (254)
Tail	0	0	8 (26)	1 (4)	9 (30)
Total tick	378	814	2570	484	4246

Numbers indicate animals harboring tick and count/burden of ticks, respectively.

Discussion

This study revealed that ixodid ticks are widespread and most significant external parasites of cattle in the district with an overall 63.02% prevalence. The animals are infested with at least a single tick. This finding is in line with previous result reported by Ammanuel and Abdu (2014) who found 62.04% infestation rate in Wolaita Zone, Ethiopia. The result is comparable with Meaza *et al.* (2014) who reported 74% at Bahirdar. However, higher prevalence (89.4%) was reported by Nigatu and Teshome (2012) in western Amhara. Mesele *et al.* (2010) reported 97.8% prevalence which is by far higher than the current prevalence rate. Lower prevalence (27.3%) and (25.6%) was reported by Addisu and Addis (2015) at Bench Maji zone and Belew and Mekonnen (2011) at Holeta, central Ethiopia, respectively. This prevalence variation is most probably attributed to the differences in the agro-climatic condition and agro-ecology among the study areas. Tick activity can be influenced by rainfall, temperature, altitude and atmospheric relative humidity (Pegram *et al.*, 1981).

Although there are different species of ticks known to be found in other parts of the country (Tsegaye *et al.*, 2014), only five species were identified in the present study. *B. decolaratus* was the most abundant tick species in the district (60.5%). This is in agreement with Sileshi *et al.* (2007) who noted *B. decolaratus* to be the commonest and most wide spread tick in Ethiopia among the ticks collected across all regions of the country, except Afar region. This is also in line with the findings of Tamiru (2008) in Asela, and Abebaw (2004) who reported the highest prevalence of *B. decolaratus* (80%). Lower prevalence of this species was reported by Alekaw (1998) in Metekel Ranch (5%), Ethiopia and Mesele *et al.* (2010) at Bedelle district (23.7%). This difference is attributable to the geographical location and altitude of the study areas since this tick species is abundant in wetter highlands and sub-highlands receiving more than 800 mm rainfall annually (Pegram *et al.*, 1981).

A. lepidium was the second most abundant tick species in this study area. This tick is an important vector of *Cowdria ruminatum* which cause heart water in cattle (Walker *et al.*, 2003). This tick was reported by several workers (Pegram *et al.*, 1981; Sileshi, 1995; Mesele *et al.*, 2010) in different part of the country and with varied prevalence. The tick is irregularly dispersed throughout most parts of the country. It occurs in arid and semi-arid areas and also in woodland, bush land as well as grassland with either trees or bushes present (Horak *et al.*, 2011).

A. varigatum was the third most abundant tick species in this study. Mesele *et al.* (2010) reported 14.1% prevalence of this species. The result reported for the survey conducted in western Shoa Bako district by Hussen (2009) indicated this tick species to be the first most abundant species with prevalence of 54.3%. Meseret *et al.* (2014) and Latif and Walker (2004) reported 38.87% and 32.2% prevalence at Haramaya district and Fiche Selale, respectively. Moreover, Solomon *et al.* (2007) noted this species to be the second abundant in cattle in Ghibe Tollay area showing seasonal peaks from April to June. The difference in result was due to the geographical location. *A. varigatum* is a potential vector of diseases caused by *C. rumintium, T. mutan*, *T. velifera* (benign bovine thelieriasis), viral disease, Nairob sheep disease and also aggravates the situation of bovine dermatophilosis (*D. congolence*) (Sileshi *et al.*, 2007).

Rhipicephalus eversi eversi is the fourth abundant tick species (7.5%) in the present study area. The prevalence of this species is lower than reports by Solomon *et al.* (2007) (21.19%) at Ghibe Tullary in central Ethiopia. This tick shows an apparent preference for any particular altitude, rainfall or season (Pegram *et al.*, 1981). It is a possible vector of *Babesia, Rickettisa* and *Theleria* (Kettle,

1995). The occurrence of this species in and around the study area was also reported by other authors (de Castro 1994; Sileshi, 1995; Sileshi *et al*., 2007). *R. mashamae* is the fifth and least tick abundant species (3.9%) identified in the area. In this study only 164 ticks were collected.

Tick infestation in the current study was significantly associated with age of the animals ($p<0.05$) in that older animals had significantly higher tick loads than adult animals. This is probably associated with a decrease in the immunity as the animals get older. Significant difference ($p<0.05$) was recorded among different body condition score groups with the higher prevalence in poor animals. Similar prevalence of tick infestation between the different sample Kebeles was due to the similarity of the environment.

With regard to distribution pattern of ticks on the animal body, *B. decolarantus* was collected from all examined body region, even though the frequency of occurrence is more in the areas extending from face to the limbs, and less on ear, back, ano-gential and tail regions. This species was also the commonest tick collected accounting for 60.5% of all ticks on the study animals. *R. evertsi* and *R. mushamae* were the most restricted tick species identified. They were restricted to the ears, the neck and ano-gential areas with very few of them observed on the scrotal/udder area. *Amblyomma* species were more fairly distributed than *Rhipicephalus* counterparts, with the exceptions of their absence from the ear, face, back and tail. *Amblyomma* spp were more concentrated in the areas of scrotum or udder. The neck, udder and scrotum areas were affected with the highest number of tick (55.4%) followed by abdomen, ano-genital and limbs. Of the total 242 animals affected 154 (63.6%) of them had ticks on the scrotum or the udder depending on the sex of the animal. The previous findings of Okello-Onen *et al.* (1999), Solomon and Kassa (2001), Walker *et al.* (2003) and Belew and Mekonnen (2011) supported the present finding regarding the attachment sites of ticks. Several factors may determine the attachment site of ticks such as host density, interaction between tick species, time and season and inaccessibility for grooming. Information on predilection sites of ticks can increase the efficiency of control methods and aids sampling.

Conclusion and Recommendations

This study showed high burden of ticks in the area with an overall prevalence of 63.02%. The most important and abundant tick species were *B. decolaratus*, *A. lepidum*, *A. varigatum*, *R. evertsi* and *R. mushamae*. The predilection sites identified for the tick species will help in designing tick control methods. Heavy infestations by different tick species can suppress the health of cattle, damage teats, hide and skin and reduce productivity of animals. Therefore, appropriate and timely strategic tick control program should be formulated and implemented based on the distribution pattern of ticks and factors responsible for their devastation.

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Conflict of Interests

The authors declare that they have no competing interests.

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Prevalence and Characterization of Hydatid Cyst in Cattle at Halaba Kulitto Municipal Abattoir, South Ethiopia

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Abstract: A cross sectional study was conducted from October 2012 to July 2013 to determine the prevalence of hydatidosis in cattle slaughtered at Halaba kulitto municipal abattoir. A total of 384 cattle organ were examined and the result revealed an overall hydatidosis prevalence of 24.21% (93/384). The distribution of hydatid cysts in different internal organ were 12.8% (49/384), 3.1% (12/384), 0%, 0.5% (2/384), 1.3% (5/384), 4.7% (18/384), 0.8% (3/384) and 1.0% (4/384), respectively in lungs, liver, kidney, heart, spleen, lung and liver, lung, liver and spleen and in lung and spleen. The lung was the most affected organ followed by the liver. Sex and age of the animal did not have statistically significant effect (P>0.05). A total of 70 cysts were examined for viability and fertility test. Accordingly, 82.86% (58/70) and 53% (37/70) cysts were fertile and viable, respectively. The findings of the present study disclosed that hydatidosis was prevalent in cattle of the study area and responsible for high level of condemnation of different organs. Hence, an integrated control approach involving strategic de-worming of the final hosts with strong surveillance system and good management practice of animals are essential for the reduction of the parasite prevalence.

Keywords: *Cattle; Hydatidosis; Municipal Abattoir; Organs; Prevalence*

Introduction

Ethiopia possesses the largest livestock population in Africa (CSA, 2014). However, the contribution of the livestock sector to the national economy has been reported to be small compared to its potential. One of the main causes of the mismatch between herd population size and production output from livestock in Ethiopia is undoubtedly the widespread occurrence of a multitude of infectious and parasitic diseases, causing morbidity, mortality and market restrictions, which drastically reduce animal production (Shapiro *et al*., 2015).

Hydatidosis is one of the major parasitic problems of domestic animals and a zoonotic disease that cause considerable economic losses and public health problems worldwide (Ekert and Deplaszes, 2004). Echinococcosis is a zoonotic infection caused by adult or larval (metacestode) stages of cestodes belonging to the genus Echinococcosis and the family Taeniidae. Hydatidosis, which is a cystic echinococcosis, also known as hydatid disease, is an infection caused by the larval stage of the flatworm Echinococcus granulosus. It has a cosmopolitan distribution and is one of the most widespread parasitic zoonoses. Hydatidosis is of importance since humans serve as incidental intermediate hosts (Jones *et al.,* 2012).

Echinococcosis has greater public health and economic impact in countries where livestock production is based on extensive grazing system and is major component of the agriculture sector. Previously there was no any data on the prevalence of the disease and its rate in different organs in the study area. Hence, the objective of this study was to estimate the prevalence of hydatidosis in cattle slaughtered at Halaba kulitto municipal abattoir.

Materials and Methods

Study Area

The study was conducted from October, 2012 to July, 2013 in Halaba district municipal abattoir southern part of Ethiopia. Halaba district is located 315 kms west to Addis Ababa in Southern Nation Nationalities People Regional State. The geographical location of the district is 7° 17' N latitude and 38° 06' E longitude. The altitude ranges from 1100 meters to 1200 meter above sea level. The annual rainfall varies from 900mm to 1200mm while the minimum and maximum temperatures are in the order of 21°C and 29°C; 18°C and 24 °C in low and highland, respectively. The area is also characterized by two seasons, the wet season from May to October and the dry season from November to April (IPMS, 2005). The livestock population of Halaba district is cattle 166,871, sheep 60,475, goats 67,163, poultry 160,515, donkey 39,379, mule 227 and horse 61,123 (CSA, 2014).

Study Animals

The study was conducted on 384 local breed cattle sourced from neighboring district such as Kulito, Arsi Neglle, Ropi, Beshno and Guba. During the study period 10 heads of cattle were slaughtered per day on average.

Study Design

A cross-sectional study were carried out from October 2012 to July 2013 by collecting data on events associated with hydatidosis in cattle slaughtered at Halaba district municipal abattoir. Sampled animals were selected using simple random sampling method from cattle registered for slaughter. The data were collected at an interval of 10 days and 20 animals were investigated at each data

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collection day. Information such as body condition and age of the slaughtered animals was determined based on Nicholson and Butter Worth (1986) and De-Lahunfa and Habel (1986), respectively.

Sample Size Determination

There was no information available in the district before this study. Hence, the sample was calculated based on the formula given by Thrusfield (2005). Accordingly, assuming 50% expected prevalence of bovine hydatidosis, the required sample size was 384 cattle at 95% confidence level and 5% expected error.

Study Methodology

Ante-mortem Examination: During ante-mortem examination details of sex, age, and origin of each individual animal was recorded. Sources of cattle for slaughter were identified based on unique identification marks made on the body of each animal using ink from source markets and the markings were transferred to all carcasses and visceral organs after slaughter.

Post-mortem Examination: During post- mortem examination organs of the abdominal and thoracic cavities, namely, liver, lung, heart, spleen and kidney were systematically inspected for the presence of hydatid cyst by applying routine meat inspection procedures which consists primary examination followed by a secondary examination when evidence of hydatid cyst were found. The primary examination involves visualization and palpation of organs and muscles, whereas secondary examination involves further incisions in each organ in case where a single or more hydatid cyst observed. When cysts were present the number of the cysts per organ per animal were recorded (FAO, 1994).

Examination of cysts for fertility and viability: Individual hydatid cysts were carefully incised and examined for the presence of protoscolices, which resembled white dots on the germinal epithelium. Such cysts were characterized as fertile cysts. Fertile cysts were further subjected to viability test (Daryani *et al.*, 2007). A drop of the sediment containing the protoscolices were placed on the microscope glass slide and covered with cover slip and observed for amoeboid like peristaltic movements 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 absorb it (Macpherson *et al.,* 1985). Furthermore, infertile cysts were classified as sterile or calcified. Sterile hydatid cysts were characterized by their smooth inner lining usually with slightly turbid fluid in their content. Typical calcified cysts produce a gritty-sound heard at incision (Soulsby, 1982).

Data Processing and Analysis

The data was entered in to MS-Excel code and analyzed using SPSS-16.0 version and descriptive statistics like percentage were used. The relative frequencies of cysts detected in various organs were calculated. Cattle harbored hydatid cyst were categorized by the number of organs involved and cysts found in different organs. Chi-square (χ^2) was used to test the existence of association between factors and prevalence. Significant association between variables was considered if the p-value is less than 0.05.

Results

The number of animals infected with hydatid cysts with respect to origin is presented in table 1. The highest prevalence was recorded for animals sourced from Arsi Negele and Kulito.

The prevalence of hydatidosis was 24.21% (93/384) (Table 2). Postmortem examination of visceral organs reveled 12.8% (49/384), 3.1% (12/384), 0%, 0.5% (2/384), 1.3% (5/384), 4.7% (18/384), 0.8% (3/384), and 1.0% (4/384), respectively in lungs, liver, kidney, heart, spleen, lung and liver, lung, liver and spleen and in lung and spleen. The present study showed the presence of hydatidosis in all origins of cattle (Table 2).

Table 1. Number of cattle infected by hydatidosis in relation to their origins

Origin	No. of Animals examined	Positive	Prevalence (%)
Kulito	77	24	31.17
Arsi Negla	77	28	36.36
Ropi	77	16	20.78
Beshno	76	11	14.47
Guba	77	14	18.18
Total	384	93	24.2

Table 2. Distribution of hydatid cyst in different organs

Organs examined	No. of organs infected	Percentage of infection (%)
Lung	49	12.8
Liver	12	3.1
Kidney	0	0
Spleen	5	1.3
Heart	2	0.5
Lung and Liver	18	4.7
Lung, liver, spleen	3	0.8
Lung, spleen	4	1.0
Total	93	24.2

The occurrences of bovine hydatidosis in different organs according to the two age group of animals slaughtered at the abattoir were 8.3% (1/12) in less than 5 years and 2.4% (92/372) in greater than 5 years of age, respectively (Table 3).

Table 3. The prevalence of hydatid cyst on different organs based on age

Organ examined	Age	No of examined animals	No of infected animals	Percentage	χ^2	P value
Lung	< 5 years	12	1	8.3	0.218	0.622
	≥5 years	372	48	12.9		
Liver	< 5 years	12	0	0	0.400	0.379
	≥5 years	372	12	3.2		
Heart	< 5 years	12	0	0	0.065	0.721
	≥5 years	372	2	0.5		
Spleen	< 5 years	12	0	0	0.163	0.572
	≥5 years	372	5	1.3		
Lung and liver	< 5 years	12	0	0	0.609	0.279
	≥5 years	372	18	4.8		
Lung, liver and spleen	< 5 years	12	0	0	0.098	0.662
	≥5 years	372	3	0.8		
Lung and spleen	< 5 years	12	0	0	0.130	0.61
	≥5 years	372	4	1.1		

The percentage distribution of hydatid cysts in different organs on sex group was depicted in table 4. The number of female animals is very small as compared to males and may not show the actual situation.

Among the 113 hydatid cysts observed, 70 were in liver, 33 in lungs, 8 in spleen and 2 in heart. Of these, 36 (31.86%), 18 (15.92%) and 16 (14.16%) and 43 (40.70%) were small, medium, large and calcified, respectively. Out of the 70 cysts subjected to fertility test, 58 (82.86%) and 2 (17.14%) were fertile and sterile, respectively. The viability test indicated that 37 (53%) to be viable. Organ wise fertility of the hydatid cysts was higher for lung, followed by liver, spleen and heart in that order, while viability of the fertile cysts were higher for liver followed by lung, spleen and heart.

Table 4. The prevalence of hydatid cyst on different organs based on sex

Organ examined	Sex	No of examined animals	No of infected Animals	Percentage	χ^2	P- value
Lung	Male	377	48	12.7%	0.015	0.0904
	Female	7	1	14.3%		
Liver	Male	377	12	3.2	0.230	0.503
	Female	7	0	0		
Heart	Male	377	2	0.5	0.037	0.786
	Female	7	0	0		
Spleen	Male	377	5	1.3	0.094	0.667
	Female	7	0	0		
Lung and liver	Male	377	18	4.8	0.351	0.410
	Female	7	0	0		
Lung, liver and spleen	Male	377	3	0.8	0.056	0.739
	Female	7	0	0		
Lung and spleen	Male	377	4	1.1	0.075	0.700
	Female	7	0	0		

Discussion

The findings of this study showed the existence of high prevalence of hydatidosis disease in cattle slaughtered at Halaba Kulitto municipal abattoir. This high prevalence of hydatidosis may be because of the presence of high number of stray dogs in the animal origin area. The high number of cyst in the lungs and liver may be due to the fact that these organs possess the first great capillaries sites which are encountered by migrating *Echinococcus* oncosphere which adopt the portal vein route and primarily dwell in the pulmonary and hepatic filtrating system sequentially before any other peripheral organ is involved (Ekert and Deplaszes, 2004). Similar finding was reported by Getachew and Jelalu (2014) with higher prevalence in lungs (19.53%) and heart (12.63%) in Addis Ababa Kara-alo PLC abattoir.

The result of this study is comparable to other findings reported in different regions of Ethiopia and other countries (Baldock *et al.,* 1985; Alemu, 2010). The prevalence recorded in the present study was greater than 11.9% reported by Zerihun (2011) from cattle in Mizan municipal abattoir, 8.28% in Al Baha region, Saudi Arabia (Ibrahim, 2010) and 11.9% by Zerihun (2011) in Mizan municipal abattoir, but lower than 54.4% reported by Ndrirangu *et al.* (2004) for cattle in Kenya, 38.3% in cattle slaughtered in the Ardabil province of North West Iran (Daryani *et al.* 2007), 38.9% for cattle in Pakistan Khan *et al.* (1990) and 32% in Niger Delta (Arene, 1985). 59% for cattle in Gondar abattoir (Nigatu, 2002); 32.1% in Tigray region (Gebretsadik, 2009); 48.9% in Debre markos municipal abattoir (Nigatu, 2009). The difference in the prevalence of hydatidosis among the different areas could be related to geography, dog population, source of cattle, dog management, de-worming practices, offal disposal habits and age of cattle slaughtered.

In this study 53% of the tests were viable. Organ wise fertility of the hydatid cysts was 45.8%, 34.2%, 12.4% and 7.6%, for lung, liver, spleen and heart, respectively. While viability of these fertile cysts were 37.84%, 45.95%, 2.7 and 13.5 from lung, liver, heart and spleen, respectively. About 27.3% fertile, 72.7% infertile, 37.8% viable and 62.2% non-viable cysts were recorded in Kara-alo PLC abattoir (Getachew and Jelalu, 2014) which is higher in all cases than the present finding finding.

Higher infection of hydatidosis was registered in adult cattle (>5 years old) compared to young cattle (<5 years) with variable prevalence among different organs examined. This is in line with Regassa *et al.* (2010) and Endrias *et al.* (2010) who found significantly higher prevalence of the disease in adult cattle. This could be due to immune status and longer exposure to the eggs of the parasite in adult than young animals.

Conclusion and Recommendations

The present study showed hydatidosis to be a prevalent disease in the study area. Lung and liver were the major organs harboring hydatid cyst than other visceral organs and most of the cysts were found to be fertile. Moreover, the prevalence of hydatidosis is somehow varied among animals from different sources. Hence, an integrated parasitic control including treatment of final hosts of the parasite should be practiced in the area.

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Conflict of Interests

The authors declare that they have no competing interests.

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Johnson, L., Lewis, K., Peters, M., Harris, Y., Moreton, G., Morgan, B. *et al.* (2005). *How Far Is Far?* London: McMillan.

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For institutions as authors

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For chapter in an edited book

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Senior, B., & Swailes, S. (2007). Inside Management Teams: Developing a Team Work Survey Instrument. *British Journal of Management, 18,* 138-153. doi:10.1111/j.1467-8551.2006.00507.x (**with doi no.)**

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