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ORIGINAL ARTICLE

BOVINE TUBERCULOSIS (BTB) AS A RISK FACTOR FOR DEVELOPING TUBERCULOSIS IN HUMANS IN THE RURAL COMMUNITY OF ETHIOPIA: A CASE-CONTROL STUDY.

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ABSTRACT

Aim: *The current study aimed at assessing BTB as a possible risk factor for human TB in the rural community of North Eastern and Western parts of Ethiopia.*

Method*: A case-control design was conducted among cattle owning households with TB and without TB. Comparative cervical intradermal test using purified protein derivatives were used to test cattle. Reading of the reaction was done 72 ± 4hrs after antigen injection. Based on the skin test reaction measurement, cattle categorized as negative, doubtful and positive. Questionnaires were used to collect the required factors.*

Results*: Thirty-five with TB and 105 households without TB participated in this study of which 49.3% and 61.4% had the habit of drinking raw milk and eating uncooked meat, respectively. About 70.7% knew about the chance of disease transmission from animals to humans. Among the TB households 31.43% shared their house with their cattle. Of the attendants, approximately 38% shared utensil. Based on >2mms as a cutoff value 23.6% an overall apparent bovine tuberculosis (BTB) and 48.6% apparent BTB in households with TB were recorded. The odds for households having bovine TB in their cattle to get tuberculosis was more than 8 times (95% CI; 2.82-24.60) higher than those owned by households without TB.*

Conclusion: *Bovine TB has been seen as an exposure to human pulmonary TB occurrence. A separate house for cattle should be constructed to minimize the fear of cross infections and further study regarding the possible infection of cattle with M. tuberculosis is suggested.*

Key words: bovine tuberculosis, households, human TB, M. tuberculosis, risk.

INTRODUCTION

Bovine tuberculosis is a debilitating disease mainly affecting cattle. The disease causing agent, *Mycobacterium bovis* (*M. bovis*) could infect a wide range of hosts including humans [1-3]. Humans could be infected by mainly taking raw milk and to some extent aerosol or droplet nuclei inhalation in situations of close contact [4,5]. Humans infected with *M.bovis* revealed extrapulmonary and to some extent pulmonary TB [1]. Research works revealed that cattle owned by TB households showed higher bovine tuberculosis skin test results [6-8]. This might be due to the possible chance of harboring *Mycobacterium tuberculosis (M. tb)*. *Mycobacterium tb* is isolated from cattle lesions as well as milk samples in different parts of the world, including Ethiopia [6,9]. *Mycobacterium tb* and *M. bovis* are very similar genetically [10-12] and purified protein derivatives (PPD) antigens shared by members of *Mycobacterium species* [13] cattle harboring *M. tb* could react positively to skin tests. In this case cattle could be considered as false positives, since the antigen (purified protein derivative, PPD) for cattle skin test purpose prepared from *Mycobacterium bovis*, strain AN-5 [14]. Cattle could get the *M. tb* infections when there is close contact with TB patients and have an access to their discharges containing the infective bacilli. Cattle may get the agent directly from feed materials contaminated with the agent as well as with aerosols [15, 16] when the patient sneezes or coughs. Drinking and feed materials could be contaminated by the disease causative agent if an infected person spits his sputum, which contains infective bacilli, particularly before treatment commences. Besides the main route of aerosol transmission, ingestion of contaminated water, feed as well as fomites have been recognized [17,18]. To look for possible risk factors that contribute to the development of human TB in households with bovine tuberculosis, the status of bovine tuberculosis was assessed in the rural community of North Eastern and Western parts of Ethiopia. For this purpose cattle owned by confirmed TB cases were compared to those cattle owned by individuals without TB.

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MATERIALS AND METHODS

Study area and design: This study used a case-control design in North Gondar and Wollo zones of Amhara regional state, North Western and Eastern Ethiopia, where the livelihood mainly is mixed farming. Dembia, Chilga, Dabat, Debark, Adarkay and Wegera from North Gondar zone and Meket, Gubalafto, Habru and Raya Kobo Districts from North Wollo zone were included in the study. Based on 2007 Census [19], the study place covered an estimated area of 58,117.13 square kilometers with a total population of 4,429,931. Of whom majorities are rural dwellers accounted about 90% and 85% in North Wollo and North Gondar zones, respectively [19]. The data collection was carried out between September and November 2013.

Study Population: Households owning cattle with at least one family member of TB case were considered as cases while those without TB were used as controls. For each case three control households owning cattle were selected. In the study populations houses could be shared and for this purpose a house share is defined as a house shared by cattle/animals and human/s or families to live together in the same house or separate house having the same main door for entrance.

Sample size determination: The sample size was calculated taking into consideration case-control with the assumption of 15%, where a study done in three districts of west Gojjam zone reported detection of 14.9% of *Mycobacterium bovis* from cattle owners [6] bovine TB among the control households and 41.38% among the cases, confidence interval of 95% with a power of 80%, a ratio of cases to control group of 1:3. Adding 10% non-response the required sample sizes were 35 households with TB and 105 households without TB.

Comparative cervical intradermal skin test: Comparative cervical intradermal skin test was performed to know the status of bovine tuberculosis (which is defined as a disease of bovine caused by *Mycobacterium bovis* and tested positive for intradermal tuberculin skin tests) in the study area. For this test AVITUBAL-25000inj. ads us.vet, *Mycobacterii avium* (strain D4 ER, 25000TU/ml) and BOVITUBAL (*Mycobacterium bovis*, strain AN-5 25.000 IU/ml) PPD antigens were used at a dose rate of 0.1ml each intradermally [14]. First the injection sites for the antigens were shaved and cleansed. Then a fold of skin within each clipped area taken between the forefinger and the thumb was measured using digital caliper and the test antigen injected using a BD1/2 tuberculin syringe (graduated) [20] based on manufacturer recommendation [14]. A correct administration was confirmed by palpating a small pea-like swelling at each site of injection. Evaluation of results was done 72 $\pm$ 4hrs after injection. The result was considered as positive, if the reaction number to bovine exceeds the reaction to avian tuberculin by >2mms and if the reaction number is the same or lower than for avian tuberculin the result considered as negative.

Questionnaire: Questionnaires were used to collect the relevant information to identify the possible factors that could be associated with the occurrence of higher BTB reaction in TB households. Most of the questionnaire was closed and organized to collect data concerning Scio-demographic, livestock related, human knowledge of zoonoses diseases as well as tuberculosis, consumption habits and human-livestock interface. The questionnaires were pretested before the actual study.

Data analysis: The data entered into access using double data entry method and transported to SPSS version 20 [21] for edition, recoding and analysis. Multivariable logistic regression was applied and a $P<0.05$ was considered as significant.

RESULTS

Socio-demographic issues: On average the age of participants was 42.13 $\pm$13.70, ranging from 15-82 years old, male contributes about 88% (120/140) and among the case groups more than half were females 51.4% (18/35). Eighty point seven percent (113/140), nearly 58% (81/140), 95% (133/140) and 97.1% (136/140) were married, illiterate and orthodox Christianity followers, respectively. Roughly a quarter, 23.6% (33/140) of herds were positive for bovine tuberculosis (Table-1).

Table-1: Socio-demographic characteristics of participants in two zones of Amhara region, Ethiopia
N= 140 households

Category	Households				Total	
	With tuberculosis (N=35)		Without tuberculosis (N=105)			
	No	%	No	%	No	%
Socio-demographic						
Sex						
Male	17	48.6	103	98.1	120	85.7
Female	18	51.4	2	1.9	20	14.3
Age (Mean)			42.13 ±13.70			
Marital status						
Married	15	42.9	98	93.3	113	80.7
Single	16	45.7	6	5.7	22	15.7
Divorced	0	0	1	1.0	1	0.7
Widowed	4	11.4	0	0	4	2.9
Educational status						
Illiterate	16	45.7	65	61.9	81	57.9
Primary	13	37.2	24	22.9	37	26.4
High school	4	11.4	6	5.7	10	7.1
College	2	5.7	2	1.9	4	2.9
Informal	0	0	8	7.6	8	5.7
Religion						
Orthodox	34	97.1	99	94.3	133	95.0
Others	1	8.9	6	5.7	7	5.0
Household (HH) BTB*						
HH with BTB	17	48.6	16	15.2	33	63.6
HH without BTB	18	51.4	89	84.8	107	76.4

*BTB: bovine tuberculosis

Purpose of cattle keeping, food consumption habit and disease knowledge: All farmers were keeping cattle for the purpose of draught and milk use. Nearly half, 49.3% (69/140) and 61.4% (86/140) were having the habit of drinking raw milk and consumption of uncooked meat, respectively. More than three fourth, 67.1% (94/140) of the participants did not know the benefit of drinking boiled milk. Among the individuals, 70.7% (99/140) and 85.7% (120/140) knew the possible transmission of disease from animals/cattle to human and human tuberculosis, respectively (**Table-2**).

Cattle related data: Individuals practiced mixed type of agriculture like other communities in the area. Twenty nine point three percent, (41/140) and 21.4% (30/140) of cattle owners responded that there was the presence of cattle having cough and showing wasting conditions, respectively. Of the participants, 78.6 % (110/140) did not isolate their cattle when they get sick and 87.1% (122/140) keep sick cattle with them **(Table-2).**

Table-2: Purpose of cattle keeping, feeding habit and disease knowledge in two zones of Amhara region, Ethiopia.

N=140

Category	Households				Total	
	With tuberculosis (N=35)		Without tuberculosis (N=105)			
	No	%	No	%	No	%
Purpose of cattle keeping						
Draught	35	100	105	100	140	100
Milk	35	100	105	100	140	100
Consumption of raw milk/meat						
Raw Milk						
No	10	28.6	61	58.1	71	50.7
Yes	25	71.4	44	41.9	69	49.3
Raw meat						
No	23	65.7	31	29.5	54	38.6
Yes	12	34.3	74	70.5	86	61.4
Knowledge assessment						
Consumption of boiled milk bene-fit						
No	22	62.9	72	68.6	94	67.1
Yes	13	37.1	33	31.4	46	32.9
Knowing zoonoses diseases						
No	17	48.6	24	22.9	41	29.3
Yes	18	51.4	81	77.1	99	70.7
Knowing Tuberculosis						
No	7	20.0	13	12.4	20	14.3
Yes	28	80.0	92	87.6	120	85.7
Cattle related						
Cattle that cough						
No	22	62.9	77	73.3	99	70.7
Yes	13	37.1	28	26.7	41	29.3
Cattle with wasting						
No	21	60.0	92	87.6	113	80.7
Yes	14	40.0	13	12.4	27	19.3
Sick cattle isolation						
No	28	80.0	82	78.1	110	78.6
Yes	7	20.0	23	21.9	30	21.4
Keeping sick cattle						
No	4	11.4	14	13.3	18	12.9
Yes	31	88.6	91	86.7	122	87.1

Possible risk factors: Among the participants, 22.1% (31/140) shared a house with their cattle/animals. Of individuals with tuberculosis 31.43% (11/35) used house sharing. Assessing some possible risk factors, nearly 18% (25/140) were using common water source, ~38% (53/140) sharing utensil, 30.0% (42/140) were giving their urine to cattle or cattle licked their urine, 7.1% (10/140) individuals had the habit of urinating on cattle feed/hay, 25.7% (36/140) answered cattle lick their hands, 32.9% (46/140) were spit sputum in the house and 44.3% (62/140) of the houses were having ventilations (window or holes) (**Table-3**).

As it is presented in **Table- 3** adjusted for taking unboild milk, sharing house, cattle licking human urine, man urinating on cattle feed, human spitting sputum in the house, house ventilation, using the same water source and knowing zoonoses diseases, the odds of households with positive bovine tuberculosis to acquire tuberculosis was more than 8 (AOR=8.32, 95% CI; 2.82-24.60) times higher compared to bovine TB negative households. Raw milk taking (OR=8. 80, 95%CI; 2.60-29.81), human urinating on cattle's feed (OR=12. 10, 95% CI; 1.80-81.20) and house ventilation (OR= 5.50, 95%CI; 1.60-18.9) were also the contributing factors for the possible circulation of *Mycobacterium tuberculosis complex* in the households.

Table-3: Risk factors associated with the occurrence of Bovine tuberculosis in two zones of Amhara region, Ethiopia using Ameni *et al*., 2010, as cut off value.

Category	Households Human TB		Total	Sig	AOR	95% CI	
	Controls (105)	Cases (35)				Lower	Upper
Bovine TB							
Negative	89 (84.8)	18 (51.4)	**107 (76.4)**		1.0		
Positive	16 (15.2)	17 (48.6)	33 (23.6)	0.0001	8.32	2.82	24.60
House sharing							
No	85 (81)	24 (68.6)	109 (77.9)		1.0		
Yes	20 (9)	11(31.4)	31 (22.1)	0.05	0.31	0.01	0.99
Urinating on hay/feed							
No	100 (95.2)	30 (85.7)	130 (92.9)		1.0		
Yes	5 (4.8)	5 (14.3)	10 (7.1)	0.01	12.10	1.8	81.22
Urine licking							
No	76(724.4)	22(62.9)	98 (70.0)		1.0		
Yes	29 (27.6)	13(37.1)	42 (30.0)	0.90	1.10	0.32	3.60
Cattle licking hand							
No	78 (74.3)	26 (74.3)	104 (74.3)		1.0		
Yes	27 (25.7)	9 (25.7)	36 (25.7)	0.008	0.14	0.03	0.60
Sharing utensils							
No	67 (63.8)	20 (57.1)	87 (62.1)		1.0		
Yes	38 (36.2)	15 (42.9)	53 (37.9)	0.22	1.97	0.70	5.90
Spitting sputum in the house							
No	75 (71.4)	19 (54.3)	94 (67.1)		1.0		
Yes	30 (28.6)	16 (45.7)	46 (32.9)	0.08	2.64	0.90	7.70
House ventilation							
No	61 (58.1)	17 (48.6)	78 (55.7)		1.0		
Yes	44 (41.9)	18 (51.4)	62 (44.3)	0.007	5.50	1.60	18.9
Using same water source							
No	89 (84.8)	26 (74.3)	115 (82.1)		1.0		
Yes	16 (15.2)	9 (25.7)	25 (17.9)	0.40	1.80	0.51	6.11
Habit of taking unboiled milk							
No	61 (58.1)	10 (28.6)	71 (50.7)		1.0		
Yes	44 (41.9)	25 (71.4)	69 (49.3)	0.0001	8.80	2.6	29.81

Number in brackets represents percentages.

DISCUSSION

As bovine tuberculosis (BTB) is endemic to Ethiopia, reaching to herd prevalence of 62.5% (7), in this case control study, nearly a quarter (23.6%) of households showed BTB. Looking BTB as a risk factor to human pulmonary tuberculosis, the adjusted odds ratio showed that households with BTB to get tuberculosis was more than 8 (AOR= 8.32, 95% CI; 2.82-24.60) times higher compared to households without BTB. As close contact is a predisposing factor for TB transmission between humans (22, 23), prolonged close contact (24-28) between the cattle with TB and human could result in longer duration of exposure that would probably lead to higher risk for the disease transmission between the two populations. Most of the houses in the study areas were not having adequate ventilation or completely closed. Having house ventilation was found as a contributing factor in the occurrence of human TB in households with bovine BTB. The odds of house ventilation for the occurrence of human TB in households with bovine TB was 5.50 (95% CI: 1.60-18.90) times to households without BTB. It is known that inadequate ventilation could result in insufficient dilution or removal of infectious droplet nuclei which enhance the transmission of *M. tuberculosis complex* (22,23). However, in this study the definition used for ventilation and the perception of ventilation by respondents might cause a bias and hence presence of ventilation seen as contributing factor for the occurrence of human TB.

Awareness about the disease transmission between cattle and humans could play a great role in the occurrence of diseases like TB. The more peoples' awareness about the diseases the better chance of reducing disease transmission. Based on this case control study, among the participants, 70.7% knew disease transmission from cattle to humans and mentioned some of the diseases, particularly anthrax and rabies, while their knowledge regarding BTB was limited to literates (few in number). Ameni and his colleagues reported 38.3% of the respondents knew about BTB (4). This difference of awareness of BTB might be attributed to lower participation of veterinary personnel's in the public health issues, little attention given by health extension workers or less literate peoples' participation in the current study compared to Wuchalie-Jida (Ethiopia) districts (4). Similarly, the study also indicated that of the households 85.7% knew human tuberculosis. The higher percentage of awareness about humans TB might be related to the existing health extension activities practiced and implemented in the country as well as in the study areas.

Since there are food-borne diseases, feeding habits of the individuals could also serve as a vehicle for disease transmission. Taking raw milk and uncooked meat is well practiced in different parts of the country. In this study, nearly half, which is 49.3% of the participants, was having a habit of consuming raw milk and 61.4% of them were eating uncooked meat. Besides, in this study more than three-fourth (67.1%) of the respondents did not know the benefit of using boiled milk. In a study conducted in rural Torodi (Niger), more than 68% of the household families consumed unpasteurized milk (29) and a study carried out in Wuchalie-Jida (Ethiopia) districts showed 52.1% of the interviewed households used to drink raw milk and 90% ate raw as well as cooked meat (4). Relatively a lower percentage of raw meat and milk consumption habit was recorded in the current study when we compared to studies done in Niger while a relatively closer figure is registered in Wuchali-Jida area and this might be related to having similar cultural settings between the study sites in Ethiopia while the higher figure in Niger could be justified as having different soil-cultural settings between the two countries. The habit of raw meat consumption in the current study was very low compared to the findings from Wuchali-Jida district and this might be a reflection of the effects of increased community awareness about the use of boiled milk and cooked meat consumption. As Ameni and his colleagues(4) reported that 44.7% of individuals practiced consumption of boiled fresh milk, individuals interviewed in the current study areas who own cattle used to take boiled milk but 67.1% of them did not know the advantages of consuming boiled milk. As a factor for the occurrence of human pulmonary TB in households with BTB, unboiled milk drinking was associated with the manifestation of TB where the risk was 8.80 (AOR= 8.80, 95%CI: 2.6-29.81) higher compared to households without BTB.

Since TB is a contagious disease, close contact or proximity between cattle and humans could create favorable condition for TB transmission. In this case control study, 22.4% (31/140) households were practicing house sharing. Although house sharing was not seen as a contributing factor in the occurrence of human TB in households with BTB, cattle which harbor *Mycobacterium tuberculosis complex species* could serve as a source of infection to humans by releasing droplet nuclei during coughing (30). Similarly Ayele and coauthors (31) reported that inhalation of dust particles or bacilli containing aerosols released from cattle could cause pulmonary TB in humans. The survival of the bacilli in the dark (while house sharing) for hours will facilitate transmission of *M. tuberculosis complex* species (23). The more droplet nuclei, space, ventilation, air circulation as well as air pressure are the factors that could increase the probability of *M. tuberculosis complex* transmission (22). The current study revealed that those households who did not share a house with their cattle and those who owned cattle that do not have the habit of hand licking were 31% (95% CI: 10-99%) and 14% (95% CI: 3-60%) less likely acquire TB infection than their counterparts, respectively.

Malpractices could also play their own role in the disease circulation. In this study, about 7.1% of the participants were urinating (Males) on animal feed and this was found to be as a contributing factor (AOR: 12.10, 95%CI; 1.80-81.20) for the occurrence of human TB in

households with BTB. As evidenced by different author's urine could contain *Mycobacterium* bacilli when a person is infected with either disseminated or localized TB. Mortier and colleagues (32) isolated *M. tuberculosis* from human urine samples and (33) detect *Mycobacterium tuberculosis* complex in HIV-infected and uninfected pulmonary and extrapulmonary tuberculosis patients in Burkina Faso. In Italy [34] *Mycobacterium tuberculosis* complex was also detected in urine using rapid gen-probe amplification test. The possible explanation of this contribution is that when infected individuals urinate on animal feed, it will be contaminated by tubercle bacilli and taken by cattle. Infected cattle might serve as a continuing source of infection to the owners thereby re-infection or new infection could be possible despite the patients' having received adequate treatment and cured of the disease.

In a nutshell, the risk of bovine TB to the occurrence of human TB in the current study could be seen in light of the detection of 6.2% and 7.4% M. *tuberculosis* prevalence in cattle in Algeria and Sudan, respectively (35, 36). Besides, *Mycobacterium species* could be excreted in the milk (37). This might suggest the possible role of cattle in the maintenance as well as transmission of tuberculosis to humans. Using 3 controls per case could have been seen as strength, however, lack of isolating the disease causing agent either from cattle owners or positive cattle's should be seen as a limitation of this study.

Conclusion: Human tuberculosis is mainly caused by *M. tuberculosis* and at times by *M. bovis,* which mostly occurred as extrapulmonary TB. In this study bovine TB has been seen as an exposure to human pulmonary TB occurrence. Prolonged close contact between human and cattle could help the disease transmission. Apart from it drinking unboiled milk and urinating on animal feeds were found as a contributing factor for the occurrence of human TB. Those who do not share their house with their cattle were less likely to reveal TB. The participant knowledge about zoonoses and TB diseases should be valued for future TB control activities. Further study regarding the possible isolation of the disease causing agent in both populations is mandatory. A separate house for cattle should be constructed to minimize the fear of cross infections. In addition to these the habit of eating raw meat and taking unboiled milk should be discouraged through vigorous health extension activities and rising the community knowledge about the risk of zoonotic diseases is paramount important.

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