Original Article

Zoonotic Tuberculosis in Occupationally Exposed Groups in the Adama Municipal Abattoir, Central Ethiopia

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Abstract

Background: Considering the socio-economic-cultural connection with livestock in African countries, zoonotic tuberculosis remains a public health threat for the public and abattoir workers.

Objective: To assess the prevalence, awareness, and associated risk factors for zoonotic tuberculosis among the Adama Municipal Abattoir workers in Central Ethiopia.

Methods: Interviews were conducted among 113 Abattoir workers, in Adama, Central Ethiopia, to assess the awareness level and predisposing factors for Zoonotic Tuberculosis. Sputum samples were collected from 54 consenting workers was for laboratory testing. Descriptive statistics and Chi-square/Fishers' Exact test were employed for analysis.

Results: Four out of the 54 abattoir workers tested positive for Mycobacteria by culture and acid-fast staining. One out of the four isolates were M. *bovis* while three were not members of Mycobacterium tuberculosis complex (MTC) by PCR analysis. Seventy-three (63.71%) respondents had no knowledge regarding the main hygiene practices in the abattoir. Most of the respondents (>90%) had the habit of consuming raw milk/meat and had contact with live animals and animal products for more than eight hour's daily. Among the considered risk factors, only age ($\chi 2$=3.3074; P=0.0003) had a significant influence over the culture's positive status of the workers.

Conclusion: Isolation of M. bovis with limited awareness and high-risk behavior for acquiring zoonotic tuberculosis is a concern. This warrants further investigation into the role of M. bovis with regards to the burdens posed by human tuberculosis in the area. [*Ethiop. J. Health Dev*. 2022; 36(1):000-000]

Key Words: Zoonotic tuberculosis, Abattoir workers, Occupational exposure, Ethiopia

Introduction

Emerging and re-emerging pathogens are threats to human health, animal health, and animal production (1) as most of them are zoonotic diseases. Due to regular contact with animals and animal products being a cultural and occupational norm, zoonotic illnesses have a greater veterinary and public health impact, especially in the developing countries. Mycobacterium bovis (M. bovis) is a zoonotic disease that causes large economic losses in livestock production, which has an impact on animal product trade and productivity. Mycobacterium bovis (M. *bovis*) also causes serious health problem in human beings, such as pulmonary tuberculosis, and extra-pulmonary tuberculosis (2,3).

In sub-Saharan Africa, zoonotic tuberculosis is endemic and there little concern for the testing and slaughter policy (4). Resultant limited control of zoonotic tuberculosis places the abattoir workers and animal handlers at high risk of contracting zoonotic tuberculosis infections (5,6). Although recent studies provide insights into the importance of zoonotic tuberculosis in Africa (7–10), attributable human tuberculosis from zoonotic tuberculosis is limited. Furthermore, in the developing countries there is poorly documented data on the spread of zoonotic tuberculosis in the human population, estimates suggest that it contributes to 2.1% and 9.4% of pulmonary tuberculosis, and extra-pulmonary tuberculosis respectively (2,10,11).

Like other developing countries, the zoonotic tuberculosis control programme is evolving over time but is also constrained by multiple contextual factors, that puts the people at risk for contracting zoonotic tuberculosis infections (12). Moreover, Studies have indicated that zoonotic tuberculosis is an on-going risk to public health in Ethiopia (13,14).

A few studies have generated biological evidence that zoonotic tuberculosis contributes to human tuberculosis in Ethiopia. Regassa et al., has demonstrated that one in six sputum and fine needle aspiration (FNA) human samples from suspected individuals had M. *bovis*(15). The information on zoonotic tuberculosis in occupationally exposed individuals and their awareness level in Ethiopia is limited. In this context, this study explored the burden of M. *bovis*, awareness levels and associated factors predisposing abattoir workers to zoonotic tuberculosis at Adama Municipal Abattoir East Shewa, Central Ethiopia.

Materials and Methods

Study site: The study was carried out in the Adama Municipal Abattoir in Adama, Central Ethiopia. This abattoir receives animals from the nearby Adama city and other regions of the country and provides slaughter and inspection services to butcher shops in Adama city.

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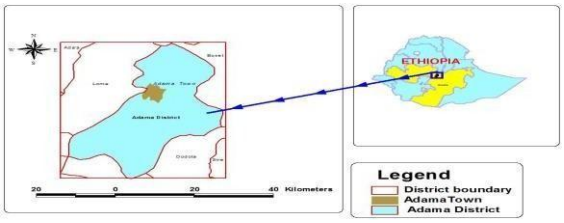

Figure 1: Location map of study site

Study population
The study populations were abattoir workers in the Adama Municipal Abattoir

Study design and sampling
This study utilized a cross-sectional survey design, which included a sample of all volunteer abattoir workers in the Adama Municipal Abattoir. The Sample consisted of a total of 113 workers from 54 abattoirs, who consented to having their sputum samples analyzed.

Questionnaire survey
A semi-structured interview was administered in the local language using face-to-face interviews and consent was obtained verbally from the participants for participation in the study.

Isolation of Mycobacteria
A total of 'on-spot' 54 sputum samples (3-5ml) were collected from the consenting abattoir workers using a 50 ml plastic container in the presence of the principal investigator in an outside space. The sputum samples were transported in reverse cold chain to 'Aklilu Lemma Institute of Pathobiology (ALIPB) TB laboratory' for processing and Mycobacterial culturing as per the World Health Organization guidelines (16). In the laboratory equal volumes of specimen and 4% Na OH of roughly 5ml each were placed in a sterile 15ml centrifuge tube with a screw cap; the contents were mixed well by shaking the tubes for a few seconds; and the tubes were then left to stand for 10 minutes at room temperature. For 15 minutes, the homogeneous solution was centrifuge at 3000 RPM. Concentrated hydrochloric acid was used for neutralization. One or two drops of phenol red were applied to monitor neutralization. Thereafter, using the drop method, the 100ml neutralized samples were inoculated on to the Lowenstein-Jensen media (a tube containing 0.6% glycerol and sodium pyruvate). For 8 to 12 weeks, the tubes were cultured at 37℃ and growth was monitored weekly. If no mycobacteria were present following the 12 weeks of inoculation, the culture was deemed negative. Ziehl- Neelsen staining confirmed the positive culture, which was then heat-killed in a water bath at 80℃ for 45 minutes. The heat-killed isolates were kept at -20℃ until molecular typing could be conducted.

Molecular typing
The nucleic acid amplification (PCR) assay kit was purchased from Integrated DNA Technology Co. Ltd. A total of 5L DNA templates was added to the PCR tube (containing a 10L prime gene expression master mix with ROX dye; 0.5L prime time qPCR Assay, and 4.5L distilled water solution). 20L was regarded as the total volume. Real time-qPCR was conducted on an applied biosystem, containing 7500 fluorescent quantitative systems with application conditions as follows; a 3-minute polymerase activation at 95℃ with one cycle; 15 seconds of initial denaturation at 95℃; and 40 cycles of 15 seconds each at 95℃ and a further 1minute at 63℃(the annealing extension step).

Data management and analysis
The data collected was entered and coded into the Excel sheet. The Epi-info 7.2.2.6 software package was used for statistical analysis. The frequency and percentages were used to present the findings. Chi-square/Fishers' Exact test was calculated to assess statistical significance.

Ethical approval
Ethical approval was obtained from the University ethics committee of Kalinga Institute of Medical Science, KIIT Deemed-to-be-University, Bhubaneswar, India with Ref. No: KIIT/KIMS/IEC/62/2019. Local permission was also obtained from the Adama City Municipality. Informed verbal consent was obtained from all the abattoir workers who participated in the study prior to the collection of data.

Results
A total of 113 abattoir workers were interviewed, and they all responded to the questionnaire. Table 1 presents a summary of the respondents' socio-demographic characteristics. The study sample included a 4:1 male to female ratio, with the majority (80%) being between the ages of 18 and 37. Two-thirds of the respondents were orthodox religious followers, and seven out of ten were married. Illiteracy accounted for 2.65% of the workforce. Six out of ten people had a permanent job, and two-thirds of the respondents were involved in the slaughtering and skinning processes.

Table 1: Socio- Demographic Characteristics of the respondents (N= 113)

Variables	Frequency (n)	Percent (LCL-UCL)
Gender		
Male	91	80.53%(72.02-87.38)
Female	22	19.47%(12.62-27.98)
Age (year)		
18-27	40	35.39%(26.63-44.95)
28-37	50	44.24%(34.91-53.89)
38-47	15	13.27%(7.62-20.95)
48-57	7	6.19%(2.53-12.35)
≥58	1	0.88%(0.02-4.83)
Marital status		
Combined (Living with boy/girlfriend)	2	1.77%(0.22-6.25)
Divorced	1	0.88%(0.02-4.83)
Married	81	71.68%(62.43-79.76)
Unmarried living with mother and/or father	15	13.27%(7.62-20.95)
Unmarried living alone	14	12.39%(6.94-19.91)
Religion		
Muslim	12	10.62%(5.61-17.82)
Orthodox Christian	75	66.37%(56.88-74.99)
Protestant Christian	26	23.01%(15.61-31.87)
Job category/job type		
Administration	7	6.19%(2.53-12.35)
Supervision	13	11.50%(6.27-18.87)
Veterinarian	3	2.65%(0.55-7.56)
Slaughtering and skinning	75	66.37%(56.88-74.99)
Cleaner	15	13.27%(7.62-20.95)
Level of education		
Illiterate	3	2.65%(0.55-7.56)
Middle	56	49.56%(40.02-59.12)
Secondary	44	38.94%(29.91-48.56)
Graduate	44	38.94%(4.33-15.67)
Employment status		
Daily laborer	1	0.88%(0.02-4.83)
Temporary	53	46.90%(37.45-56.52)
Permanent	59	52.21%(42.61-61.70)

About 105 (92.92%) of the respondents had a habit of consuming raw milk/meat. Seven in 10 respondents were working in the abattoir for more than three years. Most of the staff (95.58%) had daily contact with live animals and animal products for more than eight hours. Forty-seven (41.59 %) of the respondents had a history and evidence of BCG vaccination while 41 (36.28%) had not been vaccinated and 25 (22.12%) participants did not know their BCG vaccination history. Majority 87 (76.99%) of the respondents had not undergone any sort of formal training on tuberculosis (TB) or bovine TB (BTB). A large number, 94 (83.19%) of the participants had stated that, they had not visited the clinic/hospital for regular health check ups (Table 2).

Table 2: **Personal and medical history of the respondents**

Factors	Frequency	Percentage (%)
Habit of drinking/eating raw milk/meat		
Yes	105	92.92%
No	8	7.08%
Working experience (years)		
Less than 3	5	4.42%
1-3	28	24.78%
Above 3	80	70.80%
Daily contact with animal (hours)		
<8	3	2.65%
8	2	1.77%
>8	108	95.58%
Immunization (BCG vaccine)		
yes	47	41.59%
no	41	36.28%
I don't know	25	22.12%
Obtained training on prevention of Zoonosis and personal protection		
Yes	26	23.01%
No	87	76.99%
Tested for TB		
Yes	30	27.03%
No	81	72.97%
Visited hospital / clinic for regular check up		
Yes	19	16.81%
No	94	83.19%

About 111 (98.23 %) and 99 (87.61 %) of the respondents were aware of TB and BTB, respectively. Ninety-four percent of the workers interviewed were aware that tuberculosis might be spread from TB-infected animals to humans. About 76.99% of the abattoir workers who were interviewed correctly identified TB as the causal agent.

Regarding abattoir workers knowledge of hygiene practices to protect themselves from contracting zoonotic tuberculosis in the abattoir, seventy-three (63.71%) of the respondents did not know about the use of hot water, detergent, and sanitizers as one of the hygiene practices. None of the abattoir workers spontaneously responded to the use of sanitizers as one of the hygiene practices. Less than half (32.74%) of the abattoir workers responded to washing their hands after completing the task and washing their body before leaving the abattoir spontaneously as hygiene practices. However, awareness regarding various kinds of hygiene practices within the abattoir is depicted in Table 3.

Table 3: Summary of Abattoir workers hygiene practices

	Statements	Response n (%)		
		Prompted	Spontaneously	Do not know
1	Washing body before starting the task	77(68.14)	36(31.86)	-
2	Washing hand after touching the animal	70(61.95)	43(38.05)	-
3	Washing hand after completing the task	15(13.27)	98(86.73)	-
4	Washing body before leaving the abattoir	22(19.47)	90(79.65)	1(0.88)
5	Use of hot water	87(76.99)	23(20.35)	3(2.65)
6	Use of detergent	80(70.80)	18(15.93)	15(13.27)
7	Use of sanitizer	59(52.21)	-	54(47.79)
8	PPE-Gloves	45(39.82)	63(55.75)	5(4.42)
9	PPE- Masks	42(37.17)	54(47.79)	17(15.04)
10	PPE-Boot shoes	9(7.96)	104(92.04)	9(7.96)
11	PPE-Separate working cloth	17(15.04)	96(84.96)	

Bacteriology and Molecular typing

Out of the 54 sputum samples from the abattoir workers, 4 (7.41%) (95% CI: 2.1-17.9%) were positive for the growth of Mycobacteria on Solid Lowenstein Jensen (LJ) media and Smear microscopy (AFB). This study identified 1(1.9%) (95% CI: 0.05-9.9%) of 54 sputum samples as M. *bovis* species, but three were not members of Mycobacterium tuberculosis complex (MTC) via RD4 RT-PCR analysis.

The current study revealed that among the considered risk factors only age ($\chi 2$=3.3074; P=0.0003) had a significant influence over the culture's positive status of abattoir workers. Those who had an age of between 18-27 years were more likely to be affected by tuberculosis than those who were older (Table 4).

Table 4: Association of different risk factors to LJ culture media positivity for tuberculosis in Adama Municipal Abattoir in East Shewa, Central Ethiopia

Parameter	Negative N=50 (%)	Positive N=4 (%)	p-value
Age			
18-27	19 (90.47)	2 (9.53)	0.0003 *
28-37	24 (96.00)	1 (4.00)	
38-47	2 (66.66)	1 (33.33)	
48-57	5 (100.00)	0 (0.00)	
58 and above	0 (0.00)	0 (0.00)	
Education			
Illiterate	1(100)	0 (0.00)	0.6732
Middle	25(92.59)	2 (7.41)	
Secondary	20(95.23)	1(4.77)	
Graduate	4(80.00)	1(20.00)	
Gender			
Male	37(92.50)	3 (7.50)	0.5849
Female	13(92.9)	1(7.1)	
Duration of work (years)			
<1 yrs	5(100)	0 (0.0)	0.9050
1-3 yrs	13(92.85)	1 (7.15)	
>3 yrs	32(91.42)	3 (8.58)	
Type of work			
Administration	5(100.00)	0 (0.00)	0.8580
Supervisors/ Veterinarian	87(100.00)	0 (0.00)	
Slaughtering & Skinning	28(90.32)	3 (9.68)	
Cleaners	9(90.00)	1 (10.00)	
Employment type			
Daily laborer	0(0.00)	0 (0.00)	0.9758
Temporarily employed	27(93.10)	2 (6.90)	
Permanently employed	23(92.00)	2 (8.00)	

Discussion

The current investigation found that abattoir workers who were infected with Mycobacterium tuberculosis complex (MTC), primarily M.bovis, were at an increased risk of being exposed to zoonotic tuberculosis. M. bovis is responsible for approximately 17% of all tuberculosis cases in Ethiopia (17). The present study used culture isolates to identify the presence of M. bovis such as pulmonary tuberculosis. Getahun et al., (17), reported 2 (0.13 %) of 1,599 isolates with M.bovis were attributed to pulmonary tuberculosis, which is inconsistent with the current study's findings of 1.9% (1/54). This studys' finding is comparable with a study carried out by Balako et al(14), who reported that 2% of TB was due to M. bovis. In Nigeria 6% of human TB patients were as a result of M. bovis, which is a relatively high prevalence as compared to the study findings (18). In addition to zoonotic tuberculosis, three cases of mycobacteria infection among the abattoir workers were also confirmed. The findings of the investigation confirm the disease's zoonotic nature. It's also worth noting that the isolation of zoonotic tuberculosis from the sputum of an abattoir worker with tuberculosis falls short of proving that aerosol was responsible for the disease. The results stressed the risk posed to persons occupationally exposed, particularly abattoir workers, which is in agreement with previous research in which zoonotic tuberculosis was present in individuals who were occupationally exposed (10,19). The isolation of zoonotic tuberculosis from infected abattoir employees in Ethiopia clearly reveals that zoonotic tuberculosis is transmitted among occupationally exposed persons.

With regards to demographic and other factors, the 18-27year age group was found to be associated with the occurrence of culture positive tuberculosis. This might be since a relatively large proportion of the workers in the abattoir was in this age group (35.39%). This (18-27) age group was the youngest age group when compared to other age groups and might not follow and implement the hygiene practices properly. Earlier there were reports which suggested the association between age and Mycobacterial infection (6).

Zoonotic tuberculosis, sometimes M.bovis in cattle causes a disease in humans. According to the findings of this study, most respondents (70.8 %) had worked in an abattoir for more than three years, which could be one of the risk factors that boost cattle-to-human transmission. The practice of consuming raw animal products is primarily responsible for the transfer of the disease from cattle to humans, and transmission through inhalation is a possibility when there is prolonged contact. This is in line with Byarugaba et al.,'s studies, which stated that zoonotic tuberculosis causes human tuberculosis (20).

Limited knowledge on the risk factors associated with TB, especially with regards to occupational risk factors may be one of the leading contributors to the patients being admitted with TB(21–23). This study has found that majority of the workers were at risk for zoonotic

tuberculosis due to eating raw meat (92.92%), which was relatively higher as compared to previous findings(24). About 98.23% and 87.61% of the respondents were aware of TB and BTB respectively and 94.0% of them were aware that TB can be transmitted from animals-to- humans, which is comparable with the results of Fekadu et al (24). Many abattoir workers had limited knowledge about the source or mode of transmission of TB/BTB, majority of the respondents had no detailed and accurate knowledge about hygiene practices in the abattoir. This might be since, majority (76.99%) of abattoir workers had not received any kind of on job training on TB/BTB by health professional and/or veterinarian, however; globally, particular emphasis has been placed for the control and prevention of Zoonotic tuberculosis in occupational risk groups like abattoir workers. This emphasis particularly has been placed in African continents like Ethiopia, where a high prevalence of tuberculosis may threaten the 2030 vision of WHO, as far as their end TB strategy is concerned which aims to end the global TB epidemic by 2030. People who identify as being at risk for zoonotic tuberculosis are a neglected population in the paradigm shift strategy of WHO and require further attention (25).

As observed, only 41.59% of the participants had a history and evidence of BCG vaccination even though this vaccination is recommended for every individual at birth in Ethiopia. It is therefore worrying that 41 (36.28%) of these occupationally at-risk groups had not received their BCG vaccination. Furthermore, more than three-fourths (83.19%) indicated that they would not visit the clinic/hospital for regular check-ups. This data demonstrates that the intensity of seeking health care for abattoir employees who are at risk of zoonotic infections is still low. The current study demonstrated the disease's public importance; if those affected individuals had gone undiagnosed and untreated, the risk of disease transmission to co-workers and the public would have increased. More notably, this becomes important given the poor health care-seeking behavior in Ethiopia and other developing countries (21, 26), which may increase the prevalence of tuberculosis in these low and middle income countries.

Limitation

This study had some limitations, such as, the face-to-face semi-structured interviews which were used in this study might have increased the likelihood for respondents to give socially acceptable answers. Furthermore, this research focused on the possible transmission of pulmonary TB in occupationally exposed individuals. There could be cases of extra-pulmonary TB in these groups which were not detected as our diagnosis was only based on the analysis of sputum samples.

Conclusion

This study reported the isolation of M. Bovis and NTM in abattoir workers suspected to have sub-clinical pulmonary TB infections in the study abattoir. This study indicated that M. Bovis was responsible for TB in Abattoir workers in the current study. Abattoir workers working in other districts of the region as well as in other regions of the country should be tested on a priority basis. The study also highlighted that most of the abattoir workers in the study abattoir were not aware of hygiene practices and had a habit of consuming raw milk and meat which is a potential risk factor for the transmission of the disease. The study also highlighted the need for improved knowledge of hygiene practices of Abattoir workers, along with formal work-related training. Further investigation into the role of M.bovis with regards to the burden of human tuberculosis in the area is recommended.

Conflict of interest

Authors decaled no financial and non-financial conflict of interest.

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