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Original Research

Detection of Human and Bovine Tuberculosis Using an Existing Diagnostic Practice in Residential Districts of Tigray Region, Northern Ethiopia

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Abstract

Aim: A cross-sectional study was conducted from September 2012 to May 2013 to determine the prevalence of tuberculosis using the existing diagnosis practice and the concomitant risk factors allied with the outspread of infection in cattle and the potential risk of infection to humans.
Methods: Comparative intradermal tuberculin test was carried out to differentiate between cattle infected with *M. bovis* and those sensitized to tuberculin due to exposure to other mycobacterium or related genera.
Results: A total of 423 cattle were tested for tuberculosis and 49 (11.6%) and 31 (7.3%) were found infected with and without considering doubtful reactors as positives, respectively. The overall prevalence of bovine tuberculosis was 7.3% (31 out of 423). There was higher distribution of tuberculosis reactor animals in the herds of Adigudem district than in Hewane and Wukro districts with district level positive reactor prevalence of 8.7%, 8.1% and 6.4%, respectively. Type of herd (χ^2 = 12.98, P = 0.009), breed (χ^2 =5.68, P = 0.0001), body condition (χ^2 = 22.94, P =0.0001), presence of coughing animal in the herd (χ^2 = 139.36, P = 0.0001) and contact among herds (χ^2 = 14.74, P = 0.001) were found the major risk factors significantly associated with the occurrence of tuberculosis in cattle. A total of 228 humans were attended the health centers.
Conclusion: There was statistically significant association between the detection of acid-fast bacilli and residence in urban or rural, custom of raw milk and/or meat consumption, finding of skin nodule and origin of patients were found significantly associated with the finding of the organism responsible for extra-pulmonary tuberculosis. Awareness should be created among the people, diagnostic and treatment facilities should be established in villages and test-and slaughter policy should be designed to allow formulation of appropriate strategic control measures in order to reduce public health risks.

INTRODUCTION

Globally there is growing demand for livestock products, milk and meat a “livestock revolution”, and the livestock production is changing from a subsistence activity to a global food activity [1]. Total milk production has increased during the past 25 years, but not at the same rate as world human population (49% Vs 53%), and over 90% of the world milk supply is attributed to dairy cows [2]. Ethiopia ranked amongst African nations who possess the largest livestock population, with an estimated 41.5 million heads of cattle [3]. While the distribution and the quantity appear diverse according to the type of prevailing animal production systems and agro ecological zones, but prevailing of various contagious diseases are among the major socioeconomic drawbacks of the country’s cattle productions. Furthermore, the genetic improvement is becoming a growing concern being integrated with animal intensification, that there is introduction of diseases of various etiologies in several dairy farms concurrent with importation of exotic breeds. Tuberculosis becomes a serious problem in cattle when intensive dairying is established, particularly when European breeds are introduced [4].

The primary bacterium that causes tuberculosis in humans is *Mycobacterium tuberculosis*. Other species also cause tuberculosis in humans and in animals. *Mycobacterium bovis* (*M. bovis*) causes bovine tuberculosis and also has been shown to cause tuberculosis in humans. Because of its ability to infect both humans and animals, the control of tuberculosis is more difficult. The high incidence of tuberculosis and its associated mortality in Africa prompted investigation into the importance and implication of zoonotic tuberculosis in Africa [5]. The disease, usually characterized by formation of nodular granulomas known as tubercles in every tissue or organ of a body, of worldwide in distribution, attacking both man and animals, affecting all age groups and being ranked among disease that are responsible for more deaths than any other bacterial disease ever today. Sub-populations at risk of *M. bovis* infection include any population consuming un-pasteurized contaminated milk, abattoir workers, veterinarians, hunters, and HIV-infected or other potentially immunogenically-compromised populations [6]. Because of the chronic nature of the disease and the multiplicity of signs caused by the variable localization of the infection, tuberculosis is difficult to diagnose on clinical examination [7]. Although other tests such as the gamma interferon assay and lymphocyte transformation tests are available [8], the intradermal tuberculin test (IDT) with purified protein derivative (PPD) is the principal diagnostic test for bovine tuberculosis in animals [9]. The few studies so far conducted in the country on Bovine tuberculosis revealed prevalence rates ranging from 3.4% in small holder production systems to 50% in peri-urban intensive dairy farms. Hence, economic losses associated with bovine tuberculosis have not been determined fully [10, 11].

Bovine tuberculosis of cattle remains to be great concern due to the susceptibility of humans to the disease caused by *M. bovis* and there is increasing evidence that *M. bovis* infections may be much more significant than generally considered. In Sub-Saharan Africa, nearly 2 million tuberculosis cases in humans occur each year; yet it is unknown what role Bovine tuberculosis plays in the rising epidemic of tuberculosis fostered by HIV/AIDS [12]. A varying portion of pulmonary tuberculosis cases are considered to occur, however, almost all cases of the non-pulmonary type of tuberculosis in humans has been caused due to Bovine tuberculosis. Bovine tuberculosis in the human population mainly takes place through drinking raw milk and occurs in the extra-pulmonary form in the cervical lymphadenitis form in particular. Recently, Kidane *et al.* [13] indicated that *M. bovis* was found to be a cause for tuberculous lymphadenitis in 17.1% of 29 human tuberculosis cases in Ethiopia.

One of the main strategies to control tuberculosis is to find and treat people with active disease. Unfortunately, the case detection rates remain low in the study districts. Thus, it needs intervention to find and treat sufficient number of patients to control tuberculosis. Ailments and production loses in intensive dairy and fattening farms are increasing and the habit of consumption of raw foods of animal origin. Available studies on tuberculosis are few and even do not provide detail epidemiological information in every districts of Tigray region and the prevalence of the disease has not been well established and there is a lack of information on the epidemiology and zoonotic significance of *M. bovis* in the Tigray region in particular. The circumstances that promote the transmission of tuberculosis among different species of animals as well as between animals and man are still vague to the livestock owning society. Therefore, on the ground of the aforementioned justifications, the objectives of this study were to determine the status of tuberculosis in dairy and fattening farms and field cattle herds of the study area; to observe the occurrence of human tuberculosis based on the existing means of detection of tuberculosis in the study area and to identify risk factors associated with the occurrence of tuberculosis in human and animals and quantify their degree of association.

MATERIALS AND METHODS

Description of study area

Tigray region is the northernmost of the nine ethnic regions of Ethiopia containing the homeland of the Tigray people. Wukro, Adigudem and Hewane districts are three of the 36 districts in the Tigray Region of Ethiopia. Wukro district is located in the Eastern zone of the Tigray region and this district has a total population of 30,210. Adi-gudem district is located at 13°14′50"N and 39°-53°E with an elevation of 2100 meters above sea level and has human population is 90,674. Hiwane district is located at 13°19′54"N and 41°-57°E with an elevation of 2100 meters above sea level and the human population is 153,505 [14]. The cattle population of Wukro, Adigudem and Hewane were 24,804, 13,462 and 6,572, respectively [15].

Study populations

The study animals were cattle of household farmers and the human population of the study areas. The cattle populations of Wukro, Adigudem and Hewane districts were 24,804, 13,462 and 6,572, respectively. Calves with average ages below 6 months, cows in late pregnancy and recently calved, extremely emaciated were excluded from the study for fear of immune dysfunction, false positive and false negative results, that usually occurs in dairy cows starting around 3 weeks pre-calving to 3 weeks post-calving. Persons with mental problems and disabled patients showing clinical symptoms of tuberculosis and/or patients with skin nodule or cervical lymph node swelling which discharges pus were included in the study.

Study design

A cross-sectional study was conducted from September 2012 to May 2013 to determine the prevalence of tuberculosis and the concomitant risk factors associated with the outspread of infection in humans and animals. Tuberculosis suspected cases were included in the study.

Sampling method

Three residential districts were selected conveniently based on their ease of accessibility. The dairy farms, fattening farms, field cattle herd sites in each areas were registered and 30% of them were proportionally selected considering the feasibility and financial affordability of the study. Simple random sampling method was used to select the farms and cattle herd sites and then individual animals were then randomly selected to diagnose for Bovine tuberculosis (BTB). Herdsmen or family members with previous and current coughing symptom and cervical lymphadenitis were sent to visit the health centres for diagnosis. All patients with fever, coughing, weight loss, chest pain and night sweats or presenting with clinical signs and symptoms resembling tuberculosis were included in the study for the examination of sputum and/or aspirate samples from cervical lymph nodes of patients with suspected TB lymphadenitis (extra-pulmonary TB) and tested for the organism in target. A special medical and ethical consideration was given to under-age (<18 years old) clinical patents. After the informed consent approach has made; no refusal to participate was recorded. Proportional number of animal owners and/or patients showing symptoms of pulmonary and/or extra-pulmonary tuberculosis were included from each selected households and approached for the administration of the questionnaire (for literates) or interview (if illiterates) which result a total of 228 respondents. The questionnaire was designed to gather relevant information on the family size (less than 4, 5 to 7 or greater than 7 family members), residence (rural or urban), age, sex, educational status (literate or illiterate), consumption habit of raw milk and meat, duration of coughing (acute, sub-acute, persistent or no coughing), presence of skin nodule or swelling of cervical lymph nodes which discharges pus and finding of fever, coughing, weight loss, chest pain and night sweats.

Sample size determination

There was no previous year's prevalence of tuberculosis in the study districts. Therefore, the average expected prevalence rate was assumed to be 50% for the areas within 95% Confidence Intervals (CI) at 5% desired accuracy. Hence, the formula by Thrusfield [16] was used to calculate sample size (n). Thus the desired sample size for $P_{ex} = 0.5$ was n = 384. In order to increase the accuracy of the study results, it was increased by 10% to give 423 animals (384 + 10% of 384 = 384 + 39 = 423). These numbers of cattle were obtained proportionally from all of the randomly selected farms and field herds. The sampled animals were screened on their habitation and each individual study animal was recorded with its breed (Zebu breed, Cross (Holstein Frisian x Zebu) and Begayit breed), herd size from which animals were sampled (small: 2-5 animals, medium: 6-10 animals and large herd size: above 10 animals), age group (young: [6 months-3years], adult: (3-7 years], old: above 7 years) [17], origin (Wukro, Adigudem & Hewane districts), sex and body condition (poor, medium & good). Body condition score for each cattle was estimated according to the standard set by Nicholson and Butterworth [18]. Accordingly animals were grouped as good, medium and poor.

Comparative intradermal tuberculin test

Comparative intradermal tuberculin test (CIDT) was carried out in this study in order to differentiate between animals infected with *M. bovis* and those sensitized to tuberculin due to exposure to other mycobacterium or related genera. CIDT was performed by restraining the cattle and two sites on the skin of the mid neck were shaved and cleaned being separated by 10-15 cm on the same side of the neck; the areas were examined for the presence of any gross lesion.

The skin fold thickness at the two sites were measured in millimeters with a skin-caliper and recorded before injection. Each animal was then be injected with 0.1ml (28,000 IU/ml) of bovine purified protein derivative /PPD/ (Bovitubal, strain AN_5, Bioveta Czech Republic) and 0.1ml (28,000 IU/ml) of avian PPD (Avitubal, strain D_4ER, Bioveta, Czech Republic) intradermally using semi-automatic syringe at the anterior and posterior parts, respectively. The injection sites were examined and the skin thicknesses were measured 72 hours post injection. When the difference in skin thickness is greater at the site of avian PPD inoculation than the bovine PPD inoculation site, an animal was considered as a non-specific reactor; whereas, the skin thickness increased at both sites and/or at the site of bovine PPD injection, the result was interpreted according to manufacturer's instruction as follows: $B_2 - B1 = \triangle B$, $A_2 - A1 = \triangle A$;

$\triangle B - \triangle A \leq 2mm$: Negative for *M. tuberculosis* complex; $\triangle B - \triangle A =$ (2mm-4mm): Doubtful for *M. tuberculosis* complex, and $\triangle B - \triangle A \geq 4mm$: Positive for *M. tuberculosis* complex. Where: A1 = skin thickness before inoculation of avian PPD, B1 = skin thickness before inoculation of bovine PPD, A2 = skin thickness after inoculation of avian PPD, B2 = skin thickness after inoculation of bovine PPD, $\triangle A$= Change in skin thickness at the site of avian PPD inoculation, and $\triangle B$= Change in skin thickness at the site of bovine PPD inoculation.

Microbiological examination of samples

Animal owners and/or suspected TB patients were requested to submit sputum samples for acid fast bacilli (AFB) examination and fine needle aspirated (FNA) samples from skin nodules or cervical lymph nodes of patients with suspected TB lymphadenitis. Sputum samples were collected using sterile universal bottles and in ice box (+4^{o}C) to the laboratory and stored in a freezer (-20^{o}C) until processing. In every step of the bacteriological procedure, biohazard safety cabinet level III was used and all laboratory safety measures were followed. Examination of Acid-Fast Bacilli from Ziehl-Neelsen stained smears of sputum or fine needle aspirated (FNA) samples was used as a presumptive diagnosis of mycobacterial disease. Hence, sputum and/or cervical lymph node specimens were obtained promptly with a medical research partner for proper technique and testing the specimens for microbiological examination in the microbiology laboratory of the college of health sciences and at the local health centers of the study areas. The FNA procedure was conducted with a medical pathologist at the Ayder Referral Hospital of Mekelle University. The FNA was performed using a 20-22 gauge needle with an attached 10ml syringe which was mounted on an aspiration cameco gun. During each pass, the needle was moved throughout the lesion several times while aspirating. Cervical, axillary, inguinal and occasionally extranodal areas were aspirated. Since other objects can stain acid-fast (i.e. Nocardia, fungal spores, cellular debris, etc) a slide was not be reported out as being positive for acid-fast bacilli unless at least three morphologically correct AFB are seen in the smear. In an instance where less than three AFB were seen per field of vision, it was suggested to reexamine the smear, to make several more smears from the specimen, stain and examine.

Questionnaire approach

A structured enquiry was administered to willing potential literate animal owners and/or suspected TB patients, in the form of questionnaire, and to illiterate ones, in the form of an interview, regarding family size, residence, sex, age, education status and habit of consumption of raw foods of animal origin. A total of two hundred twenty eight (N=228) individual animal owners were sampled and the respondents were proportionally sampled from the three study districts. All sampled respondents were asked in order to gather necessary information related to the variables of interest of the study and to assess potential risk factors associated with the occurrence of zoonotic and human and they were requested to submit samples for microbiological examination.

Ethical considerations

The study with human subjects was conducted in confirmation with the guidelines of the declaration of National Health Research Ethics Guideline of the Tigray Science and Technology Agency. To ascertain confidentiality, data were collected and recorded in anonymous manner. Administrative bodies of the study areas were informed about the aim and scope of the study. Herders were asked for participation one point in time in this one year study. Written informed consent was obtained from all participants in the study and the consent forms were typed in local languages for ease of administration of the information to target groups.

Data analysis

The data were summarized and compiled by summing up the findings of the study subjects. An animal and human was said to be positive if it tested positive to CIDT and AFB and FNA smears, respectively. Individual study subject disease prevalence was calculated on the basis of test positive results divided by total number of human or cattle tested. Coded data were stored in Microsoft Excel 2007 spread sheet and transferred to SPSS® Version 20 software for statistical analysis and descriptive and analytic statistics were computed. Multinomial logistic regression and Chi-square test (χ^2) were computed to see the association of risk factors with that of test-positivity to TB and the degree of association was calculated using odds ratio (OR) and 95% confidence interval (CI). Odds ratio (OR), a measure risk estimate, was calculated for the variable with a chi-square (χ^2) >3.84 and P-value <0.05 which was considered as statistically significant association between variables. Odd ratio (OR) was used to indicate the degree of association of risk factor with the disease occurrence signified by 95% confidence intervals.

Table 1. Comparative intradermal tuberculin test reactor animals in the study districts

Study area	No. examined	Positive reactors (%)	Doubtful reactors (%)	Positive & doubtful reactors (%)
Adigudem district	127	11 (8.7)	4 (3.1)	15 (11.8)
Hewane district	62	5 (8.1)	2 (3.2)	7 (11.3)
Wukro district	234	15 (6.4)	12 (5.1)	27 (11.5)
Total	423	31 (7.3)	18 (4.3)	49 (11.6)

Table 2. Multinomial logistic regression analysis of CIDT positivity and allied risk factors (N = 423)

Variables	No. tested	Positive reactors (%)	Doubtful reactors (%)	Positive & doubtful reactors (%)	χ^2	P-value	OR (95% CI)
Study area							
Adigudem district	127	11 (8.7)	4 (3.1)	15 (11.8)			
Hewane district	62	5 (8.1)	2 (3.2)	7 (11.3)	1.50*	0.833	
Wukro district	234	15 (6.4)	12 (5.1)	27 (11.5)			
Type of herding							
Dairy farm/int/	116	17 (14.7)	4 (3.4)	21 (18.1)			5.67(1.41-22.76)
Fattening farm/int/	68	5 (7.4)	2 (2.9)	7 (10.3)	12.98*	0.009[a]	3.33(1.52-21.23)
Herds in field/ext/	239	9 (3.8)	12 (5.0)	21(8.8)			1
Sex							
Male	131	9 (6.9)	5 (3.8)	14 (10.7)	0.16**	0.924	
Female	292	22 (7.5)	13 (4.5)	35 (12.0)			
Breed							
Begayit	30	2 (66.7)	0 (0.0)	2 (66.7)			8.16 (1.32-15.1)
Cross	130	15 (11.5)	6 (4.6)	21 (16.1)	5.68*	0.0001[a]	1.75 (1.71-8.1)
Zebu	263	14 (5.3)	12 (4.6)	26 (9.9)			1
Age							
Young	87	3 (3.5)	3 (3.5)	6 (7.0)			
Adult	219	20 (9.1)	9 (4.1)	29 (13.2)	3.34*	0.500	
Old	117	8 (6.8)	6 (5.1)	14 (11.9)			
Herd size							
Small	86	4 (4.6)	3 (3.5)	7 (8.1)			
Medium	204	13 (6.4)	8 (3.9)	21 (10.3)	3.62*	0.458	
Large	133	14 (10.5)	7 (5.3)	21 (15.8)			
Body condition							
Good	110	0 (0.0)	2 (1.8)	2 (1.8)			1
Medium	134	10 (7.5)	4 (3.0)	14 (10.5)	22.94*	0.0001[a]	1.43 (1.37-5.56)
Poor	179	21 (11.7)	12 (6.7)	33 (18.4)			1.66 (1.62-16.9)
Presence of coughing animal?							
Yes	115	31 (27.0)	18 (15.6)	49 (42.6)	139.36*	0.0001[a]	2.21 (2.20-2.29)
No	308	0 (0.0)	0 (0.0)	0 (0.0)			1
Contact with other herds?							
Yes	261	9 (3.5)	12 (4.6)	21 (8.1)	14.74**	0.001[a]	2.05 (1.59-7.14)
No	162	22 (13.6)	6 (3.7)	28 (17.3)			1

[*] Fisher's exact chi-square test (χ^2): two cells have expected count less than 5. ext = extensive, int = intensive

[**] Pearson chi-square test, [a] statistically significant association at 95% Confidence Interval (CI)

[1] refers to the reference category

RESULTS

Bovine Tuberculosis (BTB)

A total of 423 cattle were tested for tuberculosis from three study districts and the results of the comparative intradermal tuberculin test (CIDT) in cattle of the study districts revealed that 49 (11.6%) and 31 (7.3%) were found TB infected with and without considering doubtful reactors as positives, respectively (Table 1).

The overall prevalence of animal tuberculosis tested positive was 7.3% (31 out of 423) and there was no statistically significant (χ^2 = 1.50, P = 0.833) association between the occurrence of tuberculosis in cattle and the origin of the study animals (Table 2). However, only positive reactors were considered in investigating the possible risk factors. There was higher distribution of tuberculosis reactor animals in the herds of Adigudem district than in Hewane and Wukro districts with district level positive reactor prevalence of 8.7%, 8.1% and 6.4%, respectively (Table 1).

The type of herding of animals (χ^2 = 12.98, P = 0.009), breed (χ^2 =5.68, P = 0.0001), body condition of cattle (χ^2 =22.94, P =0.0001), presence of coughing symptoms in the type of herding (χ^2 = 71.78, P = 0.0001), presence of coughing animal in the herd (χ^2 =139.36, P = 0.0001) and contact of the study herds with other herds (χ^2 =14.74, P =0.001) were found the major risk factors significantly associated with the occurrence of tuberculosis among the study animals of the study districts. On the other hand, herd size, sex of animal, age and the study area from which animals were sampled were not possible risk factors for the finding of comparative intradermal tuberculin test reactor animals (Table 2). The type of herding of animals in dairy farm, in fattening farm and herding in the field was found significantly (χ^2 = 12.98, 0.009) associated with the distribution of tuberculous cattle of the study districts. Dairy animals were found 5.67 times (P= 0.009, OR= 5.67, 95% C.I. = 1.41-22.76) more at risk of acquiring the disease than animals kept scattered in the field. Likewise, animals in fattening farms were 3.33 times (P= 0.009, OR= 3.33, 95% C.I. = 1.52-21.23) more at risk of acquiring tuberculosis than those always released to the field (Table 2).

There was statistically significant association (χ^2 = 5.68, P = 0.0001) between the occurrence of bovine tuberculosis and the breed of cattle. The type of breed called ‘Begayit’ was 8.16 times (P= 0.0001, OR= 8.16, 95% C.I. = 1.32-15.1) more at risk of being tuberculin reactor than the Zebu breed of the study areas. Similarly, the Cross (Zebu x Holstein Friesian) breed cattle were 1.75 times (P= 0.0001, OR= 1.75, 95% C.I. = 1.71-8.1) more at risk of acquiring the disease than the local Zebu breeds (Table 2). There was statistically significant association (χ^2 = 22.94, P = 0.0001) between the occurrence of bovine tuberculosis and the body condition of the study animals. The animals having poor body were 1.66 times (P = 0.0001, OR= 1.66, 95% C.I. = 1.62-16.9) more at risk of getting the infection than the animals having good body condition. Likewise, the animals with medium body condition score were 1.43 times (P= 0.0001, OR= 1.43, 95% C.I. = 1.37-5.56) more at risk of being tuberculin reactor than those having good body condition. The presence of coughing animals in the study herds in the field, dairy and fattening farms was significantly associated (χ^2 = 139.36, P = 0.0001) with the occurrence of tuberculosis in the study animals. The herds containing coughing animals were 2.21 times (P = 0.0001, OR= 2.21, 95% C.I. = 2.20-2.29) more at risk of acquiring the infection than those herds with no coughing animals in the group. Similarly, there was statistically significant association (χ^2 = 14.74, P = 0.001) between the herds which have made contacts with other herds in the field. Therefore, the herds which have made contacts with other herds in the field were 2.05 times (P = 0.001, OR= 2.05, 95% C.I. = 1.59-7.14) more at risk of acquiring bovine tuberculosis than those herds which do not have made close contacts (Table 2).

Tuberculosis in occupational groups

Examination of acid-fast bacilli from sputum samples

During the study period a total of 228 humans; 91 from Adigudem, 76 from Hewane and 61 from Wukro districts have been attended the health centers. Examination of acid-fast bacilli (AFB) from Ziehl-Neelsen stained smears of sputum was conducted to diagnose for pulmonary TB in three health centers. But, the fine needle aspirate (FNA) sampling from cervical lymph nodes of patients with suspected TB lymphadenitis (extra-pulmonary TB) was conducted only at the Ayder referral hospital. The origin of patient (χ^2 = 1.19, P = 0.550), sex (χ^2 = 0.004, P = 0.950), family size (χ^2 = 2.21, P = 0.340), age (χ^2 = 3.37, P = 3.37) and education status (χ^2 = 1.43, P = 0.231) of patients were not significantly associated with the finding of acid-fast bacilli (AFB) from Ziehl-Neelsen stained smears of sputum. On the other hand, there was statistically significant association between the detection of acid-fast bacilli organism and the residence of study groups in urban or rural (χ^2 = 3.86, P = 0.017), custom of raw milk consumption (χ^2 = 4.28, P = 0.027), raw meat consumption habit (χ^2 = 16.87, P = 0.0001), duration of coughing (χ^2 = 17.73, P = 0.0003) among suspected TB patients and the clinical finding of fever, chest pain, weight loss, night sweats (χ^2 = 4.39, P = 0.036) (Table 3).

Table 3. Multinomial logistic regression analysis of sputum AFB positivity and the associated risk factors (N = 228)

Independent variables	No. tested	AFB positive	Prevalence	χ^2	P-value	OR (95% CI) [b]
Origin of Patient						
Adigudem district	91	14	15.4%			
Hewane district	76	8	10.5%	1.19**	0.550	a
Wukro district	61	10	16.4%			
Sex						
Male	172	24	14.0%			
Female	56	8	14.3%	0.004**	0.950	
Family Size						
$\leq$ 4	91	12	13.2%			
5-7	124	20	16.1%	2.21*	0.340	a
$\geq$ 8	13	0	0%			
Residence						
Urban	111	12	10.8%	3.86**	0.017	1
Rural	117	20	17.1%			1.26 (1.19-1.71)
Age of patient						
Young	22	0	0%			
Adult	140	21	15%	3.37*	0.185	a
Old	66	11	16.7%			
Education status						
Literate	115	13	11.3%	1.43**	0.231	
Illiterate	113	19	16.8%			
Raw milk consumption						
Yes	47	9	19.1%	4.28**	0.027	1.45 (1.18-2.71)
No	181	23	12.7%			1
Raw meat consumption						
Yes	28	11	39.3%	16.87**	0.0001	3.96 (2.05-7.67)
No	200	21	10.5%			1
Duration of coughing						
Acute	25	7	28%			
Sub-acute	62	11	17.7%	17.73*	0.0003	
Persistent	87	14	16.1%			
No coughing	54	0	0.0%			

[*]Fisher's exact chi-square test (χ^2): two cells have expected count less than 5. [**]Pearson chi-square test.

[a]Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

[1] refers to the reference category.

[b] Odds ratio (OR), a measure risk estimate, was calculated for the variable with a chi-square (χ^2) >3.84 & P-value <0.05 which was considered as statistically significant association between variables.

Table 4. Multinomial logistic regression analysis for FNA positivity of cervical lymph nodes (extra-pulmonary TB) and the associated risk factors (N = 228)

Independent variables	No. tested	FNA positive	Prevalence	χ^2	P-value
Finding of skin nodule					
Yes	50	13	26%	22.8**	0.0001
No	178	0	0%		
Origin of patient					
Adigudem district	20	6	30%		
Hewane district	22	4	18.2%	6.32*	0.017
Wukro district	8	3	37.5%		
Sex					
Male	38	11	29%	0.59*	0.774
Female	12	2	16.7%		
Family Size					
$\leq$ 4	15	4	26.7%		
5-7	32	9	28.1%	3.39*	0.459
$\geq$ 8	3	0	0%		
Residence					
Urban	30	8	26.7%	3.30**	0.192
Rural	20	5	25%		
Age of patient					
Young	6	1	16.7%	3.46*	0.465
Adult	26	8	30.8%		
Old	18	4	22.2%		
Education status					
Literate	115	5	4.4%	1.28**	0.528
Illiterate	113	8	7.1%		
Raw milk consumption					
Yes	16	6	37.5%	6.80**	0.030
No	34	7	20.6%		
Raw meat consumption					
Yes	7	3	42.9%	1.73*	0.474
No	43	10	23.3%		

*Fisher's exact chi-square test (χ^2): two cells have expected count less than 5.

**Pearson chi-square test

The livestock owning society living in the rural residential districts were 1.26 times (P = 0.017, OR= 1.26, 95% C.I. = 1.19-1.71) more at risk of acquiring the zoonotic tuberculosis than those people living in urban areas. Similarly, those individuals having the habit of raw milk consumption were 1.45 times (P = 0.027, OR= 1.45, 95% C.I. = 1.18-2.71) more at risk to acquire the infection than those who do not. Likewise, the individuals with the custom of raw meat consumption were 3.96 times (P = 0.0001, OR= 3.96, 95% C.I. = 2.05-7.67) more at risk of developing tuberculosis infection than those who don't too (Table

3).

Examination of acid-fast bacilli from fine needle aspiration (FNA) samples

The fine needle aspirate (FNA) sampling from cervical lymph nodes of patients with suspected TB lymphadenitis (extra-pulmonary TB) was conducted at the Ayder referral hospital and 50 (22.7%; N =228) individuals were having skin nodule out of the 228 study human subjects and FNA samples were tested for mycobacterium organism and among which 13 (26%; n = 50) were found positive. Out of the total, 5.7% (N = 228) were positive for FNA. The finding of skin nodule ($\chi2$ = 22.8, P = 0.0001), origin of patients ($\chi2$ = 6.32, P = 0.017) and the habit of raw milk consumption ($\chi2$ = 6.80, P = 0.030) were found significantly associated with the finding of the organism responsible for extra-pulmonary tuberculosis and were the major risk factors contributing to the occurrence of the disease. On the other hand, sex ($\chi2$ = 0.59, P = 0.774), family size ($\chi2$ = 3.39, P = 0.459), age ($\chi2$ = 3.46, P = 0.465), education status ($\chi2$ = 1.28, P = 0.528) and custom of raw meat consumption ($\chi2$ = 1.73, P = 0.474) were not statistically significant and were not the risk factors associated with the occurrence of the infection (Table 4).

DISCUSSION

The individual animal prevalence (7.3%) was smaller than that reported previously by Ameni and Erkihun [19], Ameni *et al.* [20], and Omer *et al.* [21], who recorded 11%, 14.2% and 14.5% , in central Ethiopia, southern Ethiopia and Eritrea, respectively. Asseged *et al.* [4] reported a similar animal prevalence in and around Addis Ababa, the capital of Ethiopia. In accordance with findings from other studies [4, 22], this analysis showed that, as herd size increased, there was a corresponding increase in the prevalence of bovine TB; 4.6%, 6.4% and 10.5% for small, medium and large herd size, respectively. This is because, as stated by Radostits *et al.* [7], bovine TB is a disease of overcrowding. Thus, when the number of animals in a herd increases, the transmission of the bacillus is promoted. Animals with no grazing are at a higher risk of infection than those kept on free grazing and mixed grazing. This result agrees with the established observation that bovine TB is more common and serious in housed animals (intensive) than those at pasture (herds at field) [7]. The closer the animals are packed together, the greater the chance of transmitting the disease [23]. The present study is also consistent with previous reports [20, 24], that the prevalence of bovine TB is higher in Holstein, Cross [HFxZebu] and Begayt cattle than pure Zebu breed. Fewer reactor animals were recorded in the younger age groups (3.5%) and reactivity to the CIDT test increased with age, up to six years of age (adult [9.1%]), after which it declined (old [6.8%]). This is in accord with the findings by other researchers [4, 19, 22]. It is possible that the infection may not become established in young animals but, as they get older, their chance of acquiring infection also increases, due to the increased time of exposure.

TB caused by *M. bovis* is clinically indistinguishable from TB caused by *M. tuberculosis*. In countries where bovine TB is uncontrolled, most human cases occur in young persons and result from drinking or handling contaminated milk; cervical lymphadenopathy, intestinal lesions, chronic skin TB (lupus vulgaris), and other nonpulmonary forms are particularly common. Such cases may, however, also be caused by *M. tuberculosis*. Little is known of the relative frequency with which *M. bovis* causes nonpulmonary TB in developing nations and specifically in the study areas of Tigray region, Ethiopia, because of limited laboratory facilities for the culture and typing of tubercle bacilli. Direct smear microscopy does not permit differentiation between species of the *M. tuberculosis* complex. Agricultural workers may acquire the disease by inhaling cough spray from infected cattle; they develop typical pulmonary TB. Information on human disease due to *M. bovis* in developed and developing countries is scarce. From a review of a number of zoonotic tuberculosis studies, it was estimated that the proportion of human cases due to *M. bovis* accounted for 3.1% of all forms of tuberculosis: 2.1% of pulmonary forms and 9.4% of extra-pulmonary forms. As suggested earlier [25], the presence of both a human TB patient and reactor cattle in a household could indicate that either the human TB patient was a source of infection for the cattle or *vice versa*. The disease transmission may also be cyclical: bovine to human to bovine. Close physical contact between the owner and his or her cattle, especially at night, and the consumption of raw milk or milk products facilitate the transmission of bovine TB [26]. This was also observed in the study area. Cases of human TB of animal origin will continue to pose a serious public health problem, especially in areas where raw milk or its products are commonly consumed [26]. Kidane *et al*. [13] indicated that *M. bovis* was found to be a cause for tuberculous lymphadenitis in 17.1% of 29 human tuberculosis cases in central Ethiopia which is lower than the current finding (26% of 50 cases of cervical lymphadenitis).

The questionnaire survey has provided information regarding the knowledge and practices of livestock keepers about zoonotic diseases in the study districts of Tigray region of Ethiopia. Like in most African countries, in Ethiopia, illiteracy is yet another unsolved problem in most rural communities particularly in the study districts. High number of respondents had, therefore, no detailed and accurate knowledge about tuberculosis and hence, there was higher tuberculosis cases (7.1% FNA positive and 16.8% AFB positive) among illiterate cattle owners than those literate ones (4.4% FNA positive and 11.3% AFB positive). This low awareness is a limiting factor if control strategies are to be implemented. Inability to read and write, and failure to utilize modern methods of communication [27], and the limited knowledge of the community related to the epidemiology of BTB infection, makes prevention and control program difficult and often impossible to apply. This may indicate paucity in health education rendered to the community of the study districts. The most important practices potentially supporting transmission of zoonotic tuberculosis in the study areas were consumption of raw animal products and proximate life with their animals.

CONCLUSION AND RECOMMENDATIONS

The results of this study revealed that the livestock owning community members from the study districts had low biomedical knowledge of the cause, source of infection and the mode of transmission of tuberculosis infection. The prevalence described using AFB and FNA in human and CIDT test in cattle in this study showed that human and bovine TB is present and is well established in Wukro, Adigudem and Hewane districts of Tigray region of Ethiopia. Cattle owners in the study areas are usually in close contact with their animals and also consume raw milk regularly, both of which pose high infection risks to them and men had extra-pulmonary tuberculosis infection than women. Patients often delay initial visit during farming season and often first visit a local/traditional healer and if this doesn't work then travel to a local health center. These centers often have no capacity to diagnose the disease properly and often referred to district hospital for further diagnosis. The increased number of human and bovine TB cases in the study districts calls for stronger inter-sectoral collaboration between the medical and veterinary professions to assess and evaluate the severity of the problem, mostly when zoonotic TB could represent a significant risk in rural and urban communities. Therefore, awareness should be created among the people, to meet the standard hygienic requirement and to improve husbandry practices. In intensive dairy farms, building of the new premises needs to be done according to designs appropriate to dairy and/or fattening farms taking into account space per-animal, proper manure disposal, good ventilation and lighting systems. Diagnostic and treatment facilities should be established in strategic villages that pastoralist can reach in both dry and wet seasons. Such facilities may alleviate the observed long distance to health facilities and thus long delay in diagnosis of TB. This strategy should be compounded with a community based TB control approach, whereby basic medical training on TB management such as provision of health education and drug distribution. Test-and slaughter policy should be designed and started as a major control measure to avert the spreading of the BTB infection. There should be an approach for insuring dairy farms by encouraging owners to cull their infected cattle after testing for BTB. There should be introduction of a bi-annual testing program to establish a "provisional disease free status in some herds/farms or areas". Under this scheme a herd with a negative result in two successive tests could be declared provisionally free from BTB. If this strategy is started, it should then be strengthened with a frequent follow up to make sure that these farms are not re-infected. There should be further detailed epidemiological studies to investigate the link between bovine and human TB in the present study districts to allow formulation of appropriate strategic control measures in order to reduce public health risks.

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