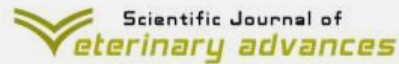


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Original article

First-time detection of tuberculosis in goats by single intradermal tuberculin test in the northern part of Ethiopia

D. Hussien*, S. Woldegebriel, B. Afera

College of Veterinary Medicine, Mekelle University, P.O. Box 231, Mekelle, Ethiopia.

*Corresponding author; College of Veterinary Medicine, Mekelle University, P.O. Box 231, Mekelle, Ethiopia.

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ABSTRACT

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A cross sectional study was conducted on randomly selected 300 goats in five Sub-districts of Kolla Tembien District from June 2012 to November 2012 to investigate the epidemiology of tuberculosis in goats using single intradermal tuberculin skin test and post-mortem examination. Well structured questionnaire was also administered to animal owners to assess their awareness about the importance of animals for transmission tuberculosis to humans. Chi-square statistic was used to analyse the data and a p-value of less than 0.05 was considered statistically significant (STATA version 11). The overall animal prevalence of TB in goats was 2.33%. From a total of 33 flocks/herds examined, 4(12.12%) contained at least a positive animal. There was significant difference ($p < 0.05$) in prevalence of caprine tuberculosis among age groups and among Sub-districts. However, the individual animal prevalence was not affected by sex and flock size ($p > 0.05$). Gross tuberculous lesions were detected in two tuberculin positive (suspected) goats that were slaughtered for confirmation of tuberculosis lesion. Acid-fast bacilli were detected in granuloma. Out of the 33 household heads interviewed, 21 (63.64%) had awareness of TB. However, 12 (36.36%) did not have the awareness of TB. Of the respondents, 29 (87.88%) replied that they consume boiled milk and 4 (12.12%) responded that they consume both raw and boiled milk. In conclusion, this study showed the occurrence of tuberculosis in goats in Kolla Tembien District. Isolation and characterization of the mycobacterial species which infect goats

are important. In addition, the zoonotic nature of the disease should be communicated to the public.

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1. Introduction

Goat population of Tigray region is estimated at about 2.77 million that shares about 15% (18.56 million) of the country's goat population (CSA, 2007). Goat is a multi-functional animal and plays a significant role in the economy and nutrition of landless, small and marginal farmers ((Tadesse et al., 2012). In spite of the large population and potential use of goats, the production system is affected by feed shortage, poor genetic makeup of the animals, and wide spread occurrence of livestock diseases such as tuberculosis which has both economic and public health significance to the communities.

TB in goat is caused by *Mycobacterium bovis* and *Mycobacterium caprae* (Aranaz et al., 1999; Crawshaw et al., 2008). It is also reported to be caused by *M. tuberculosis* (Mamo et al., 2012). Tuberculosis in goat has a wide global distribution and has been reported in Africa (Naima et al., 2011), Asia (Rahman et al., 2013), Europe (Shanahan et al., 2011) and America (Pignata et al., 2009).

In Ethiopia, the existence of TB in goats was reported by different authors earlier (Amenu et al., 2010; Mamo et al., 2012; Tafess et al., 2011). However, these studies were conducted in Oromia and Afar regions of the country. In Tigray region, where large population of goats exists, the status of the disease is unknown. The current study was, therefore, conducted to determine the prevalence of caprine tuberculosis in Kolla Tembien District of the region so that it can be used as a base for further detailed studies in goats, cattle and humans in the areas. This study also contributes data for nationwide epidemiology of the disease so that appropriate control measures can be taken.

2. Materials and methods

2.1. Description of the study area

The study was conducted in Kola Tembein district, located at the southern part of central zone of Tigray region. The district has 27 sub-districts. The administrative center for the district is Abiy Addi and located at 90 Km away from Mekelle. Topographic condition of the district is mountains, plateaus, undulating and rugged characteristics. Its elevation ranges from 1200 – 2300 m a s l. The agro climatic zone of the district is mainly lowland. Annual rainfall of the area ranges from 550-800 mm (BoPED, 1998). The study sub-districts were selected purposely based on goat population, distribution of peasant associations and accessibility to the main road. The sub-districts included in the present study were Debre Genet, Dr. Ataklti, Workamba, Begashek and Abiy-Addi.

2.2. Study animals and sampling

The study was conducted on goats above the age of six months having no clinical symptom of any disease. Study animal-related information on each tested goat (such as sex and age) was collected and recorded at the time of the test. Each animal was dewormed with antihelmintic drug after testing. A total of 300 goats were tested using single intradermal tuberculin testing. The predominant goat breeds in Tigray region are Abergelle and Central Highlands. The goats are managed under extensive production system. Traditional housing and grazing of natural pasture are the predominant husbandry practices.

2.3. Single intradermal tuberculin test

Single intradermal tuberculin test was conducted as previously reported by O'Reilly and Daborn, (1995). Briefly, the middle neck of each of the study animals was shaved, the thickness of the site was measured with caliper, and 0.1 ml of bovine tuberculin (Bovituber PPD, Symbiotic Corporation, France) containing 20,000 IU/ml of purified protein derivative (PPD) was injected into the dermis of each animal.

Three days (72 h) after injection, the site was re-measured to assess the increments or any change in skin thickness. The result was interpreted according to the manufacturer's instructions and the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2000). Hence, when the swelling was equal or less than 2 mm, between 2 mm and 4mm, and equal to or greater than 4 mm, individual result was considered as negative, doubtful and positive, respectively.

2.4. Post mortem examination and ziehl-neelsen (zn) staining

Post mortem examination was performed on two tuberculin positive goats (increment in skin thickness > 4mm) for detection of tuberculosis lesions and confirmation of tuberculosis in reactor goats. The organs examined include lymph nodes (bronchial, mediastinal, mesenteric and hepatic), lungs, liver, intestine and kidney. These organs were inspected, palpated and then incised for further investigation.

For the confirmation of the presence of acid-fast bacteria (AFB), a direct smear was prepared from the centrifuged sediments of 2 tissues (lung and intestine) with tuberculous lesions and examined by Ziehl-Neelsen (ZN) staining. Briefly, the heat-fixed smears were stained with carbol-fuchsin, heated gently to steaming and allowed to stand for about 10 min. The stain was poured-off and washed with tap water, then the slides were kept in 25% sulfuric acid and 96% ethyl alcohol solutions for one minute each (they were washed with tap water after each step). The smears were then counterstained with methylene blue for three minutes, washed with tap water, air-dried and examined by light microscope (Olivetti) with a 100× oil-immersion objective.

2.5. Questionnaire survey

Questionnaire survey was used to assess the perception of the community about the tuberculosis. During data collection, awareness about TB, transmission of TB from animals to humans, means of transmission and consumption habit of milk were recorded.

2.6. Data analysis

Individual animal prevalence was defined as the number of reactor goats out of 100 tested animals and expressed in percentage. The comparison of prevalence between different age, sex and herd size was done using chi-square test. A p-value of less than 0.05 was considered statistically significant (STATA version 11).

3. Results

3.1. Single intradermal tuberculin test

Herd/Flock Prevalence

From a total of 33 flocks/herds examined with single intradermal tuberculin test, 4(12.12%) contained at least a positive animal. The difference in the prevalence of TB among the different herd size categories was not statistically significant ($\chi^2 = 0.29$, $P=0.865$) (Table 1).

Individual animal prevalence of 2.33% was recorded when doubtful animals were considered as negative and the prevalence became 6.0% when doubtful animals were considered as positive. As shown in Table 1, statistically significant difference was recorded in prevalence among different groups of age ($\chi^2 = 19.37$, $P= 0.000$) and sub-districts of origin ($\chi^2 = 10.17$, $P= 0.038$). However, there was no statistical significance in skin reactivity between sex ($\chi^2 = 2.65$, $P=0.103$) and among flock size ($\chi^2= 0.29$, $P= 0.865$) (Table 1).



Fig. 1. Swelling at the middle neck of tuberculin reactor goat after 72 h of PPD injection.

Individual animal prevalence of 2.33% was recorded when doubtful animals were considered as negative and the prevalence became 6.0% when doubtful animals were considered as positive. As shown in Table 1, statistically significant difference was recorded in prevalence among different groups of age ($\chi^2 = 19.37$, $P= 0.000$) and sub-districts of origin ($\chi^2 = 10.17$, $P= 0.038$). However, there was no statistical significance in skin reactivity between sex ($\chi^2 = 2.65$, $P=0.103$) and among flock size ($\chi^2= 0.29$, $P= 0.865$) (Table 1).

Table1

Association of different risk factors to skin test positivity for caprine tuberculosis in Kolla Tembien District

Variables	No. animals examined	No. positive (%)	χ^2 (P-value)
Sub-district			
Debre Genet	64	0	10.17 (0.038*)
Dr. Ataklti	56	0	
Workamba	45	3 (6.67)	
Begashek	82	4 (4.88)	
Abyi-Addi	53	0	
Sex			
Female	219	7 (3.20)	2.65 (0.103)
Male	81	0	
Age			
< 2	172	2 (1.16)	19.37 (0.000*)
2 < X <4	100	1 (1.00)	
> 4	28	4 (14.29)	
Flock size			
< 10	11	0	0.29 (0.865)
10 < X < 20	117	3 (2.56)	
> 20	172	4 (2.33)	

*statistically significant

3.2. Post mortem examination

Gross tuberculous lesions were detected in the lung of one tuberculin reactor goat and intestine in the other tuberculin reactor goat. Upon incision of the lung (Fig. 2) and intestine, lesions showed a yellowish caseous material indicating a characteristic of tuberculous lesions.

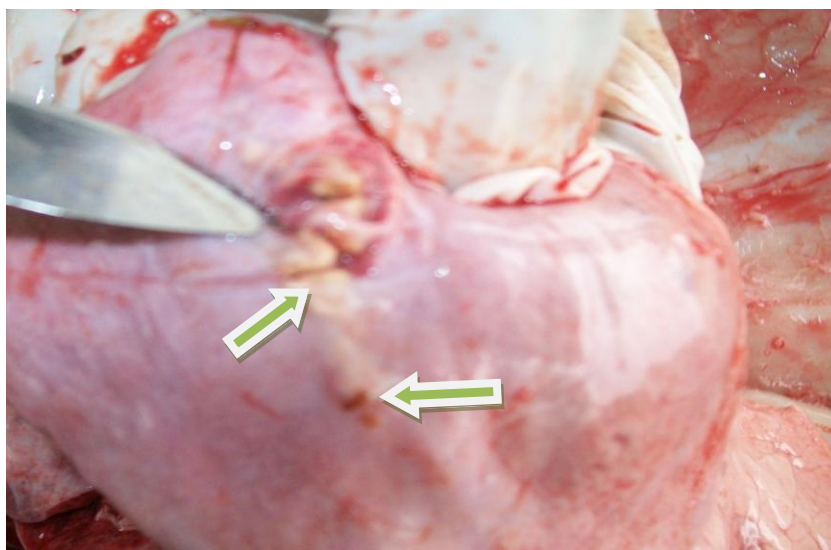


Fig. 1. A characteristic tuberculous lesion in lung of tuberculin reactor goat

3.3. Acid-fast staining

Microscopically, mycobacteria have been detected in examined lung tissue sample stained by ZN staining technique.

3.4. Questionnaire survey

In this survey, 33 household heads were interviewed and their responses were summarized as follows:

3.4.1. Awareness about tuberculosis

Out of the 33 household heads interviewed, 21 (63.64%) had awareness of TB. However, 12 (36.36%) did not have the awareness of TB.

3.4.2. Transmission of tuberculosis from animals to humans

Of the 21 interviewee who had previous awareness of TB, 11 (52.38%) had information that TB can be transmitted from animals to humans. Nevertheless, 10 (47.62%) had no the idea whether TB can be transmitted from animals to humans.

3.4.3. Means of transmission of TB from animals to humans

Out of 11 who knew TB can be transmitted from animals to humans, 7 (63.64%) replied that TB can be transmitted to humans from animas by direct contact with respiratory secretions; 1 (9.09%) reacted that humans get TB from animals by drinking raw milk; 1 (9.09%) responded that consuming raw meat from infected animals can transmit TB to humans and 2 (18.18%) replied that TB can be transmitted from animals to humans both by direct contact with respiratory secretions and drinking raw milk.

3.4.4. Milk consumption habit and method of consumption

All of the interviewed individuals, 33 (100%), replied that they consume goat and sheep milk. With regard to the method of consumption of milk, 29 (87.88%) replied that they consume boiled milk and 4 (12.12%) responded that they consume both raw and boiled milk.

4. Discussion

The prevalence of caprine TB (2.33%) obtained in the present study was comparable to the prevalence of 2.4% reported in Punjab, Pakistan (Javed et al., 2010). However, it was lower than the prevalences of 3.1%, 4.3% and 6.5% reported by Tafess et al. (2011), Mamo et al. (2012), Ashenafi et al. (2013) in Ethiopia, respectively. On the other hand, the result of the present study was higher than the prevaences of 0.47% in Brazil and 1.29% in Bangladesh reported by Pignata et al. (2009) and Rahman et al. (2013), respectively. This difference might be related to the difference in geographical location of these studies in which the epidemiology of the disease might vary between these areas.

In the present study, some animal level characteristics have been described as risk factors predisposing goats to caprine TB infection. Tuberculin reactor goats were obtained from Begashek and Workamba districts. However, goats of other districts were found to be negative which might be related to the husbandry system where goats had higher interaction with cattle in Begashek and Workamba districts than the other districts, which can favor a potential transmission of mycobacterial species between cattle and goat. Older goat showed higher proportion of positivity in tuberculin test results which might be related to the fact that older animals have longer duration and repeated chance of exposure to mycobacterial infection with their age. Similar results have been reported by Mamo et al. (2012) and Deresa et al. (2013). The prevalence of caprine TB in the present study was not seemed to be affected by sex although all tuberculin reactor goats were females. Despite conflicting ideas, other researchers reported that sex might have influence in skin reactivity to TB. Mamo et al. (2012) reported that females are more affected than males while Rahman et al. (2013) stated that males are more likely to acquire TB infection than female. Statistical analysis of the data showed that there was no significant difference in prevalence of caprine TB and flock size ($P>0.05$). Nevertheless, several studies in cattle have indicated that as herd size increases, the risk of cattle within the herd showing a positive reaction also increases (Asseged et al., 2000; Cook et al., 1996; Elias et al., 2008).

Gross tuberculous lesions were detected in the lung of one tuberculin reactor goat and intestine in the other tuberculin reactor goat (Fig. 2). Acid fast bacilli have been detected in examined lung tissue sample stained by ZN

staining technique. Mamo et al. (2012) isolated mycobacteria species from TB lesions of goats in Afar region. Further molecular characterization of the isolate revealed that it was *M. tuberculosis*. Despite further molecular characterization of the isolate was not performed, Tafess et al. (2011) also isolated mycobacteria species from TB lesions of goats in Oromia region.

Awareness of the community about TB has been assessed through interview. Accordingly, greater proportion of the respondents replied that they had previous knowledge about TB. The respondents who had information about TB were also asked about their awareness about the transmission of TB from animal to human and half of the respondents replied that they knew TB can be transmitted from animals to humans. From these respondents, majority of them said means of transmission of TB from animals to humans is by direct contact with respiratory secretion and one respondent replied that humans get infection from animals by drinking raw milk. Other two respondents replied that direct contact with respiratory secretion and drinking raw milk are means of transmission of TB from animals to humans. But, one respondent said TB can be transmitted from animals to humans by eating uncooked meat.

Milk consumption habit and method of consumption were also assessed and almost all replied that they consume sheep and goat meat. Majority of the respondent replied that they consume boiled milk. However, the response of four respondents who replied that they drink both boiled and raw milk should not be underestimated. Consumption of unpasteurized fresh and soured milk potentially infected with mycobacteria can cause milk-borne infection. This is further exacerbated by the poor understanding of animal owners' about TB.

In conclusion, this study revealed the occurrence of tuberculosis in goat in Kolla Tembien district. Detection of tuberculous lesions in the lung and intestine of tuberculin reactor goats indicates the need for further studies to isolate and characterize the causative agents, to understand the interspecies transmission of the disease, and to understand the role of goats in the epidemiology of bovine as well as human tuberculosis in the study areas. Moreover, awareness of the public about tuberculosis should be raised and the importance of boiling goats' milk before consumption should be communicated to the public.

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