

Epidemiology and Molecular Characterization of Causative Agents of Bovine Tuberculosis in Ruminants

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Abstract

A cross sectional study was conducted on 110 cattle and 397 small ruminants to determine the prevalence of bovine tuberculosis and possible risk factors, and to characterize the species of *Mycobacteria* circulating in Chifra district. Bacterial isolation and multiplex Polymerase Chain Reaction (PCR) were performed on milk and nasal swab samples of reactor animals. In tuberculin test, 13.64% cattle and 5.29% small ruminants were positive, and 31.58% and 25.00% were positive cultures on Löwenstein-Jensen media from milk and nasal swab samples, respectively. Based on PCR products, 12 were positive for genus *Mycobacterium* and none were positive for *Mycobacterium tuberculosis* complex or *Mycobacterium avium-intracellulare* complex group. The reactor rates observed for cattle under different body condition scores were poor (17.24%), medium (6.25%) and good (50.00%) Body Condition Scores (BCS) ($P=0.025$). The stepwise logistic regression analysis using independent variable medium BCS as a reference category indicated that good BCS (adjusted OR=4.29, 95% CI for OR=0.49-37.89) significantly affected tuberculin reactivity. This study showed that the prevalence of Single Comparative Intra-dermal Tuberculin (SCIDT) test positives and risk of acquiring the disease, increased with good BCS. Thus, more sensitive diagnostic techniques and control strategies should be considered on this risk group.

Keywords: Bovine tuberculosis; Molecular characterization; Single comparative intra dermal test

Introduction

Bovine Tuberculosis (BTB), caused by *Mycobacterium bovis* (*M. bovis*), is a well known zoonotic disease, which affects cattle worldwide, especially in developing countries, because of deficiencies in preventive and/or control measures, poor sanitation and health care [1,2]. It is of great socio-economic and public health importance and of significance in international trade of animals and animal products [3]. There were around 10 million new cases of human TB and 2 million deaths were reported annually [4], with sub-Saharan Africa displaying the highest annual risk of infection, probably aggravated by the expanding HIV epidemic and increasing drug resistance. Globally, TB causes more adult death than any other single infectious disease, 95% of cases and 98% of deaths occur in the developing world [5].

Ethiopia is among the 22 high TB-burden countries that accounts for 81% of estimated cases [6]. Over a third of the population has been exposed to TB. An estimated 377,030 Ethiopians (0.62% of the population) have active TB of all kinds. In 2005 alone, the number of deaths in all cases of TB, including HIV positive patients was 56,456, and that of all cases, excluding HIV positive patients, were 42,508 deaths [7]. The prevalence of BTB in Ethiopia is high and molecular typing of *M. bovis* has also indicated the existence of unique strains [8]. Human infection due to *M. bovis* is thought to be mainly through drinking of contaminated or unpasteurized raw milk. The high prevalence of TB in cattle, close contact of cattle and humans, the habit of raw milk and meat consumption, and the increasing prevalence of HIV may all increase the potential for transmission of *M. bovis* and other *Mycobacteria* between cattle and humans [9].

Very limited research has been conducted in the Afar National Regional State (ANRS) to determine the prevalence rate of Bovine Tuberculosis, except for the reports of Yalelet [10] and Hussein [11], in Afambo and Amibara districts, respectively. Despite the large number of livestock population in the district, there is lack of information on

BTB in Chifra district. In the district, there are several risk factors that promote transmission of *M. bovis* from animals to animals and animals to humans, because of consumption of raw milk, close physical contact between human and livestock, frequent animal movements across the different districts, sharing of communal grazing lands and watering points by animals from different herds. Therefore, it is important to generate epidemiological data on BTB in Chifra district. Thus, the present study was conceived with the following objectives; to estimate the prevalence of BTB in cattle, goats and sheep in Chifra district, to assess possible risk factors for infection and transmission of the causative agents, and to characterize the species of *Mycobacteria* circulating in ruminants.

Materials and Methods

Study area and population

The study was conducted in Chifra district in administrative zone one of ANRS. It is one of the 32 districts in the ANRS and located 607 km from Addis Ababa. The district, with a total area of 12,444 km² (sharing 13.13% of the total area of the region), and divided into 2 agro-climatic zones: arid (Assgura), and semiarid (Mesigidoo), each sharing 72% and 28% of the total area of the district, respectively. The district shares boundary with Werebabo district of the Amhara Regional States in the West, on the South by Mile district of zone one, on the North by

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Ewa and Awra districts of zone four, and on the East by Dubti district of zone one of the ANRS as shown in figure 1.

The district receives a mean annual rainfall of 555 mm on the Western edge of the escarpment, and 225 mm on the lava plain and volcanic ash with three rainy seasons, where only camels and goats are reared. The maximum and minimum annual temperature of the area is 38°C and 21°C, respectively. The altitude of the district ranges from 980 to 1000 m, above sea level. The district has 19 pastoralist associations, of which six of them were agro-pastoralists and the remaining were purely pastoralists. 90% of the district population is leading a pastoral life by rearing camels, cattle, goats, and sheep. Central Statistical Agency [12], estimated that a total of 352,346 cattle, 342,286 sheep, 306,720 goats, 126,349 camels and 24,977 donkeys were kept in the district. Ruminants (cattle, sheep and goats) were the targeted study animals for this study.

Study design, sample size and sampling techniques

A cross sectional study design was used to determine prevalence of bovine tuberculosis on ruminants in Chifra district. The variable of interest was the occurrence of TB on live animals. The explanatory variables considered were pastoralist associations, age, body condition, herd/flock size, sex, reproductive status, parity number, lactating status, and species in case of small ruminants. The sample size was determined following the formula by Thrusfield [13], for simple random sampling, and the sample size required was calculated based on 95% confidence interval, 5% desired precision. The calculated sample sizes were 110 and 397 for cattle and small ruminants, respectively, using the report of Yalelet, as reference prevalence rate.

Due to the clustering of animals/herds in a pastoralist association, a multi-stage random sampling technique was designed to select study animals. A complete list of pastoralist associations was obtained from the district head-quarter and five pastoralist associations were selected randomly, so as to study the prevalence of BTB and subsequently, milk and nasal swab samples were collected from reactor animals (n=36), for bacterial isolation. At each pastoralist association, one sub-division was selected at random to represent the pastoralist association.

Study methodology

Animals belonging to selected pastoralist associations were

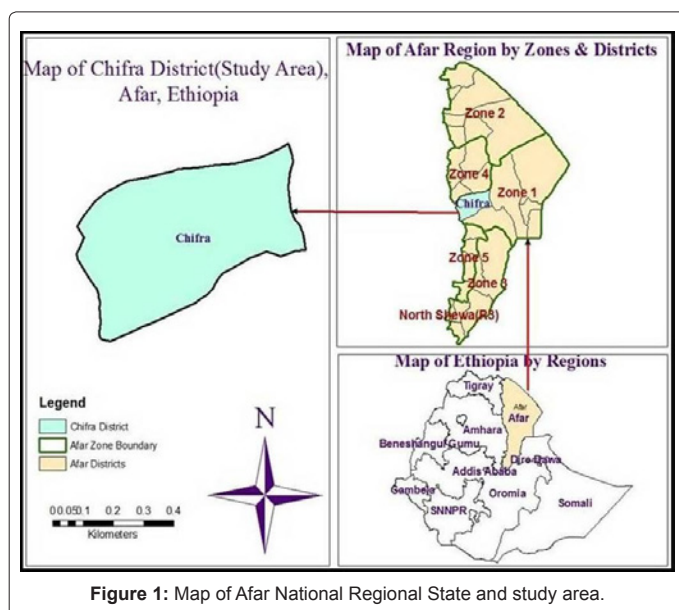


Figure 1: Map of Afar National Regional State and study area.

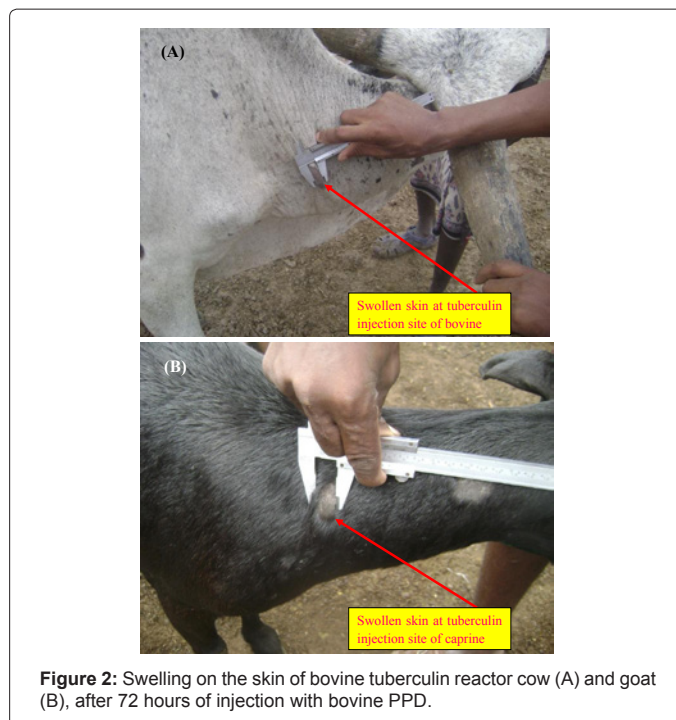


Figure 2: Swelling on the skin of bovine tuberculin reactor cow (A) and goat (B), after 72 hours of injection with bovine PPD.

individually identified by names, which were previously given by their owners or assigned on the day of tuberculin inoculation, using identification numbers temporarily written on their back. Their age, Body Condition Score (BCS), sex, lactating status, reproductive status, and skin test measurements were recorded on prepared format sheet. Body condition score of cattle was determined using the method of Nicholson and Butterworth [14], and age determination using the method of Dyce et al. [15]. For small ruminants, BCS was based on the Langston University method [16], and age determinations were based on ESGPIP method [17,18].

Single Comparative Intra-dermal Tuberculin (SCIDT) Test

Two sites on the skin of the right side of mid neck of the study animals, 12 cm apart, were shaved inspected, and skin thickness was measured in millimeters with digital caliper, before the injection of tuberculin. One site was injected with an aliquot of 0.1 ml containing 2500 IU/ml bovine Purified Protein Derivative (PPD) (Veterinary Laboratories Agency, Addle stone, and Surrey KT15 3NB, U.K.). Similarly, 0.1 ml of 2500 IU/ml avian PPD of the same agency was injected into the second site. After 72 hours, the skin thicknesses at both injection sites were measured (Figure 2). Animals showing increase in skin thickness at the injection site for bovine PPD of 2 mm more than the avian PPD were considered to be positive [19,20].

Mycobacterial culture

Nasal swab samples were collected from both nostrils of reactor animals (n=36) by using sterile swab, and placed in leak proof universal bottle, which contains sterile physiological saline solution and labeled with the animal's code number and date of collection. The samples were collected for isolation and identification of *Mycobacterium* species. The samples were kept at 4°C, until being transported to Aklilu Lemma Institute of Pathobiology (ALIPB). The samples were cultured and decontaminated using 4% NaOH solution, agitated in a vortex mixer for 15 min at room temperature, and centrifuged at 3000 rpm for 15 min.

The supernatant was removed and the sediment suspended in 2 ml of sterile physiological saline solution. One to two drops of 0.05% phenol red indicator was added to indicate pH change, and then neutralized using concentrated (0.1 N HCl), until the color changed to yellow. The sediment was inoculated onto two slants of L-J media, one enriched with pyruvate, to enhance growth of *M. bovis*, and the other enriched with glycerol, to suppress growth of *M. bovis* and stimulate the growth of *M. tuberculosis*. Finally, isolates were harvested for molecular typing analysis by scrapping the growth from a slope into 200 µL of sterile distilled water, and heating at 80°C for 45 min [21], and the harvest was kept at -20°C, until molecular analysis.

About 30 ml milk samples of the last few streams of milk were aseptically drawn into sterile universal bottle from the four quarters of each PPD reactor animals (n=19) and transported immediately, and kept at 4°C at Chifra district health center, until they were transported. Then, they were transported to ALIPB for bacteriological examination and kept at 4°C. The samples were added into sterile centrifuge tube and centrifuged at 3000 rpm for 15 min at room temperature. The sediment was suspended to 2 ml of sterile physiological saline solution and decontaminated with equal volume of sterilized 4% NaOH solution, centrifuged, and neutralized using concentrated HCl, using one or two drops of 0.05% phenol red as an indicator. After neutralization, the sediment from each sample was inoculated onto two slants of L-J media, as in nasal swabs. The culture was incubated at 37°C, and was observed for bacterial growth for 8 weeks, according to Kazwala et al. [22].

Multiplex Polymerase Chain Reaction

Multiplex PCR as molecular technique differentiates MTBC from *M. avium*, *M. intracellulareae*, and other mycobacterial species. *Mycobacterial* genus typing was conducted [23]. Heat killed AFB positive sample DNA was used as source of DNA template. The PCR targets the sequence of the genus *Mycobacterium*, within the 16S rRNA gene (G1,G2) sequences, within the hyper variable region of 16S rRNA, that is known to be specific to *M. intracellulareae* (MYCINT-F) and *M. avium* (MYCAV-R), and the MTB70 gene specific for MTBC (TB-A, TB-1B).

The primers used were MYCGEN-F, 5'-AGA GTT TGA TCC TGG CTC GA 3'; (35 ng/µL), MYCGEN-R, 5'-TGC ACA CAG GCC ACA AGG GA 3'; (35 ng/µL); MYCAV-R, 5'-ACC AGA AGA CAT GCG TCT TG 3' (35 ng/µL); MYCINT-F, 5'-CCT TTA GGC GCA TGT CTT TA 3' (75 ng/µL); TB1-F, 5'-GAA CAA TCC GGA GTT GAC AA 3' (20 ng/µL); TB1-R, 5'-AGC ACG CTG TCA ATC ATG TA 3' (20 ng/µL) [24]. The reaction was carried out using thermal cycler (Applied Biosystems, GeneAMP 9700). The mastermix was heated for 10 min at 95°C, further 35 cycles of 1 min at 95°C, 1 min at 61°C, and 1.5 min at 72°C; and 10 min at 72°C. Each PCR tube consisted of 5.2 µL H₂O Qiagen, 8 µL Hot Star Taq Master Mix, 0.3 µL of each of the six primers (concentration given above), and 5 µL of DNA templates of samples or controls, making the total volume 20 µL. *M. avium*, *M. intracellulareae*, H37Rv and 2122/97 *M. bovis* strain were used as positive controls, while H₂O Qiagen was as a negative control. The product was electrophoresed in 2% agarose gel in TAE running buffer 10X. SYBR Safe at a ratio of 1:10 in 2% agarose gel, 100 bp DNA ladder, and orange 6X loading dye were used in gel electrophoresis. All members of the genus *Mycobacterium* produce a band of 1030 bp, *M. avium* or subspecies, such as *M. avium* subsp. *paratuberculosis* produces a band of 180 bp, *M. intracellulareae* a band of 850 bp, while members of MTBC produce a band with 372 bp.

Data analysis

Descriptive statistics, chi-square (χ^2), univariate and multivariate logistic regression were performed to analyze the data, using SPSS version 15 for Windows. For all analysis performed, 95% CI and P-value<0.05 was set for statistical significance of an estimate.

Results

Bovine tuberculosis in cattle

Apparent prevalence and associated risk factors of bovine tuberculosis in cattle: Under the Single Comparative Intradermal Tuberculin test, screening of the tested animals for the other laboratory sample collection and processing of bacteriological and molecular characterization steps from the reactor animals. Of the associated risk factors considered, body condition score was found to be significant. The apparent prevalence of BTB based on a cut-off >2 mm in cattle is presented in table 1.

Logistic regression analysis of risk factors with bovine tuberculosis positivity in cattle: As shown in table 2, the univariate and multivariable logistic regression analysis of the putative risk factors

Factors (Variables)	No of animals tested	No of reactors (%)	χ^2 Value	P-value
Pastoralist associations				
Gerirona Wekello	31	5 (16.13%)		
Anderkellona Kelayitu	62	8 (12.90%)		
Wanebana Robellie	17	2 (11.76%)	0.24	0.89
Herd size				
X ≤ 20	73	10 (13.70%)		
X > 20	37	5 (13.51%)	0.01	0.98
Sex				
Male	25	2 (8.00%)		
Female	85	13 (15.29%)	0.87	0.35
Age in years				
X < 2	37	3 (8.11%)		
2 ≤ X ≤ 4	36	6 (16.67%)		
X > 4	37	6 (16.22%)	1.45	0.48
Body condition scores				
Poor	58	10 (17.24%)		
Medium	48	3 (6.25%)		
Good	4	2 (50.00%)	7.36	0.03
Lactating status				
Heifer	34	3 (8.82%)		
Lactating	28	5 (17.86%)		
Non lactating	23	5 (21.74%)	1.98	0.37
Reproductive status				
Heifer	30	3 (10.00%)		
Pregnant	27	5 (18.52%)		
Non pregnant	28	5 (17.86%)	1.01	0.60
Parity number				
X = 0	39	4 (10.26%)		
0 < X ≤ 2	20	4 (20.00%)		
X > 2	26	5 (19.23%)	1.42	0.49

Table 1: Association of assumed risk factors with single comparative intradermal tuberculin test positivity in cattle.

showed no statistically significant differences on tuberculin reactivity among animals, within each group of variables.

Bovine tuberculosis in small ruminants

Apparent prevalence and associated risk factors of bovine tuberculosis in small ruminants: The association of risk factors with tuberculin reactivity indicated a slight statistical significant difference between goat and sheep, in proportion of bovine tuberculin reactivity; while, other variables such as pastoralist associations, flock size, sex, age, BCS, parity levels, lactating and reproductive status of the animals did not show a statistically significant difference between each group considered (table 3), positive tuberculin reactor goat as shown in figure 2.

Logistic regression analysis of risk factors with bovine tuberculosis positivity in small ruminants: Multivariable logistic regression analysis showed that goats had 9.23 OR of being bovine tuberculin reactor compared to sheep (adjusted OR=9.23; 95% CI: 1.13-75.11), as shown in table 4.

Factors (Variables)	No of animals		OR	
	Examined	Positive for TB (%)	Crude (95% CI)	Adjusted (95% CI)
Pastoralist associations				
Gerirona Wekello*	31	5 (16.13%)	1	1
Anderkellona Kelayitu	62	8 (12.90%)	0.77 (0.23-2.59)	0.59 (0.14-2.53)
Wanebana Robellie	17	2 (11.76%)	0.69 (0.12-4.02)	0.47 (0.06-3.39)
Herd size				
X ≤ 20*	73	10 (13.70%)	1	1
X > 20	37	5 (13.51%)	0.98 (0.31-3.12)	0.9 (0.24-3.37)
Sex				
Male*	25	2 (8.00%)	1	1
Female	85	13 (15.29%)	2.08 (0.44-9.89)	1.68 (0.27-10.59)
Age (years)				
X < 2*	37	3 (8.11%)	1	1
2 ≤ X ≤ 4	36	6 (16.67%)	2.27 (0.52-9.86)	1.88 (0.39-8.95)
X > 4	37	6 (16.22%)	2.19 (0.5-9.53)	1.83 (0.36-9.35)
Body condition scores				
Poor*	58	10 (17.24%)	1	1
Medium	48	3 (6.25%)	0.32 (0.08-1.24)	0.32 (0.08-1.32)
Good	4	2 (50.00%)	4.8 (0.6-38.23)	4.29 (0.40-37.89)
Lactating status				
Heifer*	34	3 (8.82%)	1	1
Lactating	28	5 (17.86%)	2.25 (0.49-10.37)	
Non lactating	23	5 (21.74%)	2.87 (0.61-13.45)	
Reproductive status				
Heifer*	30	3 (10.00%)	1	1
Pregnant	27	5 (18.52%)	2.05 (0.44-9.52)	
Non pregnant	28	5 (17.86%)	1.96 (0.42-9.09)	
Parity number				
X=0*	39	4 (10.26%)	1	1
0 < X ≤ 2	20	4 (20.00%)	2.19 (0.48-9.87)	
X > 2	26	5 (19.23%)	2.08 (0.50-8.63)	

*Reference indicated as 1 in crude and adjusted odds ratios.

Table 2: Multivariable logistic regression analysis of tuberculin reactors with various host - related risk factors at 2mm cut-off point in cattle.

Factors (Variables)	No of animals tested	No of reactors (%)	X ² Value	P - Value
Pastoralist associations				
Gerirona Wekello	89	2 (2.25%)		
Anderkellona Kelayitu	187	9 (4.81%)		
Jarana Kontolla	64	5 (7.81%)		
Semsemina Addoo	57	5 (8.77%)	3.92	0.27
Species				
Sheep	77	1 (1.30%)		
Goat	320	20 (6.25%)	3.04	0.08
Flock size				
X < 75	206	11 (5.34%)		
X ≥ 75	191	10 (5.24%)	0.00	0.96
Sex				
Male	54	3 (5.56%)		
Female	343	18 (5.25%)	0.01	0.93
Age (years)				
X ≤ 2	126	5 (3.97%)		
2 < X ≤ 5	171	13 (7.60%)		
X > 5	100	3 (3.00%)	3.31	0.92
Body condition scores				
Poor	188	12 (6.38%)		
Medium	122	8 (6.56%)		
Good	87	1 (1.15%)	3.82	0.15
Lactating status				
Lamb/kid	48	2 (4.17%)		
Lactating	198	12 (6.06%)		
Non lactating	97	4 (4.12%)	0.62	0.73
Reproductive status				
Lamb/kid	31	1 (3.23%)		
Pregnant	111	5 (4.50%)		
Non pregnant	201	12 (5.97%)	0.59	0.75
Parity number				
X ≤ 1	107	7 (6.54%)		
1 < X ≤ 3	102	6 (5.88%)		
X > 3	134	5 (3.73%)	1.06	0.59

Table 3: Association of assumed risk factors with single comparative tuberculin test positivity in small ruminants.

Bacteriological culture and polymerase chain reaction

Mycobacteriological examination: Mycobacteriological culture examination on samples from SCIDT test tuberculin reactor animals (cattle, n=15 and small ruminants, n=21) showed that 31.58% (6) and 25% (9) were culture positives from milk and nasal swab samples, respectively. Acid fast bacilli were confirmed in all of culture positive colonies.

Genus typing (Multiplex-Polymerase Chain Reaction) for *Mycobacterium* species: Based on electrophoresis separation of PCR products, 12 are positive for genus *Mycobacterium* and none were positive for MTBC or *Mycobacterium avium-intracellulare* complex (MAC) group. They are Non-tuberculosis *Mycobacteria* (NTM) (Figure 3). Numbers 5, 6, 7 and 8 are milk, and 9 and 10 are nasal swab samples from cattle. Also, numbers 11 and 12 are nasal swab, and 13, 14, 15, 16, 17, 18 and 19 are milk samples from small ruminants.

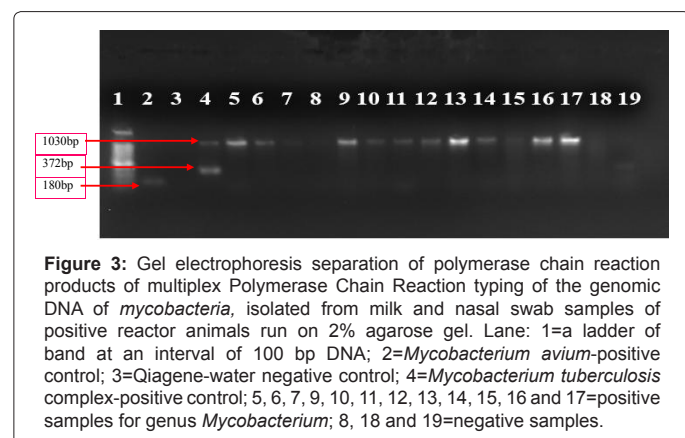
Discussion

The prevalence of BTB in the current study is similar to the report by Hussein [11] in cattle, and slightly higher than in goats of Amibara district. The overall prevalence obtained in cattle was higher than in some previous reports [10,25-28], in different pastoral areas of Ethiopia.

Factors (Variables)	No of animals		OR	
	Examined	Positive for TB (%)	Crude (95% CI)	Adjusted (95% CI)
Pastoralist associations				
Gerirona Wekello*	89	2 (2.25%)	1	1
Anderkellona Kelayitu	187	9 (4.81%)	2.2 (0.47-10.40)	4.9 (0.71-33.66)
Jarana Kontolla	64	5 (7.81%)	3.69 (0.69-19.60)	1.92 (0.34-10.92)
Semsemina Addoo	57	5 (8.77%)	4.18 (0.78-22.30)	7.54 (0.74-76.84)
Species				
Sheep*	77	1 (1.30%)	1	1
Goat	320	20 (6.25%)	5.07 (0.67-38.40)	9.23 (1.13-75.11)
Flock size				
X<75*	206	11 (5.34%)	1	1
X ≥ 75	191	10 (5.24%)	0.79 (0.29-2.16)	2.41 (0.53-11)
Sex				
Male*	54	3 (5.56%)	1	1
Female	343	18 (5.25%)	0.94 (0.27-3.31)	0.64 (0.16-2.43)
Age (years)				
X ≤ 2*	126	5 (3.97%)	1	1
2<X ≤ 5	171	13 (7.60%)	1.2 (0.69-5.74)	1.74 (0.58-5.21)
X>5	100	3 (3.00%)	0.75 (0.17-3.21)	0.68 (0.15-3.16)
Body condition scores				
Poor*	188	12 (6.38%)	1	1
Medium	122	8 (6.56%)	1.03 (0.41-2.60)	1.04 (0.40-2.71)
Good	87	1 (1.15%)	0.17 (0.02-1.33)	0.18 (0.02-1.5)
Lactating status				
Lamb/kid*	48	2 (4.17%)	1	1
Lactating	198	12 (6.06%)	1.48 (0.30-6.86)	
Non lactating	97	4 (4.12%)	0.99 (0.17-5.60)	
Reproductive status				
Lamb/kid*	31	1 (3.23%)	1	1
Pregnant	111	5 (4.50%)	1.42 (0.16-12.58)	
Non pregnant	201	12 (5.97%)	1.9 (0.24-15.19)	
Parity number				
X ≤ 1*	107	7 (6.54%)	1	1
1<X ≤ 3	102	6 (5.88%)	0.89 (0.29-2.75)	
X>3	134	5 (3.73%)	0.55 (0.17-1.80)	

*Reference category is indicated as 1 in crude and adjusted odds ratios.

Table 4: Multivariable logistic regression analysis of tuberculin reactors with various host-related risk factors at 2mm cut-off point in small ruminants.



Similarly, a lower prevalence was recorded in Uganda, as reported by Inangolet et al. [29]. The differences of the reports from the present study might be attributed to the epidemiological factors that favor

transmission of BTB in the Chifra district, as the district has a large area of communal grazing and watering points, high number of livestock from different districts of Afar region move seasonally into the district. This might increase the transmission of the diseases, and consequently, increase prevalence of the disease in the district.

On the other hand, the result of the present study was much lower than the higher prevalence of BTB reported in urban intensive dairy farms of Ethiopia, where Holstein and crossbreeds of cattle predominantly form the composition of the farms under intensive management system [8,30]. This difference might be mainly related to the intensive husbandry system practiced and breed susceptibility [8,20,31,32].

The overall prevalence of BTB in small ruminants in the present study is in agreement with some reports [33,34]. The results of the present study was different from the report on goats and sheep of central Ethiopia using SCIDT, which showed a low prevalence of BTB [35], and from the report which indicated the absence of the disease in goats of Hamar pastoral district of southern Ethiopia [26]. This difference might be related to the difference in geographical location, in which the epidemiology of the disease might vary. The prevalence of TB in goats recorded by the present study is higher than the previous report of Yalelet [10] in Afambo and Dubti, and of Hussein [11], in Amibara districts of ANRS. An assumption to explain this fact is that, the Afar pastoralists in Chifra district keep large numbers of goats together with cattle in the same grazing pasture, and this might increase the risk of transmission of the disease from infected cattle to goats. It was reported that goats acquire TB when they have close contact with cattle and share pasture with infected cattle [36].

The higher prevalence of BTB in female than male cattle could be due to the small number of observation of male cattle, as the male animal population in pastoral society is minimal. These may reflect greater productivity stress and longer life span among female animals. This study revealed lower reactor rates of age ≤ 2 years old, as compared to cattle of age >2 years old. As explained by different reports, including Radostits et al. [36], as age increases the probability of acquiring TB infection increases. The result was in agreement with previous studies where sheep TB has been reported [35,37-39]. Furthermore, in the multivariable logistic regression analysis, statistically significant difference was observed between goats and sheep.

In the present study, several animal level characteristics have been described as risk factors predisposing ruminants to BTB infection. However, none of the factors except BCS, in case of cattle, and species, in case of small ruminants, were found significantly associated with tuberculin reactivity of animals. Similarly, individual animal characteristics such as age, sex, pregnancy and lactation, were not significantly related with tuberculin reactivity of cattle.

The lower isolation rate of *Mycobacteria* may have resulted from reduced sensitivity of culture, arising from prolonged storage at field sites, and the freeze-thaw cycles that occurred during transportation and contamination of samples, as well as growth of other environmental *Mycobacteria* [40]. It is comparable with other findings [10,11], in which no growths of *M. bovis* from milk of cows positive to SCIDT test was reported. However, a predominant isolation of Non-tuberculosis *Mycobacterium* (NTM) from milk and nasal swab of tuberculin reactor animals from this study indicates the importance of these groups of *Mycobacterium* in the epidemiology of TB in Chifra pastoral district.

The current study supports the endemic nature of BTB, and further, indicates the importance of the disease in livestock of ANRS. Moreover, since Afar pastoralists predominantly depend on consumption of raw

animal products, including milk and meat, the current study highlights the potential zoonotic risk of BTB to humans in the region. In addition, identification of NTM from tuberculin reactor cattle and goats indicates their importance in the epidemiology of livestock TB, and which need to be further investigated, to design a control strategy in the region, in particular, and in the country, in general. Further study is recommended to extensively investigate the epidemiology of the diseases in wider sites, and larger sample sizes to identify the risk factors for infection and transmission of BTB among the livestock of ANRS, in order to design preventive and control strategies relevant to the pastoralists setting. Education and awareness creation among pastoralist community about the economic and public health significance of BTB will help to design a feasible community-based control programs.

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