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The Ethiopian Veterinary Journal (*Ethiop. Vet. J.*) is a multidisciplinary peer-reviewed journal intended to promote animal health and production of national and regional/international importance. The journal publishes review articles, original research articles, short communication as well as technical notes in English. Under special circumstances, articles in Amharic may be considered for publication.

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Table of Contents

Abattoir-based prevalence of avian tuberculosis in chicken slaughtered at Poultry abattoir in Bishoftu, Central Ethiopia
Gezahegne Mamo.....*1*
https://dx.doi.org/10.4314/evj.v24i1.1

Serological investigation of brucellosis and its association with abortion in sheep and goats in selected districts of Jimma zone, southwestern Ethiopia
Dereje Tulu , Abiy Gojam and Benti Deresa.....*15*
https://dx.doi.org/10.4314/evj.v24i1.2

Study on ruminant tick infestation, phytochemical analysis and ***in vitro*** acaricidal effect of ***Calpurnia aurea*** and ***Otostegia integrifolia*** extracts on ***Amblyomma variegatum***
Jelalu Kemal , Sisay Alemu, Biniam Tsegaye, Nateneal Tamerat.....*34*
https://dx.doi.org/10.4314/evj.v24i1.3

Prevalence, risk factors and bacterial causes of bovine mastitis in southern Ethiopia
Rahmeto Abebe, Mesele Abera, Yifat Denbarga, Mishamo Suleyman, Amene Fekadu, Fufa Abunna, Adugna Abebe, Demisie Wale, Erdachew Kassa, Beyene Tune.....*52*
https://dx.doi.org/10.4314/evj.v24i1.4

Contagious Bovine Pleuropneumonia: Sero-prevalence and associated risk factors in Gudeya Bila and Boneya Boshe Districts of East Wollega Zone, Oromia, Ethiopia
Tolesa Neggasa, Feyissa Begna, Moti Wakgari, Sisay Shibashi, Eshetu Gezahegn, Fikadu Bekele, Tadele Tolosa.....*69*
https://dx.doi.org/10.4314/evj.v24i1.5

Study on the prevalence and risk factors of bovine tuberculosis in dairy cattle in Adama city, central Ethiopia
Tefera Woldemariam, Samuel Bekele, Gezahegne Mamo, Gobena Ameni.....*82*
https://dx.doi.org/10.4314/evj.v24i1.6

Zoonotic helminth parasites of dog in Bishoftu Town, central Ethiopia: prevalence, dog owners' knowledge and control practice
Mitiku Tadesse, Dinka Ayana, Bersisa Kumsa and Abebe Fromsa.....*93*
https://dx.doi.org/10.4314/evj.v24i1.7

Mycobacterium tuberculosis in a Primagam negative wild caught captive olive baboon (***Papio anubis***) in Ethiopia
Gizachew Gemechu, Sosina Ayalew, Elena Hailu, Azeb Tarekegn, Rea Tschopp.....*116*
https://dx.doi.org/10.4314/evj.v24i1.8

Guidelines for authors.....128

Mamo, Ethiop. Vet. J., 2020, 24 (1), 1-14
DOI https://dx.doi.org/10.4314/evj.v24i1.1 *Ethiopian Veterinary Journal*

Abattoir-based prevalence of avian tuberculosis in chicken slaughtered at Poultry abattoir in Bishoftu, Central Ethiopia

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Abstract

A cross-sectional abattoir-based study was conducted in apparently healthy chicken in Bishoftu town, Ethiopia to estimate the prevalence of avian tuberculosis and isolate its causative agent. The occurrence of avian tuberculosis was investigated using postmortem examination, bacteriological culture and acid-fast staining methods. Of the total 648 chicken examined to detect avian tuberculosis, 42 tissue samples showing gross pathological tuberculous-like lesions were collected from liver, spleen and intestine. The overall prevalence of avian tuberculosis in poultry was 6.48% (42/648) (95%CI: 4.53-8.38) on the basis of detailed postmortem examination. Out of 42 tissue samples cultured on Lowenstein-Jensen medium, 14 (33.3%) were bacteriologically culture positive and showed growth of dough-shaped smooth colony characteristic and out of these 14 culture positive samples, 5 (35.7%) were acid-fast positive mycobacteria. Statistical significant difference was observed in the prevalence of avian TB among chicken with different body condition scoring ($\chi 2 = 23.593$, $p = 0.001$). Multivariable logistic regression analysis for risk factors showed that body condition scoring has a high statistical significant association with the prevalence of avian tuberculosis in the study area ($p<0.05$). Poor body conditioned chicken were more likely to show TB lesions (OR=4.45, 95% CI, 2.33-8.52) than good body conditioned chicken. The present preliminary study on avian TB using postmortem lesion examination and microbiological methods revealed the occurrence of avian TB in low prevalence in apparently healthy chicken originated from intensive poultry farms in Bishoftu area; hence detail poultry meat inspection should be practiced at poultry abattoirs in order to reduce the public health risk.

Keywords: Abattoir, Avian tuberculosis, Postmortem examination, Poultry, Ethiopia

Introduction

Ethiopia has large population of chicken, estimated to be 56.53 million, of which local breeds representing 94.31%, hybrid chicken 3.21% and exotic breeds of chicken 2.49% (CSA, 2017). As in most developing countries, in Ethiopia village chickens raised under backyard production system make up the largest proportion, more than 95%, while chicken in commercial production system constitute the remaining small proportion of the national population (Dessie *et al.,* 2003; Habte *et al.*, 2017).

Poultry sector has an important contribution in the provision of high quality protein food in the form of meat and egg to rural smallholder farming families in Africa (Sonaiya *et al.,* 1999) and Ethiopia (Dessie and Ogle 2001). Even though the backyard poultry production system constitutes the majority of poultry production in Ethiopia, currently large and small-scale commercial poultry farms are expanding in urban and periurban areas of Ethiopia to meet the poultry meat and egg demand of the fast growing urban population of the country. However, this growing poultry sector is constrained by various factors including infectious diseases which directly or indirectly influence the productivity of the sector (Habte *et al.*, 2017).

Avian tuberculosis (Avian TB) is one of the important infectious diseases of various species of birds including domestic chicken, pet birds, free-living and captive wild birds, and it has also public health significance (Dhama *et al.,* 2011). Avian tuberculosis in poultry is most often caused by *Mycobacterium avium* subsp *avium*. However, more than ten mycobacterial species including *Mycobacterium genavense*, *M. avium* subsp. *hominissuis, M. intracellulare*, *M. scrofulaceum*, *M. fortuitum* and other potential pathogenic mycobacterial species have been reported to infect birds (Tell *et al.,* 2003; Shivaprasad, and Palmieri, 2012; OIE, 2018).

The oral route of infection appears to be the primary mode of transmission of avian TB in birds and majority of the lesions were detected in the intestine and liver (OIE, 2018). In most cases, infected birds show no clinical signs, but they may eventually become lethargic and emaciated. Many affected birds show diarrhea along with marked atrophy of breast muscle, and comb and wattles may regress and become pale. Under intensive husbandry conditions, sudden death may occur, often associated with severe lesions in the liver; such lesions are easily observed at post-mortem examination (Tell *et al*., 2001). Unlike TB

in animals and man, lesions in lungs of birds are rare. Tubercular nodules can be seen in liver, spleen, intestine and bone marrow. Granulomatous lesion without calcification is a prominent feature. If typical lesions of tuberculous are present at necropsy, demonstration of acid-fast bacilli in smears or histopathologic sections made from affected organs is regarded as sufficient for positive diagnosis (OIE, 2018).

In Ethiopia, in spite of the existence of large population of chicken and potential future expansion of the poultry industry, infectious diseases such as avian TB has not been well studied. Few avian TB studies carried out so far on traditionally managed local chicken reported a prevalence of 6.3% in central Ethiopia (Tadesse *et al.,* 2004) and 4.23% in Shashemene district of west Aris zone of Oromia Region (Abda *et al.,* 2015). However, there is no published abattoir-based avian TB study on slaughtered exotic chicken originated from intensive commercial production system in Ethiopia. Therefore, the present study was designed to investigate the occurrence of avian tuberculosis based on pathological lesions and isolation of its causative agents in apparently healthy chicken slaughtered at a poultry abattoir in Bishoftu, central Ethiopia.

Materials and methods

Study Area

The study was conducted at Alema poultry abattoir located in Bishoftu town in East Shoa Zone of Oromia Regional State from October 2015 to June 2016. Bishoftu town is located at 47 kilometers south east of Addis Ababa. The area is located at 9°N latitude and 40°E longitude at an altitude of 1850 meters above sea level in central high lands of Ethiopia. In Bishoftu, there are few private large scale commercial poultry farms and a number of small-scale poultry farm. Alema intensive poultry farms is the second largest commercial poultry farms in the country next to ELFORA poultry farm delivering nearly half a million broilers to Addis Ababa market each year (Abera, 2018). This farm supplies broiler chicken for slaughter to Alema poultry abattoir, which is one of the modern poultry abattoirs in Ethiopia and is producing chicken meat for local market mainly to supermarkets and hotels in Addis Ababa and its surrounding.

Study animals

The study was carried out on exotic chickens which were brought for slaughter to Alema poultry abattoir. The chickens were apparently healthy raised under intensive commercial farming system supplied with formulated feed ration and the chickens obtained vaccination for major poultry diseases such as Newcastle disease and fowl cholera. The study animals were male and female of different body condition and the same age group.

Study design

A cross-sectional study design was carried out primarily to estimate the apparent prevalence of avian TB based on the presence of suspected TB lesions during postmortem examination of the slaughtered chicken in the poultry abattoir. The organs with suspected gross pathological lesions particularly liver, spleen and intestine were examined using detail postmortem examination method.

Sample size determination

The determination of sample size for this study was calculated by considering 6.3% expected prevalence as reported in previous avian tuberculosis study in backyard chicken of central Ethiopia (Tadesse *et al.,* 2004), 95% confidence interval and 5% required precision, and using the formula for estimation of sample size as given below according to Thrusfield (2007).

$$n = \frac{1.96^2 \mathrm{X}\ \mathrm{P_{exp}} \mathrm{X}\ (1 - \mathrm{P_{exp}})}{\mathrm{d}^2}$$

Where n= required sample size, d= desired absolute precision, P_{exp}= expected prevalence

Thus, the calculated sample size was 91. However, as the expected prevalence considered in the sample size calculation was from the previous study done on local chicken in backyard scavenging management system the author believed the existence of major differences from the present abattoir-based avian TB study which targeted on apparently healthy exotic breeds managed under intensive production system. Therefore, 50% expected prevalence was considered and the final calculated samples size was 384 and in order to increase the precision of the study a total of 648 animals were examined.

Sampling methods

A systematic random sampling procedure was used to pick every sixth chicken slaughtered during the visit. The abattoir was visited for four days per week depending on the regular schedule of the abattoir to collect the samples until the sample size was fulfilled. During the study on average 200-300 chickens were slaughtered per day based on local market needs. Information about the flock of chickens slaughtered in each visit days with regarding age, breed, flock health status including vaccination history; farm of origin, management system were recorded.

Study Methodology

Ante mortem examination

Ante-mortem examination was done after the chickens were hung in shackles and before bleeding occurred and individual chicken was examined visually for general condition including the presence of visible abnormalities and superficial lesions. Body condition scoring for each chicken was done as poor body condition (Score 0) and good body condition (Score 1) according to Gregory and Robins (1998).

Gross postmortem examination and tissue sampling

A total of 648 chickens were examined for suspected gross pathological lesions and tissue samples were taken from chickens with suspected pathological TB lesions. Postmortem examinations were done on each chicken carcass to detect the presence of tuberculous lesion in different visceral organs with particular emphasis on liver, intestine and spleen. During post-mortem examination, gross lesions were sliced using separate sterile surgical blades and the lesion were described grossly on characteristic and distribution of the lesion. The suspected lesions were sampled and kept in labeled universal bottle containing phosphate buffered saline solution and transported in ice box under cold chain condition to Aklilu Lemma Institute of Pathobiology (ALIPB) laboratory for mycobacterial culture isolation.

Tissue processing for Mycobacterium culture

Isolation of mycobacteria from tissues was done in accordance with OIE (2018) protocol. Approximately 3-5 grams of tissue samples from suspected TB lesions were sectioned into pieces using sterile blades, and then homogenized by pestle and mortar. The homogenate were decontaminated by adding an equal volume of 4% NaOH followed by centrifugation at 3000 rpm for 15 minutes. The supernatant were discarded while the sediment was neutralized by 1% (0.1N) HCl using phenol red as an indicator. Neutralization was achieved when the color of the solution changed from purple to yellow. Thereafter, 0.1ml of suspension from each sample was spread onto a slope of Lowenstein-Jensen (L-J) medium. Duplicates of L-J were used; one enriched with 1% sodium pyruvate while the other was enriched with glycerol. Culture tubes were kept inclined with loosened screw to facilitate the evaporation of excess moisture and inoculum fluid for one week. After one week the tubes were placed vertical with tightened screw and incubated aerobically at 37°C for 16 weeks or until macroscopic growth was observed while they were examined on a regular basis for colony growth. Cultures were first examined after 8th week of inoculation and subsequently every week up to 16 weeks for the presence of any growth. No evidence of bacterial growth after this incubation period was considered as a negative result. A culture was considered positive when white spot colonies were seen. Growths of *Mycobacterium avium* colonies were confirmed by Ziehl-Neelsen (Acid-fast) staining and its long incubation period. Whenever, colonies were seen, sub-culturing and acid-fast staining were performed to confirm the presence of acid fast bacilli.

Acid-Fast Staining Technique

The colonies which grow on the L-J media were stained with Ziehl-Neelsen method for detection of acid-fast bacilli according to Quinn *et al*. (1994). Stained slides were observed using oil immersion of 100 x lens objective of a microscope. Each slide was examined for 30 minutes to detect acid-fast bacilli in the examined microscopic fields. The findings were recorded according to the bacteria appearance in which, observation of a bacilli with pink rod-shaped appearance taken as positive while if no acid-fast bacilli were observed, the sample was regarded as negative for acid-fast bacilli.

Data management and analysis

The data collected from abattoir and laboratory examination were entered and coded into Microsoft excel spread sheets and analyzed by STATA statistical software version 11 (STATA Corp. College station, TX). The association between different risk factors with the occurrence of avian tuberculosis suspected postmortem lesions were analyzed using chi-square ($\chi 2$) and multivariable logistic regression. A p-value <0.05 was considered statistically significant and in estimating the effect of different risk factors in terms of odds ratio (OR) with corresponding 95% confidence interval, statistical significance was assumed if the confidence interval did not include one among its values.

Results

Prevalence of avian tuberculosis and associated risk factors

The prevalence of avian TB at Alema poultry abattoir was 6.48% (95% CI: 4.58 to 8.38%) based on postmortem examination for the presence of suspected gross pathological TB-like lesions. The prevalence of the disease varies with different risk factors. From the total chicken examined in the abattoir, 56.2% were female and 43.8% were male. The multivariable logistic regression analysis for the association between prevalence and various risk factors is presented in Table 1. The analysis showed that chicken with poor body condition were more likely to be positive for avian TB suspected lesions as compared to chicken with good body condition and the difference was statistically significant (adjusted OR=4.46; 95% CI: 2.33-8.53). The association of other risk factor (sex of the chicken) with the prevalence of avian TB was not statistically significant ($p > 0.05$).

Table 1. Multivariable logistic regression analysis of the effect of different risk factors on the prevalence of TB in poultry.

Variables	categories	Total No. of chicken examined	Positive for TB (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	p-Value
Chicken sex	**Male**	287	20(6.97%)	1	1	0.641
	Female	361	22(6.1%)	0.88(0.43-1.62)	0.86(0.45-1.63)	
Body condition	**Good**	188	16(3.5%)	1	1	0.000
	Poor	460	26(13.8)	4.45(2.33-8.53)	4.46(2.33-8.53)	

Antemortem findings

Antemortem examination of all the 648 chickens showed no clinical signs of any diseases and the chicken were apparently healthy. Their live body weight was estimated to range from 0.6-1kg in which 460 chickens (71%) were in poor body condition status, and 188 chickens (29%) were in good body condition status.

Gross pathological postmortem findings

During detail examination of the organs from the 648 chickens, gross lesions were observed in liver, spleen and intestine and more typical tuberculous lesions were seen in liver. The lesions were grayish-yellow to grayish-white, pinpoint to irregularly round, and few were nodules measuring up to 2mm in diameter were swollen above the surface of the affected organs. Calcification was not seen in the nodules. Spleen and liver with pathological lesion were enlarged in size. Grossly, the lesions on liver were characterized as small; multiple circular nodules with light-yellowish color with white spot appear up on dissection with no calcification seen in the nodules (Figure 1).

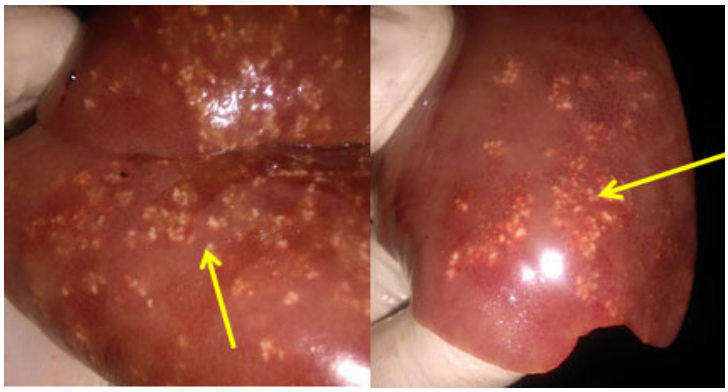


Figure 1. Small light yellowish nodular tuberculous-like lesions (yellow arrows) on the liver of chicken found during postmortem examination.

Regarding the distribution of lesions in different organs, larger proportion (88.10%) of the lesions were observed in liver, followed by spleen (9.52%) and intestine (2.38%) (Table 2). In most of the chickens that have manifested gross lesions, more than one type of organs was affected.

Table 2: Association of gross pathological lesions of suspected avian tuberculosis with distribution of the lesions in different organs of the examined chicken

Organs	Sample examined	Sample positive	%	χ^2 - value	p-value
Liver	41	37	88.10	10.43	0.108
Spleen	35	4	9.52		
Intestine	28	1	2.38		
Total	104	42	100		

Isolation of Mycobacteria from suspected TB-like lesions

From the 42 suspected TB-like lesions samples cultured for mycobacteria, growth of Mycobacterium was observed in 14 samples on pyruvate enriched L-J culture media after 3 weeks of incubation. The colonies were smooth, light yellowish and slightly raised colony. The origins of samples for the 14 isolates were liver, spleen and intestinal tissue. The culture isolates were further identified by conducting acid-fast staining and five isolates were confirmed to be acid-fast by Ziehl-Neelsen staining and they were stained bright red (pink) and had rod-shaped morphology (Figure 2).

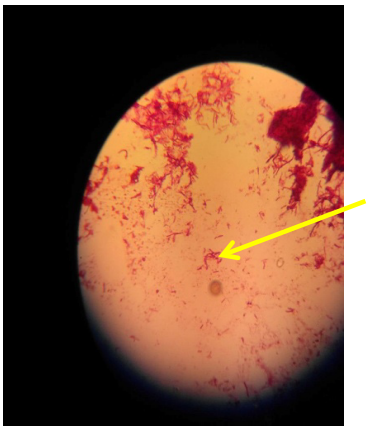


Figure.2 Detection of acid-fast bacilli of Mycobacterial culture positive samples from suspected avian TB chicken. The acid-fast bacilli were pink (indicated by yellow arrow), straight or slightly curved rods, occurring singly or in small groups in stained smears examined microscopically (oil-immersion magnification 1000X).

Discussion

In the present study an attempt was made to estimate the prevalence of avian tuberculosis based on abattoir-based postmortem examination of suspected TB-like lesions and identify the associated risk factors in chicken slaughtered at modern poultry abattoir in Bishoftu town. The study showed that the prevalence of avian TB in slaughtered exotic breeds of chicken from intensive poultry farm was low (6.48%) based detection of suspected TB-like lesions and the result was in agreement with the finding of previous study (Tadesse *et al.,* 2004) with 6.3% prevalence in Adama town from apparently healthy local chickens and (Abda *et al.*, 2015) with 4.23% prevalence in local chickens at Shashemene district. So far, no published report of avian tuberculosis in abattoir based on postmortem examination of suspected TB-lesions in exotic chicken originated from intensive commercial poultry farms in Ethiopia.

In the present study, the majority of the chickens infected had poor body condition and there was a significant association of avian TB prevalence with body condition of chickens and a number of studies indicated the association of poor body condition with avian TB prevalence (Dhama *et al.,* 2008, Miguel, 2012; Abda *et al.,* 2015). Thus, chicken with poor body condition in a farm or in antemortem inspection could be suggestive of avian tuberculosis. These studies indicated that chickens are constantly exposed to overcrowding (which may lead to stress), and unhygienic external environments may serve as sources of infection. Overcrowding within a flock can result a stressful condition for the chickens, which in turn could affect the nature and number of lesions occurring (Tell *et al.,* 2001; Fulton and Thoen, 2003; OIE, 2018). Based on postmortem examination of the TB-like lesions, majority (88.10%) of the suspected gross TB-like lesions were localized in liver and the rest were localized in spleen and intestine which might suggest that the most probable route of infection was oral route through ingestion. The route of infection helps to explain the low incidence of pulmonary lesions in birds when compared to mammals in which the lung is infected first (Gonzalez *et al.,* 2002).The overall characteristics of gross lesions and its distribution in different organs which were found in this study were in line with the previous findings (Tadesse *et al.,* 2004). The variation in size and number of lesions recorded in chickens could also be caused by successive episodes of reinfection from previously established lesions (Thoen, 1997).

In mycobacteriological culture examination, fourteen isolates were identified as positive for Mycobacterium as suspected by colony morphology and growth

characteristics; and five of the isolates were acid-fast positive as confirmed by acid-fast staining of the bacilli. Typically, species like *M. avium* produces "smooth" colonies which are virulent for chickens while variants with smooth domed or rough colonies are avirulent (Fulton and Thoen, 2003; Dhama *et al*., 2007). The recovery of Mycobacterium in culture growth was low in proportion, 33.3% (14/42) and this could be due to the non-optimal condition of the culture medium for non-tuberculous mycobacteria species including *M. avium* as this study used the available L-J media for culture of the isolates. Similar studies have also reported a low culture recovery rate for non-tuberculosis mycobacteria isolation using LJ media (Mamo *et al.,* 2011; Abda *et al.,* 2015). On the other hand, the low culture positivity might also be due to the absence of viable mycobacteria in necrotized TB-like lesion in which incompletely necrotic lesions, tubercle bacilli are dead and therefore no growth could be detected up on culture on LJ media (Quinn *et al*., 1994).

In the present study, analysis of tissues samples that resulted characteristics growth of colony of suspected *Mycobacterium avium* using acid-fast staining technique revealed that out of 42 samples only five (11.9%) were acid-fast positive. Even though, acid-fast staining method can be used for diagnosis of avian tuberculosis combined with clinical sign and mycobacterial isolation it has low sensitivity (Garg *et al.,* 2003; Dhama *et al.,* 2007; Zhu *et al*., 2018). Diseases causing nodular lesions such as leucosis, pseudotuberculosis and coligranulomas can be considered as differential diagnosis in those samples with negative acid-fast staining results (Dhama *et al.,* 2011).

Limitation of the study

This study was carried out in abattoir setting where there might be a possibility of contamination from the environment as the group in *M. avium* are ubiquitous. The other main limitation of the study was the isolates were not further analyzed by molecular method to confirm the causative agent of avian TB, *M. avium subsp. avium* using molecular techniques.

Conclusion

In general, in Ethiopia there is limited information on epidemiology of avian TB and its associated risk factors in exotic chicken reared under intensive commercial poultry production system. Hence, the present abattoir-based study revealed the occurrence of avian tuberculosis in apparently healthy exotic

chickens which originated from intensive commercial poultry farm in Bishoftu area and chicken with poor body condition were associated with the finding of suspected TB lesions; hence, it seems appropriate to recommend during antemortem inspection chicken with poor body condition should be isolated and examined thoroughly. Moreover implementation of routine detailed poultry meat inspection procedures in poultry abattoirs is important to detect the pathological lesions and produce safe poultry meat for consumers. The finding of higher proportion of TB-like lesions which were negative in culture and acid-fast staining warrants the need further investigation on the causative agents of the pathological lesions other than avian tuberculosis.

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Conflict of Interest

The author has declared that no conflict of interests exists.

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References

Abda, S., Mamo, G., Worku, A., Ameni, G., 2015. Preliminary study on avian tuberculosis and associated risk factors in domestic chickens at Shashemene district, Ethiopia. *J. Biol. Med. Sci.*, 3, 13-23.

Abera, A., 2018. Review on Chicken Production in Ethiopia with emphasis on Meat Production, Munich, GRIN Verlag, https://www.grin.com/document/385849.

CSA, 2017. Central Statistics Authority (CSA): Agricultural sample survey 2016/17. Report on livestock and livestock characteristics. Statistical Bulletin No. 585. Addis Ababa, Ethiopia.

Dhama, K., Mahendran, M. & Tomar, S., 2007. Avian tuberculosis: an overview. *Poultry Punch,* 24 (3), 38-52.

Dhama, K., Mahendran, M., & Tomar, S., 2008. Pathogens transmitted by migratory birds: threat perceptions to poultry health and production. *Int. J. Poult. Sci.,* 7 (6), 516-525.

Dhama, K., Mahendran, M., Tiwari R., Singh S.D., Kumar D., Singh S., Sawant P.M., 2011. Tuberculosis in birds: insights into the *M. avium* infections: review article, *Vet. Med. Int,* Vol 2011, 1-13. doi:10.4061/2011/712369

Fulton, R. and Thoen, C., 2003. Tuberculosis. Diseases of Poultry, Iowa State University Press, USA. Pp. 836-844.

Gonzalez, M., Rodriguez-Bertos, A., Gimeno, I., 2002. Outbreak of avian tuberculosis in 48-week-old commercial layer hen flock. *Avi. Dis.,* 46, 1055–1061.

Garg, S.K, Tiwari, R.P., Tiwari, D., Singh, R., Malhotra, D., Ramnani, V.K., *et al.*, 2003. Diagnosis of tuberculosis: available technologies, limitations, and possibilities. *J. Clin. Lab. Ana.l,* 17, 155–63.

Habte, T., Amare, A., Bettridge, J., Collins, M., Christley, R., Wigley P., 2017. Guide to chicken health and management in Ethiopia, ILRI manual 25. Nairobi, Kenya: International Livestock Research Institute (ILRI), Pp. 17-44.

Mamo, G., Bayleyegn, G., Sisay T., Legesse, M., Medhin, G., Bjune, G., Abebe, F. & Ameni, G., 2011. Pathology of Camel Tuberculosis and Molecular characterization of Its Causative Agents in Pastoral Regions of Ethiopia. *PLoS One*, 6(1), e15862.

Miguel, D.S., 2012. Mycobacteriosis: An issue of veterinary clinics: Exotic Animal Practice. *Vet. Clin. N. Am.,*15 (1), 1-10.

OIE, 2018. Avian tuberculosis. OIE Terrestrial Manual 2018: Office International des Epizooties (OIE) Chapter 3.3.6. Paris, France. Pp. 860-868.

Quinn, P.J., Carter, M.E., Marker, B. & Carter, G.P., 1994. *Mycobacterium species.* In: Clinical Veterinary Microbiology, Wolfe Publishing, London. Pp. 156-170.

Shivaprasad, H.L and Palmieri C., 2012. Pathology of Mycobacteriosis in Birds. *Vet. Clin. Exot. Anim.*, 15, 41–55

Sonaiya, E., Branckaert, R. & Guèye, E., 1999. Research and development options for family poultry. In: Gueye E. F. (Ed) The First INFPD/FAO Electronic Conference

on the Scope and Effect of Family Poultry Research and Development, (www.fao.org/ag/againfo/subjects/en/infpd/econf_scope.html)

Dessie, T. & Ogle, B., 2001. Poultry production systems in the central highlands of Ethiopia. *Trop. Anim. Hlth. Prod.,* 33(6), 521-537.

Dessie, T., Kijora, C. & Peters, K. J., 2003. Indigenous chicken ecotypes in Ethiopia: Growth and feed utilization potentials. *Int. J. Poult. Sci.,* 2, 144-152.

Tadesse, S., Woldemeskel, M., Molla, B., Tibbo, M., Kidane, D., Medhin, G., and Britton, S., 2004. Avian mycobacteriosis in domestic chickens from selected agro-climatic regions in Ethiopia. *Int. Appl. Res. Vet. Med.,* 2 (1), 17-25.

Tell, L., Leutenegger, C. and Larsen, R., 2003. Real-time polymerase chain reaction testing for the detection of *Mycobacterium genavense* and *Mycobacterium avium* complex species in avian samples. *Avi. Dis.*, 47(4), 1406–1415.

Tell, L., Woods, L. and Cromie, R., 2001. Avian tuberculosis in birds. *Rev. Sci. Tech. Off. Int. Epiz.,* 20, 180-203.

Thoen, C.O., 1997. Tuberculosis. In: Diseases of Poultry. *10th ed*, Iowa State University Press, USA, Pp. 67-178.

Thrusfield, M., 2007. Veterinary epidemiology 3rd ed., Blackwell Science Ltd, Oxford, UK, Pp. 178-197.

Zhu, L., Peng, Y., Ye, J., Wang, T., Bian, Z., Qin, Y., Zhang, H., and Ding, J., 2018. Isolation, identification, and characterization of a New Highly Pathogenic field isolate of *Mycobacterium avium spp. avium*. *Front. Vet. Sci*., 4, 243.

Serological investigation of brucellosis and its association with abortion in sheep and goats in selected districts of Jimma zone, southwestern Ethiopia

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Abstract

The occurrence of brucellosis in different species of livestock has been reported from different parts of Ethiopia, however, the serostatus and risk factors of this disease in small ruminants is not well documented in southwestern parts of the country. This study was conducted from October 2016 to October 2017 to investigate the seroprevalence and associated risk factors of brucellosis as well as its association with abortion in sheep and goats in selected districts of Jimma zone. A total of 804 small ruminants (402 sheep and 402 goats) were randomly selected and blood samples were collected for serological tests. The presence of antibody to *Brucella* was screened by Rose Bengal Plate Test and confirmed using the complement fixation test. Seroprevalence of 4.2% in sheep and 5.2% in goats was recorded in this study. An overall seroprevalence of 4.7% was recorded in small ruminants in the study areas. *Brucella* seropositivity was observed more frequently among sheep and goats with a history of abortion (6.7%) than animals that have no history of abortion (3.7%), however, the difference noted was not statistically significant. Older age (OR 3.9, CI = 1.43-9.94), pregnancy (OR 2.6, CI = 1.19-5.72), late term gestation (OR 2.4, CI = 1.54-3.78), mixed flock (OR 2.8, CI = 1.33-5.89) and larger flock size (OR 2.7, CI = 1.08-6.95) were noted to have more number of reactors. Hence, it is important to consider brucellosis as one of the diseases that needs attention and further study should be conducted to identify the circulating *Brucella* species and other causes of abortion in sheep and goats. Besides, this finding warrants the need for further investigation on its public health impact in the region.

Keywords: Brucellosis; Epidemiology; Sheep; Goat; Jimma Zone

Introduction

Ethiopia has a huge number of small ruminants with an estimated population of 30.7 million sheep and 30.2 million goats (CSA, 2017). However, the disease of reproduction is challenging to the rearing of sheep and goats in the country (ESGPIP, 2011). Infectious reproductive diseases of sheep and goats are part of the major flock health problems known to constrain the sector. These diseases usually manifest themselves through infertility, abortion, stillbirth and weak offspring (Radostits *et al*., 2007). Several infectious agents including, bacteria, viruses and protozoa are accountable for abortion in sheep and goats. Abortion is also caused by non-infectious factors such as toxic substances, metabolic, nutritional and physical injuries. These causes also have a significant impact on the overall productivity of sheep and goats (Daniel and Marley, 2008; Mirkena *et al*., 2011). Among infectious causes of abortion, *Brucella* is one of the known bacterial pathogens causing tremendous economic losses (FAO, 2003; Tegegn *et al*., 2016).

Brucella melitensis and *B. ovis* are the two important *Brucella* species known to affect sheep and goats, however; *B. abortus* is also been incremented occasionally in sheep and goats (Radostits *et al*., 2007; Akhvledian *et al*., 2010). The disease is manifested by late-term abortions, weak lambs and kids, stillbirths, infertility and characterized mainly by the retained fetal membrane (Radostits *et al*., 2007). *Brucella* infection in sheep and goats is a well-known disease worldwide. However, the disease is a serious problem in developing countries (FAO, 2003; Njeru *et al*., 2016; Shirima and Kunda, 2016). The prevalence of brucellosis is affected by several risk factors such as production system, host and environmental factors (Radostits *et al*., 2007). In sexually mature sheep and goats, brucellosis restricts to the reproductive tract and typically causes placentitis and abortion in pregnant ones. *Brucella melitensis* and *B. abortus* are zoonotic pathogens that cause disease in humans (Pappas *et al*., 2006; Radostits *et al*., 2007).

Many developing countries with a limited resource like Ethiopia are facing other priority diseases and have not yet performed an aspect of brucellosis intervention. The epidemiology, and control and prevention methods of brucellosis in sheep and goats were not well understood (McDermont and Arimi, 2002). As a result, brucellosis remains challenging in sheep and goat population and cause huge economic and public health problems in the country (Tegegn *et al*., 2016; Mohammed *et al*., 2017). Brucellosis also causes reduce the foreign cur-

rency earnings through rejection of export sheep and goats from international markets (LMA, 2005).

Ethiopia has various agro-ecological zones that have contributed to the development of different livestock production systems (Beruktayet and Mersha, 2016). Production system, breed, and environmental factors greatly influence the spread of brucellosis among the causes of abortion (Tulu *et al*., 2018). Several sheep and goats breed in Ethiopia are reared in different agro-ecological zones and production systems. The extensive production system of the country is responsible for the mixing of different livestock species for maintenance and transmission of brucellosis (Megersa *et al*., 2011b). Close animal-human contact and traditional raw animal product consumption make zoonosis among the main public health hazards in Ethiopia. Hence, detailed epidemiological studies for the implementation of applicable control strategies were needed (Addis and Desalegn, 2018). Several studies were done on sheep and goat brucellosis in different parts of the country (Teshale *et al*., 2006; Asmare *et al*., 2010; Megersa *et al*., 2011b; Dabassa *et al*., 2013; Sintayehu *et al*., 2015). Nevertheless, reports from south western part of the country are very limited. Therefore, this study aimed to estimate the seroprevalence and identify the associated risk factors of brucellosis. Besides it attempts to observe the association of *Brucella* reactors with abortion in sheep and goats in selected districts of the study area.

Materials and Methods

Description of study areas

The study was carried out in Limu Seka and Chora Boter districts of the Jimma zone. Limu Seka district located about 463 km from Addis Ababa, the capital city of Ethiopia; and 109 km from Jimma town, the capital city of Jimma zone. The district covers an area of approximately 1,694 km^2 and is divided into 38 kebeles (the smallest administrative units). The agro-ecology of the district is characterized by highland (13%), mid-highland (55%) and lowland (32%). The altitude of the district is between 1,400-2,200 meters above sea level. Chora Boter district is located 466 km away from Addis Ababa, and 112 km from Jimma town, zonal capital. This district has 19 kebeles; and agro-ecologically it is characterized by highland, mid-highland, and lowland. The altitude of the district is between 1,100-2,200 meters above sea level and has an average temperature of 22^{0}C. Chora Boter district has 228,846 head of cattle, 47,854 head of sheep, and 68,037 head of goats. Both districts have two distinct seasons.

The rainy season is starting in late March and ending in October, and the dry season is occurring from November to early March.

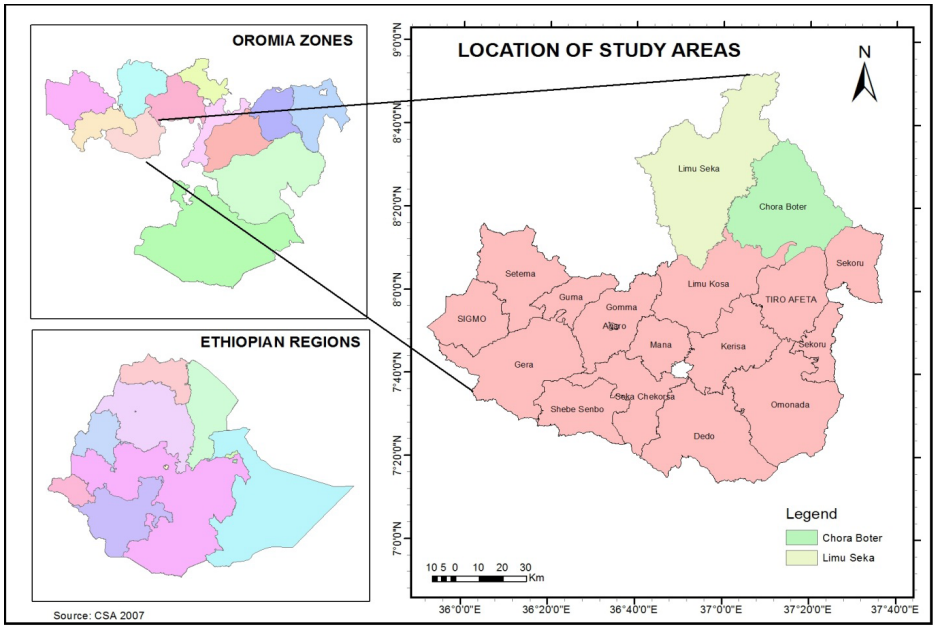


Figure 1: Map of the study areas

Study design and animals

A cross-sectional study was carried out from October 2016 to October 2017 in selected districts of Jimma zone to investigate the seroprevalence and associated risk factors of brucellosis as well as its association with abortion in sheep and goats. The target population of this study comprises female sheep and goats in study districts, namely Limu Seka and Chora Boter. Sheep and goats older than three months (to exclude maternal immunity) were randomly selected and included in the study. Age was determined using dentition and categorized as < 1 year, 1 year to 2 years and >2 years (ESGPIP, 2009). The number of sheep and goats farmers own was categorized as small (<7 sheep/goats), medium (8-12 sheep/goats) and large (>12 sheep/goats) flock sizes. Parity number was categorized as nulliparous (zero parity), monoparous (parity one) and pluriparous (≥ two parities) (Margatho *et al.*, 2019). Management systems were classified as extensive and semi-intensive based on the criteria adopted by Gizaw *et al.* (2015). Body condition scoring for sheep and goats

was conducted using the guidelines established by Langston University (Villaquiran *et al*., 2007; Kenyon *et al*., 2014) and ESGPIP (ESGPIP, Technical Bulletin No. 8) guidelines for body condition scoring and for all sheep and goats under the study their body condition grouped into three groups (poor, medium and good). Abortion was defined as loss of fetus or fetuses before 140 days of pregnancy (Margatho *et al*., 2019). The gestation stage of abortion was categorized as first trimester (<50 days), second trimester (51-100 days) and third trimester (101-154 days) (Bokko, 2011). Properly manage abortion materials when it was buried or burned whereas improperly manage was simply leave it on the ground or give to the dogs. According to the agro-altitudinal and agro-climatology classification of Ethiopia, lowland falls between 500 and 1,500 and midland falls between 1,500 and 2,300 meters above sea level (Libeau *et al*., 1995).

Sampling techniques and sample size determination

The multistage sampling method was used to select sampling units from different flocks. Jimma zone and districts (Limu Seka and Chora Boter) were selected purposively based on the dominant of sheep and goats production system, while peasant associations and flocks were selected randomly. The sampling frame of peasant associations were obtained from respective districts agricultural office. A total of ten peasant associations were selected from two districts using a random sampling technique where six peasant associations were from Limu Seka and four of them from Chora Boter based on sheep and goats population. Since there is no previous study on breeding sheep and goat brucellosis in study areas, the sample size was calculated using formula described by Thrusfield (2005) considering an expected prevalence of 50% and an absolute precision of 5% with 95% confidence interval. Substituted each gave 384 animals. Since two populations (sheep and goat) were included in this study, the sample size was double (768). However, a total of 804 small ruminants (402 sheep and 402 goats) were involved to increase the accuracy of the study.

Blood sample collection

Blood samples collected from the jugular vein, (3-5ml) aseptically using sterile plain vacutainer tubes and needle, were kept in a slanting position overnight (12 hours) at room temperature to separate the serum. Then sera were gently decanted into sterile screw cupped tubes, labeled and transported in ice packs to Jimma University, College of Agriculture and Veterinary Medicine micro-

biology laboratory and stored at -20°C until screened and tested for antibodies against natural *Brucella* microorganisms. As there has never been a history of vaccination for brucellosis, all positive results were attributed to natural infection. Parallel to blood sample collection relevant information such as agro-ecology, management system, flock size, species composition (mixed of sheep and/goats with cattle), introducing new animals, species, management of aborted material, age (years), parity, body condition, reproduction status, abortion history and frequency, gestation period at abortion and history of retained fetal membrane were collected using separate format.

Serological test

All serum samples collected were first tested using Rose Bengal Plate Test (RBPT) at the National Veterinary Institute (NVI), Debre Zeit, Ethiopia according to the technique described by Alton (1990). The sera and RBPT reagent were taken from the refrigerator and kept at room temperature for at least 30 minutes before the test was performed. A total of 30 microliters of serum sample was dispensed onto the plate and 30 microliters of RBPT antigen were dropped on the slide with sera. The interpretation of both positive and negative control results was done according to the degree of agglutination and the reaction was read in a good light source or by a magnifying glass when micro agglutination was suspected. The RBPT results were interpreted 0, +, ++ and +++ as has been described by Dohoo *et al*. (2009), where 0 indicates no agglutination, + indicates barely visible agglutination (using magnifying glasses), ++ indicates fine agglutination and +++ indicates coarse clumping. Those serums identified with no agglutination (0) were regarded as negative, while those with +, ++ and +++ were considered as positive. The whole positive serums were confirmed using the complement fixation test (CFT) using standard *B. abortus* S99 (KT153, UK). This test was also done at NVI. The preparation of the reagent was evaluated by titration and performed according to protocols recommended by OIE (2009). Sera with a strong reaction, more than 75% fixation of complement (3+) at 1:5 dilution or at least with 50% complement fixation (2+) at 1:10 dilution and above were considered as positive and lack of fixation/complete hemolysis was considered as negative (OIE, 2004).

Data management and analysis

Data obtained from this study were recorded, coded and stored in Microsoft Excel for Windows 2010 and transferred to STATA version 11.0. Seroprevalence of brucellosis was calculated by dividing the number of seropositive samples

to the total of sheep and goats samples. Association between brucellosis and presumptive risk factors was analyzed using the logistic regression analysis. The variables with p-value less than or equal to 0.25 in univariable logistic regression, after checking for multicollinearity using collinear matrix index and interaction effect using cross-product terms were taken forward for multivariable modeling. The model fitness was observed using the Hosmer-Lemeshow test. The model validate was also evaluated by using the ROC curve. For all statistical analysis, confidence intervals (CI) of 95% and p-value of 0.05 were used.

Results

Out of the total 804 sheep and goats sampled, 6.3% (n=51) of sheep and goats were tested positive on screening using RBPT. Further, confirmation using CFT identified an overall 4.7% (38/804) prevalence of *Brucella* antibodies in sheep and goats. Seroprevalence of 4.2% (17/402) and 5.2% (21/402) were recorded in sheep and goats, respectively. More seropositivity of *Brucella* antibody was observed among sheep and goats with the history of abortion (6.7%) than animals that have no history of abortion (3.7%). However, there was no difference ($p>0.05$). Likewise, sheep and goats from a large flock size category were almost three times more likely to be infected with *Brucella* organisms than small flock size (OR=2.8, $p<0.05$). Similarly, a statistically significant difference in seroprevalence of brucellosis was observed in animals herded with cattle ($p<0.05$). Mixed (sheep and/goats with cattle) species were almost three times more likely to be infected with *Brucella* organisms than those having close contact with cattle (OR=2.8). However, agro-ecology, species, introducing of a new animal, management system and properly manage of aborted material were not able to explain seroprevalence of brucellosis (Table 1).

Table 1. Univariable logistic regression of environmental-related putative risk factors in study areas

Variables	Categories	Total animals examined	Total animals positive (%)	OR (CI; 95%)	p-value
Agro-ecology	Lowland (Ref.)	176	8 (4.55)	-	0.143
	Midland	628	30 (4.78)	1.7 (0.837-3.430)	
Management system	Extensive (Ref.)	691	32 (4.63)	-	0.218
	Semi-intensive	113	6 (5.31)	0.6 (0.269-1.349)	
Flock size	Small(<7) (Ref.)	228	6 (2.63)	-	0.024
	Medium(8-12)	241	7 (2.90)	0.9 (0.429-1.912)	
	Large(>12)	335	25 (7.46)	2.8 (1.154-6.956)	
Species composition	Single species (Ref.)	419	11 (2.63)	-	0.005
	Mixed	385	27(7.01)	2.8 (1.37-5.72)	
Introducing new animals	No (Ref.)	457	19 (4.16)	-	0.893
	Yes	347	19 (5.48)	1.1 (0.541-2.024)	
Proper manage of aborted material	Yes (Ref.)	247	8 (3.24)	-	0.190
	No	527	30 (5.39)	0.6 (0.266-1.302)	
Species	Sheep (Ref.)	402	17 (4.23)	-	0.507
	Goat	402	21 (5.22)	0.8 (0.416-1.542)	

Ref. = Reference; OR=Odd Ratio; CI=Confidence Interval

Similarly, *Brucella* seropositivity was significantly varied among sheep and goat age groups with older age categories are 2.7 times more likely to be *Brucella* seropositive than younger age category (OR=2.7, $p<0.05$). The univariable logistic regression analysis shown that *Brucella* seroprevalence was a statistically significant difference with gestation stages, with the odd of seropositivity being 1.3 times higher in animal aborted in the third trimester than those have no history of abortion (OR=1.3, $p<0.05$).

Table 2. Univariable logistic regression of host-related putative risk factors in study areas

Variables	Categories	Total animal examined	Total animal positive (%)	OR (CI; 95%)	p-value
Age					0.006
	<1 year (Ref.)	236	5 (2.12)	-	
	1-2 years	217	11 (5.07)	1.6(1.42-3.45)	
	>2 years	351	22 (6.27)	2.7(1.15-6.66)	
Parity					0.136
	Nulliparous (Ref.)	168	6 (3.57)	-	
	Monoparous	207	8 (3.86)	0.7(0.32-1.77)	
	Pluriparous	429	24 (5.59)	1.6 (0.68-3.71)	
Body condition					0.569
	Good (Ref.)	135	4 (2.96)	-	
	Medium	463	24 (5.18)	0.6(0.19-1.64)	
	Poor	206	10 (4.85)	0.6(0.18-1.95)	
Reproduction status	Non-pregnant (Ref.)	342	11 (3.22)	-	0.087
	Pregnant	462	27 (5.84)	1.9(0.91-3.82)	
Abortion history	Absent	536	20 (3.7)	-	
	Present	268	18 (6.7)	0.5 (0.28-1.03)	0.064
Abortion frequency					0.104
	None aborted	536	20 (3.7)	-	-
	Once (Ref.)	199	15 (7.5)	0.5 (0.24-0.95)	
	≥2	69	3 (4.3)	0.9 (0.25-2.95)	
Gestation period at abortion					0.008
	None aborted (Ref.)	536	20 (3.73)	-	
	First trimester	41	1 (2.43)	0.2 (0.09-0.68)	
	Second trimester	68	4 (5.88)	0.3 (0.14-0.82)	
	Third trimester	159	13 (8.18)	1.3 (1.41-2.60)	
Retained placenta	Absent (Ref.)	673	29(4.31)	-	0.922
	Present	131	9(6.87)	1.0 (0.37-2.48)	

Ref. = Reference; OR=Odd Ratio; CI=Confidence Interval

In multivariable logistic regression analysis, explanatory variables with a p-value of less than or equal to 0.25 in the univariable logistic regression were included. This model showed that older sheep and goats were identified to be *Brucella* seropositive with the odds of positivity 3.9 (OR=3.9, CI; 1.43-9.94) times more likely than the younger ones. Similarly, this result also shows that sheep and goats that were from a mixed (sheep and/ goat with cattle) species were more likely to be *Brucella* antibody seropositivity than those having no contact with cattle (OR=2.8, CI; 1.33-5.89). Furthermore, sheep and goats from large flock sizes were also found to be at higher risk of *Brucella* infection, than those from small flock size (OR=2.7, CI; 1.08-6.95). Likewise, sheep and goat aborting at the third-trimester stage were higher *Brucella* seropositivity (OR=2.4, CI; 1.54-3.78) than those have no history of abortion. Moreover, reproduction status is statistically significantly different in *Brucella* antibody seropositivity with pregnant sheep and goats are 2.6 times more likely to harboring *Brucella* infection than non-pregnant ones (OR=2.6, CI; 1.19-5.72). Hosmer-Lemeshow test (0.5) indicates that the model was fit data well. ROC curve (0.76) indicated the model was good predicting ability.

Table 3. Final multivariable logistic regression model for brucellosis antibody seropositivity in study areas

Variables	Categories	Total animals examined	Total animals positive	Adjusted OR (CI; 95%)	p-value
Age(years)					0.003
	<1 year (Ref.)	236	5 (2.12)	-	-
	1-2 years	217	11 (5.07)	1.6 (1.36-3.86)	0.040
	>2 years	351	22 (6.27)	3.9 (1.43-9.94)	0.007
Species composition	Single species (Ref.)	419	11 (2.63)	-	-
	Mixed species	385	27 (7.01)	2.8(1.33-5.89)	0.007
Flock size					0.017
	Small (<7) (Ref.)	228	6 (2.63)	-	-
	Medium (8-12)	241	7 (2.90)	1.8 (1.35-2.66)	0.039
	Large (>12)	335	25 (7.46)	2.7 (1.08-6.95)	0.034
Gestation period at abortion					0.045
	None aborted (Ref)	536	20 (3.73)	-	-
	First trimester	41	1 (2.43)	0.3 (0.11-1.03)	0.056
	Second trimester	68	4 (5.88)	0.5(0.18-1.27)	0.141
	Third trimester	159	13 (8.18)	2.4(1.54-3.78)	0. 037
Reproduction status	Non-pregnant (Ref.)	342	11 (3.22)	-	-
	Pregnant	462	27 (5.84)	2.6(1.19-5.72)	0.017

Ref. = Reference; OR=Odd Ratio; CI=Confidence Interval

Discussion

In the present study, an overall 4.7% seroprevalence of brucellosis was recorded in female sheep and goats in study areas. The seroprevalence detected in this study is in line with the findings of Mengistu (2007), Wesinew *et al*. (2013) and Deddefo *et al*. (2015), who reported seroprevalence of 5.1%, 4.8%, and 4.6%, respectively. However, this result is higher than the findings of Tsehay *et al*. (2014) and Melese (2016), who reported a seroprevalence of 3.6% in eastern and 3.7% in southern Ethiopia, respectively. Higher seroprevalence

than the current study is reported by Yohanis (2012) 9.6% and Muluken (2016) 7.5% in the Afar region. Seroprevalence of 5.2% in goats and 4.2% in sheep was estimated in the present study. This result is in agreement with the findings of Feyisa *et al*. (2007), Yohanis (2012) and Deddefo *et al*. (2015), who reported 5.8% in goats and 4.9% sheep, and 4.9% in goats and 4.4% in sheep, respectively. However, the current result is higher than the findings of Teshale *et al*. (2006), who reported a seroprevalence of 1.7% in goats and 1.6% in sheep. Similarly, Mengistu (2007) reported 3.2% and 1.6% seroprevalence of brucellosis in sheep and goats, respectively. This variation may be due to differences in environmental factors, breed of sheep and goats, management and production system.

A statistically significant difference was observed in the *Brucella* seropositivity of sheep and goat among different age groups ($p<0.05$). The odds of *Brucella* seropositivity in older sheep and goats were almost four times (OR=3.9) more likely than in the younger category. This could be explained by the fact that sexually mature animals are more susceptible to *Brucella* infection than sexually immature animals since sex hormones that encourage growth and multiplication of *Brucella* organism, probable increase in concentration with age and sexual maturity (Quinn *et al*., 2004; Radostits *et al*., 2007). Significantly higher seropositivity in older small ruminants than younger can be attributed to the practice of leaving younger around home premises when adult small ruminants were taken to a communal grazing area. This decreases the risk of younger's being exposed to infection from common grazing and watering points (Kiputa *et al*., 2008). This finding is in line with the reports of Megersa *et al*. (2011a), Yohanis, (2012) and Muluken (2016), who reported a significant association between age and *Brucella* seropositivity in sheep and goats.

Reproductive status of sheep and goat is significantly associated with *Brucella* seropositivity where pregnant sheep and goat were about three (OR=2.6, $p<0.05$) times more likely than in non-pregnant ones. This could be explained by the fact that susceptibility to *Brucella* infection is increased after sexual maturity and especially with pregnancy. This is due to *Brucella* organisms prefer uterus in which allantoic fluid factors such as erythritol could stimulate the growth of *Brucella* and elevate in the placenta and fetal fluid from about the second trimester of pregnancy (Coetzer and Tustin, 2004; Radostits *et al*., 2007). This finding is in line with the report of Yohanis (2012), who stated that brucellosis was associated with the reproduction status of sheep and goats.
Gestation period at abortion is significantly associated with *Brucella* seropositivity whereby seropositivity about two (OR=2.4, $p<0.05$) times more likely in

the third-trimester stage than those have no history of abortion. This can be explained by the presence of higher concentration erythritol produced naturally by the developing fetus favors the multiplication of *Brucella* organisms, where it causes degeneration and necrosis of the cotyledons leading to abortion from the last months of gestation (Smith *et al*., 2002; Coetzer and Tustin, 2004). Besides, in highly susceptible nonvaccinated pregnant sheep and goat, abortion occurs in the last month of pregnancy is a cardinal feature of *Brucella* infection (Radostits *et al*., 2007). This finding was supported by the reports of Muluken (2016), and Coetzer and Tustin (2004), who indicated a significant association between seropositivity of *Brucella* infection and gestation stage in sheep and goats.

Species mix is also associated with *Brucella* seropositivity where sheep and/goats kept together with cattle is almost three (OR=2.8) times more likely to be positive for *Brucella* antibody than a single species flock. Cross-species infection with other *Brucella* species, especially *B. abortus*, has been documented in sheep and goat as a cause of *Brucella* infection (Glenn and Karen, 2005). Multiple livestock species herding together, especially keeping of goat and sheep along with cattle has been reported as an important determinant risk factor of *Brucella* seropositivity (Abbas and Agabu, 2002). Keeping sheep in contact with *Brucella* infected goat, also a potential risk factor for brucellosis spread among sheep flocks (Radostits *et al*., 2007). However, this finding is not supported by the reports of Coelho *et al*. (2013), who stated herding of small ruminants with cattle were not risk factors for brucellosis. This difference in *Brucella* seropositivity of species composition recorded in the different study areas may be associated with the differences in agroecology, management system and breed used in each study.

Flock size is associated with *Brucella* seropositivity in sheep and goat with large flock size was about three (OR=2.7) times more odd of brucellosis than small flock size. Flock size has previously been reported as an important determinant for transmission of *Brucella* organism between susceptible and infected animals (Omer *et al*., 2000) and because of one positive animal was at least available in large flock size compared with small flock size (Al-majali, 2005). This finding also related to a higher density of animals per flock. Keeping a large flock allows greater contact among animals. This makes a higher bacteria load in the environment and as a result, the probability of brucellosis transmission will be increased. Moreover, grazing in the communal pasture may facilitate the contact between infected and none infected flocks (Kaba-

gambe *et al*., 2001; Coelho *et al*., 2013). The association of flock size with the seropositivity in the present finding is confirmed with previous results (Kabagambe *et al*., 2001; Al-majali, 2005; Coelho *et al*., 2007; Coelho *et al*., 2013; Tegegn *et al*., 2016;).

In the current study, no statistically significant difference ($p>0.05$) was detected in seroprevalence of *Brucella* antibody between aborted and none aborted sheep and goats. This suggests that brucellosis may not be associated with abortion in sheep and goat in study areas. This could be true as abortion in sheep and goat has multiple causes, infectious (*Toxoplasma gondii*, *Leptospira spp*, *Listeria spp*, *Salmonellae spp*, *Coxiella burnetti*) and non-infectious (heat stress, nutritional deficiencies, trauma, toxic substances, etc) other than brucellosis. It is known that only seropositivity does not certainly associate with abortion in all conditions. It is important to identify the actual causes in the aborting sheep and goats and/ or in the aborted fetus or placental tissues to confirm the pathogen is accountable for abortion (Morris *et al*., 2018). This result is in line with the reports of Gebremedhin (2015) and Wubishet *et al*. (2017), who reported that the risk of *Brucella* seropositivity was not associated with abortion. However, this result is inconsistent with some previous studies in Ethiopia (Tassew and Kassahun, 2014; Asmare *et al*., 2013) that stated brucellosis was associated with abortion in sheep and goats. This variation may be due to differences in agroecology, breed, management and husbandry condition in the area. This can also be differences between the study areas regarding conditions that could favor the transmission of various causes of abortion (Radostits *et al*., 2007).

Conclusions

In the present study, the high seroprevalence of brucellosis was recorded in sheep and goat. Older age, mixed flock, pregnancy, late term gestation, and larger flock size have been noted as the most important factors for recovering higher proportion of *Brucella* reactors in sheep and goats. The present study also indicated that the presence of higher brucellosis in sheep and goat with the history of abortion than those have no history of abortion. Hence, it is important to carry out further study to identify the circulating *Brucella* species using molecular tools and other potential causes of abortion in sheep and goats. Besides, this study warrants the need for further investigation on *Brucella* infection public health impact in the study area.

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Conflict of interests

The authors have not declared any competing of interests

References

Abbas, B. and Agabu, H.A., 2002. A review of camel brucellosis. *Prev. Vet. Med.,* 55:47-56.

Addis, S.A. and Desalegn, A.Y., 2018. Comparative seroepidemiological study of brucellosis in sheep under smallholder farming and governmental breeding ranches of central and North East Ethiopia. *J. Vet. Med.*, Volume 2018, Article ID 7239156, 12 pages https://doi.org/10.1155/2018/7239156.

Akhvledian, T., Clark, D.V., Chubabria, G., Zenaishvili, O. and Hepburn, M.J., 2010. The changing pattern of human brucellosis: clinical manifestations, epidemiology and treatment outcomes over three decades in Georgia. *BMC. Infec. Dis.*, 10:346.

Al-Majali, A.M., 2005. Seroepidemiology of caprine brucellosis in Jordan. *Small Rumin. Res.*, 58, 13-18.

Alton, G.G., 1990. Brucella melitensis. In: Animal Brucellosis. Nielsen K and Duncan R (eds). CRC Press, Boca Raton, Florida, USA, pp. 383-409.

Asmare, K., Asfaw, Y., Gelaye, E. and Ayelet, G., 2010. Brucellosis in extensive management system of Zebu cattle in Sidama Zone, Southern Ethiopia. *Afr. J. Agric. Res.*, 5:257-263.

Asmare, K., Megersa, B., Denbarga, Y., Abebe, G., Taye, A., Bekele, T., Gelaye, E., Zewdu, E., Agonafir, A., Ayelet, G. and Skjerve, E., 2013. Study on seroprevalence of caprine brucellosis under three livestock production systems in southern and central Ethiopia. *Trop. Anim. Hlth. Prod.,* 45:555-560.

Beruktayet, W. and Mersha, C., 2016. Review of Cattle Brucellosis in Ethiopia. *Acad. J. Anim. Dis.*, 5(2):28-39.

Bokko, P.B., 2011. Pregnancy Wastage in Sheep and Goats in the Sahel Region of Nigeria, *Nig. Vet. J.*, 32(2):120-126.

Coelho, A.M., Coelho, A.C. and Rodrigues, J., 2013. Seroprevalence of sheep and goat brucellosis in the northeast of Portugal. *Archivos de Med. Vet.*, 45:167-172.

Coelho, A.M., Coelho, A.C., Roboredo, M. and Rodrigues, J., 2007. A case-control study of risk factors for brucellosis seropositivity in Portuguese small ruminant herds. *Prev. Vet. Med.,* 82: 291-301.

Coetzer, J.W. and Tustin, R.C., 2004. Infectious diseases of livestock, 3rd ed. South Africa Oxford University press, pp. 34–39.

CSA, 2017. Livestock and Livestock Characteristics, Agricultural sample Survey, Addis Ababa, Ethiopia. *Statistical Bulletin*, 2(583):9-13.

Dabassa, G., Tefera, M. and Addis, M., 2013. Small Ruminant Brucellosis: Serological Survey in Yabello District, Ethiopia. *Asian J. of Animal Sci.,* 7:14-21. DOI: 10.3923/ajas.2013.14.21

Daniel, G.M. and Marley, M.S.D., 2008. Infectious causes of embryonic and fetal mortality. *Theriogenology*, 70(3):270-285. doi: 10.1016/j.theriogenology.2008.04.018.

Deddefo, A., Sisay, T. and Tuli, G., 2015. Seroprevalence and risk factors of small ruminant brucellosis in selected districts of Arsi and East Shoa Zones, Oromia region, Ethiopia. *Afr. J. Microbiol. Res.*, 9(19):1338-1344. DOI: 10.5897/AJMR2015.7400

Dohoo, I., Martin, W. and Stryhn, H., (2009). Veterinary epidemiologic research, 2nd ed. AVC, Charlottetown, Prince Edward Island, pp. 239-249.

ESGPIP (Ethiopia Sheep and Goat Productivity Improvement Program), 2011. Control and prevention of common reproductive diseases of Sheep and Goats. *Technical bulletin*, no. 48.

ESGPIP (Ethiopia Sheep and Goat Productivity Improvement Program). Body condition scoring of sheep and goat, Techinical Bulletin No. 8 http://www.esgpip.org/PDF/Technical%20bulletin%20No8.html.

ESGPIP (Ethiopia Sheep and Goat Productivity Improvement Program), 2009. Technical bulletin, No. 23, Estimation of weight and age of sheep and goats, pp. 8-9.

FAO, 2003. Guidelines for coordinated human and animal brucellosis surveillance. FAO In: Proceedings of Animal Production and Health Conference, Paper 156, Rome, Italy, pp. 1-45.

Feyisa. A.., Sori, T. ., Ejeta, G., Fikru, R. and Laikemariam, Y., 2007. Distribution of brucellosis among small ruminants in the pastoral region of Afar, Eastern Ethiopia. *Rev. sci. tech. Off. int. Epiz.*, 26:731-739.

Gebremedhin, E.Z., 2015. Seroepidemiology of ovine brucellosis in East and West Shewa Zones of Oromia Regional State, Central Ethiopia. *J. Vet. Sci. Technol.,* 6:265.

Gizaw, S., Hoekstra, D., Gebremedhin, B. and Tegegne, A., 2015. Classification of small ruminant production sub-systems in Ethiopia: Implications for designing develop-

ment interventions. LIVES Working Paper 5. Nairobi, Kenya: International Livestock Research Institute (ILRI), pp.1-19.

Glenn, J.S. and Karen, W.P., 2005. Veterinary Microbiology: Bacterial and Fungal agents of animal diseases, pp. 200-203.

Kabagambe, E.K., Elzer, P.H., Geaghan, J.P., Opuda-Asibo, J., Scholl, D.T. and Miller, J.E., 2001. Risk factors for Brucella seropositivity in goat herds in Eastern and Western Uganda. *Prev. Vet. Med.*, 52:91-108.

Kenyon, P.R., Maloney, S.K. and Blache, D., 2014. Review of sheep body condition score in relation to production characteristics, *New Zealand J. Agri. Res.*, 57(1):38-64.

Kiputa, V.P.S., Kimera, S.I. and Wambura, P.N., 2008. Studies on the role of trade cattle in the transmission of brucellosis in Karagwe district, Tanzania. *Tanzania Vet. J.*, 25(1):48-59.

Libeau, G., Préhaud, C., Lancelot, R., Colas, F., Guerre, L., Bishop, D. and Diallo, A., 1995. Development of a competitive ELISA for detecting antibodies to the peste des petits ruminant virus using a recombinant nucleoprotein. *Res. Vet. Sci.*, 58: 50-55.

Livestock Marketing Authority (LMA), 2005. Brief base line information on Ethiopian Livestock resource base and its trade, Addis Ababa, Ethiopia.

Margatho, G., Vicente, R.E, Hélder, Q. and João, S., 2019. The Effects of Reproductive Disorders, Parity, and Litter Size on Milk Yield of Serrana Goats, *Animals*, 9:968.

McDermont, J.J. and Arimi, S.M., 2002. Brucellosis in sub-Saharan Africa: Epidemiology, Control and Impact. *Vet. Microbiol.,* 20:111-134.

Megersa, B., Mohammed, H., Tefera, M. and Tolosa, T., 2011a. Small ruminant brucellosis and community perception in Jijiga district, Somali Regional State, Eastern Ethiopia, *Trop. Anim. Hlth. Prod.,* 43:893-898.

Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J. and Skjerve, E., 2011b. Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. *Trop. Anim. Hlth. Prod.*, 43:651-656. doi: 10.1007/s11250-010-9748-2.

Melese, Y., 2016. Isolation, phenotype characterization and Seroprevalence Survey on small ruminant brucellosis in Arba Minch Zuria and Mirab Abaya districts of Gamo Gofa, Southern Ethiopia. MSc. Thesis, Addis Ababa University. College of Veterinary Medicine, Bishoftu, Ethiopia.

Mengistu, M., 2007. Seroepidemiology of brucellosis in small ruminants in Southern Ethiopia. MSc. Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

Mirkena, T., Duguma, D., Willam, A., Wurzinger, M. and Haile, A., 2011. Community-based alternative breeding plans for indigenous sheep breeds in four agro-ecological Zones of Ethiopia. *J. Anim. Breed Genet*, 10(1):1-10. doi: 10.1111/j.1439-0388.2011.00970.x.

Mohammed, M., Mindaye, S., Hailemariam, Z., Tamerat, N. and Muktar, Y., 2017. Sero-Prevalence of Small Ruminant Brucellosis in Three Selected Districts of Somali Region, Eastern Ethiopia. *J. Vet. Sci. Anim. Hus.,* 5(1):105.

Morris, M.J., Sookhoo, J., Blake, L., Brown, J.A., John, J., Ali, S., Sarjusingh, G., St Aime, J., Amoroso, E.H. and Oura, C.A.L., 2018. Serosurvey for Infectious Agents Associated with Subfertility and Abortion in Dairy Cattle in Trinidad and Tobago, West Indies. *Vet Sci.*, 5(2):51.

Muluken, T., 2016. Seroprevalence of Brucellosis and Isolation of *Brucella* from small ruminants that had history of recent abortion in selected Kebeles of Amibara District, Afar Region, Ethiopia. MSc. Thesis, Addis Ababa University, Bishoftu, Ethiopia.

Njeru, J., Wareth, G., Melzer, F., Henning, K., Pletz, M.W., Heller, R. and Neubauer, H., 2016. Systematic review of brucellosis in Kenya: disease frequency in humans and animals and risk factors for human infection. *BMC Public Health*, 16:853.

OIE, 2004. Manual of the diagnostic tests and vaccines for terrestrial animals, 5th Ed. Office International des Epizooties, Paris, France, pp. 409-438.

OIE, 2009. Caprine and Ovine Brucellosis (Excluding *Brucella ovis*), In: OIE (ed). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Vol. OIE-World Organization for Animal Health, Office International des Epizooties, pp. 2-8.

Omer, M.K., Skjerve, E., Woldehiwet, Z. and Holstad, G., 2000. Risk factors for Brucella spp. infection in dairy cattle farms in Asmara, State of Eritrea. *Prev. Vet. Med.,* 46:257-265.

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L. and Tsianos, E.V., 2006. The new global map of human brucellosis. *Lancet Infect. Dis.*, 6:91-9.

Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R., 2004. Clinical Veterinary Microbiology. Ireland, pp. 261-267.

Radostits, O.M., Gay, C.C., Hinclcliff, K.W. and Constable, P.O., 2007. Veterinary medicine: A text book of the disease of cattle, sheep, pigs, goat and horses, 10th ed. London, Saunders, pp. 963-1110.

Shirima, G. and Kunda, J., 2016. Prevalence of brucellosis in the human, livestock and wildlife interface areas of Serengeti National Park, Tanzania. *Onderstepoort J. Vet. Res.*, 83(1):4.

Sintayehu, G., Melesse, B., Abayneh, D., Sintayehu, A., Melaku, S., Alehegne, W., Mesfin, S., De Blas, I., Casal, J., Allepuz, A., Martin-Valls, G., Africa, T. and Abera, K., 2015. Epidemiological survey of brucellosis in sheep and goats in selected pastoral and agro-pastoral lowlands of Ethiopia, *Rev. sci. tech. Off. int. Epiz.,* 34(3):881-893.

Smith, H.A., Jones, T.C. and Hunt, R.D., 2002. Veterinary Pathology. 4th (ed.) Lea and Ferbiger, Philadelphia, USA, pp. 594-598.

Tassew, H. and Kassahun, F., 2014. Sero-epidemiological study of camel brucellosis in Mehoni District, south eastern Tigray. *J. Microbiol. Res.*, 4(1):18-23.

Tegegn, A.H., Feleke, A., Adugna, W. and Melaku, S.K., 2016. Small Ruminant Brucellosis and Public Health Awareness in Two Districts of Afar Region, Ethiopia. *J. Vet. Sci. Technol.*, 7:335.

Teshale, S., Muhie, Y., Dagne, A. and Kidanemariam, A., 2006. Seroprevalence of small ruminant brucellosis in selected districts of Afar and Somali pastoral areas of Eastern Ethiopia: the impact of husbandry practice. *Revue Med. Vet.*, 157(11): 557-563.

Thrusfield, M., 2005. Veterinary Epidemiology, 3rd Edn., Blackwell Publishing, England, pp. 345-543.

Tsehay, H., Getachew, G., Morka, A., Tadesse, B., and Eyob, H., 2014. Seroprevalence of brucellosis in small ruminants in pastoral area of Oromia and Somali regional state of Ethiopia. *J. Vet. Med. Anim. Health*, 6(11):289-294.

Tulu, D., Deresa, B., Begna, F. and Gojam, A, 2018. Review of common causes of abortion in dairy cattle in Ethiopia. *J. Vet. Med. Anim. Health*, 10(1):1-13.

Villaquiran, M., Gipson, T.A., Merkel, R.C., Goetsch, A. L. and Sahlu, T., 2007. Body condition scores in goats in Meat Goat Production Handbook, Langston University.

Wesinew, A., Moti, Y., Hailu, D. and Tadele, T., 2013. Control and prevention of brucellosis in small ruminants: time for action. *J. Vet. Res.*, 170:97-98.

Wubishet, Z.W., Sadik, K., Abdi, A., Abdallah, B., Huqa, L., Alemayehu, B., Mokonin, B., Getachew, A. and Getachew, K., 2017. Small ruminant brucellosis and awareness of pastoralist community about zoonotic importance of the disease in Yabello districts of Borena Zone Oromia regional state, southern Ethiopia. *J. Zoonotic Dis*., 4(2):27-36.

Yohanis, G., 2012. Epidemiological investigation of brucellosis in ruminants and humans in Yabello districts of Borena pastoral area, Oromia, Ethiopia. MSc. Thesis, Addis Ababa University, Bishoftu, Ethiopia.

Study on ruminant tick infestation, phytochemical analysis and *in vitro* acaricidal effect of *Calpurnia aurea* and *Otostegia integrifolia* extracts on *Amblyomma variegatum*

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Abstract

Ticks limit the productivity of livestock through decreased production, reproduction, increased mortality, downgrading and rejection of hides and skin. A cross-sectional study was conducted to estimate the prevalence of tick infestation in ruminant while experimental study was used to evaluate the *in-vitro* acaricidal efficacy of methanolic extracts: *Calpurnia aurea and Otostegia integrifolia* and the phytochemicals present in those extracts at different concentrations (200, 100, 50, 25, 12.5 and 6.25 mg/ml) against *Amblyomma variegatum*. Adult immersion was used for the *in-vitro* acaricidal efficacy test and plant extracts were subjected to qualitative phytochemical screening for the presence or absence of secondary metabolites using standard procedures. Out of the 160 goats, 152 sheep and 121 cattle, 23 (14.4%), 44 (28.9%) and 28 (23.1%) were found to be positive for tick infestation, respectively. The incidence of tick infestation was significantly different ($p<0.01$) among ruminants. Five tick spp. were identified: *A. variegatum, A. gemma, R. decoloratus, R. evertsi evertsi* and *R. pulchellus*. Extract of *C. aurea* and *O. integrifolia* was found to contain alkaloids, saponins, phlobatannin, steroids, phenolic, flavonoids, glycosides and tannins. However, both plants were found negative for triterpens. Extracts of *C. aurea* and *O. integrifolia* at 200 and 100 mg/ml concentrations showed a significantly higher ($p<0.05$) acaricidal activities compared to other treatments at 24 hrs post exposure. Mortality of ticks was increased with the increased dosage (concentration) and exposure time after treatment. Extracts of *C. aurea* showed a significantly higher ($p<0.05$) tick mortality (52%) compared to those of *O. integrifolia* (27%). This is a promising finding to have alternative means of treatment and to substitute the use of synthetic drugs which have a wide spread drug resistance especially in developing countries like Ethiopia.

Key words: *Calpurnia aurea; in-vitro* test; *Otostegia integrifolia*; Phytochemical screening; Tick infestation

Introduction

Ethiopian cattle, sheep and goat population is estimated to be about 59.49, 30.70 and 30.20 million, respectively (CSA, 2017), which plays a significant role in the socio-economic life of the people. Products and by-products of livestock such as milk, meat, cheese, and butter supply the needed animal protein that contributes to the people's nutritional improvement. They also play an immense role in providing export commodities, such as meat, live animals, hides and skins to earn foreign exchange (Abunna *et al*., 2009). Even though livestock are important components of the Ethiopian farming system, their contribution to the sector are below the expected potential because they are constrained by poor feeding, poor managements and diseases (Ashenafi *et al.,* 2013). In Ethiopia, ticks are directly or indirectly involved in causing considerable financial losses to the livestock industry with an estimate of more than 1-million-birr loss per year through rejection and down-grading of hides and skins which in turn affect the tanning industries of the country (Ashenafi *et al.,* 2013).

The World Health Organization (WHO) estimated that around 80% of the population in Africa use traditional medicines and about 85% of traditional medicine involves the use of plant extracts (WHO, 2008). In Ethiopia, plant remedies are still the most important and sometimes the only sources of therapeutics for nearly 80% of human and more than 90% in livestock population. Estimated floras of 6,500 to 7,000 species of higher plants are of medically important and out of these medicinal plants 12% are endemic to Ethiopia (Giday *et al*., 2009).

Modern livestock health care is still at its immature stage in the country due to lack of adequate clinics and supply of drugs. Besides, most modern drugs are expensive and not affordable by the majority of livestock owners. As a result, people rely on their traditional knowledge, practices and locally available materials (mainly plants) in the management of diseases of their domestic animals. However, very little of the ethnoveterinary knowledge in Ethiopia in relation to the use of medicinal plants is so far properly documented and analysed (Yineger *et al*., 2008). In one hand, even though ticks are becoming the major health, productivity and breeding concerns in the farms of the Haramaya University and the surrounding district, very limited information exists or/and none at all. On the other hand, the cost of acaricides together with loss

of enzootic stability, residues in food, undesirable effects on the environment and development of resistance by ticks are some of the problems related to utilization of acaricides (De Castro, 1997).

Calpurnia aurea and *Otostegia integrifolia* were reported to be used as a control means for livestock ticks, lice and flea infestation in ethno-veterinary practice (Teklay *et al.*, 2013). In addition, the acaricidal activity of alkaloid *C. aurea* leaves extracts against *A. variegatum* was previously reported by Amante (2016). However, the *in vitro* effect of methanolic *C. aurea* and *O. integrifolia* leaves extracts against *A. variegatum* was not investigated in the current study area. In addition, there is a need for scientific based research for testing the acaricidal efficacy of these plants in order to assess alternative herbal remedies for tick and tick associated diseases. Therefore, the objectives of this study were aimed to estimate the prevalence of ruminant tick infestation and to identify ruminant tick species in and around Haramaya University farms and to evaluate the *in vitro* acaricidal activity of *C. aurea* and *O. integrifolia* methanolic extracts against *A. variegatum*.

Material and Methods

Description of the study area

Ticks were collected from Haramaya University (HU) farms and Haramaya district. The phytochemical screening test and the *in vitro* acaricidal efficacy test experiment was conducted at the laboratory of Haramaya University, College of Agriculture and Environmental Sciences. Haramaya is located in Oromia Regional State of Eastern Hararghe zone 508 km from Addis Ababa. It is located at an altitude range of 1800 to 2345 (an average 2047) m.a.s.l, 9° 26'N latitude and 42° 3'E longitude. The mean annual rainfall is 780 mm. The mean annual minimum and maximum temperatures are 8.5 and 24.4, respectively. According to the Haramaya District Rural Development and Agricultural Bureau, the district has 63,723 cattle, 13,612 sheep, 20,350 goats, 15,978 donkeys, 536 camels and 42,035 poultry.

Study animals

Study animals were ruminants (160 goats, 152 sheep and 121 cattle). Small ruminants (n=214) and cattle (n=58) were used from HU farms while small ruminants (n=98) and cattle (n=63) were used from Haramaya district vet-

erinary clinics for tick sample collection and identification. The husbandry system of dairy cattle is semi-intensive, where animals are allowed access to grazing typically in the evening. Small ruminants in the farms are kept under semi-intensive management systems which are allowed access to graze on free range land within the University both in the morning and late in the afternoon. Study population were grouped by study area, species, sex, age, breed, body condition score (BCS) and production system. Conventional age categories were made. For cattle animals aged <3 years were considered as young and ≥3 years as adult. Sheep and goats were grouped as young (2 years) and adults (≥2 years) as stated by Gatenby (2002) and as cited in Tewodros and Dawit (2015). Body condition score categorization was done according to Nicholson and Butterworth (1986).

Study design

A cross-sectional study was conducted from September 2015 to February 2017 to estimate the prevalence of tick infestation and experimental study was carried out to evaluate the *in-vitro* acaricidal efficacy test of *C. aurea and O. integrifolia* methanolic leaves extract against *A. variegatum*.

Tick collection and identification methods

Each animal was purposively sampled and subjected to physical and clinical examination and history, such as the use of acaricide treatment, concurrent disease and tick infestation associated signs including pain, lameness, and loss of appetite was recorded. Ticks were collected using forceps from the main body sites namely: head, dewlap, brisket, belly and back, udder or scrotum, anogenital, leg and tail. Adult ticks were collected and were maintained in universal bottles separately and then transported to the parasitology laboratory of College of Veterinary Medicine, HU for identification and *in vitro* acaricidal efficacy test. Date and place of collections, body sites of collection, and breed of host were recorded duly. Identification and recording of tick samples were taken place within few hours of collection. Ticks were identified using stereomicroscope following the standard identification procedures described by Walker *et al*. (2014).

Plant material collection and extraction

The leaves of plant species *C. aurea* and *O. integrifolia* were selected based on the information obtained from botanical surveys (Teklay *et al.,* 2013) in which

the community traditionally used those plants for the control of ticks. These plants were collected around from eastern Hararghe and identified and verified with taxonomical studies as reported by Zorloni (2008). The plant materials (leaves) were spread out on paper sheets in the shade at room temperature separately to dry for two weeks. The dried plant material was crushed in an electric grinder to coarse powder consistency. One hundred gram (100 gm) of powder was soaked in 200 ml of methanol separately for each plant for 48 hrs on an orbital shaker. Extracts were filtered using a Buckner funnel and Whatman (No 1 filter paper). Each filtrate was concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator and the residue obtained was stored at 4°C (Eloff, 1999). The extraction rate (%) was calculated as given below:

$$\text{Extraction rate (\%)} = \frac{\text{Weight of extracts (gm)}}{\text{Weight of the plant material (gm) before extraction}} \times 100$$

Phytochemical screening of solvent extracts

The crude methanol extract was screened for the presence or absence of secondary metabolites such as alkaloids, saponins, phlobatannin, steroids, flavonoids, glycosides, phenolic compounds, tannins and triterpens using standard procedure (Tiwari *et al.,* 2011).

***In vitro* acaricidal efficacy test**

Adult immersion test

The dried extracts of C. aurea and O. integrifolia were diluted in distilled water and 3% methanol respectively, at the concentrations required for the bioassays and six concentrations were prepared arithmetically viz. 200, 100, 50, 25, 12.5 and 6.25 mg/ml by serial dilution based on FAO (2004). The in vitro tests were started within one hour after tick collection and identification. Ten active unsexed adult ticks and 3ml of each extract concentration were directly added in to each Petri-dish of the three replications. Petri-dishs were incubated at 27-28 °C and 75-80% relative humidity for 24 hrs (Sanis et al., 2012). Distilled water and 3% methanol were used as negative control for C. aurea and O. integrifolia, respectively whereas 0.1% diazinon 60 EC (Kat Relzayat Pesticides and Chemicals Co. Ltd, Egypt) was used as positive control (Jadhav et al., 2007). The test solutions, 0.1% diazinon 60 EC and distilled water were removed just

after two-minute contact time using Whatman filter paper No 1. Each tick in each Petri-dish was closely observed for death under stereomicroscope at 30 minutes, 1 hr, 2 hrs., 3 hrs., 6 hrs., 12 hrs., and 24 hrs. time intervals (Nanaa et al., 2010). The criteria used for identifying tick death were extremely strict. If any minor signs of life such as movement of head part, gut cells or minimal legs movements were observed with stimulation by forceps, the ticks were categorized as alive. The ticks were judged as dead, if there were no vital signs at all (Jadhav et al., 2007). The plant extracts were compared with ticks treated by different extract dosage, controls and different time of exposure. The numbers of fatalities were recorded in prepared format. The percent mortality rate of the ticks was calculated based on Krishnaveni and Venkatalakshmi (2014).

$$\text{Mortality } \% = \frac{\text{No. of mortality}}{\text{Total number of parasites}} \times 100$$

Accordingly, acaricidal effect was classified as strong (mortality > 80%), moderate (60-80% mortality), weak (40-60% mortality), little or no activity (mortality < 40%). Mortality in the Petri-dishes treated with extract was corrected to take account of control mortality using Abbott's correction (Pamo *et al.,* 2005).

Data entry and analysis

All data recorded in this study was entered into Microsoft excel and subsequently analyzed using STATA version 11.0 computer program. Chi-square test was used to determine the presence of association between the prevalence of tick infestation and study area, ruminant species, sex, age, breed, BCS and production system. Analysis of variance (one-way ANOVA test) was used to compare the means of tick mortality between treatments. The difference between treatments was considered significant at the ($P < 0.05$) level. Descriptive statistics was also used to review the different tick species.

Results

Identification of tick species in domestic ruminants

A significantly different ($p<0.01$) incidence of tick infestation was observed among examined domestic ruminants (cattle, sheep and goat) and Body Condition Score (Table 1). A total of 1518 ticks were collected from infested animals. Three genera and 5 species of ticks were identified. Of which, *A. variegatum*

was the dominant tick species (Table 2). The prevalence of species level tick infestation in domestic ruminants also reported (Table 3).

Table 1: Prevalence of ruminant tick infestation and associated factors

Variables	Category	Number examined	Number tick infested (%)	OR (95% CI)	p-value
Source	HU[a]	272	57 (20.9)	Reference	
	HVC[b]	161	38 (23.6)	0.6(0.31-.62)	0.520
Species	Goat	160	23 (14.4)	Reference	
	Sheep	152	44 (28.9)	2.5(0.16-1.34)	0.013
	Cattle	121	28 (23.1)	2.0(0.01-1.28)	0.046
	Total	433	95 (21.94)		
Sex	Male	60	11 (18.3)	Reference	
	Female	373	84 (22.5)	0.96(1.11-.38)	0.337
Age	Young	96	17 (17.7)	Reference	
	Adult	337	78 (23.1)	1.2(0.23-.29)	0.233
Breed	Local	325	74 (22.8)	Reference	
	Exotic	108	21 (19.4)	0.21(-.66-.54)	0.833
BCS[c]	Poor	116	47 (40.5)	Reference	
	Medium	229	38 (16.6)	4.6(-1.74-.71)	0.000
	Good	88	10 (11.4)	3.9(-2.36-.79)	0.000
Farming system	Semi intensive	272	57(21.0)	Reference	
	Extensive	161	38(23.6)	0.6(-.31-.62)	0.520

[a]Haramaya University, [b]Haramaya District Veterinary Clinic, [c]Body Condition Score

Table 2: Prevalence of tick species identified in the study areas

Tick genera	Proportion
Amblyommavariegatum	971 (64%)
Rhipicephalus (Boophilus) decoloratus	366 (24%)
Amblyommagemma	107 (7.5%)
Rhipicephalusevertsievertsi	53 (3.5%)
Rhipicephalus pulchellus	21 (1.5%)
Total	**1518 (100%)**

Table 3: Species-level tick infestation in domestic ruminants (cattle, sheep and goat)

Tick species	Cattle (n=121) (%)	Sheep (n=152) (%)	Goat (n=160) (%)	Overall prevalence
A. variegatum	14 (11.6)	20 (13.1)	12 (7.5)	46 (10.6)
Rh (B) decoloratus	6 (5.0)	13 (8.5)	8 (5.0)	27 (6.2)
A. gemma	4 (3.3)	6 (3.9)	3 (1.9)	13 (3.0)
Rh.evertsi evertsi	2 (1.7)	3 (2.0)	0	5 (1.2)
Rh. Pulchellus	2 (1.7)	2 (1.3)	0	4 (0.9)
Total	28 (23.1)	44 (28.9)	23 (14.4)	95 (21.9)

Physicochemical characteristics and yield of plant extracts

Methanolic crude extract of *C. aurea* (leaves) was green powder. The plants extract was soluble in organic solvents and fairly soluble in distilled water. The crude leaves extract of *O. integrifolia* was semi solid greenish brawn. *Otostegia integrifolia* extracts were soluble in 3% methanol (Table 4). Extract of *C. aurea* and *O. integrifolia* was found to contain different active ingredients (Table 5).

Table 4: Physical characteristics and percentage yield of *C. aurea* and *O. integrifolia* different crude extracts

Scientific names	Local name	Plant part extracted	Extraction solvent	Colour of extract	Yield (%)
C. aurea	Degeta	Leaf	Methanol	Green	17
O. integrifolia	Tunget	Leaf	Methanol	Greenish brown	19.3

Table 5: Qualitative determinations of active ingredients in crude extract of *C. aurea* and *O. integrifolia*

Scientific names	*C. aurea*	*O. integrifolia*
Alkaloids	+	-
Saponin	+	+
Phlobatannin	+	+
Steroids	+	-
Phenolic Cpds.	+	+
Flavonoids	+	-
Glycosides	+	+
Tannins	+	+
Triterpens	-	-

In vitro acaricidal activity of *C. aurea* and *O. integrifolia* against *A. variegatum*

Mortality of ticks was increased with the increased dosage (concentration) and exposure time after treatment. Extracts of *C. aurea* at 200 mg/ml and 24 hrs post exposure showed increased tick mortality (52%) compared to other exposure times (6 and 12 hrs.) within the treatments and compared to the negative control. *Calpurnia aurea* extract at 200 and 100 mg/ml concentrations showed comparable acaricidal efficacy to the reference drug (Fig 1).

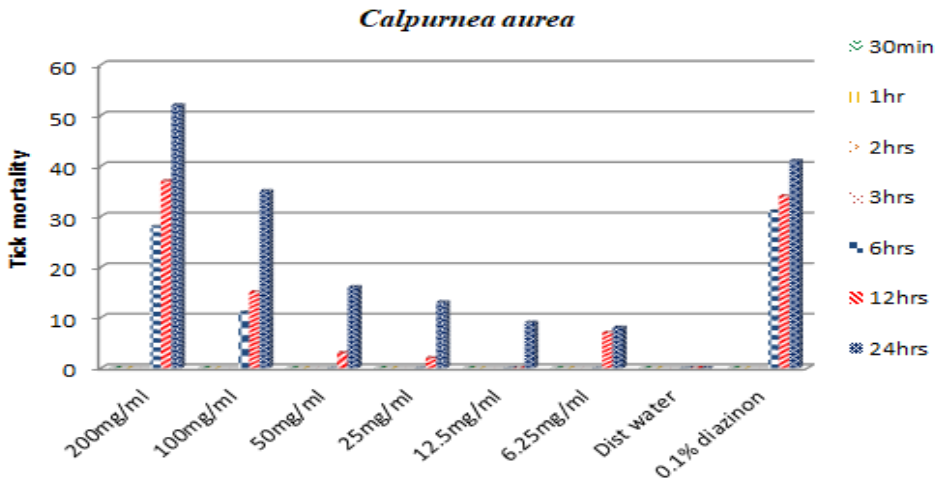


Figure 1: Mortality rate of *ticks* treated with different crude extract concentrations of *C. aurea*

Mortality of ticks was increased with the increased dosage (concentration) and exposure time after treatment. Extracts of *O. integrifolia* extract at 200 and 100 mg/ml and 24 hrs post exposure showed increased tick mortality about (26%) compared to other exposure times (6 and 12 hrs) within the treatments and compared to the negative control (Fig 2).

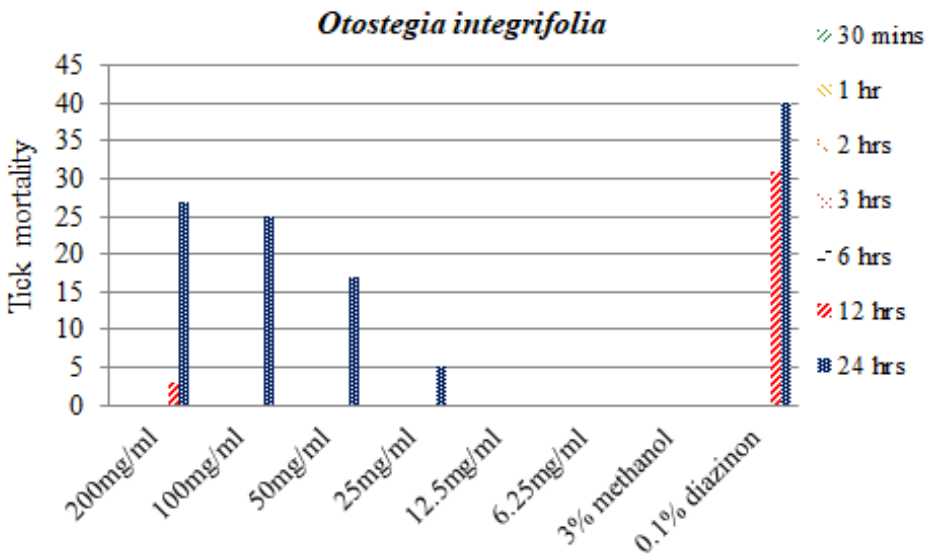


Figure 2: Mortality rate of ticks treated with different crude extract concentrations of *O. integrifolia*

The extracts of *C. aurea* at 200 mg/ml concentrations and 24 hrs after exposure showed a significantly ($p<0.05$) higher tick mortality than those of *O. integrifolia* (Table 6)

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Table 6: *In-Vitro* acaricidal efficacy evaluation of *C. aurea* and *O. integrifolia* methanolic extracts at different concentrations and time exposure against *A. variegatum*

	Mean mortality rate (%)					
	6 hrs		12 hrs		24hrs	
Dose (mg/ml)	C. aurea	O. integrifolia	C. aurea	O. integrifolia	C. aurea	O. integrifolia
200	5.3±5.3^{b}	00±00^{b}	16.3±9.5^{a}	5.5±00^{a}	51.5±5.5^{a}	26.5±5.7^{a}
100	16.3±.9.5^{a}	00±00^{b}	16.3±9.5^{a}	00±00^{b}	38.7±5.7^{b}	23±5.7^{a}
50	00±00^{c}	00±00^{b}	3.3±3.3^{d}	00±00^{b}	21.7±5.7^{c}	16±00^{b}
25	00±00^{c}	00±00^{b}	5.3±5.3^{c}	00±00^{b}	16±00^{a}	5.3±5.3^{b}
12.5	00±00^{c}	00±00^{b}	00±00^{e}	00±00^{b}	16±00^{b}	00±00^{c}
6.25	00±00^{c}	00±00^{b}	11±5.3^{b}	00±00^{b}	11±5.3^{c}	00±00^{c}
- control	00±00^{c}	00±00^{b}	00±00^{e}	00±00^{b}	00±00^{b}	00±00^{c}
+ control	5.3±5.3^{b}	6.7±3.3^{a}	11±5.3^{b}	13.3±6.7^{a}	33±3.6^{a}	38.7±5.7^{a}

Values are expressed as mean ± SD. Mean values with different letters in the same column are significantly different (P<0.05)

Discussion

In this study 21.9% of the study animals were found infested by ticks. Similar finding was also reported by Tiki and Addis (2011) who found 25.64% tick infestation around Holeta. In contrary, higher prevalence of ticks was reported from different parts of the country including 81.25% (Getachew *et al*., 2014), 74% (Meaza *et al*., 2014) and 65.5% (Wolde and Mohamed, 2014). The inconsistency among these studies could be attributed to a wide range of factors including agro-ecological, animal health practice, or managemental differences within their respective study areas.

The free-range nature of the animals most probably made significant differences of tick infestation in which cattle were two times more likely infested than goats. Whereas, animals with poor and medium body condition were more at risk for tick infestation than animals with good body condition. According to Manan *et al.* (2007), this could be due to the fact that poorly conditioned animals had low resistant to tick infestation and lack enough body capacity to build resistance whereas animals with good body condition had reasonable resistance to combat tick infestation.

Amblyomma variegatum was found to be the most abundant tick species found which accounts 64% of the total collected ticks. Similarly, prevalence data was also reported by Pawlos and Derese (2013). This finding is also in agreement with that of Ayalew *et al.* (2014) in central Oromia and Yehualashet *et al.* (1995) at Haramaya University. This was probably due to the geographic location and its being relatively active throughout the year (Hussen, 2009). Likewise, several researches, which had been conducted in different parts of Ethiopia, indicated that *A. variegatum* is the most abundant tick species with the highest prevalence. This tick species is responsible for the greatest damage to the hide and skin, because of its long mouth parts (Solomon *et al.,* 2001; Tessema and Gashaw, 2010; Tiki and Addis, 2011; Ayalew *et al.,* 2014).

The methanolic crude extract of leaves *O.integrifolia* comparatively yielded higher percentage (19.3%) than *C. aurea.* This output was supported by the report of Zewdneh *et al*. (2015) who reported similar percentage of *O. integrifolia* methanolic leaf extract. The difference on percentage yield of these extract products among the plants might be due to the difference on the nature of plant species and solvents used.

In order to know the active ingredients, present in crude extracts of the selected plants phytochemical screening test was conducted. Extract of *C. aurea* and *O. integrifolia* was found to contain alkaloids, saponins, phlobatannin, steroids, phenolic, flavonoids, glycosides and tannins. However, both plants were found negative for triterpens while *O. integrifolia*was found negative for alkaloids, steroids and flavonoids. This finding is in consistency with Umer *et al.* (2013) who reported the presence of alkaloids, tannins, flavonoids and saponins in *C. aurea* crude extract. Other study showed extract of *C. aurea* leaves was found positive for alkaloids (Zorloni *et al*., 2010).

This finding is in line with Zewdneh *et al.* (2015), who reported that methanolic extract of *O. integrifolia* (leaves) was positive for saponins and phenolic compound while negative for alkaloids, steroids and flavonoids. Parts of plant extracted and solvents used for extraction are important to determine medicinally active portions of plant (Solomon *et al*., 2013). Because the use of a different part of plant and solvent can yield in a different way in their chemical metabolites as it involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents (Ncube *et al*., 2008).

The current results revealed that the average *A. variegatum* mortality 25.5% recorded within 24 hrs of exposure of all extract concentrations of *C. aurea* at higher concentrations of 200 mg/ml and 100 mg/ml was comparable to the reference drug (positive control).This finding is different from some previous studies that reported as higher and lower average mortality of ticks. Zorloni *et al.* (2010), reported 85% of tick mortality by acetone extracts of *C. aurea* leaf at 5% concentration while Regassa (2000), found 10% of tick mortality at 5 hrs exposure of the aqueous extracts of *C. aurea* leaf and bark. The differences among these studies might be due to the difference in solvent used for extraction as studies have shown that organic solvent extracts show greater biological activity than the aqueous extract (Parekh *et al.,* 2007).

O. integrifolia showed average mortality of *A.variegatum* 12.3% at all concentrations. There is no available published scientific document on the effect of this plant against ticks. However, previous study showed the *in vivo* potent activity of hydro alcoholic leaf extract of *O. integrifolia* against *Plasmodium berghei,* malaria parasite with a maximum percent of chemo suppression of 80.5 at a dose of 600 mg/kg/day (Endale *et al*., 2013). Zorloni (2008) also reported the mosquito repellency, antimicrobial, anti-hyperglyceamic and antioxidant activities of *O. integrifolia* leaf.

Comparative *in-vitro* acaricidal activity of crude extracts of the plants revealed that *C. aurea* showed higher mortality of ticks (52%) than *O. integrifolia* (27%) after 24 hrs of exposure at 200 mg/ml concentration. The difference in mortality percentage of these plants might be due to variability in the amount of secondary metabolites among the plant extracts. The phytochemical analysis in this study showed that *C. aurea* had more secondary metabolites than *O. integrifolia.* In our study the relatively lower acaricidal activity of *O. integrifolia* is might be due to lower quantity of secondary metabolites and the absence of alkaloids, which act synergistically with glycosides when present and gave anti-tick activity (Ghosh *et al.,* 2015). Studies indicated that the presence of alkaloid, glycosides and phenol are important chemicals to initiate the mechanism of *in-vitro* and *in-vivo* action causing tick mortality (Kumar *et al.,* 2011).

Conclusion

The prevalence of tick infestation was significantly different among ruminants and body condition. Five tick species were identified: *A. variegatum, A. gemma,*

R. decoloratus, R. evertsi evertsi and R. pulchellus. *Amblyomma variegatum*. Extract of *C. aurea* and *O. integrifolia* were found to contain alkaloids, saponins, phlobatannin, steroids, phenolic, flavonoids, glycosides and tannins. Crude extracts of *C. aurea and O. integrifolia* at the higher concentrations and exposure times showed a significantly higher acaricidal activities compared to lower concentrations, exposure times and negative control. Both plants had showed comparable acarcidal effect to that of synthetic drugs (0.1% diazinon) that have been routinely used in the country. We can conclude that this is a promising finding to have alternative means of treatment to substitute the use of synthetic drugs which has a wide spread drug resistance and associated risks on the animals and human. Further detailed study on the economic losses associated with tick infestation as well as designing efficient method of tick control would have great importance. More investigation needs on their safety and efficacy *in vivo* as well as cost effectiveness of the products that exhibited considerable acaricidal activity with a view of substituting the conventional synthetic acaricide drugs. This also calls for further studies on characterization of the active ingredients of the selected plant materials. A need for further studies should be initiated to evaluate the effect of plants that showed low acaricidal effect by using a different extraction solvent and on other tick species.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

Abunna, F., Kasasa, D., Shelima, B., Megersa, B., Regassa, A. and Amenu, K., 2009. Survey of tick infestation in small ruminants of Miesso district, West Harergie, Oromia Region, Ethiopia. *Trop. Anim. Health Prod*.,41, 969–972.

Amante, M. 2016. *In vitro* louscidal and acaricidal activities of alkaloid of *Calpurnia aurea* and fractions of *Ricinus communis* Extracts against *Linognathus ovillus* and

Amblyomma variegatum. MSc. Thesis, Addis Ababa University, College of Veterinary Medicine, Bishoftu, Ethiopia.

Ashenafi, H., Hailu, Y., and Yebegaeshet, M. 2013. Impact of sheep and goats ectoparasites on the tanning industry in Tigray Region, Ethiopia. *Eth. Vet. J.,* 17(2), 63-76.

Ayalew, T., Hailu, Y., and Kumsa, B. 2014. Ixodid ticks infesting cattle in three agro-ecological zones in central Oromia: species composition, seasonal variation, and control practices. *Comp. Clin. Path*., 23(4), 1103–1110. DOI: 10.1007/s00580-013-1748-y.

Central Statistical Agency (CSA), 2017. Agricultural Sample Survey. Report on Livestock and Livestock Characteristics (Private Peasant Holdings), vol. 1, Central Statistical Agency (CSA), Addis Ababa, Ethiopia.

De Castro, J.J. 1997. Sustainable tick and tick-borne disease control in livestock improvement in developing countries. *Vet. Parasitol*.,71, 77-97.

Eloff, J.N. 1999. It is possible to use herbarium specimens to screen for antibacterial components in some plants, Short communication. *J Ethnopharmacol*. 67, 355–360. https://doi.org/10.1016/S0378-8741(99)00053-7.

Endale, A., Bisrat, D., Animut, A., Bucar, F., and Asres, K., 2013. *In-vivo* antimalarial activity of a labdane diterpenoid from the leaves of *Otostegia integrifolia* benth. *Phytother. Res*., 27, 1805–1809. https://doi.org/10.1002/ptr.4948.

FAO, 2004. Resistance management and integrated parasite control in ruminants: Guidelines. http://www.fao.org/ag/aga.html.

Gatenby, R.M., 2002. Sheep In: The tropical agriculturalist. Basin stock, 2nd edition. Macmillan Education Publisher, London, UK.

Getachew, A., Mersha, C., Desalegne, M., and Basaznew, B. 2014. Prevalence of ixodid ticks on cattle in Northwest Ethiopia, *Acta Parasitologica Globalis*., 5(2), 139–145. DOI: 10.5829/idosi.apg.2014.5.2.84292.

Ghosh, S., Shashi, S.T., Bhanu, K., Sharad, S., Anil, K., Sachin, K., Bandyopadhyay, A., Sanis, J., Rajesh, K. and Rawat, A.K.S. 2015. Identification of potential plant extracts for anti-tick activity against acaricide resistant cattle ticks, *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *Exp. Appl. Acarol*., 66**,**159–171.

Giday, M., Asfaw, Z., and Woldu, Z. 2009. Medicinal plants of the Meinit ethnic group of Ethiopia: An ethnobotanical study. *J. Ethnopharmacol*. 124(3), 513-521. doi.org/10.1016/j.jep.2009.05.009

Hussen, Y. 2009. Preliminary survey of cattle tick species and burden in and around Bako Town. DVM Thesis, school of Veterinary Medicine Jimma University, Jimma, Ethiopia.

Jadhav, V., Kore, A., and Kadam, V.J. 2007. *In vitro* pediculicidal activity of *Hedychium spicatum* essential oil. *Fitoterapia*,78 (7-8), 470–473.

Krishnaveni, S., and Venkatalakshmi, P. 2014. Antimicrobial, larvicidal and acaricidal activities of the ethanolic extract of *Andrographis paniculata* and Carica papaya leaves. *World J. Pharm. Res*., l3, 660-669.

Kumar, A., Singh, S., Kanhiya, M., Vihan, V. S. and Gururaj K. 2011. Phytochemical Analysis of Some Indigenous Plants Potent Against Ectoparasite. *Asian J. Exp. Biol. Sci.,* 2(3), 506-50.

Manan, A., Khan, Z., Ahmad, B. and Abdullah, 2007. Prevalence and Identification of Ixodid. Ministry of Economic Development and Cooperation Survey of Livestock and Fisheries Development, Agricultural Development Department, Livestock Team, Addis Ababa, Ethiopia.

Meaza, G., Abdu, M. and Yisehak, K. 2014. Determination of the prevalence of ixodid ticks of cattle breeds, their predilection sites of variation and tick burden between different risk factors in Bahir Dar, Ethiopia. *Global Veterinaria*, 13(4), 520–529.

Nanaa, P.B., Manianiaa, N.K., Marangab, R.O., Kutimab, H.L. Bogab, H.I., Nchuc, F. and Eloff, D. 2010. Attraction response of adult Rhipicephalus appendiculatus and *Rhipicephalus pulchellus* (Acari: Ixodidae) ticks to extracts from *Calpurnia aurea* (Fabaceae). *Vet. Parasito.*, 174 (1-2), 124–130. doi: 10.1016/j.vetpar.2010.08.016. Epub 2010 Aug 21.

Ncube, N.S., Afolayan, A.J., and Okoh, A.I. 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Afr. J. Biotechnol*.7 (12), 1797-1806.

Nicholson, M.J. and Buttrworth, M.H. 1986. A guide condition scoring of zebu cattle. International Livestock Center for Africa (ILCA), Addis Ababa, Ethiopia.

Pamo, E.T., Tendonkeng, F., and Kana, J.R. 2005. A study of the acaricidal properties of an essential oil extracted from the leaves of *Ageratum houstonianum*. *Vet. Parasitol.*, 128(3-4), 319–323. https://doi.org/10.1016/j.vetpar.2004.10.022.

Parekh, J., Karathia, N. and Chanda, S. 2007. Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegate* L. bark. □*Afr. J. Biomed*. Res., 9, 53-56.

Pawlos, W., and Derese, D. 2013. Study on prevalence and identification of ticks in Humbo district, Southern Nations, Nationalities, and People's Region (SNNPR) Ethiopia," *J. Vet. Med. Anim. Health* 5(3), 73–80.

Regassa, A. 2000. The use of herbal preparations for tick control in western Ethiopia. *J. S. Afr. Vet. Assoc.,* 71(4), 240–243.

Sanis, J., Reghu, R., Sunil, A., Ramankutty, A., Kumar, K.G., Suresh, N.N., Amithamol, K.K., Amitabh, B.A., Kumar, S.R. and Srikanta, G. 2012. *Jatropha curcas* (Linn) leaf extract a possible alternative for population control of *Rhipicephalus (Boophilus) annulatus*. *Asian Pac. J. Trop. Dis.,* 2(3), 225–229. https://doi.org/10.1016/S2222-1808(12)60051-6.

Solomon, G., Nigist, M. and Kassa, B. 2001. Seasonal variation of ticks on calves at Sebeta in Western Shoa zone. *Ethiop. Vet. J.,* 7(1), 1730.

Teklay, A., Abera, B. and Giday, M. 2013. An ethnobotanical study of medicinal plants used in Kilte Awulaelo District, Tigray Region of Ethiopia. *J. Ethnobiol. Ethnomed*., 65(9), 1-23. doi: 10.1186/1746-4269-9-65.

Tessema, T. and Gashaw, A., 2010. Prevalence of ticks on local and crossbreed cattle in and around Asela Town, South East, Ethiopia, Amber Animal Health Department, East Gojam, *Ethiop. Vet. J.*, 14, 79-89.

Tewodros, A.E., Dawit, A.A. 2015. Sero-prevalence of small ruminant brucellosis in and around Kombolcha, Amhara Regional State, North Eastern Ethiopia. *J. Vet. Sci. Med. Diagn*. 4(5). doi:10.4172/2325-9590.1000173.

Tiki, B. and Addis, M. 2011. Distribution of ixodid ticks on cattle in and around Holeta town, Ethiopia. *Global Veterinaria*, 7(6) 527–531.

Tiwari, P., Kumar, B., and Kaur, M. 2011. Phytochemical Screening and Extraction: A review. *Int. Pharm. Sci.* 1, 98-106.

Umer, S.U., Alemu, T., and Nigatu, K. 2013. Antidiarrhoeal and antimicrobial activity of *Calpurnia aurea* leaf extract. *BMC Complement Altern Med*. 13(21), 1-5.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Peña, A., Horak, I.G., Latif, A.A., Pegram, K. G., and Preston, P.M. 2014. Ticks of Domestic Animals in Africa: A Guide to Identification of Species. Revised version, published by Bioscience Reports. Edinburgh Scotland, U.K.

World Health Organization (WHO), 2013. Traditional Medicine Fact Sheet No.134. Report by the Secretariat. Geneva, Switzerland.

Wolde, A. and Mohamed, A. 2014. Prevalence of ixodid ticks on Bovine in Soddo zuria districts, Wolaita zone, Ethiopia, *Acta Parasitol. globalis*, 5(3), 188–197.

Yehualashet, T., Gebreab, F., Wakjira, A. and Tsega, T. 1995. Preliminary observation on ticks, Seasonal dynamics and resistance of three indigenous and three cross breed cattle in Ethiopia. *Bull. Anim. Health Prod. Afr*., 43, 114.

Yineger, H., Yewhalaw, D., and Teketay, D. 2008. Ethnomedicinal plant knowledge and practice of the Oromo ethnic group in southwestern Ethiopia. *J. Ethnobiol. Ethnomed*., 4(11). 1-10. doi:10.1186/1746-4269-4-11.

Zewdneh, S., Mohammed, B., and Zewdu, B. 2015. Methanolic leaf extract of *Otostegia integrifolia* benth reduces blood glucose levels in diabetic, glucose loaded and normal rodents. *BMC Complement Altern Med*., 15(19), 1-27. doi: 10.1186/s12906-015-0535-5.

Zorloni, A. 2008. Evaluation of plants used for the control of animal ectoparasitoses in southern Ethiopia (Oromiya and Somali regions). MSc (Veterinary Science) Dissertation. Phytomedicine Programme, University of Pretoria.

Zorloni, A., Penzhorn, B. L., and Eloff, J.N. 2010. Extracts of *Calpurnia aurea* leaves from southern Ethiopia attract and immobilise or kill ticks. *Vet. Parasitol.*, 168 (1-2), 160–4. doi: 10.1016/j.vetpar.2009.10.026. Epub 2009 Nov 10.

Prevalence, risk factors and bacterial causes of bovine mastitis in southern Ethiopia

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Abstract

Mastitis is one of the most economically important diseases in dairy farms worldwide. It is particularly important in Ethiopia where no routine prevention and control practices are in place. This cross-sectional study was carried out between October 2017 and June 2018 to estimate the prevalence of mastitis, identify the associated risk factors and isolate bacterial causes in dairy farms located in southern Ethiopia using standard microbiological methods and questionnaire survey. A total of 686 lactating cows which were found in 122 selected dairy farms were investigated by physical examination and California mastitis test. The overall cow-level prevalence of mastitis was 54.2% (95% CI: 50.5 – 57.9%). Based on the study site, the prevalence was 55.7% in Hawassa, 54.3% in Arsi Negele, 52.6% each in Wondo Genet and Wolayta Soddo towns with no significant ($p > 0.05$) difference among the sites. The majority of mastitis cases were subclinical (48.1%) while the clinical mastitis was only 6.1%. Of the 122 herds tested, 109 (89.3%; 95% CI: 82.1 – 93.9%) had at least a cow positive for mastitis. The study showed that high parity number (OR = 1.6; $p = 0.015$), flat (OR = 4.5; $p < 0.001$) and round (OR = 2; $p < 0.001$) teat end shape, history of mastitis in preceding lactation (OR = 3.3; $p < 0.001$), and slightly (OR = 3.5; $p < 0.001$), moderately (OR = 4.9; $p < 0.001$), and very dirty (OR = 9.2; $p < 0.001$) udder and legs were the major risk factors which are significantly associated with higher prevalence of mastitis. Based on the available media and reagents, the major bacteria isolated from subclinical

mastitic milk samples were *Staphylococcus* spp. (57.3%), *Streptococcus* spp. (18.6%), *E. coli* (17.3%) and *Bacillus* spp. (7.5%) in order of their abundance. The present study revealed a high prevalence of mastitis, particularly the subclinical one, and the associated risk factors. Enhancing the awareness of dairy farmers, regular screening of cows for subclinical mastitis, proper treatment of the clinical cases, improving the hygienic condition of the cows, and culling of chronically infected cows are critically important to prevent and control bovine mastitis.

Keywords: Mastitis, Prevalence, Risk factors, Southern Ethiopia

Introduction

Mastitis is an inflammation of the parenchyma of mammary glands characterized by physical, chemical and usually, bacteriological changes in milk and pathological changes in glandular tissues. The most important changes in the milk include discoloration, the presence of milk clots and large number of leucocytes in milk (Radostits *et al*., 2007). It is a multi-etiological and complex disease resulting from the interaction of three major factors: infectious agents, host resistance, and environmental factors (Gera and Guha, 2011).

Mastitis is a global problem adversely affecting animal health, quality of milk and the economics of milk production (Sharma and Sindhu, 2007). It is the most widespread infectious disease in dairy cattle (Tiwari et al., 2000; Elango et al., 2010; Sharma et al., 2012). Mastitis can occur either in clinical or subclinical forms. The clinical mastitis is characterized by changes in the udder and milk that are directly observable, whereas the subclinical mastitis is characterized by an increase in somatic cell count in the milk and absence of visible clinical signs (Kivaria et al., 2004).

Mastitis is of a particular concern for farmers in developing countries like Ethiopia. In Ethiopia, there are several bovine mastitis studies showing spatial variations in prevalence and risk factors. According to most recent studies, the prevalence of mastitis ranges from 39.5 – 62.6% (Belayneh *et al*., 2013; Tolosa *et al*., 2013; Abebe *et al.,* 2016; Birhanu *et al*., 2017). Since Ethiopia is a country with diverse agro-ecological conditions, it is obvious that mastitis prevalence and associated risk factors can vary from region to region. Thus, it is important to investigate the causes and risk factors of the disease in parts

of southern Ethiopia to formulate mastitis control program adapted to the specific local situation. The present study aims to estimate the prevalence of bovine mastitis, associated risk factors, and identify the major bacterial causes of mastitis in dairy farms in southern Ethiopia.

Materials and Methods

Study area

The study was conducted in small scale dairy farms located in Hawassa, Wendo Genet, Wolayita Sodo and Arsi Negelle towns which are high potential areas for dairy production in southern Ethiopia. The first three towns are found in Southern Nations, Nationalities and People's Regional State (SNNPRS) whereas Arsi Negelle is in Oromiya Regional State. Hawassa is located 275Kms south of Addis Ababa at 7º3´N latitude and 38º48´E longitude. It is situated at an elevation of 1708 meters above sea level. Hawassa receives an average annual rain fall of 900 mm and has mean annual temperature of 20°C. Wendo Genet is located at 30 Kms west of Hawassa. It is situated at about 1723 meters above sea level, 7°5'N latitude and $38^0$37'E longitude. The average annual rainfall of the town is 1372 mm while mean annual temperature is 19 °C. Wolayta Sodo is situated at 6°54′N latitude and 37°45′E longitude, and has an elevation between 1600 and 2100 meters above sea level. The average annual rainfall of the town ranges from 450 mm to 1446 mm while the mean annual maximum and minimum temperature of the town are 26.6 ^{0}C and 11.4 ^{0}C, respectively. Arsi Negelle is found in the West Arsi zone of the Oromia regional state at a distance of 225 Kms from Addis Ababa. The town is situated at about 2043 meters above sea level at 7°21'N latitude and 38°42'E longitude. The average annual temperature of the area varies from 10 to 25 °C while rainfall varies between 500 and 1000 mm.

Study population

The study population covers lactating dairy cows raised under semi-intensive or intensive management system. In intensive farms, cattle were kept indoors all the time and provided with roughages and concentrates. The semi-intensive farms are characterized by outdoor grazing at day time and provision of supplementary feed in the morning and evening before milking. House construction design in the study areas also varied from farm to farm. In some of the farms, the wall was made of bricks while in others it was built from wood and

mud. The floors of houses were constructed from concrete, wood or soil compact with or without beddings. In most of the houses, drainage system was not sufficient enough to remove slurry. Although it was difficult to get the actual figure, the approximate number of dairy farms found in the towns were 107 in Hawassa (Libiyos, 2018, unpublished data), 35 in Arsi Negelle (Teherku, 2018, unpublished data), 33 in Wolayta Sodo (Dema, 2018, unpublished data) and 63 in Wendo Genet (Wendo Genet Wereda Livestock and Fisheries Development Office, 2018, unpublished data). The size of the herds in the study towns ranged from 2 to 131 cattle with average herd size of 7 cattle per herd.

Study design and sample size

The study employed a cross-sectional study design. The required sample size was determined using the recommended formula (Thrusfield, 2005) with 95% confidence interval, 5% absolute precision, 0.15 between cluster variance (Vc) and 62.6% expected cow level prevalence (Abebe *et al*., 2016). Based on the given formula, the number of farms calculated was 122 and with predicted average number of five cows per farm, the minimum sample size was determined as 610 cows. The sample size was allocated proportionally to each of the study areas based on their dairy cattle population size. Accordingly, a sample size of 305, 152, 135, and 94 dairy cows was allocated for Hawassa, Wolayta Sodo, Wendo Genet, and Arsi Negelle towns, respectively. The dairy farms were selected randomly but all lactating cows found in the selected farms were included in the study.

Clinical examination

A thorough physical examination of the udder and teats was conducted on all lactating cows for evidence of clinical mastitis. Clinical findings such as secretions, abnormalities on size and shape of the udder, its consistency and temperature were assessed by visual inspection and palpation. Then, the cows negative for clinical mastitis were subjected to California Mastitis Test (CMT) for detection of subclinical mastitis. CMT was carried out according to the procedure described by Quinn et al (2002). In brief, a squirt of milk, about 2 ml from each quarter, was placed in each of the four shallow cups in the CMT paddle. An equal amount of CMT reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 seconds. The CMT result was scored as negative (0 and trace), 1 (weak positive), 2 (distinct positive) and 3 (strong positive) based on gel formation.

Bacteriological analysis

A total of 307 milk samples were collected from sub clinically affected (CMT positive) cows aseptically based on the procedure described in NMC (1999). The teats were wiped thoroughly with 70% ethyl alcohol and approximately 10 ml of milk were collected into a sterile bottle after discarding the first 3 milking stream. After collection, samples were transported in an icebox to Microbiology Laboratory, Faculty of Veterinary Medicine of Hawassa University. In the laboratory, samples were cultured immediately or stored at +4°C for a maximum of 24 hours until inoculated on a standard bacteriological media (NMC, 1999).

A loopful of the milk samples was streaked on blood agar base (Himedia, India) which was enriched with 5% sheep blood, and MacConkey agar (Himedia, India). Bacterial growths were identified and recorded after incubation for 24 to 48 hours at 37°C aerobically. Identification of bacterial isolates was done based on colony morphological features and hemolytic reactions (primary cultures), gram staining reactions and biochemical tests (INVIC, Catalase and Coagulase tests) on pure cultures (Quinn *et al*., 2002).

Questionnaire survey

During farm visits, a semi-structured questionnaire was used to collect data about herd and cow level variables thought to influence the prevalence of mastitis. The questionnaire was administered to farm owners/attendants through a face-to-face interview by four final year undergraduate veterinary students (one in each site) who were conducting research for graduation. Some of the variables were recorded by direct observation of the milking and husbandry practices. The data collectors had received training before initiation of the research to ensure that recording was consistent. The herd level variables recorded were herd size, management (intensive or semi-intensive), floor type (concrete, wood or soil), bedding (yes or no), pre or post milking teat dipping, udder washing practices (whole udder or teats only), housing (stall barn or group barn), use of towel for drying (yes or no), whether mastitic cows milked last or not, culling chronically infected cows, and dry cow therapy. Cow level data included age, parity, stage of lactation, udder position (normal or pendulous), teat end shape (pointed, round or flat), cow dirtiness (clean, slightly dirty, moderately dirty or very dirty), and previous history of mastitis.

Data analysis

Data collected through questionnaire survey and CMT were entered into Microsoft Excel spread sheet and then exported to Stata 14.2 statistical software (StataCorp, 4905 Lakeway Drive, College Station, Texas) for analysis. The prevalence of cow-level mastitis was calculated by dividing the number of mastitis-positive cows (clinical and subclinical) by the total number of animals tested while herd-level prevalence was determined by dividing positive herds by total number of herds. A herd was considered as positive if at least one cow tested positive for clinical or subclinical mastitis. Possible risk factors for mastitis were selected using univariable mixed effect logistic regression analysis with farm ID as a random effect to account for clustering at herd level. All variables having p-value <0.25 in the initial univariable analysis were further checked for co-linearity using Kruskal gamma statistics before multivariable analysis, and those variables whose gamma value ranged between −0.6 and +0.6 were considered in a multivariable logistic regression model. During multivariable mixed effect logistic regression analysis, all non-significant variables were removed sequentially by backward elimination where the model with the lowest Akaike Information Criterion (AIC) value was chosen as the best model. At every step during model development, the confounding effect of herd size was assessed by checking for changes in parameter estimates, and changes >25% were considered to indicate confounding (Dohoo *et al*., 2009). To compare differences in cow level prevalence in the four sampling towns, logistic regression with sampling town as a categorical variable and a farm as a random effect was performed. In all analyses, confidence levels were calculated at 95% and a p value < 0.05 was used for statistical significance level.

Results

The overall prevalence of mastitis at cow level was 54.2% (95% CI: 50.5 – 57.9%). The majority of mastitis cases in the cows tested was subclinical (48.1%) while the prevalence of clinical mastitis was only 6.1%. No significant (p > 0.05) difference was noted in the prevalence of mastitis across the four sampling towns (Table 1). Among the 122 herds examined in the study, 109 (89.3%; 95% CI: 82.1 – 93.9%) of them had at least a cow positive for mastitis. All the herds examined in Wolayta Sodo, 95.8% in Arsi Negele, 85.4% in Hawassa and 83.3% in Wendo Genet were positive for mastitis. The prevalence varied between 0% and 100% within a herd at an average herd prevalence of 52% (Table 2).

Table 1. Prevalence of cow-level clinical and subclinical mastitis in dairy cows in the southern Ethiopia

Sampling town	No examined	Clinical mastitis	Subclinical mastitis	Overall	95% CI
		Positive (%)	Positive (%)	Positive (%)	
Wendo Genet	135	17 (12.6)	54 (40)	71 (52.6)	44.1 – 60.1
Wolayita Sodo	152	4 (2.6)	76 (50)	80 (52.6)	44.7 – 60.5
Arsi Negele	94	10 (10.6)	41 (43.6)	51 (54.3)	44.1 – 64.1
Hawassa	305	11 (3.6)	159 (52.1)	170 (55.7)	50.1 – 61.2
Total	**686**	42 (6.1)	330 (48.1)	**372 (54.2)**	**50.5 – 57.9**

Table 2. Herd-level prevalence of mastitis in dairy farms in the southern Ethiopia

Sampling town	Herds tested	Positive herds	Prevalence (%)	95% CI
Wendo Genet	36	30	83.3	66.5 – 93.0
Wolayita Sodo	21	21	100	80.8 – 100
Arsi Negele	24	23	95.8	76.8 – 99.8
Hawassa	41	35	85.4	70.2 – 93.9
Overall	122	109	89.3	82.1 – 93.9

Out of the 2744 quarters examined, 111 (4.1%) were found blind and nonfunctional. The frequency of blind teats was slightly higher on the hind quarters than front quarters, and the overall quarter level prevalence of mastitis was 29.4% (Table 3).

Table 3. Proportion of blind quarters and prevalence of mastitis at quarter level in dairy cows in southern Ethiopia

Quarter	No quarters	Blind quarters, n (%)	No quarters tested	No positive	Prevalence (%)
RH	**686**	**35 (5.1)**	**651**	**184**	**28.3**
LH	686	24 (3.5)	662	203	30.7
RF	686	29 (4.2)	657	195	29.7
LF	686	23 (3.4)	663	191	28.8
Total	**2,744**	**111 (4.1)**	**2,633**	**773**	**29.4**

RF = Right front; LF = Left front; RH = Right hind; LH = Left hind;

In the present study, various possible risk factors at cow and farm levels were evaluated for their effect on mastitis prevalence. Univariable mixed effect logistic regression was performed on age, parity, lactation stage, udder position, teat end shape, history of mastitis, cow dirtiness, herd size, milking mastitic cow, management (intensive/semi-intensive), floor type (concrete, wood or soil), bedding (yes or no), washing udder before milking (whole udder or teats only), housing (stall barn or group barn), use of towel for drying (no, separate or common) and sampling town (Table 4). Out of 16 possible risk factors analyzed, parity, lactation stage, udder position, teat end shape, history of mastitis, cow dirtiness, milking mastitic cow and herd size had $p < 0.25$ and thus selected for multivariable analysis. Co-linearity was checked between variables before multivariable analyses. Accordingly, age was dropped from further analysis due to co-linearity with parity (gamma = 1) and history of mastitis in preceding lactation (gamma = 0.64). Parity was retained in the analysis due to its higher biological importance than age in relation to mastitis. The best fit model included parity, teat end shape, cow dirtiness, and history of mastitis as significant factors associated with prevalence of mastitis in cows. The final model showed that higher parity (OR = 1.6; $p = 0.018$), history of mastitis in preceding lactation (OR = 3.4; $p < 0.001$), round (OR = 2.2; $p < 0.001$) and flat teat ends (OR = 4.6; $p < 0.001$), and slightly dirty (OR = 2.9; $p < 0.001$), moderately dirty (OR = 3.2; $p < 0.001$) and very dirty (OR = 8; $p = 0.001$) udder and legs were significant factors associated with cow-level mastitis prevalence (Table 5). Herd size showed no association with mastitis prevalence ($p = 0.109$). However, the role of herd size as a confounder was investigated by fitting models for mastitis prevalence with and without herd size included. None of the coefficients for the other variables changed substantially when herd size was excluded, so we concluded that any confounding effect of herd size was minimal.

Table 4. Results of univariable mixed effect logistic regression analysis of potential animal and herd level risk factors with mastitis prevalence

Risk factors	Category	No cows examined	No cows positive (%)	Crude OR (95% CI)	p-value
Age	≤ 8yrs	576	289 (50.2)	1	
	> 8yrs	110	83 (75.5)	3.1 (1.9 – 4.9)	<0.001
Parity	Primiparous	177	69 (40)	1	
	Multiparous	509	303 (59.5)	2.4 (1.6 – 3.4)	<0.001
Lactation	≤ 4 month	209	102 (48.8)	1	
	> 4 to 8 month	335	185 (55.2)	1.3 (0.9 – 1.9)	0.127
	> 8 month	142	85 (59.9)	1.6 (1.0 – 2.5)	0.038
Udder position	Normal	569	287 (50.4)	1	
	Pendulous	117	85 (72.7)	2.7 (1.7 – 4.2)	<0.001
Teat end shape	Pointed	288	117 (40.6)	1	
	Round	331	199 (60.1)	2.5 (1.7 – 3.6)	<0.001
	Flat	67	56 (83.6)	8.1 (3.9 – 16.7)	<0.001
History of mastitis	No	458	196 (42.6)	1	
	Yes	228	176 (77.2)	4.7 (3.2 – 6.9)	<0.001
Cow dirtiness	Clean	229	81 (35.4)	1	
	Slightly dirty	285	176 (61.8)	3.5 (2.3 – 5.4)	<0.001
	Moderately dirty	149	97 (65.1)	4.9 (2.8 – 8.7)	<0.001
	Very dirty	23	18 (78.3)	9.2 (2.9 – 29.2)	<0.001
Herd size	≤ 10	162	78 (48.2)	1	
	> 10	524	294 (56.1)	1.4 (0.9 – 2.0)	0.109
Management	Semi-intensive	105	58 (55.2)	1	
	Intensive	581	314 (54)	0.97 (0.6 – 1.7)	0.920
Floor type	Wood	17	6 (35.3)	1	
	Earth/soil	37	19 (51.4)	0.5 (0.1 – 1.95)	0.336
	Concrete	632	347 (54.9)	1.1 (0.5 – 2.4)	0.750
Housing	Stall barn	214	117 (54.7)	1	
	Group barn	472	255 (54)	0.95 (0.6 – 1.4)	0.803
Bedding	Yes	136	72 (52.9)	1	
	No	550	300 (54.6)	1.0 (0.7 – 1.6)	0.927
Udder washing before milking	Teats only	46	22 (47.8)	1	
	Whole udder	635	348 (54.8)	0.76 (0.4 – 1.5)	0.430
Towel use	No	152	75 (49.3)	1	
	Common	142	76 (53.5)	1.3 (0.8 – 2.0)	0.244
	Separate	392	221 (56.4)	1.2 (0.7 – 1.97)	0.515
Milking mastitis cow last	No	270	139 (51.5)	1	
	Yes	416	233 (56)	0.8 (0.6 – 1.2)	0.268

CI = Confidence interval; OD=Odds ratio

Table 5. Best-fit multivariable model for risk factors associated with cow-level mastitis prevalence using mixed effect logistic regression modelling with farm as random effect

Risk factors	Category	Crude OR (95% CI)	Adjusted OR (95% CI)	p-value
Parity	Primiparous	1	1	
	Multiparous	2.3 (1.6 – 3.3)	1.6 (1.1 – 2.5)	0.018
History of mastitis	No	1	1	
	Yes	4.5 (3.2 – 6.5)	3.4 (2.3 – 5.1)	<0.001
Teat end shape	Pointed	1	1	
	Round	2.2 (1.6 – 3.0)	2.2 (1.5 – 3.2)	<0.001
	Flat	7.4 (3.7 – 14.8)	4.6 (2.1 – 9.9)	<0.001
Cow dirtiness	Clean	1	1	
	Slightly dirty	2.9 (2.1 – 4.2)	2.9 (1.9 – 4.4)	<0.001
	Moderately dirty	3.4 (2.2 – 5.3)	3.2 (1.8 – 5.6)	<0.001
	Very dirty	6.6 (2.4 – 18.4)	8.0 (2.4 – 26.1)	0.001
Constant			0.1 (0.04 – 0.21)	<0.001

CI = Confidence interval; OD=Odds ratio

Bacteria isolated

Bacterial examination was performed on 307 milk samples collected from cows with subclinical mastitis. However, due to lack of media and other facilities, isolation was limited to only certain bacteria. Accordingly, growth of different types of bacteria was observed in 299 (97.4%) of the samples cultured. *Staphylococcus* spp. were the most prevalent bacteria isolated from 57.3% of the samples cultured. *Streptococcus* spp., *E. coli* and *Bacillus* spp. were the other bacteria isolated in decreasing order of their prevalence (Table 6).

Table 6. Bacteria isolated from mastitic milk samples (N = 307)

Type of bacteria	No isolates	Prevalence (%)
Staphylococcus spp.	176	57.3
Streptococcus spp.	57	18.6
E. coli	53	17.3
Bacillus spp.	23	7.5

Discussion

Mastitis is the most costly disease in the dairy industry worldwide. It is a complex disease that results from the interaction of many factors involving the host, agent and environment. This study investigated the prevalence, risk factors and certain bacterial causes of mastitis in four towns of southern Ethiopia known for their dairy cattle potential. Unlike most of the previous studies in the region, the information presented in this paper was derived from a large sample of 686 lactating cows found in 122 dairy farms. An overall cow-level prevalence of 54.2% and overall herd-level prevalence of 89.3% was recorded. The cow-level prevalence of this study is comparable to two previous studies in Ethiopia: 52.9% in and around Areka town (G/Michael *et al*., 2013) and 56.5% in Batu and its environs (Duro and Taddele, 2011). However, it is higher than those reported in other studies that used CMT viz. 23.18% in Doba district, West Hararghe Zone (Girma *et al*., 2012), 39.5% in Adama town (Belayneh *et al*., 2013), 46.9% in and around Gondar town (Alemu *et al*., 2013) and 40.1% in Bishoftu town (Birhanu *et al*., 2017). In contrast, the present prevalence is lower than a previous report of 66.6% in Asella (Abera *et al*., 2013), 64.3 % in Adigrat (Zenebe *et al*., 2014), 62.6 % in Hawassa milk shed (Abebe *et al*., 2016) and 62% in North-West Ethiopia (Mekonnen *et al*., 2017). The first meta-analysis of the prevalence of mastitis in dairy cattle in Ethiopia conducted by Getaneh and Gebremedhin (2017) indicated that the variation of mastitis prevalence between studies might be due to variation in the locality and period of study, number of animals sampled, breeds, stages of lactation, parity number, and management practices.

In the present study, higher prevalence of subclinical mastitis (SCM) (48.1%) was observed compared to the clinical form (6.1%), a finding that is in line with previous studies (Debele, 2010; Moges *et al*., 2011; Abebe *et al*., 2016; Kebebew and Jorga, 2016). Risk factors including higher number of parity, history of mastitis in preceding lactation, flat or round teat end shapes in cows, and cow dirtiness were the likely attributable factors to the observed high prevalence of SCM. Furthermore, absence of mastitis prevention and control practices such as post milking teat disinfection, culling chronically infected cows and dry cow therapy by most of the dairy farms are the other possible reasons. Due to lack of mastitis monitoring program and absence of visible clinical signs, cows infected with SCM remain undetected for a long time. This increases the probability of mastitis transmission from infected to uninfected cows within herds

by the hands of milker's without the notice of the farmers (Radostits *et al*., 2007).

The odds of mastitis was 1.6 times higher in multiparous than primiparous cows. This finding is consistent with several previous studies (Abunna et al., 2013; Belayneh et al., 2013; Katsande et al., 2013; Zeryehun et al., 2013; Abrahmsén et al., 2014; Abebe et al., 2016). Higher prevalence of mastitis in multiparous cows might be ascribed to loosening of sphincter and patency of teat canal in older cows. Moreover, the median ligaments, which provide support to the teat, also get relaxed with age leading to hanging of udder and thus it makes more prone to mastitis (Boujenane et al., 2015; Bhat et al., 2017).

In the present study, cows with flat teat ends (OR = 4.5) and round teat ends (OR = 2) were more likely to have mastitis compared to those with pointed teat ends. This is perhaps because flat or round teats have wider streak canals, which can give greater chance for the entry of infectious agents, than pointed teat ends (Appleman, 1970). A similar finding was also reported by other studies like Belayneh et al. (2013), Nakov et al. (2014) and Abebe et al. (2016).

Cows with a history of mastitis in the preceding lactation were 3.3 times more likely to have mastitis than those without mastitis history. This finding is in agreement with previous studies (Houben *et al*., 1993; Berry and Meaney, 2005; Abebe *et al*., 2016). According to Elmaghraby et al. (2017), the reason for recurrent mastitis can be a persistent infection of the mammary gland by a mastitis pathogen.

The odds of finding a cow with mastitis increased as the degree of cow dirtiness increased. It was noted that the likelihood of mastitis was 8, 3.2 and 2.9 times higher in cows with very dirty, moderately dirty and slightly dirty udder and legs as compared to those with relatively clean udder and legs, respectively. It is obvious that the dirtiness of udder and hind legs is the result of poor hygiene of the cow's environment and facilities in the cows' barn. As stated by Rajabi *et al*. (2017), poor cow hygiene can contribute to presence of mastitis pathogens on teat ends and increasing the rate of new infections. Similar to the current finding, other researchers have also reported a significant association between mastitis prevalence and poor udder and leg hygiene (Abrahmsén *et al*., 2014; Iraguha *et al*., 2015; Abebe *et al*., 2016; Mureithi and Njuguna, 2016)

Various types of contagious and environmental pathogens have been reported to cause bovine mastitis in Ethiopia. However, only four types of bacteria were isolated in the present bacteriological study due to lack of the required media and reagents. *Staphylococcus* spp. were the most dominant organisms isolated followed by *Streptococcus* spp. As stated by Mdegela et al. (2009), the dominance of *Staphylococcus* spp. in bovine mastitis is possibly a result of poor milking hygiene. The predominance of *Staphylococcus* spp. particularly *S. aureus* has also been reported by several bovine mastitis studies in Ethiopia (Mekibib *et al.,* 2010; Abera *et al*., 2013; Belayneh *et al*., 2013; Zeryehun *et al*., 2013).

Conclusions

This study demonstrated a high prevalence of mastitis. Subclinical mastitis was the major form prevalent among the dairy farms in southern Ethiopia. Higher number of parity, history of mastitis in the preceding lactation, teat end shapes and cow dirtiness were important risk factors of bovine mastitis in the study area. The study also showed *Staphylococcus* spp., *Streptococcus* spp., *E. coli* and *Bacillus* spp. as possible causes of mastitis in the dairy farms. Therefore, raising awareness of dairy farmers, making the animals' environment clean and dry as possible, post milking teat dipping and regular screening and culling chronically infected cows are recommended as feasible interventions.

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References

Abebe, R., Hatiya, H., Abera, M, Megersa, B., and Asmare, K., 2016. Bovine mastitis: prevalence, risk factors and isolation of Staphylococcus aureus in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Vet. Res.,* 12(1):270

Abera, B., Lemma, D., and Iticha, I., 2013. Study of bovine mastitis in Asella government dairy farm of Oromia Regional state, South Eastern Ethiopia. *Int. J. Curr. Res. Aca. Rev*. 1(2), 134-145.

Abrahmsén, M., Persson, Y., Kanyima, B.M. and Båge, R., 2014. Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda. *Trop. Anim. Hlth. Prod*., 46:99–105.

Abunna, F., Fufa, G., Megersa, B. and Regassa, A., 2013. Bovine mastitis: Prevalence, risk factors and bacterial isolation in smallholder dairy farms in Addis Ababa city, Ethiopia. *Global Veterinaria.* 10 (6): 647-652.

Alemu, S., Tamiru, F., Almaw, G., and Tsega, A., 2013. Study on bovine mastitis and its effect on chemical composition of milk in and around Gondar Town, Ethiopia. *J. Vet. Med. Anim. Hlth*., 5(8):215-221

Appleman, R. D., 1970. Quantifying the genetic effects on the anatomy of streak canal. In: Proceedings of the VI International Conference on Cattle Disease.

Duro, B. D. and Taddele, H. M., 2011. Study on Prevalence of Mastitis and its Associated Risk Factors in Lactating Dairy cows in Batu and its Environs, Ethiopia. *Global Veterinaria.* 7 (6):632-637.

Belayneh, R., Belihu, K. and Wubet, A., 2013. Dairy cows mastitis survey in Adama Town, Ethiopia. *J. Vet. Med. Anim. Hlth.,* 5(10): 281-287.

Berry, D. P. and Meaney, W. J., 2005. Cow factors affecting the risk of clinical mastitis. *Irish* J. Agr. *Food.* Res. 44: 147-156

Bhat, A.M., Soodan, J.S., Singh, R., Dhobi, I.A., Hussain, T., Dar, M. Y., and Mir, M., 2017. Incidence of Bovine Clinical Mastitis in Jammu Region and Antibiogram of Isolated Pathogens. *Vet. World*, 10(25): 984-989.

Birhanu, M., Leta, S., Mamo, G. and Tesfaye, S., 2017. Prevalence of bovine subclinical mastitis and isolation of its major causes in Bishoftu town, Ethiopia. *BMC Res. Notes*, 10:767

Boujenane, I., El Aimani, J., By, K., 2015. Incidence and Occurrence Time of Clinical Mastitis in Holstein Cows. *Turkish J. Vet. Ani. Sci*.,39: 1-8.

Dohoo, I., Martin, W. and Stryhn, H., 2009. Veterinary Epidemiologic Research. 2nd ed. Charlotte town: AVC Inc; p. 27–407.

Elango, A., Doraisamy, K. A., Rajarajan, G. and Kumaresan, G., 2010. Bacteriology of sub-clinical mastitis and anti-biogram of isolates recovered from cross-bred cows. *Indian J. Anim. Res.* 44(4): 280–284.

Elmaghraby, M. M., El-Nahas, A. F., Fathala, M. M., Sahwan, F. M., and EL-Dien M. A. T., 2017. Incidence of Clinical Mastitis and its influence on reproductive performance of dairy cows. Alex. *J. Vet. Sci.* 54 (2): 84-90.

G/Michael, L., Deressa, B., Begna, F. and Mekuria, A., 2013. Study on prevalence of bovine mastitis in lactating cows and associated risk factors in and around Areka town, Southern of Ethiopia. *Afr. J. Microbiol. Res*., 7(43): 5051-5056

Getaneh A.M., Gebremedhin E.Z., 2017. Meta-analysis of the prevalence of mastitis and associated risk factors in dairy cattle in Ethiopia. *Trop. Anim. Hlth. Prod*., 49: 697-705.

Gera, S. and Guha, A. 2011. Assessment of acute phase proteins and nitric oxide as indicator of subclinical mastitis in Holstein × Haryana cattle. *Indian J. Anim. Sci*., 81(10): 1029–1031.

Debele, G. D., 2010. Study on prevalence of mastitis on cross breed dairy cow around Holeta areas. *Global Veterinaria*, 5(6): 318-323.

Girma, S., Mammo, A., Bogele, K., Sori, T., Tadesse, F. and Jibat, T., 2012. Study on prevalence of bovine mastitis and its major causative agents in West Harerghe zone, Doba district, Ethiopia. *J. Vet. Med. Anim. Hlth*., 4(8): 116-123

Houben, E. H. P., Dijkhuizen, A. A., van Arendonk, J. A. M. and Huirne, R. B. M., 1993. Short-term and long-term production losses and repeatability of clinical mastitis in dairy cattle. *J. Dairy Sci*., 76:2561–2578.

Iraguha, B., Hamudikuwanda, H. and Mushonga, B., 2015. Bovine mastitis prevalence and associated risk factors in dairy cows in Nyagatare District, Rwanda. *J. S. Afr. Vet. Assoc*., 86:1228.

Katsande, S., Matope, G., Ndengu, M. and Pfukenyi, D. M., 2013. Prevalence of mastitis in dairy cows from smallholder farms in Zimbabwe. *Onderstepoort J. Vet. Res*., 80:523.

Kebebew, G. and Jorga, E., 2016. Prevalence and risk factors of bovine mastitis in Ambo town of West Shewa Zone, Oromia, Ethiopia. *Ethiop. Vet. J*., 20 (1), 123-134

Kivaria, F. M., Noorhuizen, J. P. and Kapaga, A. M., 2004. Risk indicators associated with subclinical mastitis in smallholder dairy cows in Tanzania. *Trop. Anim. Hlth. Prod*., 36 (6): 581-592.

Mekibib, B., Furgasa, M., Abunna, F., Megersa, B. and Regassa, A., 2010. Bovine mastitis: prevalence, risk factors and major pathogens in dairy farms of Holeta town, central Ethiopia. *Vet. World*, 3 (9): 397–403.

Mekonnen, S. A., Koop, G., Melkie, S. T., Getahun, C. D., Hogeveen, H., and Lama, T. J. G. M., 2017. Prevalence of subclinical mastitis and associated risk factors at cow and herd level in dairy farms in North-West Ethiopia. *Prev. Vet. Med*., 145: 23–31.

Mdegela, R. H., Ryoba, R., Karimuribo, E.D., Phiri, E. J., Løken, T., Reksen, O., Mtengeti, E., Urio, N.A., 2009. Prevalence of clinical and subclinical mastitis and quality of milk in smallholder dairy farms in Tanzania. *J. S. Afr. Vet. Assoc*., 80(3): 163–168

Moges, N., Asfaw, Y. and Belihu, K., 2011. A Cross Sectional Study on the Prevalence of Sub- Clinical Mastitis and Associated Risk Factors in and around Gondar, Northern Ethiopia. *Int. J. Anim. Veter. Adv*., 3(6): 455-459

Mureithi, D. K. and Njuguna, M. N., 2016. Prevalence of subclinical mastitis and associated risk factors in dairy farms in urban and peri-urban areas of Thika Sub County, Kenya. *Livestock Res. Rural Dev*., 28 (2) :13

Nakov, D., Hristov, Andonov,S. and Trajchev, M., 2014. Udder-Related Risk Factors for Clinical Mastitis in Dairy Cows. *Vet. Arhiv*. 84 (2):111-127.

National Mastitis Council (NMC), 1999. Current Concept in bovine mastitis, National mastitis Council 3rd, 1840 Wilson Blvd, Arlington, VA 22201. pp: 397-432.

Quinn, P. J., Markey, B. K., Carter, E. M., Donnelly, W. J. and Leonard, F. C., 2002. Veterinary Microbiology and Microbial Disease. Blackwell Science Ltd, Blackwell Publishing Company pp: 465-474.

Radostits, O. M., Gay, C. C., Hinchcliff, K. W. and Constable, P. D., 2007. Veterinary Medicine: A text book of the disease of cattle, horses, sheep, pigs and goats. 10th ed. London: Elsevier Ltd.

Rajabi, I., Taghavi Razavizadeh, A., Azizzadeh, M. and Khoramian, B., 2017. The investigation on the relationship between dairy cow hygiene scores and intramammary infections. *Iranian J. Vet. Sci. Tech*., 9 (1): 12-16

Sharma, A. and Sindhu, N., 2007. Occurrence of clinical and sub-clinical mastitis in buffaloes in the State of Haryana (India). *Italian J. Anim. Sci*., 6 (Suppl. 2): 965–967.

Sharma, N., Rho, G. J., Hong, Y.H., Kang, T.Y., Lee, H. K., Hur, T.Y. and Jeong, D. K., 2012. Bovine mastitis: an Asian perspective. *Asian J. Anim. Vet. Adv*., 7: 454–476.

Thrusfield, M., 2005. Veterinary Epidemiology. 3rd ed. Oxford: Blackwell science Ltd; pp. 183–189.

Tiwari, A., Sisodia, R. S., Sharma, R. K., Misraulia, K. S. and Garg, U. K., 2000. Incidence of sub-clinical mastitis in cows of Malwa Region of Madhya Pradesh. *Indian J. Dairy Sci*., 53(4): 328–331.

Tolosa, T., Verbeke, J., Piepers, S., Supré, K., and De Vliegher, S., 2013. Risk factors associated with subclinical mastitis as detected by California Mastitis Test in smallholder dairy farms in Jimma, Ethiopia using multilevel modelling. *Prev. Vet. Med*., 112: 68-75.

Zenebe, N., Habtamu, T., and Endale, B., 2014. Study on bovine mastitis and associated risk factors in Adigrat, Northern Ethiopia. *Afr. J. Microbiol. Res*., 7 (48), 5400-5417.

Zeryehun, T., Aya, T. and Bayecha, R., 2013. Study on prevalence, bacterial pathogens and associated risk factors of bovine mastitis in small holder dairy farms in and around Addis Ababa, Ethiopia. *J. Anim. Plant Sci*. 23 (1): 50-55.

Contagious Bovine Pleuropneumonia: Sero-prevalence and associated risk factors in Gudeya Bila and Boneya Boshe Districts of East Wollega Zone, Oromia, Ethiopia

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Abstract

Contagious bovine pleuropneumonia (CBPP) remains a huge threat to cattle production in sub Saharan African countries in general and in Ethiopia in particular. A cross sectional study was conducted between November, 2017 and June, 2018 to estimate the seroprevalence and associated risk factors of CBPP in the Gudeya Bila and Boneya Boshe districts of East Wollega Zone, Oromia Regional State. The study was conducted on 384 cattle with no history of vaccination against CBPP, using systematic random sampling technique. Blood samples were collected from the jugular vein of each animal and tested by competitive ELISA. Information on risk factors influencing the occurrence of CBPP was collected using questionnaire survey. Data obtained from both serological and questionnaire surveys were analyzed by using SPSS software version 20. Logistic regression was used to analyze the association of exposure variables with anti-mycoplasma mycoides subspecies small colony antibodies. The results indicated that, the overall seroprevalence of CBPP at individual animal-level and herd-level was 8.6% and 26.3%, respectively. There was a statistically significant association in the sero-prevalence of Mycoplasma mycoides subsp. mycoides SC (MMmsSC) antibody ($P< 0.05$) with the poor body condition score, origin of animals (purchasing from outside of herd) and previous history of CBPP disease at individual animal and large herd size at herd level. This study showed that the overall prevalence of CBPP in study area was high. This warrants the implementation of appropriate preventive and control practice.

Keywords: Boneya Boshe, Bovine, CBPP, c-ELISA, Ethiopia, Gudeya Bila, Sero-prevalence

Introduction

Agriculture is the backbone of Ethiopian economy. Livestock is very notable in their contribution to agriculture. They contribute 13-16% of the total gross domestic product (GDP), 30- 35% of agricultural gross domestic product (GDP) and more than 85% of farm cash income (Tsedeke and Endrias, 2011; Abera *et al.*, 2016). Despite the fact that this magnificent figure is achieved from livestock sector and making the gap of economy very narrow thereby alleviating food insecurity, diseases of animals like contagious bovine pleuropneumonia (CBPP) is playing a principal role for not to achieve the real asset expected from this sector (Lesnoff *et al.*, 2004; Adugna, 2017). Contagious bovine pleuropneumonia is a highly infectious acute, sub-acute and chronic disease of cattle caused by *Mycoplasma mycoides subspecies mycoides* small colony (MmmSC). It is one of the diseases recognized by OIE that needs to be controlled or eradicated through a national surveillance protocol (John, 2016; Dereje and Shawul, 2017).

Although CBPP was worldwide in its distribution, it was eradicated from most continents, by the mid-20th century. However, because of the economic and financial difficulties that affected the ability of governments to adequately fund veterinary services, the disease is still widely distributed in sub-Saharan African countries. Contagious bovine pleuropneumonia directly impacts economies through cattle mortality and morbidity (Dereje and Shawul, 2017) and also by being a barrier to trade and reduces the value of livestock and the income of value chain stakeholders in many African countries (Tambi *et al.*, 2006; Joerg, 2014). In recent years, CBPP has been reported from countries like Botswana, where it was previously eradicated (Alemayehu *et al.*, 2015). Contagious bovine pleuropneumonia is one of the major diseases in Ethiopia that hampering export of livestock and livestock products to the international markets since long time (Farmer, 2010). Among the exacerbating risk factors of contagious bovine pleuropneumonia in Ethiopia are; lack of knowledge of the disease by farmers, vaccine shortage, poor diagnostic assays, management system, limitation of epidemiological information about the disease, concentration of livestock at watering points and grazing area and difficulty to control of cattle movements are the principal things which have been cited by many literatures (Ebisa *et al.*, 2015).

In western Oromia farming communities there are different animal diseases in which their etiological agent was not identified and affecting production and

productivity of livestock and threatening the livelihood of small scale farmers. East Wollega zone is one of west Oromia zone in which previously study based on food security was conducted and reported that feed shortage and massive cattle death was a main problem of the zone. The disease that caused massive cattle death at reporting time was tentatively diagnosed as pasteurellosis and it might be other respiratory disease like CBPP (Mersha, 2016). However, there was no systematic study conducted to investigate the status of this disease in the area, insufficient epidemiological information and limited resources to apply control measures; thus study was planned to determine seroprevalence and its associated risk factors CBPP in Boneya Boshe and Gudeya Bila districts.

Materials and methods

Description of the study area

The study was conducted in Gudeya Bila and Boneya Boshe districts of East Wollega zone. Gudeya Bila district is found in the East Wollega zone of Oromia Regional State, Western Ethiopia which is located at 274 km West of Addis Ababa, at 09º 17'363"N latitude and 037º 01'460" E longitudes with an altitude ranging from 1876 -2092 meters above sea level. The area is characterized by humid tropical climate with annual rainfall that ranges from 1000-2200 millimeter per annum with average temperature of 20ºC (CSA, 2017). The district has 121, 081 cattle population (GBLFRDO, 2017). The district has fifteen Peasant associations (PAs). Boneya Boshe district is found in the East Wollega zone of Oromia State, Western Ethiopia which is located at 307 km West of Addis Ababa, at 08º 54'045"N latitude and 037º 00'136" E longitudes with an altitude ranging from 1613-1641 meters above sea level. The area is characterized by a humid tropical climate with annual rainfall that ranges from 1000-1200 millimeter per annum with average temperature of 20.9ºC (CSA, 2017). The district has cattle population of 102, 917 (BBLFRDO, 2017). The district has ten PA's (Figure 1).

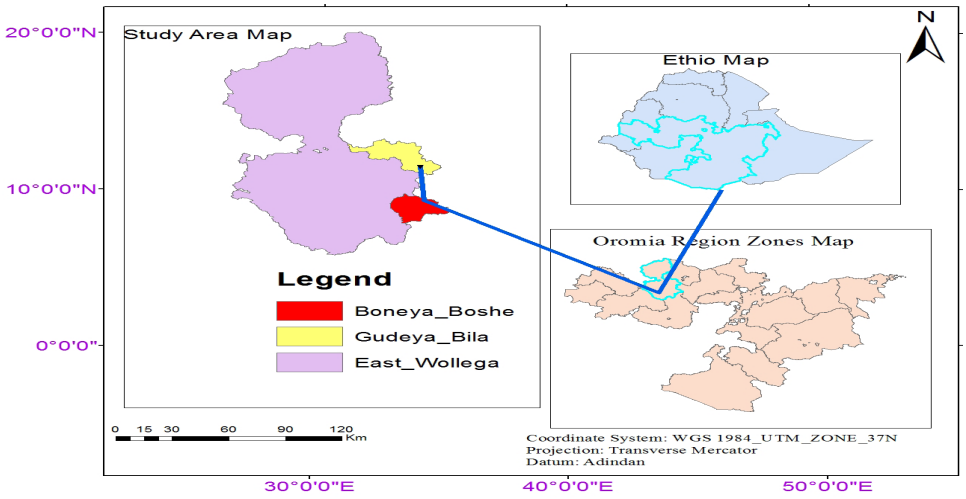


Figure 1: Study area map (CSA, 2017)

Study Design

A cross-sectional study was conducted using a systematic random sampling technique to select the study cattle. The size of the households' and list of herd distribution were identified from PAs and then both blood sample collection and questionnaire survey were conducted. Pre-tested semi-structured questionnaire was used to collect information on factors influencing the occurrence of CBPP within or between herds by face-to face interview. Data on sex, age, origin of animal, herd size, previous infection history, body condition scores of animal, animal management, introduction of new animal, herd contact and herd contact area were recorded. The body condition scores of animals were scored according to DEFRA (2001).

Target and study population

The target populations in this study were all local Horo breed cattle above six months of age of both sexes with no history of vaccination in selected PAs of Gudeya Bila and Boneya Boshe districts of Oromia Regional State and the study populations in this study were all cattle selected for purpose of this study.

Sampling strategy

Four PAs form Boneya Boshe and 4 PAs from Gudeya Bila district were selected based on cattle population and access to road facility and individual animals were selected by using systematic random sampling technique.

Sample size determination

The sample size was determined using the formula described by Thrusfield (2007) by considering an expected prevalence of 50% with an absolute precision of 5% with 95% confidence level.

$$N = \frac{1.96^2 X\ Pexp\ (1-Pexp)}{d^2}$$

Where N = sample size of the study population
d = Absolute desired precision
p = expected prevalence in the study area

$$N = \frac{1.96^2 X\ 0.5(1-0.5)}{0.05^2} = 384$$

Therefore 384 cattle were selected from both districts (186 cattle from Boneya Boshe and 198 cattle Gudeya Bila) using proportional allocation based on the cattle population in each district.

Questionnaire survey

Ninety-five households were interviewed during sampling of study cattle. The questionnaire was covering information on the name of the owner, location, sex, age, origin of animal, herd size, previous infection history of contagious bovine pleuropneumonia, animal management, contact of herds with one or more animals or herds at grazing areas or watering points, introduction of new animals. This questionnaire was administered by face-to-face interview with the owner of animals using the local language (Afaan Oromo).

Sample collection and Laboratory test (Competitive ELISA (c- ELISA))

About 10 milliliters of blood samples were collected from the jugular vein of each cattle using sterile vacutainer tubes and needles by following aseptic procedure after cattle restrained by owner and each sample was properly labeled (include all necessary information like owner name, species of animal, sex, age, breed, body condition etc.). The samples were kept protected from sun light in a slanting position for 6-8 hours. The serum was separated manually and transfered to a sterile tube and stored at -20ºC and analysis with Competitive ELISA at Bedele Veterinary Regional Laboratory. The serum samples were tested by competitive enzyme-linked immunosorbent assay (c-ELISA) to detect MmmSC antibodies based on the manufacturer instruction. Competitive ELISA is an OIE prescribed test and can be used for official CBPP testing (OIE, 2014).

Data analysis

Data obtained from both serological tests and questionnaire surveys were entered and stored in Microsoft (MS) Excel spreadsheet program and analyzed using SPSS software programs version 20. The total seroprevalence of individual animals was calculated by dividing the number of c-ELISA positive animals by the total number of animals tested and herd prevalence was calculated by number of herd positive to total number of herds tested. A herd was considered seropositive, if at least one animal in the herd was found seropositive. Individual animal risk factors like age, sex, body condition scores, history of disease and origin of animal and herd level risk factors like herd size, management, herd contact with other herds, contact area and introduction of new animal were analyzed.

Univariable logistic regression was used to select the exposure variables forward for multivariable analysis. Factors were selected for final multivariable logistic regression analysis if the p-value was ≤ 0.25 (sex, age, body condition, disease history and origin at individual animal level and large herd size and management at herd level). The strength of association between the risk factors and the occurrence of the disease was assessed using Odds Ratio (OR). Pearson correlation coefficients were used to check the variables for co-linearity. Then, multivariable analysis was conducted and non-significant variables were removed sequentially using backward elimination at $p< 0.05$.

Results

Prevalence

From the total animals of 384 examined 33 of them were found to be positive for anti-MmmSC antibody. The overall sero-prevalence of CBPP at individual animal-level was 8.6% (n=33/384) (95% CI: 5.8% -11.4%). From the total of 95 herds examined 25 of them were found to be positive to CBPP antibodies with an estimated seroprevalence of CBPP at herd-level 26.3% (n=25/95) (95% CI: 17.5%-35.2%). The positive animal for MmmSC antibodies in the Boneya Boshe and Gudeya Bila were 10.8% (20/186) and 6.6% (13/198), respectively (Table 1).

Table 1: Sero-prevalence of contagious bovine pleuropneumonia in study area

Factor	Categories	No of examined	Prevalence (%)	95%CI	
				Lower	Upper
Districts	Gudeyabila	198	13(6.6)	3.1	10.0
	Boneyaboshe	186	20(10.8)	6.3	15.2
Gudeyabila	Harogudisa	50	1(2.0)	1.9	5.9
	Henajawaja	52	5(9.6)	1.6	17.6
	Jare	48	4(8.3)	0.5	16.1
	Agalogidami	48	3(6.3)	0.6	13.1
Boneyaboshe	Ejersagute	51	12(23.5)	11.9	35.2
	Gala gore	43	5(11.6)	2.1	21.2
	Bilo	45	2(4.4)	1.6	10.5
	Jawis	47	1(2.1)	2.0	6.3

Risk factors

Animal- level risk factors

Male animals have high sero-prevalence 11.3% (17/151) than female 6.9% (16/233). Prevalence of CBPP in animals with age > 5 years 10.6% (19/180) were higher than animals with age ≤ 5 years 6.9% (14/204). Animal with poor body condition score 12.1% (28/ 231) has high sero-prevalence than animal with good body condition score 3.3% (5/153) and significantly associated ($p<0.05$) with anti-MmmSC antibodies. Prevalence of disease among animal

with history of disease 13.3% (23/173) were higher than cattle with no disease history 4.5% (10/211) and statistically associated ($p<0.05$) ant-MmmSC antibodies. Animal replacement from outside the herd was statistically associated with anti-MmmSC antibody where the herds with animals replaced was found to be higher 17.1% (9/52) sero prevalent than animal with own source origin 7.2% (24/332) (Table 2 and 3).

Table 2: Sero-prevalence of CBPP antibody with potential risk factors

Factor	Categories		No of examined	Prevalence (%)	95%CI Crude OR (95% CI)	Univariable analysis p-value	
Individual animal level	Sex	Male	151	17(11.3)	6.2-16.3	0.4(0.2-1.0)	0.06
		Female	233	16(6.9)	3.6-10.1		
	Age	≤5years	204	14(6.9)	3.4-10.3	1.7(0.7-4.1)	0.20
		>5 years	180	19(10.6)	6.0-15.0		
	BCS	Poor	231	28(12.1)	7.9-16.3	5(1.6-15.8)	0.09
		Good	153	5(3.3)	0.5-6.1		
	Disease history	Yes	173	23(13.3)	8.2-18.3	5.6(2.0-15.9)	0.001
		No	211	10(4.5)	1.9-7.6.0		
	Origin	Own source	332	24(7.2)	4.4-10.0	12.4(3.0-51.9)	0.001
		Outside	52	9(17.1)	7.0-27.6		
Herd level	Herd size	≤36 herd size	64	12(18.8)	9.2	28.3	
		>36 herd size	31	13(41.9)	24.6	59.3	0.02
	Management	Extensive	67	19(28.4)	17.6	39.2	0.2
		Semi extensive	28	6(21.4)	6.2	36.6	
	Herd contact animal introduction	Yes	92	24(26.1)	17.1	35.1	0.60
		No	3	1(33.3)	20.0	86.7	
	Animal introduction	Yes	46	10(21.7)	9.8	33.7	0.90
		No	49	15(30.6)	17.7	43.5	
	Contact area	Watering	28	9(32.1)	14.8	49.4	0.90
		Water & grazing	61	15	13.8		
			3	1	20		

Table 3: Results of multivariable analysis of potential risk factors

Factors	Categories	No of examined	Prevalence (%)	Multivariable analysis	
				Adjusted OR(95% CI)	P-value
Bcs	Poor	231	28(12.1)	4.6(1.6-13.2)	0.01
	Good	153	5(3.3)	*	
Disease history	Yes	173	23(13.3)	4.9(1.9-12.9)	0.01
	No	211	10(4.7)	*	
Origin	Outside	52	9(17.3)	12.5(3.1-51.0)	0.01
	Own herd	332	24(7.2)	*	

*= reference

Herd level risk factors

Among the potential risk factors assessed at herd level prevalence of CBPP was higher in large herd size 41.9% (13/31) than lower herd size 18.8% (12/64) and significantly associated with anti-MmmSC antibodies (OR: 3.6 (1.3-10.3; $p<0.05$). Herds from extensive management system 28.4% (19/67) have high prevalence than herds found in semi extensive management system 21.4% (6/28). Herd with history of contact with other herds 26.1% (24/92) has lower prevalence than herds with no history of contact with other herds 33.3% (1/3). Prevalence of CBPP in animals with history of new animal introduction 21.7% (10/42) was lower than herds with no history of new animal introduction 30.6% (15/49). Herds which had contact with other herds at watering point has 32.1% (9/28) high prevalence than herds contact with other herds both at watering and grazing point has 24.6% (15/61) (Table 2).

Discussion

The overall seroprevalence of MmmSC antibodies was estimated to be 8.6%. Related result was reported by other investigators, 9.4% in Borena (Ahmed, 2004), 8.1% by Mamo *et al.*(2018) and 9.7% in south western Kenya (Schnier *et al.*, 2006). The higher seroprevalence was previously reported from different regions of the country and outside of the country by other investigators, 39% in Somali Regional State (Gedlu, 2004), 28.5% in western Oromia (Daniel *et al.*, 2016), 28% in the Bodji district (Fikru, 2001), 25.3% in Sidama Zone (Malicha *et al.*, 2017) and 16% in Kajiado district of Kenya (Matua-Alumira *et al.*,

2006) and on the other hand the lower seroprevalence also previously reported by others investigator, 4% in and around Adama (Kassaye and Molla, 2012), 6.14% in Southern Ethiopia (Asmamaw, 2003), 1.4% in Bale zone (Dereje and Shawul, 2017). The variation in prevalence of CBPP reported from different parts of Ethiopia and other countries might be due to differences in agro ecological systems, cattle management and production systems, population density, sample size and the types of tests used to determine the seroprevalence.

In this study, poor body condition was found significantly (P<0.05) associated with anti-MmmSC antibodies which agrees with finding of Atnafie *et al*. (2015) in Bishoftu abattoir which reported that poor body condition was significantly associated (p<0.05) with occurrence of CBPP. This might be due to the fact that animals with poor body conditions are more susceptible to the disease due to low immunity to resist disease.

Animals with history of disease was significantly (P<0.05) associated with anti- MmmSC antibodies. This might be due to the fact that previous diseased animals are carrier of MmmSC in lung sequestra. There was no any suggestion on previous studies that is parallel with this finding.

The statistical significant (p< 0.05) association between the large herd sizes and sero-prevalence of anti- MmmSC antibodies was in agreement with study on bulls at finishing phase for export in East Shewa zone brought from Borena Pastoral Area of Southern Ethiopia in which there was significant association between prevalence of the CBPP antibodies as herd size increases reported (Alemayehu *et al*.,2015) The statistical significant (p< 0.05) association between the large herd sizes and sero-prevalence of anti- MmmSC antibodies was in agreement with report of Alemayehu *et al. (*2015) which reported that the number of seropositive animals increases as the herd size increases (p<0.05) in both at herd and individual level, the highest CBPP prevalence was recorded in herd size >1000 and the difference was found statistically significant (p<0.05) in Borena pastoral area of Southern Ethiopia. This might be related to the health management of large herd size and risks of an individual animal become infected with disease increases as herd size increase due to overcrowding of animals.

Conclusion

The sero-prevalence of anti-MmmSC antibodies in the study areas at individual animal and herd level was relatively high as compared with different reports of the disease which indicated that the disease was prevalent in the study areas. Animals kept in these study areas are always at the risk of contracting CBPP because of their uncontrolled replacement of animals from outside origin and related management problem.

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References

Abera, Z., Mengistu, D., Batu, G. and Wakgari, M. 2016. Review on contagious bovine pleuropneumonia and its economic impacts. *Acad. J. Anim. Dis.*, 5(1):1-15.

Adugna, T., A. 2017. Contagious bovine pleuropneumonia (CBPP): Literature review on distribution, sero-prevalence, and associated risk factors which plays major role in an economic loss of this sector. *Austin J. Vet. Sci. Anim. Husb*., 4(2).

Ahmed, I. 2004. Epidemiological study of contagious bovine pleuropneumonia in Borena pastoral areas using complement fixation test and competitive enzyme-linked immunosorbent assay. MSc thesis, Addis Ababa University, Debrezeit, Ethiopia.

Alemayehu, G., Leta, S. and Hailu, B. 2015. Sero-prevalence of contagious bovine pleuropneumonia (CBPP) in bulls originated from Borena pastoral area of Southern Ethiopia. *Trop. Anim. Hlth. Prod.*, 47 (5): 983-987.

Asmamaw, M. 2003. Situation of CBPP in selected district of Southern Ethiopia. MSc thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debrezeit, Ethiopia.

Atnafie, B., Goba, H., Sori, H., and Kasaye, S. 2015. Sero-prevalence of contagious bovine pleuropneumonia in abattoirs at Bishoftu and export-oriented feedlots around Adama. *Global Veterinaria*, 15 (3): 321-324.

BBLSRDO. 2017. Boneya Boshe woreda Livestock and Fishery Resource and Development office. Animal population annual report. Unpublished woreda report.

CSA, (2017). Federal Democratic Republic of Ethiopia, central statistical agency. Agricultural sample survey. Report on Livestock and Livestock Characteristics (Private Peasant Holdings).

Daniel, G., Abdurahaman, M., Tuli, G. and Deresa, B. 2016. Contagious bovine pleuropneumonia: Seroprevalence and risk factors in Western Oromia, Ethiopia. *Onderstepoort J. Vet. Res.,* 83 (1):1-5. doi: 10.4102/ojvr.v83i1.958.

DEFRA (Department for Environment, Food and Rural Affairs). 2001. Condition scoring of dairy cows. DEFRA Publications ONLINE: www.defra.gov.uk/corporate/publications/pubfrm.htm, London, UK.

Dereje, L., and Shawul, W. 2017.A Sero-prevalence study of contagious bovine pleuropneumonia (CBPP) in Bale zone, Asella Regional Veterinary Laboratory, Asella, Ethiopia. *Acad. J. Anim. Dis.*, 6 (3): 83-87.

Ebisa, T., Hirpa, H. and Aklilu, F. 2015. Study on seroprevalence and risk factors contagious bovine pleuropneumonia in Southern Nation and Nationality People of Ethiopia Regional State in Amaro special district. *Sci. Tech. Arts Res.J.,* 4 (4**):**106.

Farmer, E. 2010. End market analysis of Ethiopian livestock and meat. *A Desk Study Micro report*, Published by The United States Agency for International Development (USAID).

Fikru, R. 2001. Herd prevalence of contagious bovine pleuropneumonia (CBPP), Bovine Tuberculosis and Dictyocaulosis in Bodji woreda, West Wollega. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debrezeit, Ethiopia.

GBLSRDO, 2017. Gudeya Bila woreda Livestock and Fishery Resource and Development office. Animal population annual report. Unpublished data.

Gedlu, M. 2004. Serological, clinical and participatory epidemiological survey of Contagious Bovine Pleuropneumonia in Somali Region, Ethiopia. MSC Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debrezeit, Ethiopia.

Joerg J. 2014. Developing an improved vaccine to control contagious bovine pleuropneumonia. Deutsche GesellschaftfürInternationaleZusammenarbeit (GIZ) GmbH, Bonn and Eschborn, Germany, in cooperation with Kenya Agricultural Research Institute (KARI), Kenya and Central Veterinary Laboratory Windhoek, Namibia.

John, C. 2016. Contagious bovine pleuropneumonia. In large animal clinical sciences, Western College of Veterinary Medicine, University of Saskatchewan. Merck and Co., Inc., Kenilworth, NJ, USA.

Kassaye, D., and Molla, W. 2012.Seroprevalence of contagious bovine pleuropneumonia at export quarantine centers in and around Adama, Ethiopia. *Trop. Anim. Hlth. Prod.*, 45 (1):275-279.

Lesnoff, M., Laval, G., Bonnet, P., Chalvet-Monfray, K., Lancelot, R. and Thiaucourt, F. 2004. A mathematical model of the effects of chronic carriers on the within-herd spread of contagious bovine pleuropneumonia in African mixed crop–livestock system. *Prev. Vet. Med.*, 62 (2):101-117.

Malicha, G., Alemu, S., Aklilu, F. and Abraha, A. 2017. Study of seroprevalence and associated risk factors of contagious bovine pleuropneumonia in Sidama Zone, Southern Ethiopia. *J. Vet. Sci. Tech.*, 8 (4):2.

Mamo, Y., Bitew, M., Teklemariam, T., Soma, M., Gebre, D., Abera, T., Benti, T. and Deneke, Y., 2018. Contagious bovine pleuropneumonia: seroprevalence and risk factors in Gimbo District, Southwest Ethiopia. *Vet. Med. Internal.* Volume 2018, Article ID 5729296, 7 pages. https://doi.org/10.1155/2018/5729296.

Matua-Alumira, R.W., Ng'ang'a, Z., Kiara, H., Matere, C., Mbithi, F., Mwirigi, M., Marobella-Raborogwe, C. and Sidiadie, S. 2006. The prevalence of CBPP in cattle under different production systems in Kajiado district, Kenya. In Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics. Available at www.sciquest.org.nz.

Mersha, T. 2016. Sero-prevalence of contagious bovine pleuropneumonia and its potential risk factors in selected sites of Western Oromia, Ethiopia. *Ethiop. Vet. J.*, 20 (2):31-41.

OIE. 2014.Office of International des Epizooties. Terrestrial Animal Health Code (Chapter 11.8.1). Paris, France.

Schnier, C., Mtui-Malamsha, N.J., Cleaveland, S., Kiara, H., Grace, D., McKeever, D.J. and Zadoks, R. 2006. Contagious bovine pleuropneumonia seroprevalence and associated risk factors in the Maasai ecosystem of south-western Kenya. In proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics.

Tambi, N.E., Maina, W.O. and Ndi, C. 2006. An estimation of the economic impact of CBPP in Africa. *Rev.Sci. Tec.*, 25 (3): 999-1011.

Thrusfield, M. (2007). Veterinary Epidemiology. 2nd edition, Blackwell Science Ltd. Edinburgh, UK, Pp: 178-197.

Tsedeke, K., and Endrias, G. 2011. Agro-ecologic mapping of livestock system in smallholder crop-livestock mixed farming of Wolayita and Dawuro districts, Southern Ethiopia. *Livest. Res. Rural Dev.*, 23(3).

Study on the prevalence and risk factors of bovine tuberculosis in dairy cattle in Adama city, central Ethiopia

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Abstract

Bovine tuberculosis (bTB) is a serious infectious disease of cattle with significant economic impact and public health risk. It is particularly important in Ethiopia where effective control measures are lacking. This cross-sectional study was carried out between April and July 2016 on 1038 cattle selected from 206 dairy farms in Adama city located in central Ethiopia to estimate the prevalence of bTB and identify the potential risk factors using comparative intradermal tuberculin skin test. Accordingly, the individual animal level prevalence was found to be 2.1% (95% CI: 1.35 – 3.22). Of the 206 dairy farms included in the study, 7.3% (95% CI: 4.29 – 11.98) had one or more 15 cattle positive for the tuberculin test. Furthermore, 5.5% of the examined cattle were found reactive for atypical *Mycobacterium*. Among the risk factors considered, age and lactation status of the animals were significantly associated ($p< 0.05$) with the prevalence of bTB. It was noted that the apparent prevalence of bTB positive reactivity was greater in young and non-lactating cattle than their counterparts. In conclusion, the present study revealed 2.1% prevalence of bTB in the dairy farms investigated and culling of positive reactors is recommended as a feasible control intervention.

Keywords: Adama; Bovine tuberculosis; CIDT; dairy cattle; Ethiopia

Introduction

Ethiopia has the largest cattle population in Africa which is estimated to be 60,392,019 heads (CSA, 2018). The vast majority of the national herd is of

indigenous zebu cattle maintained in rural areas under extensive husbandry systems. However, in response to the increasing demand for milk products and the Ethiopian government's efforts to improve productivity in the livestock sector, recent years have seen increased intensive husbandry settings holding exotic and cross breeds. This drive for increased productivity is however threatened by animal diseases that thrive under intensive settings, such as bTB (Firdessa *et al.,* 2012).

Bovine tuberculosis (bTB) is a disease of zoonotic and economic importance caused predominantly by *Mycobacterium bovis*. The disease is transmitted between animals primarily by inhalation of aerosols although transmission through ingestion is also common in cattle grazing on pasture contaminated with *M. bovis*.The disease in cattle is characterized by the formation of granulomas in tissues and organs, more significantly in the lungs, lymph nodes, intestine, kidneys and others. The economic loss of bTB in dairy cattle include reduction in productivity, movement restrictions, screening costs, culling of affected animals, and trade restrictions (OIE, 2016).

Over the years several studies have been conducted in Ethiopia to show the importance of bTB. Published studies revealed that the prevalence of bTBin the country ranges from 2 to 47% (Ameni *et al.,* 2003; Ameni *et al.,* 2007; Tschopp *et al.,* 2010; Gumi *et al.,* 2012; Firdessa *et al.,* 2012; Nega *et al.,* 2012; Mamo *et al.*, 2013; Romha *et al.,* 2014; Dejene *et al.,* 2016). The studies have shown that bTB is an endemic disease in Ethiopian dairy farms due to lack of effective control programs and needs due attention. Although previous studies have indicated that the disease is endemic in the country, there is paucity of information about the status of bTB at Adama city where dairy farming with different levels of intensification is flourishing rapidly in response to a higher demand for milk and milk products. Therefore, this study was planned to estimate the current prevalence of bTB in the dairy farms of the town and identify the potential risk factors.

Materials and Methods

Study area

This study was conducted between April and July, 2016 in dairy herds found at Adama city, central Ethiopia. Adama is located at 8.54°N latitude and 39.27°E longitude and situated at an elevation of 1712 meters, 99 km southeast of Ad-

dis Ababa. The city is situated along the road that connects Addis Ababa to Harar and Dire HYPERLINK "http://en.wikipedia.org/wiki/Dire_Dawa"Dawa. According to data obtained from Adama city Animal and Fisheries Department (ACAFD, 2016), there are about 10,000 dairy cattle in Adama and its suburbs. These animals are mainly cross-breed (Zebu X Holstein- Friesian) and kept under semi-intensive to intensive management system.

Study design and sample size

A cross sectional study design was followed throughout the course of the study. The sample size was determined according to Thrusfield (2005) considering 13.50% expected prevalence based previous report from cross and local breed animals from central Ethiopia (Ameni *et al.,* 2007), 5% absolute precision and 95% confidence level.

Adama town is divided into 14 kebeles (a kebele is the smallest administrative district in Ethiopia. In Adama, there are about 500 dairy farms. In the current study, 10-20 dairy farms were selected from each kebele considering the number of dairy farms existing in each kebele; willingness of the owners and availability of the road for transport during the study period. Accordingly, 206 farms were selected. Each selected farm has got 3-50 dairy cattle kept for milk production purpose mainly for family income. All cattle in the farm except sick and less than six months age have been tested. Most of dairy cattle were cross breeds and in each farm one local breed or no local breed was found, so breed differentiation was not reasonable. Based on above mentioned reasons a total of 1038 cattle were required for the study. These animals were obtained from 206 dairy farms found in the city. The sampling frame which represents the list of dairy farms in the city was constructed in collaboration with the veterinary department. All animals were included in farms with small herd size (≤10 animals) in the study, while at least 10% were sampled from large farms (>10 animals). The study animals were identified by their ID numbers. During sampling, information about animal and herd- level factors, such as breed, age, body condition score, herd size, pregnancy status, stage of lactation, and parity was collected on especially designed format. Based on herd size, 185 (89.8%) herds were small and only 21 (10.2%) considered large. According to their lactation status the study animals were categorized as dry cows, lactating cows, and heifers and calves while in terms of parity they were classified in four categories (1-2, 3-5, ≥6, and calf + heifer) (Vanholder *et al.,* 2015). Body condition scoring (BCS) was made using a method developed for Zebu cattle

(Nicholson and Butterworth, 1986). Accordingly, on the basis of observation of anatomical parts such as vertebral column, ribs, and spines, the study animals were classified as lean (1), medium (2) and good (3 & 4). Age of the study animals was determined by using the dental eruption and wear as described by de Lahunta and Habel (1986). Accordingly, animals were categorized in three age groups: <2, 2-5 and ≥ 6 years. In all the farms, young animals under six months of age, cows in late gestation and those who had recently calved were not included in the study for fear of immune suppression that usually occurs in dairy cows starting from three weeks pre-calving to three weeks post calving (Radostits *et al.,* 2001).

Tuberculin skin testing

Comparative intradermal tuberculin skin test (CIDT) was carried out by injecting both bovine purified protein derivative (PPD) and avian PPD (Prionics Lelystad B.V., The Netherlands). Two sites on the skin of the mid-neck of the cattle, 12 cm apart were shaved and skin thickness was measured with a caliper. One site was injected with an aliquot of 0.1 ml of 3000 IU/ml bovine PPD into the dermis, and the other was similarly injected with 0.1ml of 2,500 IU/ml avian PPD. After 72 hrs., the skin thickness at the injection sites was measured and recorded. Results were interpreted according to the recommendations of the Office International des Epizooties (OIE, 2009) at ≥ 4 mm cut-off and also at ≥ 2 mm cut-off (Ameni *et al*., 2008). Thus, at cut-off ≥ 4 mm, if the increase in skin thickness at the injection site for bovine PPD (PPD-B) was greater than the increase in skin thickness at the injection site for avian PPD (PPD-A) and PPD-B minus PPD-A was less than 2 mm, between 2 and 4 mm, or 4 mm and above, the animal was classified as negative, doubtful, or positive for BTB, respectively. At cut-off ≥ 2 mm, if the difference between B and A was greater or equal to 2mm, the animal was considered as positive, while if the difference is less than 2 mm, the animal was considered as negative. When the change in skin thickness was greater at PPD-A injection site, the animal was considered positive for mycobacterium species other than Mycobacterium tuberculosis complex. A herd was considered as positive if it had at least one tuberculin reactor animal.

Data analysis

All the data collected were coded and entered into Microsoft Excel Spreadsheet. All statistical analyses were performed on STATA version 11 (4905

Lakeway Drive, College Station, Texas) using survey command. The apparent individual animal prevalence of bovine tuberculosis was calculated as the number of positive tuberculin reactors divided by the total number of cattle tested. Herd level prevalence was calculated as the number of herds with at least one-reactor positive animal divided by the total number of herds tested. Pearson chi- square was used to evaluate the statistical significance of the association between the independent variables (herd size, age, breed, BCS, pregnancy, parity and lactation) and dependent variable (tuberculin skin test result). Multivariable logistic regression analyses was performed to account for confounding effects and interaction between variables. P-value less than 5% was considered statistically significant.

Results

A total of 1038 cattle were screened for bTB and all animals were followed up for the second reading after 72 hours. Based on single CIDT result, the apparent individual animal prevalence of tuberculin reactors was 2.1% (95% CI:1.35 – 3.22) using 2 mm cut-off point. Of the 206 herds tested, 15 (7.3%, 95% CI: 4.29 - 11.98) had one or more animals reactive to bovine PPD. The prevalence of bTB in individual herds ranges from 0 –55.6%.

Of the 206 herds tested, 15 (7.3%, 95% CI: 4.29 – 11.98) had one or more animals reactive to bovine PPD. The prevalence of bTB in individual herds ranges from 0 –55.6% (Table 1).

On the other hand the change in skin thickness was found greater at avian PPD injection site than bovine PPD infection site in 5.5% (57/1038) cattle showing that these animals are positive for *Mycobacterium* spp. other than *M. tuberculosis* complex (Table 1).

Table 1: Apparent prevalence of bovine and avian tuberculin reactors in Adama dairy farms, central Ethiopia

Tuberculin test type	No. of cattle tested	No. of positive reactors	Prevalence (%)	No of farm tested	No of positive (%)	95% Confidence Interval	X^2	P
Bovine PPD	1038	22	2.1	206	15(7.3)	1.35 – 3.22		
Avian PPD	1038	57	5.5	206	39(18.9)	4.32 – 7.22	17.2	<0.001

In univariable analysis of the potential risk factors, age of the animals was the only variable significantly contributed to positive reactivity to bTB (P<0.05) (Table 2).

Table 2: Univariable analysis of risk factors for bovine tuberculin reactors in Adama dairy farms using χ^2–test

Variables	Category	No of cattle examined	No of positive	Prevalence (%)	χ^2	p- value
Herd size					2.5	0.116
	Small (<10)	741	19	2.6		
	Large (≥10)	297	3	1.0		
Age					8.0	0.018
	<2 years	156	7	4.5		
	2-5 years	486	12	2.5		
	>5 years	396	3	0.8		
BCS					0.14	0.934
	Poor	5	0	0		
	Medium	105	2	1.9		
	Good	928	20	2.2		
Pregnancy status						
	Non-pregnant	993	22	2.2		
	Pregnant	45	0	0		
Parity					0.94	0.816
	1-2	448	11	2.5		
	3-5	273	4	1.5		
	>5	5	0	0		
	Calves and heifers	312	7	2.2		
Lactation					5.3	0.070
	Dry	68	4	5.9		
	Lactating	659	11	1.7		
	Calves and heifers	311	7	2.3		

However, in multivariable analysis, age and lactation were found to be significantly associated with tuberculin positive reaction (Table 3).

Table 3: Multivariable logistic regression analysis of risk factors for bovine tuberculin reactors in Adama dairy farms

Variable	Odds Ratio (OR)	Std. Err.	Z-values	p-values	95% CI for OR
Herd size	0.31	0.23	-1.56	0.118	0.07 – 1.34
Age	0.11	0.07	-3.43	0.001	0.03 – 0.38
BCS	1.35	1.03	0.39	0.695	0.30 – 6.04
Parity	0.68	0.23	-1.14	0.254	0.35 – 1.32
Lactation	0.32	0.18	-2.01	0.044	0.10 – 0.97
Cons	20.57	74.17	0.84	0.402	0.02 - 24100

Discussion

Bovine tuberculosis is known to be endemic in dairy farms of Ethiopia. The present study has shown an apparent individual animal prevalence of 2.1% in the dairy farms of Adama city. This finding is comparable to a previous report of 2% in Ethiopia (Gumi *et al.,* 2012) and 2.4% in Tanzania (Katale *et al.,* 2013). However, it is considerably lower than the previous studies in Ethiopia, viz: 7.9% (Ameni *et al.,* 2003), 13.5% (Ameni *et al.,* 2007), 3% (Tschopp *et al.,* 2010), 30% (Firdessa *et al.,* 2012), 7.1% (Nega *et al.,* 2012), 18% (Mamo *et al.,* 2013), 4.3% (Romha *et al.,* 2014) and 5.5% (Dejene *et al.,* 2016). Out of the 206 herds tested, only 15 (7.3%) had one or more tuberculin positive animals and the number of tuberculin reactors varied widely (0 – 55.6%) between the herds. The present herd-level prevalence was considerably similar range of estimates (12.5 - 56%) documented in the previous studies done by (Kemal *et al.,* 2019) herd level prevalence of 51.2%; by (Abie *et al.,* 2019) the herd prevalence was 22.4% in Gondar, Hawassa and Mekelle; by (Dejene *et al.,* 2016) herd prevalence of 46% in Awash national park and Afar regional state. The herd range variation in the prevalence of tuberculous reactors was thought to be attributed to differences in the herd size of the study animals. As the majority of the herds in the present study were small comprising less than 10 animals, management conditions favoring the spread of bTB, such as overcrowding and poor ventilation, were less likely to have influenced the prevalence of infection. In the present study, age of the animals was found to be significantly associated with positive tuberculin reaction.

In the multivariable analysis of the risk factors considered, lactation status of the animals was another factor found to be significantly associated with tuberculin reactivity although this was not evident in the univariable analy-

sis. The apparent prevalence was higher among dry cows than lactating cows or calves and heifers. It is difficult to give a plausible biological explanation for this finding and perhaps it might be due to the confounding effect of some other variables unnoticed. In contrast to the present finding, previous studies in Ethiopia that considered lactation status in their analysis did not find significant variation in the prevalence of positive reactors between lactating and non-lactating cattle (Mamo *et al.,* 2013; Romha *et al.,* 2014; Zeru *et al.,* 2014; Dejene *et al.,* 2016). However, the present finding is consistent with Tschopp *et al.* (2010) and Admasu *et al.* (2014).

Previous studies in Ethiopia have shown that the native zebu cattle are more resistant to bTB than exotic breeds or their crosses (Vordermeier *et al.,* 2012; Admasu *et al.,* 2014; Sibhat *et al.,* 2017). Consistent to this, the entire tuberculin positive reactors in the current study were Holstein-Friesian X zebu cross breed while none of the local breeds were found reactive. However, the number of local cattle included in the study was too small to compare with the cross breeds. Because the milk yield of cross breed is higher than the local ones, owners prefer to keep the cross breed animals for dairy farms. Therefore, the current evidence is not sufficient to declare breed differences in susceptibility to bTB.

None of the pregnant cows tested in the current study were positive for tuberculin reaction and the entire test positive animals were non-pregnant. However, previous studies have shown lack of significant difference between pregnant and non-pregnant cows (Romha *et al.,* 2014; Zeru *et al.,* 2014). Likewise, no significant association ($p>0.05$) was found between parity numbers and positive reaction to tuberculin test and this is consistent with previous studies (Romha *et al.,* 2014; Zeru *et al.,* 2014).

In the present study about 5.6% of the study animals were reactive for avian *Mycobacterium* PPD. This is much higher than a previous report of 0.7% in Ethiopia (Gumi *et al.,* 2012). Reaction bias to *M. avium* PPD could be due to infection with *Mycobacterium avium* subsp. *avium* and *Mycobacterium avium* subsp. *paratuberculosis*. The latter causes a chronic debilitating disease known as paratuberculosis in cattle, which are most susceptible to infection when they are young. Apart from its economic impact on cattle production, *M. avium* sub spp. *paratuberculosis* has a zoonotic significance (Radostits *et al.,* 2007).

In conclusion the present study has shown a very low prevalence of bTB in the dairy farms studied compared to previous reports in the country. As the proportion of cattle affected with bTBin each herd was small, culling of the positive reactors could be a feasible control intervention. Thus owners of the positive herds need to be advised in this regard. Furthermore, the observation of atypical TB with a proportion higher than bTB warrants the need for future studies to focus on *Mycobacterium* spp. other than *M. tuberculosis* complex, particularly *M avium* sub spp. *paratuberculosis* due to its zoonotic and economic importance.

Conflict of interests

The authors declare that they have no conflict of interests.

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References

Abie, M, Conlan, A, Berg, S, Ayele, B, Alemu, A and Guta S., 2019. Prevalence of bovine tuberculosis and its associated risk factors in the emerging dairy belts of regional cities in Ethiopia. *Prev. Vet. Med.,* 168: 81-89. doi.org/10.1016/j.prevetmed.2019.04.010

ACAFD, 2016. Adama city Animal and Fisheries Department report. Unpublished report.

Admasu, P., Berihun, W. and Niguse, A., 2014. Prevalence of bovine tuberculosis in dairy cattle of Yeki district, Southern Ethiopia. *African J. Basic & Appl. Sci*., 6 (5): 135-140.

Ameni, G., Bonnet, P. and Tibbo, M. 2003. A cross-sectional study of bovine tuberculosis in selected dairy farms in Ethiopia. *Int. J. Appl. Res Vet. Med*. 1(4):1-8.

Ameni, G., Aseffa, A., Engers, H., Young, D., Gordon, S., Hewinson, G., and Vordermeier, M., 2007. High prevalence and increased severity of pathology of bovine

tuberculosis in Holsteins compared to Zebu breeds under field cattle husbandry in central Ethiopia. *Clin. Vaccine Immunol.*14:1356-1361.

Ameni, G., Hewinson, G., Aseffa, A., Young, D. and Vordermeier, M., 2008. Appraisal of interpretation criteria for the comparative intradermal tuberculin test for diagnosis of tuberculosis in cattle in central Ethiopia. *Clin. Vaccine Immunol.*15 (8):1272–1276.

CSA, 2018. Central Statistical Agency Agricultural Sample Survey Volume II

Dejene, S.W., Heitkönig, I.M.A., Prins, H.H.T., Lemma, F.A., Mekonnen, D.A., Alemu, Z.E., Kelkay, T.Z. and de Boer W.F., 2016. Risk factors for bovine tuberculosis (bTB) in cattle in Ethiopia. *PLoS ONE* 11(7): e0159083. https://doi.org/10.1371/journal.pone.0159083

de Lahunta, A. and Habel, RE., 1986. Teeth - Applied Veterinary Anatomy; USA. W. B. Sounders Company. Philadelphia, PA, pp. 4-16.

Firdessa, R., Tschopp, R., Wubete, A., Sombo, M., Hailu, E., Erenso, G., Kiros, T., Yamuaha, L., Vordermeier, M., Glyn-Hewinson, R., Young, D., Gordon, SV., Sahile, M., Aseffa, A. and Berg, S., 2012. High prevalence of bovine tuberculosis in dairy cattle in Central Ethiopia: Implications for the dairy industry and public health. *PLoS ONE* 7(12): e52851. doi:10.1371/journal.pone.0052851.

Gumi, B., Schelling, E., Firdessa, R., Erenso, G., Biffa, D., Aseffa, A., Tschopp, R., Yamuah, L., Young, D. and Zinsstag, J., 2012. Low prevalence of bovine tuberculosis in Somali pastoral livestock, southeast Ethiopia. *Trop. Anim. Hlth. Prod.*,44 (7): 1445-1450. doi: 10.1007/s11250-012-0085-5

Katale, BZ., Mbugi, EV., Karimuribo, ED., Keyyu, JD., Kendall, S., Kibiki, GS., Godfrey-Faussett, P., Michel, AL., Kazwala, RR., van Helden, P. and Matee, MI., 2013. Prevalence and risk factors for infection of bovine tuberculosis in indigenous cattle in the Serengeti ecosystem, Tanzania. *BMC Vet. Res.*, 9:267. doi: 10.1186/1746-6148-9-267

Kemal, J., Sibhat, B., Abraham, A., Terefe, Y., Tafess, K., Welay, K., Getahun, N. 2019. Bovine tuberculosis in eastern Ethiopia: prevalence, risk factors and its public health importance. *BMC Infect Dis.*, 19: 39. doi: 10.1186/s12879-018-3628-1

Mamo, G., Abebe, F., Worku, Y., Hussein, N., Legesse, M., Tilahun, G., Medhin, G., Bjune, G. and Ameni, G. 2013. Bovine tuberculosis and its associated risk factors in pastoral and agro-pastoral cattle herds of Afar Region, Northeast Ethiopia. *J. Vet. Med. Anim. Hlth.* 5 (6): 171-179.

Nega, M., Mazengia, H., and Mekonen, G., 2012. Prevalence and zoonotic implications of bovine tuberculosis in Northwest Ethiopia. *Int. J. Med. Med. Sci.* 2 (9):188-192.

Nicholson, MJ., and Butterworth, MH., 1986. A guide to condition scoring of zebu cattle; ILCA, Addis Ababa, Ethiopia.

OIE, 2009. Office International des Epizooties (OIE) Terrestrial manual: Bovine Tuberculosis, World Health Organization for Animal Health, Paris, France.

OIE, 2016. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Office Internationale des Epizooties *(*OIE), Paris, France.

Radostits, OM., Leslie, KE., and Fetrow, J., 2001. Herd health: Food Animal Production Medicine, 3rd edition. W.B. Saunders Company*,* PA, 296.

Radostits, OM., Gay, CC., Hinchcliff, KW., and Constable, PD., 2007. Veterinary Medicine: A textbook of the disease of cattle, horses, sheep, pigs and goats. 10th edition. Elsevier Ltd. London, UK.

Romha, G., Gebreegziabher, GM. and Ameni, G., 2014. Assessment of bovine tuberculosis and its risk factors in cattle and humans, at and around Dilla town, southern Ethiopia. *Anim. & Vet. Sci*., 2(4): 94-100.

Sibhat, B., Asmare, K., Demissie, K., Ayelet, G., Mamo, G. and Ameni, G., 2017. Bovine tuberculosis in Ethiopia: A systematic review and meta-analysis. *Prev. Vet. Med*., 147: 149-157.

Thrusfield, M., 2005. Veterinary Epidemiology, 3rd edition. Pp.182-187. Blackwell Publishing Company. Oxford, UK.

Tschopp, R., Aseffa, A., Schelling, E., Berg, S., Hailu, E., Gadisa, E., Habtamu, M., Argaw, K. and Zinsstag, J., 2010. Bovine tuberculosis at the wildlife-livestock-human interface in Hamer Woreda, South Omo, Southern Ethiopia. *PLoS One*. 5(8): e12205, doi: 10.1371/journal.pone.0012205

Vanholder, T., Papen, J.; Bemers R., Vertenten, G. and Berge, A. 2015. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. *J. Dairy Sci.,*98(2) 880-888. doi: 10.3168/jds.2014-8362

Vordermeier, M., Ameni, G., Berg, S., Bishop, R., Robertson, BD., Aseffa, A., Hewinson, RG. and Young, DB., 2012. The influence of cattle breed on susceptibility to bovine tuberculosis in Ethiopia. *Comp. Immunol. Microbiol. Infect. Dis*., 35: 227- 232.

Zeru, F., Romha, G., Berhe, G., Mamo, G., Sisay, T. and Ameni, G., 2014. Prevalence of bovine tuberculosis and assessment of Cattle owners' awareness on its public health implication in and around Mekelle, Northern Ethiopia. *J. Vet. Med. Anim. Hlth*. 6(6): 159-167

Zoonotic helminth parasites of dog in Bishoftu Town, central Ethiopia: prevalence, dog owners' knowledge and control practice

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Abstract

Many dog helminth parasites are endemic in many countries of the world posing public health threat. However, they were neglected and less studied in the developing countries such as Ethiopia. This cross-sectional study carried out from November 2016 to April 2017 in Bishoftu town aims at estimating the prevalence of major gastrointestinal tract (GIT) zoonotic helminth parasites of dogs and assessing dog owner's knowledge and control practice against zoonotic dog parasites. Accordingly, the whole area of Bishoftu town was divided into 60 blocks, of which 10 were randomly selected for the study. A structured questionnaire was prepared in English, pretested and administered face to face to 140 dog-owning respondents using two local languages: Amharic and Affan Oromo. Fecal samples collected from 238 dogs after administration of ivermectin and praziquantel at recommended doses to increase sensitivity of detection and to get favorable cooperation of owners. For case detection flotation technique is used for parasite egg identification and parasite morphology for parasites observed in the feces. Pearson's Chi-square ($\chi 2$), p-values and 95% confidence intervals calculated to measure association. Four zoonotic helminthic parasites detected with combined infection prevalence of 59.24% (95% CI: 52.84-65.35). The prevalence of each was 33.61% (95% CI: 27.86 – 39.90) *Ancylostoma (A.) caninum*, 29.41% (95% CI: 23.93 – 35.56) *Toxocara (T.) canis*, 19.75% (95% CI: 15.14 – 25.34) *Dipylidium (D.) caninum*, and 2.10% (95% CI: 0.87 – 4.98) *Echinococcus (E.) granulosus*. The prevalence of *A. caninum* and *T. canis* was significantly higher than the prevalence of *D. caninum* and *E. granulosus*. Mixed infection with two parasites recorded in 13.87% whereas concurrent infection with three parasites registered in 5.88% of the dogs. Statistically significant association ($p<0.05$) was observed between overall infection prevalence and the sex of dogs, where infection prevalence of *A. caninum*

(*p*<0.001) and *T. canis* (*p*<0.001) were significantly higher in male than in female dogs. The questionnaire survey revealed that only 40% of the respondents were aware of the transmission of zoonotic helminthes to humans while none know the route of transmission. Of all the respondents, 58.57% reported regular deworming of their dogs, at least twice per annum, whereas 47.86% of respondents clean and dispose dogs' excrement with household garbage and 88.57% of dog owners remove dogs' excrement without using glove, facemasks, boots and/or coverall/gown for personal protection In relative terms high prevalence of zoonotic helminth parasites infestation was observed in owned dog population with poor awareness about route of transmission. Thus, it is advisable to create awareness of dog owners in waste management and use of Personal Protective Equipment's (PPE).

Keywords: *Ancylostoma*; Bishoftu; *Dipylidium*; *Echinococcus*; Ethiopia; *Toxocara*

Introduction

Man has had a close association with dog since long before the dawn of history (Morey, 1994). In the modern society, dogs are used for diverse purposes such as military functions, guiding the blind, guarding property and being companion often considered as part of the family by households (Westgarth *et al*., 2013). In Ethiopia, dogs are important animals in many urban and rural households mainly kept as house guards, but also sometimes kept as hunting and companion animals (Hailu *et al*., 2007). However, despite the benefits of pets to their owners, there are also health hazards to humans. Animal health professionals may also have high risks of exposure to zoonotic diseases including parasites. A questionnaire survey conducted in Australia involving 344 veterinarians revealed 44.9% contracting a zoonotic disease including parasites during their careers, 63.2% of these have worked in companion animal practice indicating the high risk of animal health professionals' exposure to dog borne zoonosis (Dowd *et al.,* 2013).

Thus, dogs are responsible for the spread of several zoonotic diseases having serious health consequences to humans. A number of parasitic infections that are capable of transmission from pets to humans include cutaneous larva migrans, visceral larva migrans and ocular migrans caused by *Ancylostoma* spp. and *Toxocara* spp. (Gawor *et al*., 2008; Paul *et al*., 2009; Bowman *et al.*, 2010; Finsterer and Auer, 2013). Dogs are definitive hosts for a number of cestodes

including *Taenia hydatigena (Cysticercus tenuicollis)*, *Taenia multiceps (Coenurus cerebralis)*, *Taenia ovis (Cysticercus ovis)* and *Echinococcus granulosus (hydatid cyst*) that causes severe economic losses due to downgrading or condemnation of various visceral organs and carcasses of livestock (Macpherson, *et al*., 2005; Fromsa and Jobre, 2012; Elsheikha and Patterson 2013). Some of these zoonotic parasitic diseases of dogs are very important causes of human mortality and morbidity in developing countries due to the extensive existence of uncontrolled dog population in close proximity to increasing density of human population in both rural and urban environment (Dutta, 2002). Human infection with hydatid cysts can cause serious disease and death (Zajac and Conboy, 2012). The persistent occurrence and transmission of these diseases in the poorer countries attributed to poor level of hygienic conditions, lack of sufficient veterinary attention to parasitic zoonotic diseases of dogs and lack of awareness of the public about the presence and transmission of these diseases (Traub *et al.,* 2002).

Gastrointestinal helminths may also have a serious impact on health of dogs. Parasitized animals may show a variety of symptoms depending on the infesting parasite species and density. Severe cases such as heavy hookworm infestation can cause severe anemia and death due to their voracious blood sucking habits (Barutzki and Schaper, 2003; Zajac and Conboy, 2012). Mild to moderate cases of dog parasitism may impede the successful rearing of dogs due to lowered resistance to infectious diseases, retarded growth, reduced work and feed efficiency, and general ill health (Little *et al*., 2009).

Several research reports have documented the prevalence of canine intestinal parasites in various locations around Ethiopia and the world (Hailu *et al*., 2007; Little *et al*., 2009; Zewdu *et al.,* 2010; Joffe *et al.,* 2011; Abere *et al*., 2012; Getahun and Addis, 2012; Gebreselasie *et al*., 2013). However, due to regional variations in parasite prevalence, such information is often of limited value outside the specific study areas. Therefore, to devise a successful control program against any disease of public health importance, understanding epidemiology of the diseases as well as knowledge, practice and attitude of the community with regard to disease transmission route is crucial (Macpherson, 2005). Identification of the knowledge gap and increasing the awareness and knowledge of the community based on the observed gap and deficiency is considered reliable for making informed intervention that will result in changing attitudes and practices of the society to minimize disease burden.

However, there is lack of sufficient information about the magnitude of zoonotic parasitic disease prevalence among dogs. Little is known about the awareness and practice of the dog-owning community of Bishoftu area with regard to the practice of regular dog deworming, prevention of the communal environment from contamination by zoonotic parasite laden dog fecal material and self-protection against potential zoonotic parasitic infection from their dogs (Hailu *et al.,* 2007; Zewdu *et al.,* 2010; Merga and Sibhat, 2015). To fill the existing information and knowledge gap, all relevant data and information should be collected including the prevalence of zoonotic parasites in dogs' feces and habitual practices of dog owners in the management of their dog's excreta. Therefore, this study conducted with specific objective to estimate the prevalence of major zoonotic helminth parasites in the feces of dogs and to assess the knowledge and practices of dog owners in Bishoftu Town.

Materials and methods

The study area

The study was conducted from November 2016 to April 2017 in Bishoftu town located southeast at a distance of 47.9 Kilometers away from the capital, Addis Ababa. The town of Bishoftu is situated at an elevation of 1978 meters above sea level and 8°35' N latitude and 39°06' E longitude experiencing a bimodal pattern of rainfall. The main rainy season extends from June to September contributing for 84% of the expected annual rainfall. A short rainy season occurs from March to May with an average annual rainfall of 800 mm. The mean annual minimum and maximum temperatures are 12.3°C and 27.7°C, respectively, with an overall average of 18.7°C. The mean relative humidity is 61.3% (Mengistu *et al.,* 2002).

Study design and area selection

The study design is cross-sectional. Google map of Bishoftu town downloaded from Google website: http://earth.google.com/streetview/. Then then the whole area of the town divided into non-overlapping approximately equal-sized blocks surrounded by wide asphalt or cobble stone roads on the map. Then each equal-sized adjacent block was marked using four different colors: green, red, yellow and light blue (Fig.1A). Accordingly, the whole of Bishoftu town divided into 60 blocks, each adjacent area having approximately equal size and marked with one of the four colors producing 15 blocks marked by each

color. The four colors were written on a piece of paper and entered for a pick by lottery system. By the lottery system, the green color was selected. Since there were 15 green color marked blocks of Bishoftu town and limited capacity to cover all, 10 blocks reselected randomly by the lottery system (Fig. 1B). The selected areas were then visited to mark their boundaries on the ground using turning roads and fixed permanent posts such as hotels, buildings, and long fences as markers. Then all the households within the marked boundaries of the selected blocks that are having one or more dogs were identified, the number of owned dogs counted and color marks were put on the outer door or fence of the dog-owning households (Fig.2). Accordingly, from 469 households located within the selected blocks, 334 households were marked as dog-owning households.

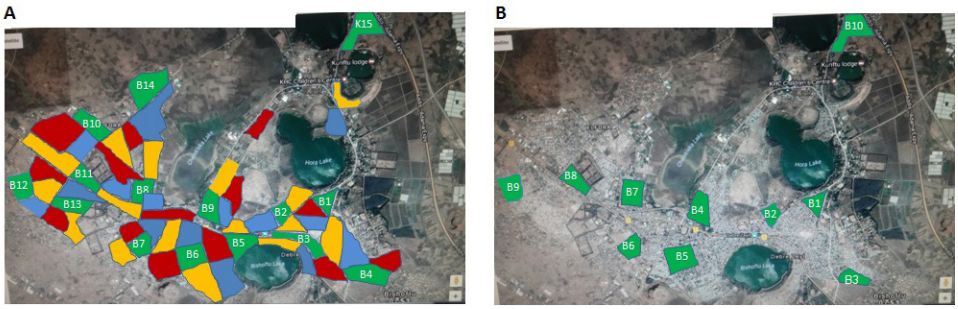


Fig.1: Map of Bishoftu town, A) map divided into 60 approximately equal-sized non-overlapping blocks marked with four different colors. B) The randomly selected 10 green colored blocks and included in the study.

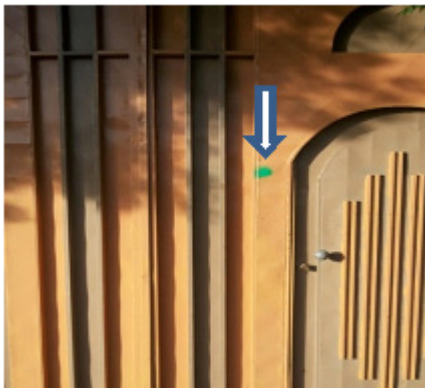


Fig.2: The outer fence door of dog-owning household marked with green color (open arrow) for ease of differentiating it from other households that do not have dog.

Fecal sample collection

Each dog-owning household identified by color marks were visited and owners were explained about the study and asked if they could agree to participate in the study and get their dogs dewormed with ivermectin at 200 µg/kg and praziquantel at a dose rate of 10 mg/kg as an incentive to them and as a supplementary diagnostic tool for detection of helminth infection. When the households agree, then a dog is given the treatments based on estimated weight and fecal samples were collected the next day in the morning. The variables studied include age and sex recorded with similar identification numbers on both the sample vials and recording sheets. For the purpose of analysis, dogs were classified in to three groups based on age difference as puppies (≤ 6month), young (6 months to 1 year) and adult (≥1 year of age) as applied by Zewdu *et al.,* (2010).

From a total of 469 households registered within the 10 randomly selected blocks, 334 were dog-owning and all were considered for inclusion provided consent is given and collection of fecal sample is possible after deworming. Finally, fecal sample collection made possible only from 238 dogs. The remaining excluded since obtaining fecal sample was impossible either due to lack of owners' interest to participate or failure to submit fecal sample after deworming. For the 238 household dogs, the expelled adult parasites (Fig. 3d) and/or approximately 6 grams of fresh fecal samples collected, either immediately after voided from target animal or directly from the rectum. Then, the samples labeled, preserved in 10% formalin and transported to Parasitology Laboratory of College of Veterinary Medicine and Agriculture, Addis Ababa University for processing.

Laboratory test

At the laboratory, the fecal samples were examined using flotation techniques in saturated sodium chloride (NaCl; SG 1.18–1.20; to identify the eggs of nematodes) and zinc sulphate (ZnSO4; SG 1.20 or sucrose solution (SG 1.27) for cestode egg detection (Mirzaei and Fooladi, 2013). Three grams of the sample measured and added to mortal then 42 ml of floatation fluid added to fecal sample contained in mortar then crushed well with pestle and mixed thoroughly. The solution sieved by using tea strainer into beaker to remove rough materials. The filtrate added to centrifuge tube and centrifuged at 3000 rpm for 3 minutes, then a top-up of floatation fluid was added until a cone shape is

formed at the top of the centrifuge tube, then coverslip was placed on the top of the tube and allowed to stand for 15-20 minute. The coverslip raised up gently and placed on the microscopic slide. The sample is positive when an adult parasite expelled and/or when at least one egg of the specific parasite is detected (Lorenzini *et al.,* 2007). The eggs were identified by observing the slides under 10x magnification of compound microscope using ova identification keys to the level of genera or species based on the key provided by Hendrix (2003) and adult parasites were identified based on the keys provided by Zajac and Conboy, (2012). Only the presence or absence of a given parasite is assessed to calculate prevalence; neither egg nor adult parasite count was performed. When an egg and/or adult parasite is seen, the case is recorded as positive, when both egg and adult parasite of any species is absent, the case is recorded as negative. For *D. caninum*, all case detections were based on morphologic characteristics of expelled adult parasites, except in two dogs in which eggs were also seen in fecal floatation.

Questionnaire survey

A structured questionnaire was prepared to gather information on dog ownership, feeding, cleaning and deworming practices, the extent of awareness on zoonotic parasites of dogs, their transmission routes to human, and other related factors (Table 4). The questionnaire developed in English was translated either to Amharic or Oromifa and administered by members of the research team to gather information on dog ownership; feeding, cleaning and deworming practices; knowledge on zoonotic parasites' routes of transmission and other related factors. The questionnaire administered in Amharic and Oromifa then translated back to English to record. The questionnaire was pretested before administration via a face-to-face interview with adult person responsible for the care of the dog/s at the household. The questionnaires included in the study analysis were those administered to 140 dog-owning residents, who gave their consent to participate and answered all questions.

Data Management and Analysis

The raw data were first entered into Microsoft excel database system and imported to STATA statistical software version 13 (Stata Corp. 2013) for computation of descriptive statistics such as percent prevalence and frequency distributions. Pearson's Chi-square ($\chi 2$) used to measure association between parasite prevalence and age and sex of dogs. In all the analysis, the confidence

level was held at 95% and the results were considered significant when p-value was <0.05.

Results

Adult parasites and/or eggs of four intestinal zoonotic parasite species of dogs were identified and 141 (59.24%), (95% CI: 52.84-65.35) of dogs had ≥ 1 species of zoonotic parasite from a total of 238 canine fecal samples examined where the maximum number of parasites detected in mixed infection was three (Table 1, 2). The prevalence of each was 33.61% (95% CI: 27.86 – 39.90) *A. caninum*, 29.41% (95% CI: 23.93 – 35.56) *T. canis*, 19.75% (95% CI: 15.14 – 25.34) *D. caninum*, and 2.10% (95% CI: 0.87 – 4.98) *E. granulosus*. The prevalence of *A. caninum* and *T. canis* was significantly higher (p<0.001) than the prevalence of *D. caninum* and *E. granulosus*. *Ancylostoma caninum* was the most prevalent, while *Echinococcus granulosus* was the least detected in this study (Table 1).

Table 1: Intestinal helminth parasites of zoonotic importance identified in dog fecal samples based on expelled adult parasites and/or eggs in Bishoftu town

Helminth parasites	Number examined	Number (%) positive	SE (%)	95% CI
Echinococcus granulosus	238	5 (2.10)	0.93	[0.87 – 4.98]
Dipylidium caninum	238	47 (19.75)	2.59	[15.14 – 25.34]
Ancylostoma caninum	238	80 (33.61)	3.07	[27.86 – 39.90]
Toxocara canis	238	70 (29.41)	2.96	[23.93 – 35.56]
Total	238	141 (59.24)	3.19	[52.84 – 65.35]

Mixed infection with two parasites recorded in 13.87% whereas concurrent infection with three parasites registered in 5.88% dogs.

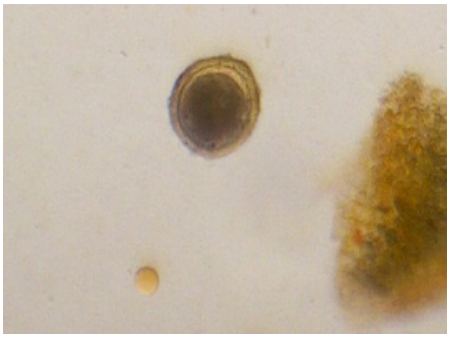
Table 2: Prevalence of mixed helminths infection in dogs (dogs harboring zoonotic dog parasite infection with at least one detected parasites).

Number of parasites per infested dogs	Number examined	Number (%) Positive	SE (%)	95% CI
One parasite	238	94 (39.49)	3.18	[33.44 – 45.89]
Two parasites	238	33 (13.87)	2.24	[10.00 – 18.90]
Three parasites	238	14 (5.88)	1.53	[3.50 – 9.72]
Total	**238**	**141 (59.24)**	**3.19**	**[52.84 – 65.35]**

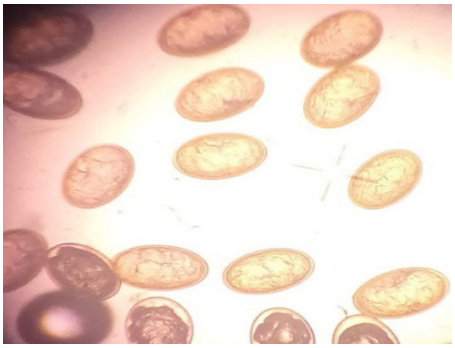
Although parasites detected in fecal samples of every age and sex groups of dogs examined in this study, parasitism more frequently detected in fecal samples of adult dog ≥ 1 year of age and in males than in females (Table 3, $p < 0.001$).

Table 3: The prevalence of intestinal zoonotic helminth parasites in different age and sex groups of dogs in Bishoftu town.

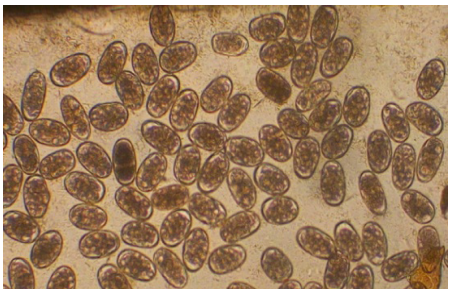
Parasites	Risk factors	Categories (Groups)	Frequency (%)	χ^2	p-value
E. granulosus	Age	Puppy (≤ 6 month)	0	1.7992	0.407
		Young (6 month-1 year)	0		
		Adult (≥1 year)	5(2.10)		
	Sex	Male	5(2.10)	1.1584	0.282
		Female	0		
D. caninum	Age	Puppy	6(2.52)	0.7835	0.676
		Young	4(1.68)		
		Adult	37(15.55)		
	Sex	Male	35(14.71)	1.9286	0.165
		Female	12(5.04)		
A. caninum	Age	Puppy	12(5.04)	0.3521	0.839
		Young	7(2.94)		
		Adult	61(25.63		
	Sex	Male	56(23.53)	10.5988	0.001
		Female	24(10.08)		
T. caninum	Age	Puppy	13(5.46)	0.3303	0.848
		Young	7(2.94)		
		Adult	50(21.01)		
	Sex	Male	48(20.17)	11.0208	0.001
		Female	22(9.24)		
Overall	**Age**	**Puppy**	**26(10.92)**	**11.3927**	**0.318**
		Young	**10(4.20)**		
		Adult	**105(44.12)**		
	Sex	**Male**	**105(44.12)**	2.2939	0.001
		Female	**36(15.13)**		



a) Eggs of *Toxocara canis detected* **in the feces of a local dog from Bishoftu (X10)**

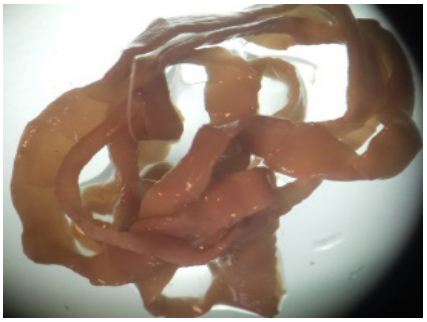


b) Mixed infection *with T. caninum* **and** *A. caninum* **(X10)**



c) Eggs of *A. caninum*, **in a single microscopic field, in 2 years old owned dog from Bishoftu town (X10)**





d. Top) *D. caninum*, **purged with feces after praziquantel treatment of 8-month-old local owned dog. Bottom) the parasite viewed under stereomicroscope.**

Figure 3: The adult parasites and eggs of zoonotic helminths identified from dogs of Bishoftu.

Questionnaire survey result

Ninety percent (90%) of the respondents keep dogs to guard them against thieves and wildlife whereas the remaining 10% keep dogs as companion animals. From respondent households, who have dogs, 61.43% (86/140) had a single dog whereas 38.57% (54/140) had two or more dogs with 60% of the respondents reporting free roaming of their dogs outside their property. Of all respondents, 73.57% knew that dogs can have gastro-intestinal parasites and 52.52% of them have observed parasites in their dog's excrement. The characteristics description given about the observed parasite by 34.29% of respondents fits to tapeworms' morphology (locally called "*Yewusha Koso"*). Out of the total respondents, 40% had information about parasitic diseases transmitted from dogs to human, but none of them has information about routes of exposure for humans. About 37.14% of the respondents do let their dogs to defecate on the public fields whereas 47.86% of owners dispose dog's feces with household garbage out of their compound. The questionnaire survey revealed that 88.57% of dog owners clean and dispose their dogs' excreta with household garbage without using glove, facemasks, boots and/or coverall/gown for personal protection predisposing themselves for potential contamination and infection. Out of all the respondents, 58.57% reported deworming of their dogs at least twice per year. The assessment of the feeding practice of dog owners revealed that 83.67% (82/98) of the dog owners either feed uncooked offal from butcher house or back yard slaughter to their dogs or release them to search for their own feed with a potential access to infected raw condemned offal. (Table 4).

Table 4: Knowledge, and practice of dog owners regarding potential zoonotic parasitic disease of dogs in the Bishoftu town (N=140).

Variable	Number (%)_respondents	SE (%)	95% CI
Dog ownership			
One dog	86 (61.43)	4.12	[53.02-69.21]
Two and more dogs	54 (38.57)	4.12	[30.79-46.98]
Purpose of keeping			
Guard against theft	123 (87.86)	2.77	[81.23-92.36]
Guard against wild animal	3 (2.14)	1.23	[0.68-6.51]
As companion pet	14 (10.00)	2.54	[5.97-16.27]
Housing of dogs at night			
In the cage	47 (33.57)	4.00	[26.16-41.89]
In the house	2 (1.43)	1.00	[0.35-5.62]
With the children	2 (1.43)	1.00	[0.35-5.62]
Roam in and outside of the compound	84 (60)	4.15	[51.57-67.86]
In the compound only	5 (3.57)	1.57	[8.37-8.37]
Feeding of dog/s			
Uncooked offal/head from butcher house	15 (10.71)	2.62	[6.52-17.10]
Uncooked offal/head from backyard slaughter	5 (3.57)	1.57	[1.47-8.37]
Left over from family dish	23 (16.43)	3.14	[11.11-23.61]
From all of the above three sources	85 (60.71)	4.14	[52.23-68.53]
Roam and gets its own feed	6 (4.29)	1.71	[1.91-9.23]
From all above sources	6 (4.29)	1.71	[1.91-9.23]
Usual place of defecation of dog/s			
In public field	52 (37.14)	4.09	[29.45-45.53]
In their cage only	32 (22.86)	3.56	[16.57-30.63]
In the compound	39 (27.86)	3.80	[20.98-35.95]
In all of the above	17 (12.14)	2.77	[7.64-18.76]
Way of dogs' excrement cleaning and disposal			
Clean and bury in the ground	8 (5.71)	1.96	[2.85-11.09]
Clean and add to the toilet	28 (20.00)	3.34	[14.11-27.54]
Clean and dispose with house garbage	67 (47.86)	4.23	[39.61-56.21]
Do not clean	37 (26.43)	3.74	[19.71-34.44
Use of protective equipment during cleaning			
Uses glove, facemasks boots and/or coverall/gown	16 (11.43)	2.69	[7.07-17.93]
No use of glove, facemasks, boots and/or coverall/gown	123 (88.57)	2.69	[82.06-92.92]
Owners viewpoint towards dog parasites			
Knows that dogs can have parasites	103 (73.57)	3.74	[65.55-80.28]
Observed a parasite in their dogs' excrement	73 (52.52)	4.25	[44.12-60.77]
Know parasites transmitted from dogs to humans	56 (40.00)	4.15	[32.13-48.42]
Knows transmission route	0 (0)	-	-
Deworm their dogs regularly	82 (58.57)	4.17	[50.14-66.52]

Discussion

Toxocara canis, *Ancylostoma caninum*, *Dipylidium caninum*, and *Echinococcus granulosus* are well-known zoonotic parasites worldwide, resulting in high public health risks (Moskvina and Ermolenko, 2016). This study revealed the presence of four species of endoparasites with overall prevalence of 59.24% (95% CI: 52.84 – 65.35) in owned dog population of Bishoftu town. This relatively high prevalence is recorded despite regular deworming at least twice per year being practiced as reported by 58.57% (95% CI: 50.14-66.52) of the interviewed dog-owning households of the town. This indicates the inadequacy of twice-yearly treatments to control dog parasitism effectively in Bishoftu signifying the need to increase treatment frequency. Comparatively, similar overall infection prevalence reported by previous studies conducted in Ethiopia (Hailu *et al.,* 2007; Zewdu *et al.,* 2010). The overall prevalence observed in this study is also in concordance with previous reports of other countries (Cardoso *et al.,* 2014; Quyen *et al.,* 2015). However, the overall prevalence recorded in this study is much lower than the previously reported prevalence that ranged from 73 to 90.7% in some parts of Ethiopia (Jones *et al.,* 2011; Abere *et al*., 2012; Paulos *et al*., 2012; Gugsa *et al.,* 2015; Merga and Sibhat, 2015; Tamerat *et al*., 2015). A recent review on canine helminths in sub-Saharan Africa revealed high pooled prevalence of 71% (95% CI: 63–79%) across 36 studies (Chidumayo, 2018). A study conducted in Canada reported an overall infection prevalence of 16.5% (Joffe *et al*., 2011). These differences might be attributable to local variations such as different climatic conditions; different population structure of studied dogs and sample size. Moreover, it might be due to different levels of public awareness on the effect of these parasites on the health of dogs and humans, ease of access to veterinary services, awareness about the availability of anthelminthic drugs to treat dogs and lack of knowledge in the transmission routes. Regional variation in the prevalence of canine intestinal parasitism was well documented (Little *et al*., 2009; Moskvina, and Ermolenko, 2016).

In this study, the recorded prevalence of each of these gastrointestinal zoonotic helminths parasites of dogs were 33.61% *Ancylostoma caninum,* 29.41% *Toxocara canis,* 19.75 % *Dipylidium caninum,* and 2.10% *Echinococcus granulosus.* The prevalence of *Ancylostoma caninum* and *Toxocara canis* was significantly higher ($p<0.001$) than the prevalence of *Dipylidium caninum* and *Echinococcus granulosus.* There is no single diagnostic technique capable of detecting all kinds of parasitic species present in dog feces (Mateus *et al*., 2014). The lower detection of *Dipylidium caninum* and *Echinococcus granulosus* egg might be

due to the poor sensitivity of floatation technique for taeniidae egg detection. Using flotation technique, tapeworm eggs detected when free in the feces. However, tapeworm eggs passed from the host being contained in tapeworm segments and rarely found free in the feces. Therefore, fecal flotation tends to be a poor detector of tapeworm infection status (Zajac and Conboy, 2012). Less than 1% detection of Taeniidae and *Dipylidium caninum* eggs was reported in faecal samples analyzed using sedimentation/flotation techniques compared to higher than 7% detection of *Ancylostoma* spp. and *Toxocara* spp. (Mateus *et al*., 2014; Kostopoulou *et al.,* 2017).

The infection prevalence of *Ancylostoma caninum* and *Toxocara canis* was significantly higher ($p<0.001$) in male than in female dogs. This is in agreement with the previous reports (Ramirez-Barrios *et al*., 2004; Maria *et al*., 2006; Davoust *et al.* 2008; Zewdu *et al.,* 2010). This could be attributable to hormonal factors and sex-associated behaviors such as roaming and aggressive temperament possibly due to testosterone (Kirkpatrick, 1988). Studies have also demonstrated movement behavior frequently observed in male dogs. Androgens or male sex hormones, such as testosterone have a number of behavioral influences in adult males contributing to manifestation of sexually dimorphic behaviors, which are behaviors more common in one sex than in the other. Sexually dimorphic behaviors manifested in male dogs include roaming away from home, urine or scent marking, aggressiveness and fighting with other male dogs. Castration reduced roaming in 90% of dogs through reduction of testosterone production without affecting behaviors that are similar between males and females such as hunting, playfulness, house guarding and seeking affection (Hopkins *et al*., 1976). In a demographic study conducted on a stray dog population in India, higher prevalence of male stray dogs of >3 months old age group was observed yielding a male-biased sex ratio of 1.3:1 to 1.4:1 (Totton *et al*., 2010). The male-biased sex ratio in roaming dog population might be attributed to the choice of male dogs as pets by the society. Most people generally prefer male dogs for their aggressive temperament to be used as guardian. In contrary, most people tend to avoid having female dogs due to the nuisance of a bitch in estrus attracting groups of intact males and some people hate to deal with unwanted puppies (Totton *et al*., 2010; Cortez-Aguirre *et al*., 2018).

Ancylostoma caninum was the most prevalent intestinal helminth parasite of dogs identified in this study. The predominance of *Ancylostoma* spp in dog fecal examination has been documented in prior studies reported from various

geographical locations in Ethiopia (Hailu *et al*., 2007; Zewdu *et al.,* 2010; Abere *et al*., 2012; Getahun and Addis, 2012; Gebreselasie *et al*., 2013; Tamerat *et al*., 2015), USA and Canada (Little *et al*., 2009; Joffe *et al*., 2011). The reported prevalence by previous studies from different geographic locations of Ethiopia ranged from 40-70% in necroscopic study (Hailu *et al*., 2007; Zewdu *et al.,* 2010; Merga and Sibhat, 2015). *Ancylostoma* spp. were also among the most commonly identified intestinal parasites with reported prevalence of 2.5% in the United States (Little *et al*., 2009) and 0.81% in Canada (Joffe *et al*., 2011). These differences in prevalence of *Ancylostoma* spp. infection in dogs of different localities could be attributed to variations in the diagnostic technique employed, deworming practices, level of awareness and ecological factors. The studies that had reported prevalence of 50% and 70% from Ethiopia have used necropsy methods for diagnosis.

Toxocara canis is the second most prevalent zoonotic helminth parasites of dogs found in 29.41% of 238 dogs examined in this study. This is in agreement with the reported prevalence (25-32%) from some parts of Ethiopia (Paulos *et al.,* 2012; Gebreselasie *et al*., 2013; Abere *et al.,* 2013; Tamerat *et al*., 2015)), but higher than the previous reported prevalence of 21% from Bishoftu (Hailu *et al*., 2007) and 11.72% from Adama (Merga and Sibhat, 2015). This indicates that the prevalence *Toxocara canis* has increased from the previous study conducted 10 years earlier in Bishoftu. Similar prevalence (30.0-35.7%) with this study reported from Bangladesh (Shubhagata *et al*., 2012). Nevertheless, high incidence of Toxocara infection is reported from other developing countries (Dutta, 2002; Traub *et al.,* 2002; Shubhagata *et al*., 2012; Fang *et al*. 2015). Toxocariasis is one of the most common parasitic zoonoses in the world capable of causing visceral and ocular larva migrans in humans, particularly in children. Routine year-round use of monthly anthelmintic to deworm regularly dogs significantly reduces prevalence of these parasites (Little *et al*., 2009; Joffe *et al*., 2011).

Dipylidium caninum is an intestinal parasite of domestic dog, domestic cat, wild carnivores and occasionally humans (East *et al.,* 2013; Fang *et al*., 2015). *D. caninum* was the third most prevalent parasite purged from 19.41% of examined dogs after administration of praziquantel in this study. This result is in agreement with the prevalence of 22.4-23.7% reported from some parts of Ethiopia (Getahun and Addis, 2012; Merga and Sibhat, 2015). The prevalence of this parasite recorded in this study is much higher than the prevalence of 6.56% reported from northern part of Ethiopia (Negash *et al.,* 2014) and 2.3%

from Harar (Tamerat *et al.*, 2015) indicating variation in the prevalence of *D. caninum* in different geographical areas of the country. This variation could be associated with variation in the diagnostic techniques used, the study population and the geographical area affecting the distribution of flea intermediate hosts that may be present relatively in a higher density in feral dogs than in owned dog population. Although infections with *D. caninum* are rare, it is a zoonotic parasite and humans, especially children can acquire the infection by accidentally ingesting infected fleas due to their playing habits and proximity with pet dogs and cats (Jiang *et al.*, 2017).

Echinococcus granulosus was the least detected parasite in owned dog population in this study. The recorded low prevalence of 2.10% (5/238) *E. granulosus* in this study might be due to the low sensitivity of the fecal egg detection method used in this study. Similar low prevalence (3.6-6%) reported by previous studies from Ethiopia using similar diagnostic method (Paulos *et al.,* 2012; Gebreselasie *et al.*, 2013; Gugsa *et al.,* 2015; Merga and Sibhat, 2015). The prevalence *E. granulosus* is relatively low in reports from other parts of the world (Kostopoulou *et al.,* 2017). This is partly associated with limitations of microscopic detection of *E. granulosus* eggs in fecal samples by routine coprological methods that suffers from a low sensitivity (Deplazes *et al.*, 1992) and even the sensitivity of arecoline hydrobromide purgation may be as low as 39% for *E. granulosus* (Ziadinov *et al.*, 2008). The other factor that might have contributed to the low detection rate of eggs is the intermittent release of eggs by the parasite (East *et al.*, 2013). However, higher prevalence (17.3-61.5%) was reported using necropsy as a diagnostic method (Zewdu *et al.*, 2010; Jones *et al.,* 2011). Comparative study conducted in Brazil and Ethiopia showed variability in the sensitivity of diagnostic tests, necropsy being more sensitive than coprology (Hailu *et al.*, 2007; Klimpel *et al.*, 2010) attributed to inability of coprological technique to detect immature parasites that do not lay eggs.

From a total of 469 households found registered within the territories of 10 Bishoftu town blocks randomly selected for this study, 334 (71.2%) owned one or more dog. Of all households who responded to the questionnaire, 61.43% had single dog while the other 38.57% had two or more dogs. The questionnaire revealed 90% of the respondents keep dogs to guard them against thieves and wildlife whereas only 10% of the dog-owning population keeps dogs as companion animals. Dogs play a diversity of roles in different societies, cultures, social interests, religious convictions and occupation (Macpherson, *et al.,* 2005).

Households keep dogs for a purpose and are over-concerned for their safety. In this study, some households that had a previous negative experience from regular practice of strychnine-baited killing of roaming dogs by town's municipality became suspicious of the drug given for deworming might be lethal and refused to participate. Some households agreed to deworm their dogs but failed to submit fecal sample due to releasing their dogs to free roam and inability to observe where their dog defecated.

Owners often observe tapeworm segments in the perianal area or in feces (Zajac and Conboy, 2012). Around half of participants in this questionnaire survey have also observed a parasite in their dogs' excrement and knew that dogs can have parasites, but none of them has information about routes of transmission to humans indicating a need for public awareness creation to prevent potential exposure of people to dog borne parasitic zoonosis. Similar questionnaire survey conducted in Jimma reported that 19.4% (34/175) of the respondents of that study had noticed parasitic disease in dogs, but had no information whether it can be transmitted to humans or not. The dog-owning population included in this study has relatively better awareness about potential transmissibility of dog parasites to humans than the reported 0% from Ambo by Zewdu *et al.* (2010), 3% from Hawassa by Gebreselasie *et al.* (2013), 22% from Wondoganet by Jones *et al. (*2011) and 27.1% from Harar by Tamerat *et al.*, (2015). The current study population has better awareness as compared to the lower level of awareness reported by previous studies conducted in other geographic areas of Ethiopia. This might be due to exposure to and existence of long years of veterinary services in Bishoftu town that might have created relatively better awareness about the importance of animal treatment and ease of access to anti-helminthic drugs used for treatment of dogs and other animal species. This is also partly attributable to the awareness of the population about animal health and welfare created through National Veterinary Institute, Animal Welfare Projects and Veterinary Teaching Hospital of Addis Ababa University College of Veterinary Medicine and Agriculture as well as the existence of better access to formal education and relatively better living standard of the people.

This study indicates the existence of serious environmental pollution due to contamination by dog fecal material. More than one-third of dog owners either let their dogs to defecate in the public fields or dispose their dogs' excreta out of their compound. Nearly 90% of the respondent's clean dog excreta without

wearing glove, facemasks, boots and/or coverall /gown indicating high risk of exposure to potentially dangerous zoonotic parasitic infection such as *E. granulosus*. The assessment of feeding practice also showed that more than 80% of the dog owners either feed uncooked offal from butcher houses or backyard slaughter or release their dogs to search for their own feed with a potential access to infected raw condemned offal. The practice of feeding raw or improperly cooked uninspected offal to dogs may increase the risk of public exposure since it could serve as a source of infection and perpetuate many parasitic zoonoses.

Limitations of the study

We have used flotation techniques for parasitic egg detection and collected fecal materials as well as expelled adult parasites 24 hours after treatment with ivermectin and praziquantel. All *D. caninum* detections except in two cases were based on expelled adult parasite, but we did not have record and do not remember the numbers of parasite-based detection versus egg-based detection separately for others parasites. The reported detection of *E. granulosus* egg solely based on egg detection might be an underestimation of the true prevalence. The questionnaire response rate was around 59% and needs caution to generalize the interpretation as those who declined to respond may vary from those who responded in wealth status and educational level.

Conclusion and recommendations

The finding of this study revealed that zoonotic intestinal helminth parasites remain common in owned dogs of Bishoftu town. This indicates the existence of potential threat to the health of affected dogs and the public as well. Despite the zoonotic importance of these parasites, the dog-owners awareness and practice to protect themselves and the society from potential zoonotic infection of these parasites is at low level. Therefore, it is recommended to conduct a systematic study of these parasites on humans and the free-roaming dog population to elucidate the true extent of the problem in the dog population and the public to take appropriate actions towards awareness creation of dog-owning population and promote dog-deworming programs and to reduce the public's exposure. Following treatment with anthelminthic drugs used in this study, many dogs expelled adult parasites in their feces except for *E. granulosus*. Thus, future research needs to objectively evaluate the importance of anthelminthic treatment in improving diagnostic sensitivity to helminthic infection in dogs.

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References

Abere, T., Bogale, B., and Melaku, A. 2013. Gastrointestinal helminth parasites of pet and stray dogs as a potential risk for human health in Bahir Dar town, northwestern Ethiopia. *Vet.World.* 6(7): 388-392.

Barutzki, D. and Schaper, R. 2003. Endoparasites in dogs and cats in Germany 1999 – 2002. *Parasitol. Res*., 90(3): S148–S150.

Bowman, D.D., Montgomery, S.P., Zajac, A.M., Eberhard, M.L., and Kazacos, K.R. 2010. Hookworms of dogs and cats as agents of cutaneous larva migrans. *Trends Parasitol.,* 26(4): 162–167.

Cardoso, A.S., Costa, I.M.H., Figueiredo, C., Castro, A.; Conceicao, M.A.P. 2014. The occurrence of zoonotic parasites in rural dog populations from Northern Portugal. *J. Helminthol.,* 88(2): 203–209.

Chidumayo, N.N. 2018. Epidemiology of canine gastrointestinal helminths in sub-Saharan Africa. *Parasit. & Vect.*, 11:100, https://doi.org/10.1186/s13071-018-2688-9

Cortez-Aguirre, G. R., Jiménez-Coello, M., Gutiérrez-Blanco, E., and Ortega-Pacheco, A. 2018. Stray Dog Population in a City of Southern Mexico and Its Impact on the Contamination of Public Areas. *Hindawi Vet. Med. Int*., https://doi.org/10.1155/2018/2381583.

Davoust, B., T. Normand, O. Bourry, H. Dang, E. Leroy, and G. Bourdoiseau. 2008. Epidemiological survey on gastro-intestinal and blood-borne helminths of dogs in North-East Gabon. *Onderstepoort* J. Vet. Res., 75: 359-364.

Deplazes, P., Gottstein, B., Eckert, J., Jenkins, D.J., Ewald, D., Jimenezpalacios, S., 1992. Detection of Echinococcus coproantigens by enzyme-linked immunosorbent assay in dogs, dingoes, and foxes. *Parasitol. Res.,* 78(4): 303–308.

Dowd, K., Taylor, M., Toribio, J.L.M.L., Hooker, C. and Dhand, N. K. 2013. Zoonotic disease risk perceptions and infection control practices of Australian veterinarians: Call for change in work culture, *Prev. Vet. Med*., 11(1-2): 17–24.

Dutta, J.K. 2002. Disastrous results indigenous methods rabies prevention in developing countries. *Int. J. Infect. Dis.,* 6: 236.

East, M.L., Kurze, C., Wilhelm, K., Benhaiem, S., Hofer, H., 2013. Factors influencing Dipylidium sp. infection in a free-ranging social carnivore, the spotted hyaena (Crocuta crocuta*). Int. J. Parasitol. Parasites Wildl* 2: 257–265.

Elsheikha, H.M., and Patterson, J.S. 2013. Self-Assessment Colour Review Veterinary Parasitology. Manson Publishing Ltd, London, UK, pp 256.

Fang, F., Li, J., Huang, T., Guillot, G., and Huang, W., 2015. Zoonotic helminths parasites in the digestive tract of feral dogs and cats in Guangxi, China. *BMC Vet. Res*. 11:211

Finsterer, J. and Auer, H. 2013. Parasitoses of the human central nervous system. *J. Helminthol.*, 87(3): 257–270.

Fromsa, A. and Jobre, Y. 2012. Estimated annual economic loss from organ condemnation, decreased carcass weight and milk yield due to bovine hydatidosis (*Echinococcus granulosus*, Batsch, 1786) in Ethiopia. *Ethiop. Vet. J.,* 16 (2):1-14

Gawor, J., Borecka, A., Zarnowska, H., Marczynska, M. and Dobosz, S. 2008. Environmental and personal risk factors for toxocariasis in children with diagnosed disease in urban and rural areas of central Poland. *Vet. Parasitol*., 155(3-4): 217–222.

Gebreselasie, D., Geyola, M., Dagne, E., Asmare, K. and Mekuria, S. 2013. Gastrointestinal Helminthes in Dogs and Community Perception on Parasite Zoonosis at Hawassa City, Ethiopia. *Glob. Vet.*, 11 (4): 432-440.

Getahun, Z. and Addis, M. 2012. Prevalence of gastrointestinal helminthes among dogs in Bahir Dar town, Ethiopia. *World Appl. Sci. J.,* 19 (5): 595-601.

Goggle Map, http://earth.google.com/streetview/, accessed on December 20, 2017.

Gugsa, G., Hailu, T., Kalayou, S., Abebe, N. and Hagos, Y. 2015. Study on gastro-intestinal helminth parasites of dogs in Mekelle city, Tigray Ethiopia. *J. Parasitol. Vect. Biol.* 7(2): 26-36.

Hailu, Y.., Taddese, A.., Regassa, F., and Basu, A.K. 2007. Gastrointestinal nematodes in dogs from Debre Zeit, Ethiopia. *Vet. Parasitol*., 148(2): 144-148.

Hendrix, C.M. 2003. Laboratory procedures for veterinary technicians, 4th edition, Mosby publishing, USA, Pp 364.

Hopkins, S.G., Schubert, T.A., and Hart, B.L. 1976. Castration of adult male dogs: effects on roaming, aggression, urine marking, and mounting. *J. Am. Vet. Med. Assoc.* 168 (12):1108-1110.

Jiang, P., Zhang, X., Liu, R.D., Wang, Z.Q. and Cui, J. 2017. A Human Case of Zoonotic Dog Tapeworm, *Dipylidium caninum (Eucestoda: Dilepidiidae*), in China. *Kor. J. Parasitol*., 55 (1): 61-64.

Joffe, D., Niekerk, D. V., Gagné, F., Gilleard, J., Kutz, S., Lobingier, R., 2011. The prevalence of intestinal parasites in dogs and cats in Calgary, Alberta. *Can. Vet. J.,* 52(12): 1323–1328

Jones, O., Kebede, N., Kassa, T., Tilahun, G., and Macias, C. 2011. Prevalence of dog gastrointestinal parasites and risk perception of zoonotic infection by dog owners in Wondo Genet, Southern Ethiopia. *J. Pub. Hlth. Epidemiolo*., 3 (11): 550-555.

Kirkpatrick, C.E., 1988. Epizootiology of endoparasitic infections in pet dogs and cats presented to a veterinary teaching hospital. *Vet. Parasitol.,* 30(2): 113-124.

Klimpel, S., Heukelbach, J., Pothmann, D., and Ruckert, S. 2010. Gastrointestinal and ectoparasites from urban stray dogs in Fortaleza (Brazil): High infection risk for humans? *Parasitol. Res.,* 107(3): 713-719.

Kostopoulou, D., Claerebout, E., Arvanitis, D., Ligda, P., Voutzourakis, N., Casaert, S. and Sotiraki, S. 2017. Abundance, zoonotic potential and risk factors of intestinal parasitism amongst dog and cat populations: The scenario of Crete, Greece. *Parasit & Vectors.,* 10:43 DOI 10.1186/s13071-017-1989-8.

Little, S.A., Johnson, E.M., Lewis, D., Jaklitsch, R.P., Payton, M.E., Blagburn, B.L., *et al*., 2009. Prevalence of intestinal parasites in pet dogs in the United States. *Vet. Parasitol*. 166(1-2): 144–152. doi: 10.1016/j.vetpar.2009.07.044.

Lorenzini, G., Tasca, T., and De Carli, G.A. 2007. Prevalence of intestinal parasites in dogs and cats under veterinary care in for to Alegre, Rio Grande do Sul, Brazil. *Braz. J. Vet. Res. Anim. Sci*. 44(2): 137-145.

Macpherson, C.N.L. 2005. Human behaviour and the epidemiology of parasitic zoonoses, *Int. J. Parasitol*., 35(11-12):1319–1331

Maria, F., Fontanarrosa, V., Dar´O, B., Julia, B., and Diego, E. 2006. An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): Age, gender, breed, mixed infection and seasonal and spatial patterns. *Vet. Parasitol.,* 136(3-4): 283-295.

Mateus T. L., Castro, A., Ribeiro, J. N., and Vieira-Pinto, M. 2014. Multiple Zoonotic Parasites Identified in Dog Feces Collected in Ponte de Lima, Portugal - A Potential Threat to Human Health. *Int. J. Environ. Res. Pub. Hlth.*, 11(9): 9050-9067, doi:10.3390/ijerph110909050.

Mengistu, S., Keftasa, D. and Yami, A. 2002. Productivity of four Sesbania species on two soil types in Ethiopia. *Agroforest. Syst.* 54: 235-244.

Merga, T., and Sibhat, B. 2015. Prevalence of gastrointestinal helminth parasites of dogs and associated risk factors in Adama Town, Central Ethiopia. *Ethiop. Vet. J.,* 19 (2): 91-103.

Mirzaei, M., and Fooladi, M. 2013. Coproscopy survey of gastrointestinal parasites in owned dogs of Kerman city, Iran. *Vet. Ital.,* 49(3): 309-13.

Morey, D.F., 1994: The early evolution of the domestic dog. *Am. Sci.,* 82(4): 336–347.

Moskvina, T. V., and Ermolenko, A. V. 2016. Helminth infections in domestic dogs from Russia. *Vet. World*, 9 (11): 1248-1258.

Negash, G., K/Yohannis, T., Afera, B., Mengiste, B., Weldu, K. and W/Mariam H.S. 2014. Zoonotic Importance and Prevalence of Parasites in Dogs: Pet Owner's Knowledge Assessment. *Euro. J. Appl. Sci.,* 6 (3): 45-49.

Paul, A.M., Overgaauw, Linda-van, Z.B., Denise, H., Felix, O., Yaya, J.R., Elena, P., *et al*., 2009. Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands. *Vet. Parasitol*., 163(1-3): 115-122.

Paulos, D., Addis, M., Fromsa, A. and Mekbib, B. 2012. Prevalence of gastrointestinal helminths among dogs and owner's perception about zoonotic dog parasites in Hawassa Town, Ethiopia. *J. Pub. Hlth. Epidemiol*., 4 (8), 205-209.

Quyen, N.T., Lan, N.T.K, Van, C. and Nang, N.T. 2015. A Study on Prevalence of Intestinal Nematodes in Dogs in Phutho Province. *J. Agri. Tech*., 11 (8): 2563-2576.

Ramirez-Barrios, R.A., Barboza-Mena, G., Munoz, J., Angulo-Cubillán, F., .and Hernandez, E. 2004. Prevalence of intestinal parasites in dogs under veterinary care in Maracaibo, Venezuela. *Vet. Parasitol.,* 121(1-2): 11-20.

Shubhagata, D.A.S., Abdul A.L., Suchandan, S.I.K. and Aungshuman, D. G.U. 2012. Prevalence and Worm Load of Enteric Helminthiasis in Stray Dogs of Chittagong Metropolitan, Bangladesh. *YYU Vet Fak. Derg.,* 23 (3): 141 – 145.

Tamerat, N., Shimelis, D., Dagim, N. and Terefe, Y., 2015. Gastrointestinal parasites of pets and zoonosis awareness assessment of owners in Harar Town, Eastern Ethiopia. *World. Appl. Sci. J*., 33 (8): 1348-1354,

Totton, S. C., Wandeler, A. I., Zinsstag, J., Bauch, C. T., Ribble, C. S., Rosatte, R.C., McEwen, S.A. 2010. Stray dog population demographics in Jodhpur, India following a population control/rabies vaccination program. *Prev. Vet. Med*., 97(1): 51–57. doi: 10.1016/j.prevetmed.2010.07.009.

Traub, R.J., Robertson, I.D., Irwin, P., Mencke N., Monis, P. and Thompson, R.C. 2002. Humans, dogs, and parasitic zoonoses - unraveling the relationships in a remote endemic community in northeast India using molecular tools. *Parasitol. Res.,* 90: 5156-5157.

Westgarth, C., Boddy, L. M., Stratton, G., German, A.J., Gaskell, R.M. and Coyne, K.P., 2013. Pet ownership, dog types and attachment to pets in 9–10-year-old children in Liverpool, UK. *BMC Vet. Res.,* 9: 11-86. doi: 10.1186/1746-6148-9-102.

Zajac, A. M., and Conboy, G. A. 2012. Veterinary Clinical Parasitology, 8th edition John Wiley & Sons, Inc., Ames, Iowa, USA, Pp 354

Zewdu, E., Semahegn, Y. and Mekbib, B. 2010. Prevalence of helminth parasites of dogs and owner's awareness about zoonotic parasites in Ambo town, Central Ethiopia. *Ethiop. Vet. J.,* 14(2): 17-30.

Ziadinov, I., Mathis, A., Trachsel, D., Rysmukhambetova, A., Abdyjaparov, T.A., Kuttubaev, O.T., Deplazes, P. and Torgerson, P.R. 2008. Canine echinococcosis in Kyrgyzstan: using prevalence data adjusted for measurement error to develop transmission dynamics models. *Int. J. Parasitol*. 38(10):1179–1190. doi: 10.1016/j.ijpara.2008.01.009.

Short Communication

Mycobacterium tuberculosis in a Primagam negative wild caught captive olive baboon (*Papio anubis*) in Ethiopia

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Abstract

A free-roaming wild olive baboon (*Papio anubis*) was caught in the compound of a hospital and kept in captivity pending reintroduction to the wild. The animal had a sporadic dry cough but was TB negative on the blood-based assay PRIMAGAM (IFN-γ test). Six years later, the animal was found dead without any prior clinical signs. The lungs were severely affected. Laboratory analysis included Ziehl-Neelsen staining, GenExpert, culture, deletion typing and spoligotyping. *M. tuberculosis* was isolated. The spoligotype was SIT 53 (lineage 4) and no Rifampicin resistance was detected. This case report raised challenges on accurate diagnosis of TB in Non-Human Primates in Ethiopia, the question of latency in baboon and the lack of spread of a highly virulent TB strain in the Non-Human Primate colony. It also highlighted the potential role of TB transmission between Non-Human Primates and people in Ethiopia with impacts as well on public health as on primate conservation.

Key words: Baboon; diagnostics; Ethiopia; Mycobacterium tuberculosis; tuberculosis

Introduction

TB remains worldwide a leading cause for morbidity and mortality. Ethiopia has a high TB burden, ranking third in Africa and eighth globally among the 22 countries with highest TB prevalence (WHO 2018). Moreover, Multi-drug

resistant TB (MDR-TB) has been increasingly observed in Ethiopia, with prevalence ranging from 3.3 and 46.3%, providing additional challenges to the national TB control program (Biadglegne *et al*., 2014).

Non-human primates (NHP), like their human counterparts, can get infected with TB and show identical disease epidemiology (e.g. transmission, immunology, type and course of disease) hence they are often used as model for human TB (Gormus *et al*., 2004; Scanga and Flyn 2014). Old world monkeys are considered to be the most susceptible species for TB among all NHP species (Montali *et al*., 2001). NHP are primarily affected by *M. tuberculosis* but *M. bovis* infections have also been observed. The disease does not exist naturally in free-ranging NHP (Montali *et al*., 2001). However, TB becomes a major health risk for NHP when they are exposed to humans with TB (Fourie and Odendaal 2003) or when they consume BTB infected meat (Tarara *et al.,* 1985; Thorel *et al*., 1998; Keet *et al*., 2000). TB in captive NHP colonies has major health and economic implications (human exposure, animal losses, and cost for disease control and staff therapy) but also in terms of conservation (risk of TB to wild populations during reintroduction programs). Animals with active TB may be symptomless for weeks and months while they transmit the disease (Gibson 1998). Latent TB, although not infectious can be reactivated at any time of the animal's life. Latent TB is not detected by traditional screening methods such as the skin test (Lerche *et al*., 2008). Therefore, early TB detection in captive NHP is crucial. Unfortunately, there is currently no diagnostic gold standard test for TB. Traditionally, NHP are tested by skin test (eye-lid or abdominal skin PPD skin injections). This can be stressful for the animal, and requires two anesthesia within 72 hours. In addition, the skin test lacks specificity (84-87%) and sensitivity (84%), does not always account for high environmental TB and does not detect latency (Garcia *et al*., 2004; Lerche *et al*., 2008; Lin *et al*., 2009). Rapid blood based *in-vitro* assay such as the PRIMAGAM test offer an alternative, with relatively good sensitivity (68%) and excellent specificity (97%) and able to detect TB at a very early stage (Garcia *et al*., 2004). PPD antigens are presented to lymphocytes in whole blood culture and the resulting production of interferon-γ (IFN-γ) by the TB-exposed lymphocytes is detected using a monoclonal antibody-based sandwich enzyme immunoassay (EIA). Lymphocytes of non-infected animals does not produce any IFN-γ. We present here a case of *M. tuberculosis* detected in a captive wild caught olive baboon, who tested negative with the blood-assay TB-PRIMAGAM test and discuss the TB diagnostics challenges in NHP in Ethiopia.

Material and methods

History

A juvenile female olive baboon (*Papio Anubis*) was roaming freely on the premises of a large hospital in Addis Ababa, was then captured by the Ethiopian Wildlife and Conservation Authority and kept in captivity in a large enclosure close to Addis Ababa, Ethiopia before considering a potential release into the wild. Upon arrival, the animal underwent quarantine. She looked healthy with normal body condition. No visible clinical signs were observed with the exception of a very sporadic dry cough that would persist over the following years. The animal was housed with other rescued olive baboons. The large enclosure was on natural ground (shrubs, dirt ground, grass and rocks) surrounded by a meshed cage. Diet consisted on scattered mix of various fresh fruits and vegetables as well as supplemental grass and acacia tree branches with pods or flowers. All animals were screened shortly after arrival for tuberculosis (TB) using the commercially available primate interferon-γ test (Primagam® blood essay; Prionics AG). All tested animals were negative to the TB test. Six years later, the baboon was found dead in the enclosure without showing any prior signs of disease except a very sporadic dry cough that she kept throughout the years.

Post-mortem examination

A standard post-mortem examination was carried out within 2 hrs. of death. Specimens of lung tissues were collected into sterile containers (one third without transport media, which were frozen at -80°C, one third contained 10% formalin solution and one third contained PBS (phosphate buffered saline) and brought to the laboratory at the Armauer Hansen Research Institute (AHRI), Addis Ababa within 15 minutes of collection.

Laboratory diagnosis

Suspicion of TB lead to follow all diagnostic procedures according to the institute and national TB SOP's (TB program quality assurance) (e.g. use of P3-TB lab for all diagnostic procedures; wearing of face masks and gloves). Smears from lung tissue samples were prepared followed by Ziehl- Neelsen (ZN) staining and observation for acid fast bacilli under the light microscope. *Mycobacterium* culture was done on Lowenstein-Jensen (LJ) medium following the procedure described in Mycobacteriology Laboratory Manual (Global Labo-

ratory Initiative, 2014). Tissue sample was homogenized with 3 ml phosphate buffered saline (PBS) using sterile mortar and pestle. *Mycobacterium* culture was done on Lowenstein-Jensen (LJ) medium. Digestion-decontamination of tissue sample was performed by N-acetyl L-cysteine /Sodium Hydroxide method (NALC/NAOH) with a final NaOH concentration of 1%. An equal volume of standard NALC/NaOH solution was added to tissue sample and incubated for 15 minutes. After neutralization by PBS and centrifugation (15 minutes at 3000g), the sediment was re-suspended in 1ml sterile PBS. Finally, 200µl of sediment was used to inoculate on two LJ slants. The remaining sediment was used for smear preparation followed by Ziehl- Neelsen (ZN) staining, and examined for acid-fast bacilli (AFB) using regular light microscopy (Federal Democratic Republic Ethiopia Ministry of Health, 2014). The AFB grading was done according to WHO smear grading scale for ZN staining (WHO, 1998). Culture was checked daily during the first week, then weekly thereafter. Colonies from positive culture were removed from the surface of LJ medium and suspended in 300 µl of Molecular grade water and the mixture heated at 80°C for 1 hr. in water bath. After centrifugation, the supernatant was collected and used for RD9 deletion typing and spoligotyping.

RD 9 Deletion Typing

Mycobacterial species was identified using polymerase chain reaction (PCR) based region of difference (RD9) typing as described by Brosch *et al*. (2002) using previously published RD9_FlankF, IntR and FlankR primer sequence (Berg *et al*., 2009). PCR was performed on heat-killed cells. *M. tuberculosis* H37Rv, *M. bovis*, and Qiagen water was used respectively as positive and negative controls.

Spoligotyping

Spoligotyping was performed following the method described by Kamerbeek *et al*. (1997).The spoligotype pattern was entered in an Excel spreadsheet, compared with those in the International Spoligotyping Database (SITVITWEB) of the Pasteur Institute of Guadaloupe (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/), and a spoligotype international type (SIT) was assigned.

In addition, for the sake of diagnostic speed, 0.5 ml of homogenized tissue sample was subjected for GeneExpert analysis by mixing with the supplied sample reagent in a 1:3 ratio, vortexed and incubated for 15 minutes. Two ml of the reagent-sample mix was then transferred to an Xpert cartridge using a

pasteur pipette and the cartridge loaded onto Expert machine. The GeneXpert automates all following steps, including sample work-up, nucleic acid amplification, detection of the target sequence and result interpretation (Geleta *et al*., 2015).

Further investigation of personnel

Four primate animal personnel, who had close physical contact to the TB positive animal over the years but showed no clinical signs of TB, wished to be tested for TB as well following the result of the animal. The gamma interferon test (QuantiFERON-TB Gold Plus) was used. Four ml of venous blood was drawn into lithium-heparin tubes and processed within 2 hours at AHRI. One ml of blood was dispensed into each QFT plus tubes (Nil, TB1, TB2, mitogen) followed by mixing by inversion and incubation at 37°C for 20 hrs. After incubation, the tubes were centrifuged at 3000 RPM for 10 minutes, the supernatants removed and the amount of IFN gamma measured by ELISA. IFN-γ ELISA was performed according to the QFT-Plus protocol and the result was interpreted using the software supplied by the manufacturer.

Results

The animal showed a normal to thin body condition based on the body scoring system by Clingerman and Summers (2005). No external changes were observed with the exception of hyperaemic conjunctivae. Superficial lymph nodes were normal. Intra-abdominal examination showed that all organs were hyperaemic without other visible pathological abnormalities. Lungs were severely enlarged and abnormally shaped, having entirely lost their normal lung shape, with hard bumpy sections and ballooning. The lungs filled the entire thorax cavity with fibrous adhesions to the pleura that made extraction from the thorax cavity difficult. The lungs were removed en-toto using sharp and blunt dissection. The lung texture was hard fleshy for most part of the organ with hardly any normal looking lung tissue left. Granulomas, soft in texture could be felt throughout the lungs. Upon dissection, the lungs were entirely filled with various sized cavities (from 1 to 5 cm in diameters) sometimes coalescent/confluent filled with little liquid creamy looking pus and ill-defined walls (Figure 1). When pressure was applied to the lungs, pus would ooze out from everywhere, including bronchi. No calcifications were observed. Lung lymph nodes could not be identified. The heart was enlarged with pericardial fluid.

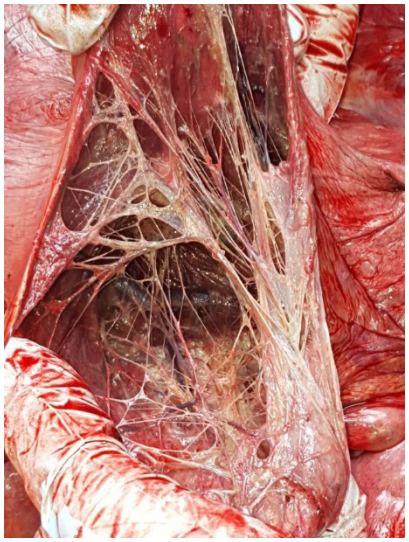


Figure 1. Macroscopic close-up of a dissected lung lesion in an olive baboon (*P. anubis*)

Laboratory results

Baboon sample

Ziehl-Neelsen staining of three slides (direct lung smear) and one slide with colonies from culture were graded as +++, implying the presence of more than 10 Acid Fast Bacilli per field. The result of the GenExpert was positive for *M. tuberculosis* and no rifampicin resistance was detected. Results from culture showed colony growth within two weeks of inoculation that were creamy white dry, rough, raised, irregular with wrinkled surface and confluent growth was observed. Figure 2 shows the results of RD9 deletion typing. The isolate had an intact RD 9 region identifying it as *M. tuberculosis*. The spoligo pattern was *M. tuberculosis* SIT 53, belonging to Lineage 4.

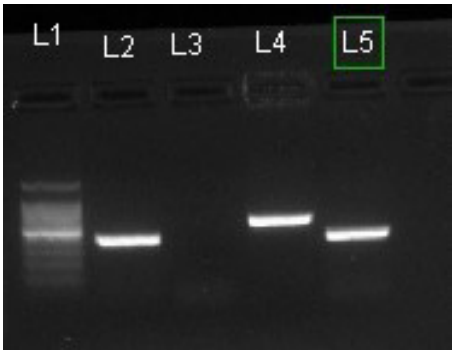


Figure 2. Electrophoretic pattern of the amplified element of PCR products, Lane=100bp ladder; Lane 2= H37Rv (*M. tuberculosis* positive control), Lane 3= Qiagen H2O (negative control); Lane 4 = *M. bovis* (positive control); Lane 5= DNA from heat killed culture

Human samples

One out of 4 people were positive to the quantiferon test, hence making *M. tuberculosis* infection likely. However, due to the limitation of the test, definitive diagnosis of whether the infection is active or most probably latent since the person did not show any signs of active TB, requires further combination of epidemiological, historical, medical and complementary diagnostic findings. Furthermore, the study could not link with certainty the infection in the animal and the person due to the lack of *M. tuberculosis* isolation in the sampled people.

Discussion

This case-study highlighted two major issues regarding TB in NPH, namely: 1) poor detection of TB by the blood-based assay PRIMAGAM and 2) the unusual very long disease course and lack of further spread. In hindsight, we assume that the animal had likely already an active TB, when he was tested 6 years ago considering the chronic cough she had. Animals with active TB can be symptomless for weeks to months (Gibson, 1998). However, although TB can be a chronic debilitating disease, having an active TB for so many years has, to our knowledge not been observed. Furthermore, the animal kept his good body condition to the end and no disease outbreak occurred in the colony. TB is usually described as highly contagious within a NHP colony. All primates

underwent monthly health checks during these 6 years (assessment of body condition, cough, appetite etc). Furthermore, autopsies were performed as a standard procedure on several NHP during these 6 years that died from any cause. No visible lesions suggestive for TB were ever observed, and smears of samples collected routinely from autopsies were ZN negative. In addition we know that the isolated strain belonged to a virulent most commonly found lineage in Ethiopia (modern lineage), which makes it more surprising for not having spread fast. It is well possible that the animal was already in an advanced state of anergy during the testing. However, it would be unlikely she would have survived another 6 years. TB latency is a growing concern in the NHP TB epidemiology. Studies have shown that a latent TB can be reactivated for example by a pregnancy and develop quickly into a fatal active TB (Martino *et al*., 2006). Hence, the importance to diagnose TB at the latent stage. The last possibility is that the animal was negative at the time of the testing and it acquired the disease while in captivity. However, this scenario is the least plausible since no other baboons sharing the enclosure became clinically sick with TB, and later performed autopsies did not reveal any visible TB-like lesions and were ZN negative. A TB latency could not to be ruled out in the remaining animals of the group, However, latency over several years in a captive primate colony has never been previously described.

The standard testing procedure in NHP remains the Tuberculin Skin testing (TST), which detects delayed type hypersensitivity (DTH) to tuberculin antigens. Classically, the SIDT test is performed in the eyelid and swellings observed after 72 hours. The procedure requests two anesthesia and is subject to the observer's interpretation leading to subjectivity. The required antigen concentration to detect reactivity for TB is higher than in humans but the exact quantity of tuberculin units (TU) is often not provided in PPD for NHP and same testing is often used as in humans. The TST is known for its poor sensitivity and specificity and leads to false negative animals (Lerche *et al*., 2008). Moreover, the TST does not detect TB latency. Various blood tests have been used to screen NHP with diverse results (Parsons *et al*., 2004; Vervenne *et al*., 2004). More recently, non-invasive TB screening was done among chimpanzees and baboons in Tanzania, using fecal IS6110 PCR (Wolf *et al*., 2016). Blood tests, such as the PRIMAGAM, reduces the stress in animals since only one anesthesia is required. Unlike the TST, it is a quantifiable test. It detects TB reactivity by measuring the IFN-γ production by lymphocytes that had contact to Mycobacteria. It is highly specific (97%) and can detect TB at a very early stage (Garcia *et al*., 2004). However, studies have clearly shown that no

single test has the high enough sensitivity and specificity to detect correctly all positive animals in a group and a combination of tests might be necessary, highlighting the still existing challenge to diagnose accurately TB.

TB in NHP is a concern in Ethiopia in light of the high numbers of illegally kept primates with close contact to humans, rehabilitated/rescued primates that are set to be released into the wild and the many free-ranging NHP in close vicinity to human dwellings, particularly urban wild NHP. The baboon of this study was a wild baboon initially roaming freely in a hospital compound. In light of the high TB burden in Ethiopia and the emergence of MDR-TB, these wild roaming NHPs can become infected by TB and can then also potentially act as vehicle for further spread to humans and other NHP. Awareness campaigns are warranted. They are a crucial step in controlling the spread of TB between humans and NHP but also for overall conservation purposes, by promoting the stop of illegal ownership of NHP as pets and banning illegal wildlife trade.

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Conflict of interest

The authors declare that there is no conflict of interest

Reference

Berg, S., Firdessa, R., Habtamu, M., et al. 2009.The burden of mycobacterial disease in Ethiopian cattle: implications for public health. *PLOS One* 4(4): e5068. https://doi.org/10.1371/journal.pone.0005068.

Biadglegne, F., Sack, U., Rodloff, A.C. 2014. Multidrug-resistant tuberculosis in Ethiopia: efforts to expand diagnostic services, treatment and care. *Antimicrob. Resist. Infect. Control*, 3**(**31). doi: 10.1186/2047-2994-3-31.

Brosch, R., Gordon, S. V., Marmiesse, M., Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K., Garnier, T., Gutierrez, C., Hewinson, G., Kremer, K., Parson, L.M.,

Pym, A.S., Samper, S., Van Soolingen, D., and Cole S. T. 2002. A new evolutionary scenario for the M. tuberculosis complex. *Proc. Natl. Acad. Sci.,* 99(6), 3684–3689.

Federal Democratic Republic Ethiopia Ministry of Health. 2014. AFB smear microscopy manual. Addis Ababa: Ethiopian Public Health Institute.

Clingerman, K.J., Summers, L., 2005. Development of a body condition scoring system for nonhuman primates using *Macaca mulatta* as a model. *Lab Anim*., 34(5),31-36.

Fourie, P.B., Odendaal, M.W. 2003. *Mycobacterium tuberculosis* in a closed colony of baboons (*P. ursinus*). *Lab Anim.,* 17(2)**,**125-128. doi.org/10.1258/002367783780959376.

Garcia, M.A., Yee, J.A.L., Bouley, D.M., Moorhead, R., Lerche, N.W. 2004. Diagnosis of Tuberculosis in Macaques, Using Whole-Blood In Vitro Interferon-Gamma (PRIMAGAM) Testing. *Comp. Med*., 54(1), 86-92

Geleta, D.A., Megerssa, Y.C., Gudeta, A.N., Akalu, G.T., Debele, M.T., Tulu, K.D. 2015. Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis in sputum specimens in remote health care facility. *BMC Microbiol*., 15: 220. doi: 10.1186/s12866-015-0566-6

Gibson, S.V. 1998. Bacterial and Mycotic Diseases: Non-human Primates in Biomedical research. San Diego: Academic Press. Pp 59-110.

Global Laboratory Initiative: 2014: Mycobacteriology Laboratory Manual. 1st Edition. A publication of the Global Laboratory Initiative a Working Group of the Stop TB Partnership PP: 19-22.

Gormus, B.J., Blanchard, J.L., Alvarez, X.H., Didier, P.J. 2004. Evidence for a rhesus monkey model of asymptomatic tuberculosis. *J. Med. Primatol*., 33(3),134-145.

Kamerbeek, J., Schouls, L., Kolk, A., Van Agterveld, M., Van Soolingen, D., Kuijper, S., Bunschoten, A., Molhuizen, H., Shaw, R., Goyal, M., Van Embden, J. 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J. Clin. Microbiol*., 35(4), 907–914.

Keet, D.F., Kriek, N.P.J., Bengis, R.G., Grobler, D.G., Michel, A. 2000. The rise and fall of tuberculosis in a free-ranging chacma baboon troop. *Onderstepoort J. Vet. Res*., 67(2), 115-122.

Lerche, N.W., Yee, J.A.L., Capuano, S.V., Flynn, J.L. 2008. New Approaches to Tuberculosis Surveillance in Nonhuman Primates. *ILAR J*., 49(2), 170-178

Martino, M., Hubbard, G.B., Schlabritz-Loutsevitch, N. 2006. Tuberculosis (*Mycobacterium tuberculosis*) in a pregnant baboon (*Papio cynocephalus*). *J. Med. Primatol*., 36(2), 108–112.

Montali, R.J., Mikota, S.K., Cheng, L.I. 2001. Mycobacterium tuberculosis in zoo and wildlife species. *Rev. Sci. Tech*., 20(1) : 291-303

Parsons, S.D.C., Gous, T.A., Warren, R.M., De Villiers, C., Seier, J.V., Van Helden, P.D. 2009. Detection of *Mycobacterium tuberculosis* infection in chacma baboons (*Papio ursinus*) using the QuantiFERON-TB Gold (In-Tube) assay. *J. Med. Primatol*., 38(6), 411–417.

Scanga, C.A., Flynn, J.A.L. 2014. Modeling Tuberculosis in Nonhuman Primates. *Cold Spring Harb. Perspect. Med*., 4(12): a018564. doi: 10.1101/cshperspect.a018564

Tarara, R., Suleman, M.A., Salolsky, R., Wabomba, M.J., Else, J.G. 1985. Tuberculosis in wild olive baboons, *Papio cynocephalus anubis* (Lesson), in Kenya. *J.Wildl. Dis*., 21(2), 137-140

Thorel, M.F., Karoui, C., Varnerot, A., Fleury, C., Vincent, V. 1998. Isolation of *Mycobacterium bovis* from baboons, leopards and a sea-lion. *Vet. Res*., 29(2), 207-212.

Vervenne, R.A.W., Jones, S.L.J., Van Soolingen, D., Van der Laan, T., Andersen, P., Heidt, P.J., Thomas, A.W., Langermans, J.A.M. 2004. TB diagnosis in non-human primates: comparison of two interferon-g assays and the skin test for identification of *Mycobacterium tuberculosis* infection. *Vet. Immunol. Immunopathol*., 100(1-2), 61–71

WHO. 1998. Laboratory services in tuberculosis control. Part II: Microscopy. Geneva, Switzerland.

WHO 2018: https://www.who.int/tb/country/data/profiles/en/. Accessed 12.12.2018

Wolf, T.M., Sreevatsan, S., Singer, R.S., Lipende, I., Collins, A., Gillespie, T.R., Lonsdorf, E.V., Travis, D.A. 2016. Noninvasive Tuberculosis Screening in Free-Living Primate Populations in Gombe National Park, Tanzania. *EcoHealth*, 13(1),139–144.

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This section should be separately presented with supporting evidences based on the major findings of the study. Appropriate recommendations can be made if necessary

Acknowledgements

Acknowledgments should be briefly stated after the Conclusions. Under this, technical, financial and material support can be mentioned.

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Jobre, Y., Malone, J. B., McCarroll, J. C., Erko, B., Mukaratirwa, S. and Xinyu, Z., 2001. Satellite climatology and the environmental risk of Schistosoma mansoni in Ethiopia and east Africa. *Acta Trop*., (*In press*).

C) Papers in proceedings

Tegegne, A., Wirtu, G., Mukasa-Mugerwa, E. and Kassa, T., 1994. Oestrus phenomenon and oestrus detection efficiency using androgenized cows and entire bulls in Boran and Boran x Friesian crossbred cows. In: Proceedings, Advances in Tropical Agriculture in the 20th Century and Prospectus for the 21st: TA 2000, 4-9 September 1994, Port-of- Spain, Trinidad.

D) Books or chapters in books:

Thrusfield, M., 1995. Veterinary Epidemiology, 2nd edition, Blackwell Science Ltd, Oxford, UK.

Malone, J. B and Jobre, Y., 1999. Predicting outbreaks of fasciolosis from Ollernshaw to satellites. In: Dalton, J.P. (Ed.), Fasciolosis. CBA International Publications, Cambridge, Pp. 151-183.

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OIE, 1992. Bovine tuberculosis. OIE manual for diagnostic techniques of livestock diseases. Office International des Epizooties (OIE), Paris, France. Pp. 287-296.

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