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Original Article

Burden and factors associated with occupational tuberculosis infection among high-risk workers in Lahore District, Pakistan

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Abstract

Introduction: The main objective of the study was to estimate the burden of occupational tuberculosis infection in high-risk occupational workers and to identify risk factors associated with the prevalence of *Mycobacterium tuberculosis* complex (MTBC).

Methodology: An analytical cross-sectional study was conducted among high-risk occupational workers including veterinarians, abattoir workers, animal handlers, livestock farmers, and microbiology laboratory workers. Sputum samples were collected from 100 participants and polymerase chain reaction (PCR) tests were done to diagnose tuberculosis (TB) infection. Data on potential risk factors was collected in a pre-designed questionnaire. The MTBC prevalence ratio was estimated. Logistic regression analysis was conducted to identify risk factors and the crude odds ratio (OR) was calculated.

Results: Among the 100 enrolled high risk occupational workers, the prevalence of MTBC was 46% (95% CI: 35.98–56.25). Living in a joint family (OR 3.85, 95% CI: 1.58–9.37), and use of unpasteurized milk (OR 3.42, 95% CI: 1.4–8.39), were significantly associated with MTBC infection.

Conclusions: Tuberculosis is a significant health burden in high-risk occupational groups, especially animal handlers and laboratory workers, in Lahore, Pakistan. The study also emphasized the need for formal work-related training, and enhanced zoonotic TB awareness among occupational workers.

Key words: MTBC; occupational workers; Pakistan; tuberculosis; zoonosis.

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Introduction

Tuberculosis (TB) is among the top ten diseases with high mortality despite the availability of cures and vaccines. Every year about 10 million people are affected by TB and approximately 1.5 million people die due to TB, making it the world's top infectious killer. Although TB is a global issue, the burden of disease is highest among low- and middle-income countries (LMICs). In 2020, 86% of all new TB cases were found in 30 countries with the highest TB burden. Eight countries were responsible for two-thirds of the total burden of TB. Pakistan ranked fifth among these eight countries [1]. Tuberculosis is caused by the *Mycobacterium tuberculosis* complex (MTBC). MTBC includes the species *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. caprae*, *M. canettii*, *M. microti*, *M. pinnipedii*, *M. suricattae*, *M. orygis*, and *M. mungi* [2]. Zoonotic TB is a serious problem that has remained neglected on the global level [3]. The World Health Organization (WHO) global tuberculosis report estimates that in 2019 among the 10 million TB cases, 140,000 new cases were diagnosed with zoonotic TB and resulted in 11,400 deaths [4]. In developing countries, data on zoonotic TB is scarce [5]. Zoonotic TB (zTB) is a form of tuberculosis in humans that is predominately caused by *M. bovis* and to a lesser extent by *M. tuberculosis*, *M. caprae,* and *M. orygis* [6].

Zoonotic TB caused by *Mycobacterium bovis* is relatively common in LMICs, and the most common route of transmission is the consumption of unpasteurized dairy products. Humans may also acquire bovine TB by eating infected cattle meat, through

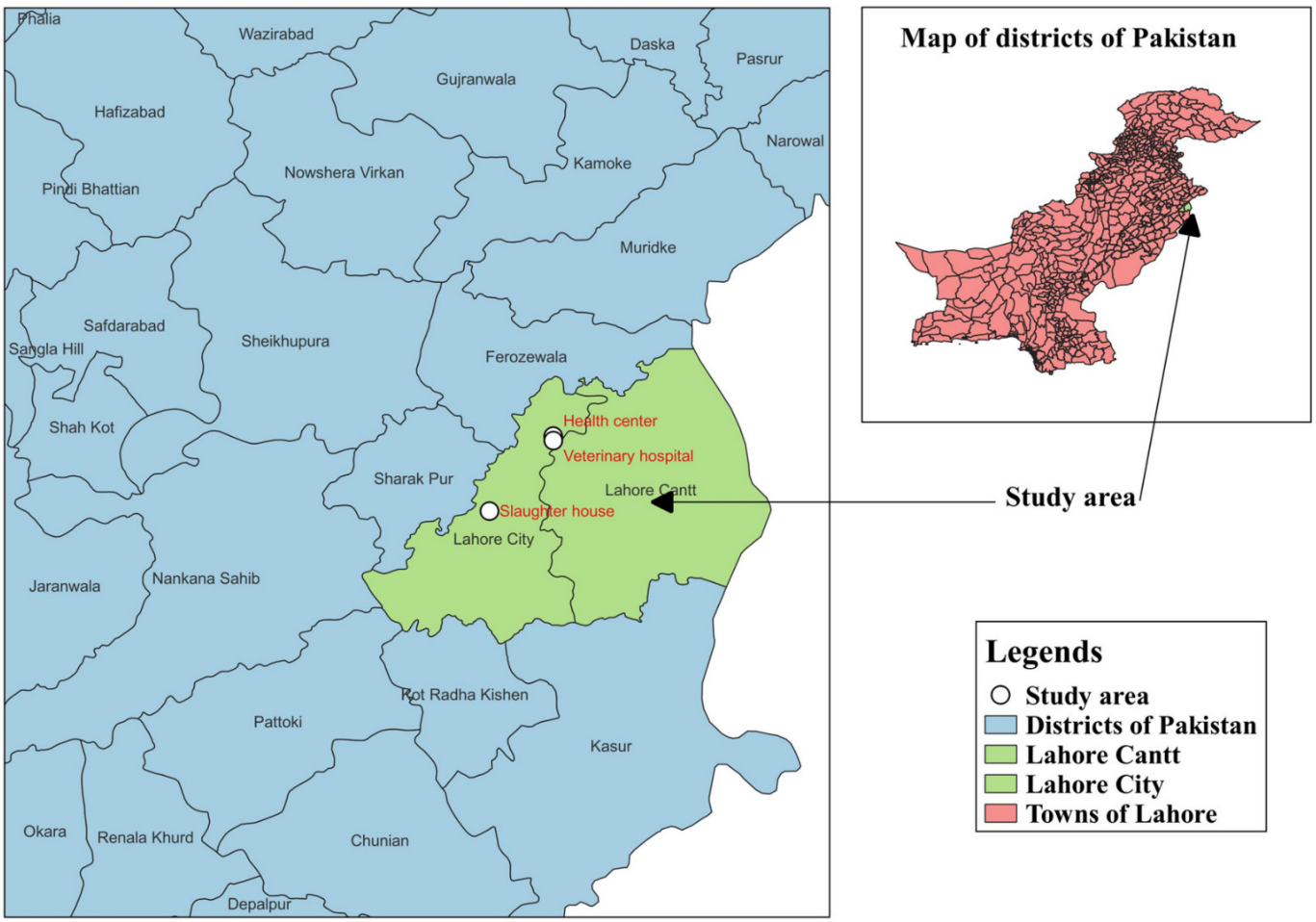
inhalation of infectious droplets exhaled by infected humans or infected animals, or through direct contact with infected animal in the presence of wound. Clinically, the symptoms are identical in cases that are positive for *Mycobacterium tuberculosis* and *Mycobacterium bovis* [7,8]. Cattle are the main reservoir of *M. bovis* but other animal species are also involved in disease transmission, making it a complex process [9]. Zoonotic TB due to *Mycobacterium bovis* has been reported previously in the Khyber Pakhtunkhwa province of Pakistan [5,10]. No data are available on the occurrence of zoonotic TB in high-risk occupational workers including veterinarians, abattoir workers, animal handlers, livestock farmers, and microbiology laboratory workers. Unfortunately, the trends and status of zoonotic TB in Pakistan are unknown due to the absence of currently implemented national zoonotic TB control and prevention policies. Considering the gaps in zoonotic TB data availability, the current study was designed to estimate the burden of occupational tuberculosis infection (*Mycobacterium tuberculosis* and *Mycobacterium bovis*) among high-risk occupational groups (veterinarians, abattoir workers, animal handlers, livestock farmers, and microbiology laboratory workers) in Lahore, Pakistan; and identified various risk factors associated with infection. The health authorities in LMICs face significant data challenges while developing zoonotic TB control programs. Surveillance is crucial for the identification of vulnerable groups and the estimation of disease burden to establish effective TB control measures [11].

Methodology

Study design

This analytical cross-sectional study was conducted from March to July 2022 in Lahore (31.5204° N, 74.3587° E) Pakistan, which is the second largest city after Karachi, and the 26th largest city in the world. The study sites were the health centers, teaching veterinary hospitals, and slaughterhouses in Lahore (Figure 1).

Figure 1. Map of the study area.



Study population and sample collection

A total of 100 occupational workers were enrolled in the current study. The occupational workers included 13 veterinarians, 69 abattoir workers, 7 animal handlers, 6 livestock farmers, and 5 microbiology laboratory workers with age ≥ 15 years. All participants gave their informed consent to be included in the study. Occupational workers were selected using the convenience sampling technique. Random sputum samples were collected by taking a deep breath, holding it for 5 sec, and then exhaling; this was followed up by holding the breath again and coughing up the sputum in a plastic container. The samples were stored at 2–8 °C. All laboratory work was done in the Biosafety level 2 (BSL-2) laboratory in the Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore, Pakistan. Information on patients' socio-demographic characteristics and risk factors for occupational TB infection were collected by using a pre-designed structured questionnaire [5,12] in the local Urdu language (Supplementary document 1).

Inclusion and exclusion criteria

Abbatoir workers, farmers, animal handlers, lab workers, and veterinarians who were ≥ 15 years old and gave consent were included in the study. Those who were < 15 years of age and already diagnosed with TB were excluded.

DNA extraction and conventional PCR

DNA was extracted by the phenol chloroform isoamyl alcohol method, followed by ethanol precipitation [13]. Briefly, 300 µL normal saline was added to the sputum sample, and the sample was transferred to an Eppendorf tube. Then, 400 µL extraction buffer, 40 µL protinase K, and 20 µL lysozyme were added, and the mixture was vortexed for a few seconds. The mixture was incubated in a thermoblock at 56 °C overnight. On the next day, 500 µL phenol chloroform isoamyl alcohol (PCI) was added and mixed thoroughly. The mixture was subjected to chilled centrifugation at 14,000 rpm for 15 min. Three layers were formed in the mixture. The uppermost layer was collected carefully into a separate Eppendrof tube. Next, 500 µL chilled isopropyl alcohol was added and mixed. This mixture was centrifuged at 14,000 rpm for 10 min. The supernatant was removed and 1 mL chilled 70% ethanol was added to the pellet. It was again centrifuged at 8,000 rpm for 15 min. The supernatant was discarded and the pellet was dried on a thermoblock at 65 °C. When the pellet dried completely, it was suspended in 20 µL diethyl pyrocarbonate (DEPC) water. This suspension was given a heat shock at 60 °C for 15–20 minutes. Then, the extracted DNA was stored at -20 °C.

Polymerase chain reaction (PCR) assays previously established and validated at McGill University (Montreal, QC, Canada) [14] were performed with some modifications. Two deletion-based PCR assays were developed for this screening. A three-primer PCR was designed to detect the presence or absence of the region of difference 9 (RD-9), which is present in *M. tuberculosis* and absent in other MTBC subspecies. The PCR amplified a product of 209 bp in *M. tuberculosis* and 410 bp in other MTBC [6], thus leading to identification of the species.

The three primers used were: RD9_Forward, CCGATACCATGCAACAACGG; RD9_Reverse1, CGGTCTCTCCGAGCATTC; and RD9_Reverse2, GCTCGAGCTAGACCTGCAC.

A Thermo Fischer Scientific Veriti 96 well Thermal Cycler (Applied Biosystem, Woodland, Singapore) was used for the PCR amplifications. The PCR reaction volume was 25 µL and contained 12.5 µL Thermo Scientific Dream *Taq* Green Master Mix (2x) (Thermo Scientific™ K 1081, USA), 1 µL RD9_Forward primer, 1 µL RD9_Reverse1 primer, 1 µL RD9_Reverse2 primer, 7.5 µL DNAase-free deionized water, and 2 µL sample DNA. The PCR amplification protocol included initial denaturation (94 °C for 3 min); followed by 35 cycles of denaturation (94 °C for 30 sec), annealing (55 °C for 1 min), and elongation (72 °C for 1min); and a final extension step (72 °C for 10 min). The PCR amplified products were visualized with UV illumination.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki. The participants who fulfilled the inclusion criteria and gave informed consent were enrolled for data and sample collection. The Institutional Review Committee for Biomedical Research, University of Veterinary and Animal Sciences, Lahore approved the study protocol (Letter no. 075/IRC/BMR).

Statistical analysis

The questionnaire data were collected in Epidata version 3.1 (available at http://www.epidata.dk), validated for errors and inconsistencies by random checking of digital data with the hard copy record, and then exported to Microsoft Excel (version 2013, Microsoft Office, USA) for further processing. All statistical analyses were conducted in R software

(version 4.2.1, R Foundation for Statistical Computing, Vienna, Austria). Univariable logistic regression analysis was applied to test the association of independent risk factors with desired outcomes (TB infection positive and negative cases). Variables that fulfilled the selection criteria (i.e., $p \leq 0.25$) on univariable analysis qualified for further analysis by multivariable logistic regression. Odds ratios (ORs) and corresponding 95% confidence intervals with p values were estimated. Multivariable models were developed using a forward manual stepwise addition process, adding the one with the largest p value sequentially. If a variable was no longer statistically significant after adjustment for other variables it was removed (p value = 0.05) [15].

Results

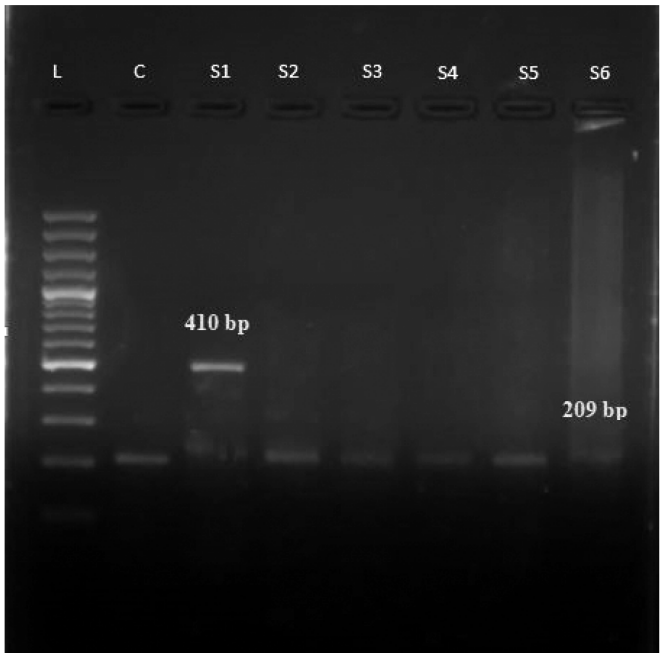
Detection and differentiation of M. tuberculosis and M. bovis by PCR

DNA was extracted from 100 sputum samples. All samples were subjected to PCR amplification using RD9 primers. A total of 45 samples tested positive for *M. tuberculosis* with an amplification product of 209 bp, while only one sample tested positive for *M. bovis* with a 410 bp amplification product (Figure 2).

Prevalence of MTBC

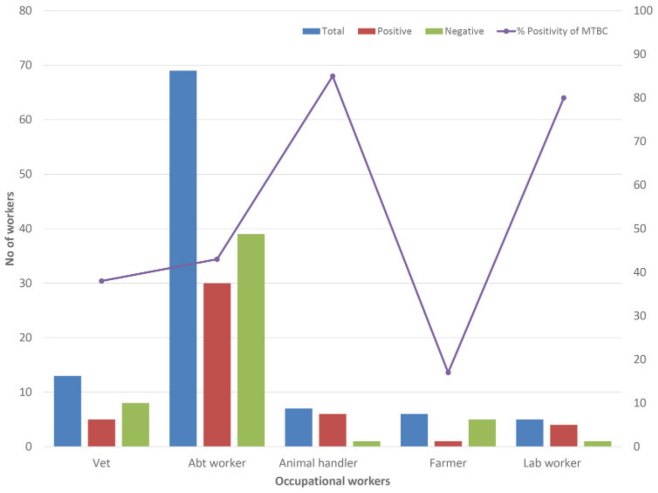
The prevalence of MTBC was 46% (95% CI: 35.98–56.25). Out of 100 participants, 5 veterinarians, 30 abattoir workers, 6 animal handlers, 1 farmer, and 4 laboratory workers were positive for MTBC (Figure 3).

Figure 2. Amplified polymerase chain reaction (PCR) Products.



L: Ladder; C: Control; S1, ... , S6: Samples.

Figure 3. Prevalence of MTBC infection among different occupational groups.



MTBC: Mycobacterium tuberculosis complex; Vet: veterinarians; Abt: abattoir workers.

Characteristics of occupational workers

A total of 100 participants were screened of which 99% were males and 1% was female. The mean age of enrolled patients was 34.4 years. Among the TB patients (n = 46), 100% were male and none of the females were positive. Among the TB patients, 33% (15/46) were < 30 years of age, 30% (14/46) were between 30–40 years of age, and 37% (17/46) were ≥ 41 years. Seventy-eight percent of TB patients were married and 22% were unmarried. Most of the TB patients had primary education (33%), 26% had secondary education and 4% had intermediate education. Most TB patients (65%) were abattoir workers, 11% were veterinarians, 13% were animal handlers, 9% were laboratory workers, and 2% were farmers. The highest number of TB patients lived in urban areas (80%), and only 20% lived in rural areas. 63% TB patients had a monthly income of 15,000–24,000 PKR (67.98–108.77 USD). Furthermore, 65% of TB patients lived in a joint family system, 39% had a family size of 6–10 members; and 80% lived in 1–3 rooms. Most of the TB patients (78%) did not use any personal protective equipment (PPEs) such as gloves, mask, etc. Only 26% of TB patients were vaccinated with the Bacillus Calmette-Guérin (BCG) vaccine, 22% were regular smokers, and 41% had contact with another TB person (Table 1).

Table 1. Characteristics of occupational workers.

Variable	Response	Total N = 100	Positive	Negative	Proportion
Gender	Male	99	46	53	0.464
	Female	1	0	1	-
Age (years)	< 30	39	15	24	0.384
	30–40	33	14	19	0.424
	≥ 41	28	17	11	0.607
Marital status	Married	82	36	46	0.439
	Unmarried	18	10	8	0.555
Education	Illiterate	25	11	14	0.440
	Primary	29	15	14	0.517
	Secondary	30	12	18	0.400
	Intermediate	3	2	1	0.667
	Graduate	13	6	7	0.461
Occupation	Veterinarian	13	5	8	0.385
	Abattoir Worker	69	30	39	0.435
	Animal Handler	7	6	1	0.857
	Farmer	6	1	5	0.167
	Lab Worker	5	4	1	0.800
Residence	Rural	24	9	15	0.375
	Urban	76	37	39	0.487
Monthly income (PKR)	15,000–24,000	62	29	33	0.346
	25,000–34,000	25	10	15	0.400
	35,000–44,000	8	3	5	0.375
	≥ 45,000	5	4	1	0.800
Work experience (years)	1–5	48	16	32	0.333
	6–10	30	20	10	0.667
	11–15	12	6	6	0.500
	16–19	2	1	1	0.375
	≥ 20	8	3	5	0.375
Family type	Nuclear	55	16	39	0.291
	Joint	45	30	15	0.667
Family size (members)	1–5	26	9	17	0.346
	6–10	45	18	27	0.400
	11–15	20	12	8	0.600
	≥ 16	9	7	2	0.778
Number of rooms	1–3	81	37	44	0.457
	4–6	14	5	9	0.357
	≥ 7	5	4	1	0.8
Use of gloves/face mask/head cover	Yes	13	10	3	0.769
	No	87	36	51	0.414
Consumption of unpasteurized milk	Yes	42	28	14	0.667
	No	58	18	40	0.310
Having chronic cough	Yes	8	8	0	1.000
	No	92	38	54	0.413
Fever less than 38 °C	Yes	18	18	0	1.000
	No	82	28	54	0.341
Pain in chest	Yes	3	2	1	0.667
	No	97	44	53	0.453
Coughing up blood or sputum	Yes	18	18	0	1.000
	No	82	28	54	0.341
Weakness/fatigue	Yes	29	24	5	0.828
	No	71	22	49	0.309
Weight loss	Yes	8	8	0	1.000
	No	92	38	54	0.413
Sweating at night	Yes	4	4	0	1.000
	No	96	42	54	0.4375
Diarrhea	Yes	3	3	0	1.000
	No	97	43	54	0.443
Vaccinated against BCG	Yes	60	12	39	0.200
	No	23	13	11	0.565
	Unknown	17	13	04	0.765
Wash hands	Yes	95	44	51	0.463
	No	5	2	3	0.400
Perceived health status	Good	98	44	54	0.449
	Not Good	2	2	0	1.000
Smoke	Yes	23	10	13	0.435
	No	77	36	41	0.468
Contact with TB Person	Yes	21	19	2	0.905
	No	79	27	52	0.342

TB: tuberculosis; BCG: Bacillus Calmette-Guérin.

Table 2. Univariable analysis of potential factors for MTBC infection among occupational workers.

Variable	Response	OR	95% CI	*p* value
Age (years)	< 30	Ref		
	30–40	1.18	0.46–3.03	0.173
	≥ 41	2.47	0.91–6.69	
Family type	Nuclear	Ref		
	Joint	4.87	2.08–11.41	< 0.001***
Use of PPEs	No	Ref		
	Yes	0.21	0.05–0.82	0.015**
Use of unpasteurized milk	Yes	Ref		
	No	4.44	1.9–10.39	< 0.001***
Vaccinated against BCG	Yes	Ref		
	No	3.1	1.35–7.11	0.007**

Ref: The reference level of a categorical predictor variable is often considered the baseline or usual value that is observed for the given variable. ***denotes highly significant with *p* value < 0.001 and ** denotes significant results with *p* value < 0.05. MTBC: *Mycobacterium tuberculosis* complex; OR: odds ratio; CI: confidence interval; PPE: personal protective equipment; BCG: Bacillus Calmette-Guérin.

Occupational workers (veterinarians, abattoir workers, animal handlers, farmer and laboratory workers) presented with the symptoms of tuberculosis (having chronic cough, feeling feverish, coughing up blood or sputum, pain in chest, weakness or fatigue, weight loss, sweating at night and diarrhea) (Figure 4).

Risk factors of MTBC in high-risk population

The univariable analysis identified five independent risk factors for zoonotic TB with *p* value ≤ 0.25 (Table 2). Among these, family type, use of PPEs, consumption of unpasteurized milk, and BCG vaccination were significantly associated risk factors; while age was not statistically significantly associated with MTBC infection. The final multivariable model was established using the epiDisplay package of R software (available at https://posit.co/download/rstudio-desktop/). The forward elimination method was used starting with the most significant factors with the lowest *p* value in the univariable analysis to determine independent risk factors. The variables were retained or removed from the model after considering the Wald Statistic (or log likelihood ratio test for categorical variables with 3 or more levels) with a *p* value of 0.05. Family type and consumption of unpasteurized milk were best fitted to the model, and were considered independent risk factors of MTBC infection. The models derived manually were compared to those from the automated model selection procedure in the function step AIC in the MASS package in R, which uses Akaike's information criterion to trade goodness-of-fit against model complexity (Table 3).

Discussion

The importance of zoonotic diseases in human health and the economy continues to be high due to their impact. In developed countries, an extensive animal TB control and elimination program, together with the pasteurization of milk has reduced the disease in animals and humans drastically [16]. However, in developing countries, animal TB control programs are not very extensive, and there is no effective strategy of pasteurization of milk and dairy products [17].

Figure 4. Symptoms reported in tuberculosis patients.

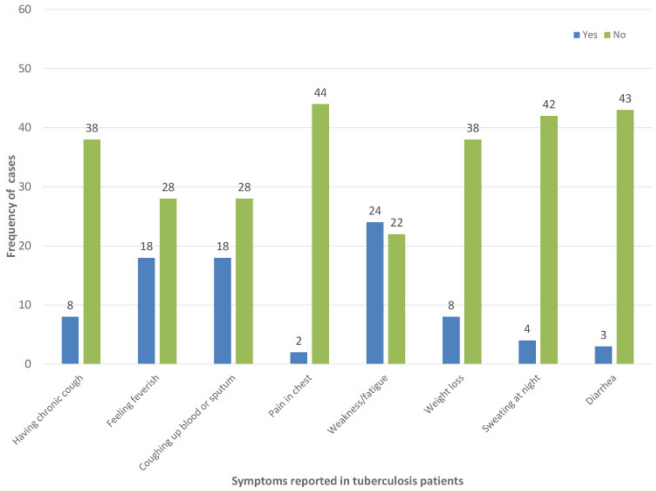


Table 3. Multivariable analysis of potential risk factors for MTBC infection among occupational workers.

Variable	Categories	Positive	Negative	Crude OR	95% CI	*p* value
Family type	Nuclear	16	39	Ref	1.58 - 9.37	0.003***
	Joint	30	15	3.85		
Use of unpasteurized milk	No	36	51	Ref	1.4 - 8.39	0.007***
	Yes	10	3	3.42		

***denotes highly significant results with *p* value < 0.001. MTBC: *Mycobacterium tuberculosis complex*; OR: odds ratio; CI: confidence interval.

This was one of the initial studies conducted in the Lahore district of Pakistan, to assess the prevalence of MTBC (*Mycobacterium tuberculosis* and *Mycobacterium bovis*) among high-risk workers and populations. A high TB burden was found among animal handlers (0.85) and laboratory workers (0.80), compared to the other professionals included in this study. MTBC is endemic in Pakistan, both in animals and humans. These findings are opposed to the previous studies conducted in Pakistan and Australia in which abattoir workers were the most affected by TB [5,18]. *M. bovis* can also cause pulmonary TB among slaughterhouse workers [19].

In this study, respondents living in joint family systems were the most affected by TB. People living in overcrowded places are at higher risk of contracting TB, as overcrowding is a risk factor for the progression of TB infection to TB disease [20]. Thus, inadequate housing is a strong environmental factor that is associated with TB disease [21]. Overcrowding has been previously defined as more than two people living in a single room [22]. In our study more than two people lived in one room, as most people lived in joint families in houses with 1–3 rooms.

In this study, a strong association was found between MTBC prevalence and lack of PPEs (gloves, head cover, and mask) among workers and professionals. The use of PPEs was identified as a protective factor (OR = 0.21, p = 0.015). Abbatoir workers were most affected because they were not using gloves and masks during their work. Awareness about the transmission route of zoonotic TB is very limited in Pakistan. Workers are not provided with any formal training for health and safety. This leads to a higher burden of TB in these professionals. PPEs protect against respiratory diseases, as has been reported in the case of influenza [23].

Another significant factor for TB prevalence was consumption of raw milk (OR = 4.82, p = 0.011). Previous studies have reported inadequate disease control measures, consumption of unpasteurized milk, and frequent human-animal contact as the risk factors of zoonotic tuberculosis [3,12,24]. Similarly, Doran *et al.* reported that the households that consumed raw milk from infected cattle tested positive with Mantoux test. This showed a strong association between ingestion of raw milk and TB [25]. The most common route of *Mycobacterium bovis* transmission is the consumption of raw milk and untreated meat products. Transmission through inhalation and direct contact with skin abrasion and mucous membranes is also possible [26]. Studies in Tanzania and Ethiopia have identified various factors related to bovine TB which include lack of awareness, lack of PPE, unhygienic work conditions, and consumption of unpasteurized milk [27,28].

Close contact with TB patients is a significant exposure factor for TB infection. Previous studies have demonstrated that close contact with index cases leads to TB infection among healthy people within 3 months after the diagnosis of the index patient. It is important to conduct contact investigations to prevent further spread of active and latent TB [29].

The limitation of our study is that we included only cases with pulmonary TB based on the examination of sputum. It is possible that there were cases of latent TB and extra-pulmonary TB in these populations that went undetected.

Bovine tuberculosis is a major public health concern among certain occupational workers in Lahore. PCR may be used in medical and veterinary laboratories to identify *Mycobacterium bovis* in clinical samples taken from both people and animals. Despite its high cost, PCR is used as a standard diagnostic test in Pakistan to determine the prevalence of bovine tuberculosis in cattle and/or buffalo. Based on our findings, the province of Punjab in Pakistan needs surveillance, and preventive and control strategies for this neglected zoonotic disease that spreads as a result of the ongoing and uncontrolled migration of animals; and can spread from animals to humans.

Conclusions

In our study, long-term work exposure, living in joint family systems, lack of use of PPE, and consumption of unpasteurized milk were strongly associated with occupational TB among high-risk groups. The results show that a bovine tuberculosis control program is necessary to improve public health. It also highlights the need for formal work-related training, and enhanced zoonotic TB awareness among veterinarians, abattoir workers, animal handlers, livestock farmers, and microbiology laboratory workers.

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Authors' contributions

CJ, RM, MC: conceptualization; CJ, GU, MC: data curation; CJ, MC: methodology; MHM, SS, MSS, JAK, MA, KI, SA: project administration; CJ, visualization; CJ, MC: writing–original draft; HBR: writing–review and editing. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

The data presented in this study are available upon request from the corresponding author.

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Annex – Supplementary Items

Supplementary Document 1. Translated questionnaire used to collect data on patients' socio-demographic characteristics and risk factors for occupational TB infection.

Serial No:_____
Phone#_____
Sputum Sample_____
Address:_____
Latitude:_____ Longitude:_____

1. **Gender**
   a.□ Male
   b.□ Female
2. **Age**
   a.□ < 30 years
   b.□ 30–40 years
   c. □ ≥ 41years
3. **Marital status**
   a.□ Unmarried
   b.□ Married
4. **Education**
   a.□ Illiterate
   b.□ Primary
   c.□ Secondary
   d.□ Intermediate
   e.□ Graduate
5. **Occupation**
   a.□ Veterinarian
   b.□ Lab worker
   c.□ Farmer
   d.□ Animal handler
   e.□ Abattoir worker
6. **Residence**
   a.□ Rural
   b.□ Urban
7. **Monthly income (PKR)**
   a.□ 15,000–24,000
   b.□ 25,000–34,000
   c.□ 35,000–44,000
   d.□ ≥ 45,000
8. **Work experience (years)**
   a.□ 6–10
   b.□ 11–15
   c.□ 16–19
   d.□ ≥ 20
9. **Family type**
   a.□ Nuclear
   b.□ Joint
10. **What is your family size (number of members)?**
    a.□ 1–5
    b.□ 6–10
    c.□ 11–15
    d.□ ≥ 16
11. **How many rooms do you have in your house?**
    a.□ 1–3
    b.□ 4–6
    c.□ ≥ 7
12. **Do you use facemask, head cover, and gloves during your work?**
    a.□ Yes
    b.□ No

13. Do you consume unpasteurized milk?

a.□ Yes

b.□ No

14. Symptom at any stage of course of infection	**Yes**	**No**
a. Chronic cough lasts for more than 3 weeks	□	□
b. Fever less than 38°C	□	□
c. Pain in the chest	□	□
d. Coughing up blood or sputum	□	□
e. Weakness or fatigue	□	□
f. Weight loss	□	□
g. Sweating at night	□	□
h. Diarrhea	□	□

15. Are you vaccinated with BCG

a.□ Yes

b.□ No

c. □ Unknown

16. Do you wash your hands with soap before and after work?

a.□ Yes

b.□ No

17. What is your perceived health status?

a.□ Not good

b.□ Good

18. Do you smoke?

a.□ Yes

b.□ No

19. Do you have contact with tuberculosis-infected person?

a.□ Yes

b.□ No