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Research Article

The Prevalence of Caprine Tuberculosis in Adami Tulu, Jido-Kombolcha District and its Surroundings, Oromia, Ethopia

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Keywords

• Goat; M Bovis; M Caprae; Prevalence; Tuberculosis

Abstract

Caprine tuberculosis (TB) is important science milk is usually consumed raw particularly by Ethiopian pastoralists. An attempt was undertaken to investigate the occurrence of bovine and avian tuberculosis in goat population in Adami Tulu Jidokombolcha district. A total of 665 animals were screened for bovine and avian tuberculosis by comparative intradermal tuberculin test. The overall percentage of reactors to CIDT test 7.51% for both bovine and avian tuberculosis. Prevalence in females (11.7%) reactors was 3.71 times higher than males (3.15%). Gross tuberculosis lesions were detected in two of the four strong tuberculin positive goats, on the liver, large intestine, lung and museum. The study indicates that bovine and avian tuberculosis prevalence were high in female animals, older age, poor body condition score and in female with high parity number. Tuberculosis also got zoonotic effect on human health, especially in areas that consume raw goats' milk like pastoral and agro-pastoral areas. Therefore, at least awareness for community concerning the transmission of the disease from goats' milk and meat can be created.

ABBREVIATIONS

BCG: Bacilli Calmett –Guerin**, BCS:** Body Condition Score**, CSA:** Central Statistical Agency, **ESGPIP:** Ethiopia Sheep and Goat Productivity Improvement Program, **LJ:** Löwenstein-Jensen, **MTBC:** Mycobacterium tuberculosis complex, **OIE:** Office International des Epizooties, **PPD:** Purified protein derivative, **PZA:** Pyrazinamide, **TB:** Tuberculosis, **WHO:** World Health Organization

INTRODUCTION

Tuberculosis (TB) is an infectious, granulomatous, contagious, and chronic debilitating disease caused by acid fast bacilli of the genus *Mycobacterium* [1] and occurs in several species of animals and in humans [2]. TB occasionally assumes acute, rapidly progressive courses and major health problem worldwide and a single leading cause of human mortality due to infectious disease [3]. The disease affects all age groups of susceptible hosts and is accountable for more deaths throughout the world than any other bacterial disease ever today [4].

Tuberculosis in cattle and other domestic animals is above all caused by two members of *Mycobacterium tuberculosis* complex (*MTC*): *M. bovis*and *M. caprae* [5-7]. *M. bovis*is a member of the *M. tuberculosis* complex (MTC), which also includes *M. tuberculosis*, *M. africanum*, *M. canettii, M. bovis*ba cille Calmette- Guérin (BCG), *M. microti*, *M. caprae*and *M. pinnipedii* [8-11]. These pathogens are causative agents of TB in humans and animals, and are taxonomically closely related. Biochemical and molecular variations among MTC species enable their differentiation and identification [2].

Differentiation of *M. bovis*is based on a number of phenotypic characteristics and biochemical tests, *M. bovis*shows dysgonic growth on Löwenstein- Jensen (LJ) medium, and is negative for nitrate reduction and niacin accumulation. Although intrinsic resistance of *M. bovis*to pyrazinamide (PZA) had been described, PZA-susceptible strains are found in several countries. In former taxonomy, *M. bovis*was divided into *M. bovis*subsp. *bovis*, which showed resistance to PZA, and *M. bovis*subsp. *caprae,* which was PZA sensitive [2, 12]. The pronounced PZA resistance of some MTC members imposes the need for precise member differentiation due to its therapeutic and epidemiological importance in human infections [13-15].

Caprine tuberculosis (TB) caused mainly by **Mycobacterium bovis** and **M. Caprae [15,16]** poses a risk to goat health and production in developing world [3,17]. In Spain, *M. caprae* represents 7.4% of all *M. tuberculosis* complex isolates from domestic and wild animals. The routine application of molecular diagnosis and typing techniques in clinical laboratories has enabled its real role as a pathogen for several species to be recognized [18].

In past, TB has been considered rare in goats and it was thought that this species is naturally immune to tuberculosis; however, long time ago, it was reported that goats had no particular resistance to the disease [19]. Recently, reports of caprine TB have increased in several countries; even in those practicing a long standing test and slaughter policy [3, 16, 17, 20]. It is reported that the infection is widespread in Africa where goats co-graze with cattle that are not subject to TB testing and slaughter regimes [4, 6]. Goats may become infected with **M.**

bovis when sharing pastures with infected cattle, at watering points, market places and shared night shelters [21].

In Ethiopia, mixed farming of cattle and goats is a common practice with no restriction of livestock between regions and farms. Since, bovine TB is endemic in Ethiopia [22] this practice poses a high risk of inter- and interspecies transmission and spread of **M. bovis** infection [23]. A previous study [24] reported a prevalence of 4.2% TB in Ethiopian goats and reported the occurrence of the disease for the first time in Ethiopia. Other study [3] reported a prevalence of 3.1 and 9.6% in goats kept at Adami Tulu Agricultural Research Centre and house hold farmers, respectively.However, this study does not use comparative intradermal tuberculin test which was more confirmatory test than single interadermal tuberculin test. Therefore, the present study was designed to determine the prevalence of caprine tuberculosis in Adami Tulu Jido-kombolcha and its surroundings.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Adami Tulu Jiddo-kombolcha district, located in the East Showa zone of Oromia regional state. The zone covers the largest part of the mid-rift valley, which is about 165 km south of Addis Ababa. The district lies at latitude of 7.58ºN and 38.43ºE longitudes. Its agro-ecological zone is semi-arid and sub-humid in which 90% of the area is lowland while the remaining 10% is intermediate with altitude ranges from 1500 –2000 meter above sea level. The mean annual rainfall ranges from 750-1000mm and the distribution is highly variable between and within years [25].

The area has average maximum and minimum annual temperature of 27.2 and 12.7 ^{0}C respectively and a relative humidity of 60% [26-27].The total land area of the district is estimated to be about 75,223 hectares of which, 36,661 hectares is used for crop production, whereas, 17113 hectares is used for grazing. Livestock production is the dominant economic activity followed by crop production [28].

Study Animals

The study population represented goats from different smallholder farmers of Adami Tulu Jido-Kombolcha district. In the study area the predominant goats are managed under extensive production system. Traditional housing and grazing of natural pasture were the predominant husbandry practices, farmers who own more than five goats keep them in barns while those who own less than five goats keep them in their own house [3].

Study design

A cross-sectional study was conducted from November 2013 up to April 2014. The study was designed with the aim to determine the prevalence of caprine tuberculosis in Adami Tulu Jidokombolcha and its surroundings. Study methodology included comparative intradermal tuberculin test and post mortem examination of goat that were positive to the tuberculin test. Therefore, the present study was designed to determine the prevalence of caprine tuberculosis in Adami Tulu Jido-kombolcha and its surroundings by using comparative intradermal tubercline test.

Sample Size Determination

The sample size required for this study was determined according to Thrusfield [29].

$$n = 1.96^2 \times P_{exp} (1-P_{exp})$$

$$d^2$$

Where:n = the required sample size

Pexp= expected prevalence

D = desired absolute precision.

Therefore, by using estimated prevalence of 9.6% caprine tuberculosis in individual animal recorded by Tafesse *et al*. [3] on household farmer's goats and taking a confidence interval of 95% and 5% absolute precision, the minimum sample size required was 133 caprine sample. However, based on the above prevalence it was difficult to know the actual prevalence. So that, to be accurate and increase the probability of estimating the actual population prevalence for this study, it was mandatory to increase the former sample size by five folds which result in 665 goats at study area.

Sampling Strategy

A combination of multistage and purposive sampling method was used to select the study areas. First, target administrative zone was chosen based on its livestock potential mainly in goat. Secondly, one study district was selected from the administrative zone; the main criteria used were goat population. Thirdly, villages and farmers were selected purposively based on the inclusion criteria (accessibility, willingness of the farmers to participate in research and based on the previous information on problems). Finally, the required amount of caprine sample population was selected by simple random sampling technique.

Study Methodology Study methodology includes comparative intradermal tuberculin test and post mortem examination of goat that were positive to the tuberculin test. All procedure in selected study animal was carried out after properly restrained and thorough examination. During that all course of action was carried out aseptically using disposable gloves with great care to the animal.

Comparative Intradermal Tuberculin Test

This test is a confirmatory skin test to determine if a responder's positive CIDT test is more likely due to *M. bovis*or *M. avium* and carried out by injecting both bovine purified protein derivative (PPD) and avian PPD. Two sites on the skin of the mid-neck of the animal, 12 cm apart, were shaved, and skin thickness was measured with a caliper. One site was injected with an aliquot of 0.1mL of 2,500-IU/mL bovine PPD into the dermis, and the other was similarly injected with 0.1mL of 2,500-IU/mL avian PPD [30].

After 72 h, the skin thickness at the injection site was measured and recorded. Results were interpreted according to the recommendations of the Office International des Epizooties

at ≥ 4 mm cut-off and at ≥ 2 mm cut-off [31]. 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, 2<x ≤ 4 mm, and above, the animal was classified as negative, doubtful, or positive reactor based on CIDT test, respectively [32].

Post mortem examination

Post mortem examination was carried out on two tuberculin positive goats (increment in skin thickness >4mm) for detection of tuberculosis lesions and confirmation of tuberculosis in reactor goats at Bulbula veterinary clinic. The animals were purchased after we obtain permission from the owner to conduct the necropsy and use safeguards necessary for proper disposal of the carcass. The rubber boots, gloves, and coveralls were wearied before performing necropsy. The animal was slaughtered without any euthanasia by sharp knife at the jugular vein and flaying was done to access the internal organ. Then the abdominal and thoracic cavities were carefully opened to prevent contamination from the outside or from a cut organ.

The organs examined include lymph nodes (bronchial, mediastinal, mesenteric and hepatic), lungs, liver, omentum, intestine and kidney. These organs were inspected, palpated and then sliced finely for further investigation. Decontaminate self (e.g., disinfect and remove boots, gloves, and coveralls) and instruments before cleaning them, clean and disinfect all work surfaces and recording the necropsy findings were post-necropsy activities.

Data Analysis

The collected data was compiled, tabulated and analyzed in accordance with the objectives of the study. The raw data were entered and managed in Microsoft Excel worksheet and descriptive statistic is utilized to summarize the data. The point prevalence is calculated for all data by dividing positive samples by total number of examined samples and multiplied by hundred.

RESULTS

Result on PPD Test

In the current study, 665 goats of different sexes and ages were examined (comprising 538 were female and 127 were male) for the prevalence of bovine and avian tuberculosis by CIDT test. Out of 665 goats, 50 (7.51%) respond to CIDT test for both bovine and avian PDD and suspected or doubtful cases were 12 (1.8%). Among the positive case responded for bovine and avian tuberculosis CIDT test 46 (11.7%) were females and 4 (1.65%) were male. Moreover among the suspected case or doubtful bovine and avian tuberculosis CIDT test result 11 (2%) were female goats and 1 (0.8%) were males. The prevalence and positive and doubtful distribution result is summarized in (Table 1 and 2).

The present study results indicated that, out of 665 examined goats, 87 goats were young animals with the age less than one and half year and the rest 578 goats were adult with the age greater than one and half year, result in young goats indicated 2 (2.3%) and 48 (8.3%) adult goats were respond to CIDT test. Also 10 (11.5%) and 2 (0.35%) case were indicated doubtful result for young and adult goats respectively. The prevalence of positive and doubtful distribution result is summarized in (Table 3)

Bovine and avian tuberculosis CIDT test in relation to body condition score indicated, out of 665 examined goats, 148 goats had poor body condition, 396 goats had medium body condition and 121 goats had good body condition, the positive PPD test in body condition score indicated, 7 (21.1%), 38 (9.6%) and 5 (4.13%), were positive for poor, medium and good body condition goats were respond to PPD reagent, respectively. The prevalence of positive and doubtful distribution PPD results in relation to body condition score is summarized in (Table 4).

Moreover, this study result was indicated PPD test result in relation to parity of goats, out of 665 examined goats, 187 goats had no delivery, 211 goats had two parities, 193 had three parity and 74 goats had four parities. The overall percentage indicated 7 (3.7%), 11 (5.2%), 22 (11.4%) and 10 (13.5%) were reactor cases for zero parity, two parities, three parities and four parities respectively and also finding indicated 3 (1.6%), 3 (1.4%), 4 (2.1%) and 0 (0%) were suspected cases for zero parity, two parities, three parities and four parities respectively. The prevalence of positive and doubtful distribution PPD results in relation to parity number is summarized in (Table 5).

Postmortem Lesions in Tuberculin Reactor Goats

Of the total 665 goats subjected to CIDT test, 4 were found to be strong reactors and two were slaughtered for post mortem investigation (Figure 1). The lungs and lymph nodes of these 2

Table 1. Skin test positivity at ≥ 4mm and ≥ 2mm cut-off point for avian PPD based on sex.

Sex	№tested	№ Positive (%)	Doubtful (%)
Female	538	46 (11.7)	11 (2)
Male	127	4 (3.15)	1 (0.8)

Table 2. Skin test positivity at ≥ 4mm cut-off point for bovine PPD based on sex.

Sex	№ tested	№ Positive (%)	Doubtful (%)
Female	538	46 (11.7)	11 (2)
Male	127	4 (3.15)	1 (0.8)

Table 3. Skin test positivity at ≥4 mm and ≥ 2 mm cut-off point for based on age.

Age	№tested	№ Positive (%)	Doubtful (%)
<1.5	87	2 (2.3)	10 (11.5)
>_1.5	578	48 (8.3)	2 (0.35)

Table 4: Skin test positivity at ≥4 mm and ≥ 2 mm cut-off point for BCS difference.

Body condition score	№tested	№ Positive (%)	Doubtful (%)
Poor	4	87 (21.1)	4 (2.7)
Medium	9	638 (9.6)	5 (1.3)
Good	2	15 (4.13)	3 (2.5)

Table 5. Skin test positivity at ≥ 4 mm and ≥ 2 mm cut-off point based on parity number.

Parity	№tested	№ Positive (%)	Doubtful (%)
0	187	7 (3.7)	3 (1.6)
>_2	211	11 (5.2)	3 (1.4)
2 < x >_ 4	193	22 (11.4)	4 (2.1)
> 4	74	10 (13.5)	0

Figure 1 Skin fold after 72h injection of PPD.

CIDT positive goats were removed and examined for the presence of gross TB lesions. The carcass including internal organs and lymph nodes were examined under a bright-light source. Each of the seven lobes of the lungs were thoroughly inspected and palpated for any suspicious gross TB lesions. Similarly, mandibular, retropharyngeal, cranial and caudal mediastinal, left and right bronchial, hepatic, and mesenteric lymph nodes were sliced into thin sections and inspected for the presence of visible lesions.

The lungs, liver and lymph nodes were cut into approximately 2 cm thick slices using separate sterile scalpel blades. The cut surfaces were examined visually under bright light for the presence of lesions compatible with TB. Accordingly, liver (Figure 5), large intestine wall (Figure 2), omentum (Figure 4), both right and left lung lobes (Figure 3), cranial mediastinal lymph nodes and mesentry tissues were among the tissues with gross nodular lesions suggestive of TB. When gross lesions suggestive of TB were found in any of the tissues, the animal was classified as having lesions.

DISCUSSION

Little information is available on TB of goats in Ethiopia even though bovine TB is known to be endemic in cattle of Ethiopia [33]. In this study, out of 665 goats, 50 (7.51%) respond to CIDT test for both bovine and avian PDD and suspected or doubtful cases were 12 (1.8%). The result was slightly in agreement with Tafesse *et al*. [3] having prevalence of 9.6% at the same study area on household level. However, this result was different from a prevalence of 3.1% in goats with single intradermal tuberculin skin test in Adami Tulu Research Center. This difference might be related with the difference in study methodology in which the former study uses single intradermal tuberculin test and the area is known for goat production and they keep large number of goats in barn with young cattle at night. This increases the risk of transmission of the disease from infected cattle to goat [3] and from goat to goat. In contrast, recent study [33] carried out in central Ethiopia using CIDT showed a low prevalence of tuberculosis (0.41% at 2 mm cut-off point). This difference might be related, the difference in geographical location of the two studies in which the epidemiology of the disease might vary between these areas and difference in population of study animal. Even though, a prevalence of TB in such study area was low, Hiko [34] and Nigussie [20] have shown the importance of the disease at different abattoirs in Ethiopia. **The prevalence of TB in goats recorded by the present study is different from the previous study [35]** in small ruminants at Chifra district of Afar pastoralist area.

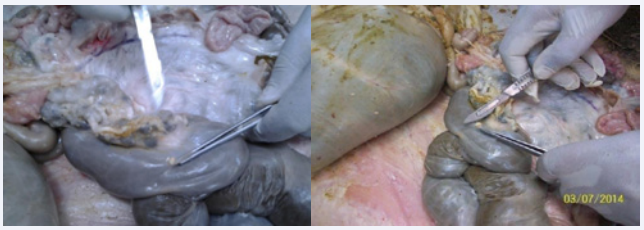

Figure 2 Tuberculin lesion on intestinal wall.

Figure 3 TB lesion on right diaphragmatic lung of CIDT in positive goat.

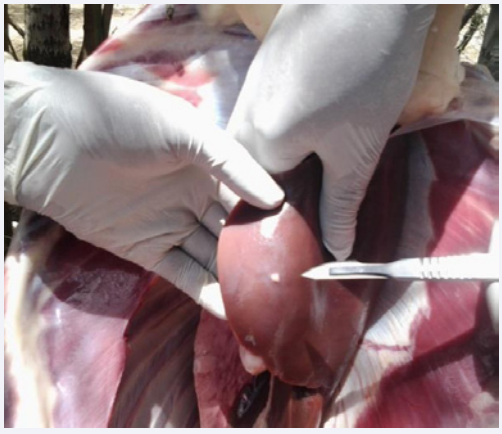

Figure 4 Tuberclin lesion on liver.

Figure 5 Tubercle lesion on omentum.

Different studies show different prevalence with corresponding time and study area or country. Abattoir based studies show the prevalence of 4.2% [20] and 3.5% [36] in study conducted at Mojo Modern Export Abattoir and Luna Modern Export Abattoir, respectively. A similar prevalence (4.3% in 1,152 goats) was recorded based on gross lesions at Helmex Export Abattoir [20], which were different (low prevalence) from present study. This might be because of the study animals in abattoir comes from different geographical areas and which the epidemiology of the disease might vary between these areas.

Females in the present study have higher prevalence than males, which was in agreement with that of bovine TB prevalence based on sex difference **could be due to the small number of observation of male cattle, as the male animal population in pastoral society is minimal [35]. In the present study the difference might be also related to sample size difference** which estimated to 81% of sample size were females and the fact that higher life expectancy and stress due to pregnancy and milk production that depress their immunity making them susceptible to the disease.

In this study, older goat showed higher proportion of positivity as summarized in Table: 3 of 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. Other researchers in cattle [32] have reported similar results. In addition, Female animals with more parity number showed higher proportion of positivity as indicated in Table: 5 of tuberculin test results than in those with lower parity number. This might be related to the age of the animals, as animals with high parity number were older in their age which increases their chance of exposure to mycobacterial infection in their longer life time [35].

The current study supports the endemic nature of Caprine TB and since midrift valley pastoralists predominantly depend on consumption of raw animal products, including milk and meat, that reminds the potential zoonotic risk of TB to humans in the district.

CONCLUSIONS AND RECOMMENDATIONS

The overall percentages of reactor animals in CIDT test were 7.51% for avian TB and bovine TB. In general, the overall percentage indicated, 46 (11.7%), 48 (8.3%), 10 (13.5) and 7 (21.1%) were reactor cases for the female, the older, female with high parity number and the poor BCS animals, respectively. The CIDT test is the primary screening test used to identify potentially infected with bovine tuberculosis. Since it is a screening test, results are not considered absolute proof that an animal or herd has bovine TB. Therefore, it is used to identify animals and herds that need to undergo further testing with Culturing and identification of Mycobacteria, and Serum immunoglobulin detection for bovine and avian TB. Further investigation should be done to elucidate its epidemiological significance for public health and control of the disease in the district in addition to conducting immediate and appropriate control program against bovine tuberculosis infection.It is highly important to integrate research and disease surveillance programs in-between the medical and veterinary institutions, to minimize zoonotic threat of tuberculosis and the government should work on behavioral and cultural changes of the people from consuming raw milk and meat to minimize zoonotic infection of tuberculosis.

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