JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

A retrospective analysis of tuberculosis in livestock farmers in Lahore district, Pakistan

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Abstract

Introduction: *Mycobacterium tuberculosis* is the main cause of tuberculosis in humans, accounting for numerous illnesses and thousands of fatalities globally. Data regarding the association of various risk factors and TB in livestock farmers in Pakistan is scarce.

Methodology: A retrospective matched case-control study of TB cases was performed in Lahore, Pakistan to investigate the potential risk factors that lead to the development of TB in Pakistani livestock farmers. A total of 170 participants were included in the study. The case was matched with control based on neighborhood and the case-control ratio was kept 1:1. Data were statistically analyzed using R version 4.2.1. Conditional logistic regression was conducted to identify biologically and statistically plausible risk factors associated with the TB outcome among livestock farmers.

Results: In univariable analysis, 10 risk factors were identified ($p < 0.05$). Gender, age, being married, family type, living in a big family, BCG vaccination status, history of smoking, working at a cattle farm, co-housing with cattle at night, , consumption of raw milk. The multivariable model identified four risk factors i.e., consumption of raw milk (Odds Ratio [OR]: 7.7; 95% Confidence Interval [CI]: 1.95-30.68), living in big family (OR: 6.2; 95% CI: 1.25-30.82) and working at cattle farm (OR: 4.2; 95% CI: 1.08-16.56), while gender was found to be a protective factor with OR < 1 (OR: 0.06; 95% CI: 0.01-0.26).

Conclusions: This study demonstrated that sociodemographic risk factors and exposure to infected cattle can influence the development of TB in farmers.

Key words: TB Risk factors; farmers; Pakistan; case-control study.

J Infect Dev Ctries 2024; 18(8):1249-1257. doi:10.3855/jidc.18296

(Received 30 March 2023 – Accepted 04 September 2023)

Introduction

Tuberculosis (TB) is a re-emerging disease at the global level and its main causative agent is *Mycobacterium tuberculosis*, the lead member of Mycobacterium Tuberculosis Complex (MTBC) [1]. In 2016, there were 12,500 confirmed global deaths due to zoonotic tuberculosis and nearly 147,000 new cases, making up roughly 1.4% of the global tuberculosis burden [2]. Annually, over $12 billion is incurred in terms of the financial cost of TB [3]. With an estimated 1.3 million deaths annually, tuberculosis ranks among the top 10 causes of death worldwide. It is a significant cause of illness and death in low and middle-income countries (LMICs) [4]. Pakistan is ranked fifth among the countries with the highest TB burden in the world. In Pakistan, the numbers for prevalence, incidence, and TB-related deaths per 100,000 people per year are 340, 259, and 20, respectively [5].

Other than *Mycobacterium tuberculosis*, MTBC also includes *Mycobacterium bovis* which causes zoonotic TB [6]. *Mycobacterium bovis* is usually transmitted to humans through drinking unpasteurized milk and breathing in an environment with droplets from infected animals. It is estimated that almost 10-15% of new cases of TB are caused by *Mycobacterium bovis* in LMICs. The reason behind this is the frequent interaction of humans and animals due to a shared microenvironment [7]. Thus, various occupational workers such as livestock farmers, abattoir workers, veterinarians, and hunters are at increased risk of

becoming infected with *Mycobacterium bovis* because they have direct or in direct contact with wild and domestic animals [8,9]. However, evidence of reverse zoonosis (i.e. *Mycobacterium tuberculosis* transmission from humans to animals) is also possible, and numerous investigations have shown *M. tuberculosis* in both domestic and wild animals [10,11].

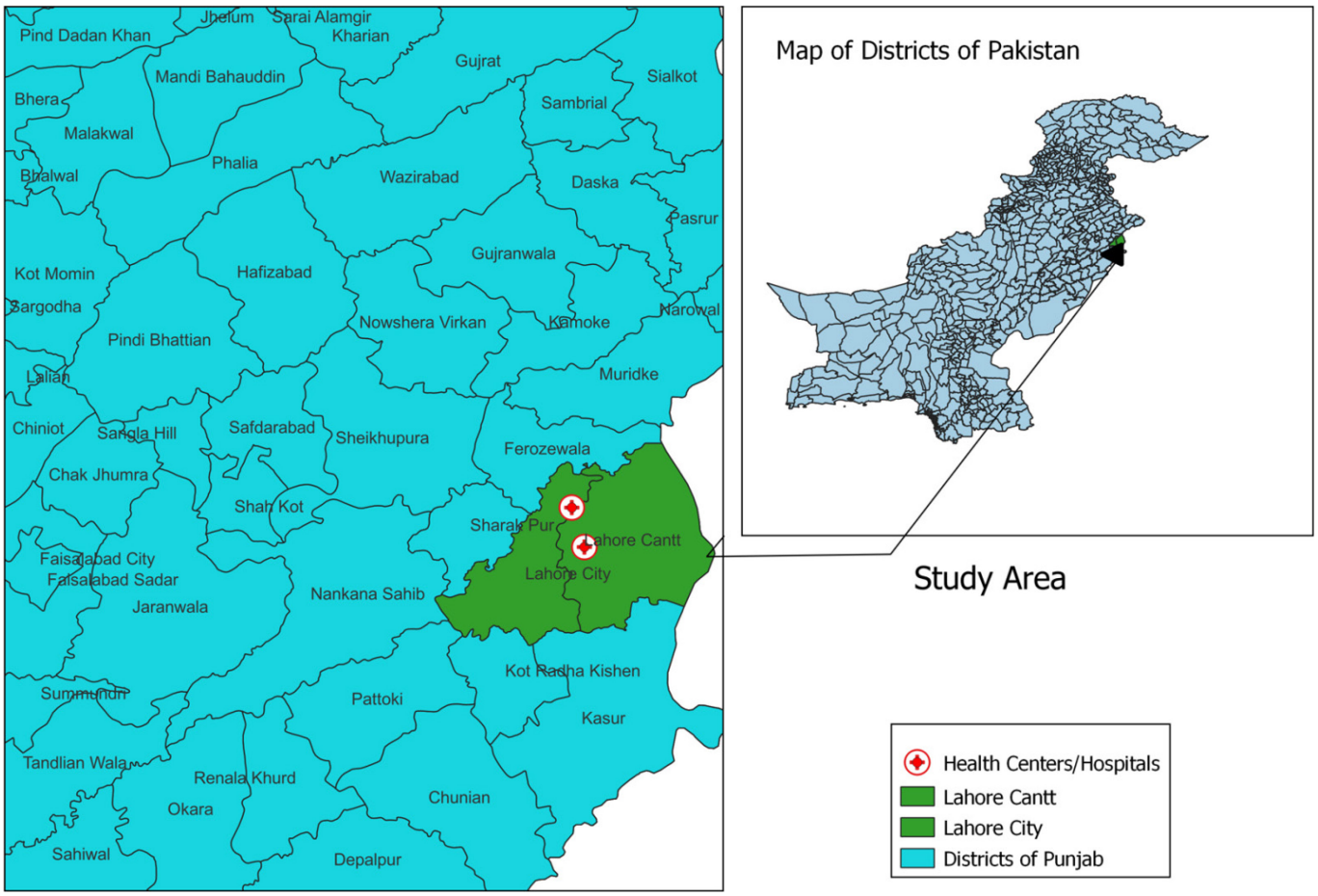
Despite the heavy dependence of LMICs on agriculture, there is no comprehensive data available about zoonotic TB prevalence and its associated risk factors. Several factors facilitate the spread of zoonosis including poor dietary habits, poverty, close physical contacts between human and animals, and a lack of disease control measures [12]. The likelihood of transmission between farmers and their cattle is thus suggested by the prevalence of Mycobacterium Tuberculosis Complex in both humans and cattle in the selected geographical area [10]. In LMICs where disease control activities are poor and there is higher interaction between humans and cattle, data is poorly documented about the transmission of *Mycobacterium bovis* and its associated risk factors [13]. For these reasons, data on TB transmission and its associated risk factors at the human-animal interface is important for designing One Health approach for controlling the disease in rural settings [14]. To modify and adapt new TB control strategies in response to the re-emergence of TB, particularly in developing nations, it is necessary to reassess the role of potential risk factors associated with the TB agent, host, and environment that may affect the development of tuberculosis in farmers. This study explores the sociodemographic risk factors such as age, gender, occupation, level of education, and animal-associated risk factors such as contact with diseased or coughing cattle and ingestion of unpasteurized milk associated with TB outcomes in farmers.

Methodology

Study Design and Setting

A retrospective matched case-control study was performed to identify potential risk factors of TB in cattle farmers. The objective of this study was to identify the potential risk factors of TB among cases and controls. Pakistan is an agricultural country, with livestock accounting for 11.8% of national GDP and 55.9% of total agricultural value added. There are

Figure 1. Study area and location of cases.



around 41.2 million cattle and 35.6 million buffaloes in the country [15]. Lahore is the capital city of Punjab Province and the second-most populous city in Pakistan. It is situated in the northeastern part of the country at 31° 32′ 59″ latitude and 74° 20′ 37″ longitude [16]. The geographical locations of the hospitals were located on Google Maps. Shape files of Pakistan boundaries were available in the repository [17]. A dot map was produced using the QGIS software Version 3.2.2 (Open Source Geospatial Foundation Project, Boston, MA, USA) (available at http://qgis.org/).

Study Population

The cases were selected from two tertiary healthcare hospitals in Lahore, Pakistan (Mayo Hospital and Infectious Disease Hospital) (Figure 1) and controls were selected from the neighborhood of cases living in the same village. The data was recorded in Microsoft Excel. The study's power was kept at 80%, the level of significance at 5%, the proportion of controls with exposure at 40% [18], the odds ratio at 2.5, and the case-to-control ratio at 1: 1. This resulted in 170 samples, 85 cases, and 85 controls. Winpepi software was used to calculate the sample size (http://www.brixtonhealth.com/pepi4windows.html).

Definition of Cases and Controls

Medical professionals in the selected hospitals confirmed the diagnosis of human TB cases. A confirmed case of tuberculosis was defined as a livestock farmer diagnosed with TB by the clinician or other medical practitioner, and was under complete course of TB treatment at a designated health facility (Mayo or Infectious Disease Hospital) [19]. The trained medical officer clinically diagnosed TB (based on symptoms of TB and chest x-ray) followed by a confirmatory diagnosis by Gene Xpert. The controls were an equal number of livestock farmers without TB who lived in the same village in the neighborhood of TB-positive cases. A household which did not have any history of tuberculosis over the last ten years and the members were clinically confirmed to have no clinical signs of TB like illnesses at the time of visit were declared as negative households and selected as controls.

Enrollment of cases and controls

A total of 170 participants were registered. The cases (n = 85) were livestock farmers of 15 years of age or above and the controls (n = 85) were livestock farmers matched at a location (same village) with a case-control ratio of 1:1.

Study Procedure

Informed consent was obtained from all the participants. Both cases and controls included in the study were interviewed face-to-face in the local language (Urdu) and all information was collected on a predesigned questionnaire [18,20] (Supplementary Item 1). The questionnaire comprised open and close-ended questions. The questionnaire comprised of two sections: the first section had demographical information related to sex, age, marital status, education, residence, and monthly income, and the second section included questions about various potential risk factors i.e., family size, number of rooms in the house, numbers of windows in the house, BCG vaccination, perceived health status, smoking history, drinking raw milk, chronic diseases, etc. A unique code identification number was given for each questionnaire to keep data confidential by a trained research team member. Each interview lasted approximately 20 minutes. The study protocol followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

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 collection. The institutional review committee for biomedical research, University of Veterinary and Animal Sciences, Lahore (Letter no. 075/IRC/BMR) approved study protocol.

Statistical Analysis

Data sets were entered into EpiData software (version 3.1, Odense, Denmark, 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 2016, Microsoft Office, USA) for further processing. The data were statistically analyzed using R version 4.2.1. Using the survival package in R software, conditional logistic regression was conducted to identify biologically and statistically plausible risk factors related with the TB among livestock farmers. Multivariable analysis was performed on variables with $p < 0.25$ in the univariable analysis. Forward elimination approach was used in multivariable logistic regression to create the final model, starting with the factors that had the lowest p value in the univariate analysis to identify independent risk factors. Variables were kept or removed from the model based on the p value > 0.05 calculated for the Wald Statistic (or log-

likelihood ratio test for categorical variables with 3 or more levels). The odds ratios with 95% confidence intervals (CI) were computed to determine the degree of the association for significant variables. All statistical tests were performed with a significance level of 0.05.

Results

Characteristics of participants

A total of 170 participants were included in this study. Of which, 111(65%) were males and 59 (34.7%) were females. Among all participants, 29 (17.1%) individuals were in the age group 15 to 24 years, while 34 (20%) were in the age group 55 years and above (Figure 2).

Figure 2. Ages of the participants in case and control groups.

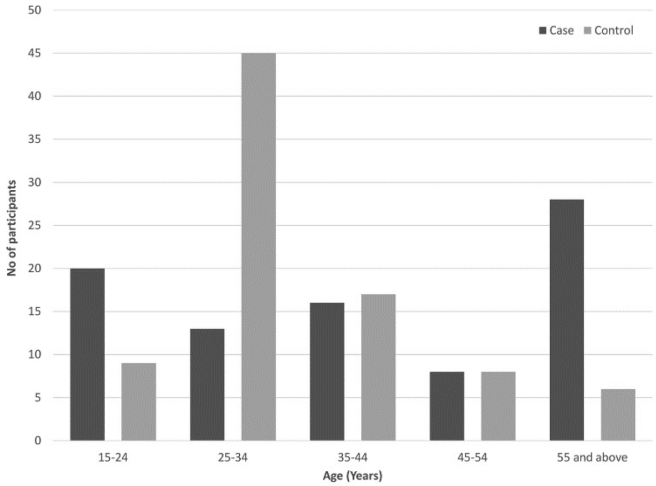


Table 1. Socio-demographical & Behavioral Characteristics of the cases and control participated in the study.

Variable	Response	Total number	Cases	Control
Gender	Female	59 (34.7%)	45 (52.95%)	14 (16.47%)
	Male	111(65%)	40 (47.05%)	71 (83.52%)
Age	15-24	29 (17%)	20 (23.52%)	9 (10.58%)
	25-34	58 (34.11%)	13 (15.29%)	45 (52.94%)
	35-44	33 (19.4%)	16 (18.82%)	17 (20%)
	45-54	16 (9.41%)	8 (9.41%)	8 (9.41%)
	55 and above	34 (20%)	28 (32.94%)	6 (7.05%)
Marital status	Married	34 (20%)	21 (24.70%)	13 (15.29%)
	Unmarried	136 (80%)	64 (75.29%)	72 (84.70%)
Income	10,000-15,000 pkr	134 (78.8%)	69 (18.17%)	65 (76.47%)
	16,000 pkr and more	36 (21%)	16 (18.82%)	20 (23.52%)
Education	Illiterate	59 (35%)	41 (48.23%)	18 (21.17%)
	Primary	45 (26%)	19 (22.35%)	26 (30.58%)
	Secondary	56 (33%)	20 (23.52%)	36 (42.35%)
	Intermediate	10 (5.9%)	5 (5.88%)	5 (5.88%)
Family type	Joint	115 (67.6%)	65 (76.47%)	50 (58.82%)
	Nuclear	55 (32%)	20 (23.52%)	35 (41.17%)
Family size	1-10	133 (78.2%)	55 (64.70%)	78 (91.76%)
	11 and more	37 (22%)	30 (35.29%)	7 (8.23%)
Windows	4 and more	42 (24.7%)	17 (20%)	25 (29.41%)
	1to 3	99 (58%)	43 (50.58%)	56 (65.88%)
	No	29 (17.1%)	25 (29.41%)	4 (4.7%)
Duration of window open	Window open half day	54 (32%)	34 (40%)	20 (23.52%)
	Window open Never	31 (18.2%)	27 (31.76%)	4 (4.7%)
	Window open whole day	85 (50%)	24 (28.23%)	61 (71.76%)
BCG vaccine	No	46 (27%)	37 (43.52%)	9 (10.58%)
	Yes	124 (72.9%)	48 (56.47%)	76 (89.41%)
History of smoking	No	143 (84.1%)	66 (77.64%)	77 (90.58%)
	Yes	27 (16%)	19 (22.35%)	8 (9.41%)
Current smoking	No	164 (96.5%)	81 (95.29%)	83 (97.64%)
	Yes	6 (4%)	4 (4.70%)	2 (2.35%)
Work at cattle farm	No	91 (53.5%)	32 (37.64%)	59 (69.41%)
	Yes	79 (46%)	53 (62.35%)	26 (30.58%)
Cattle at home	No	128 (75.3%)	54 (63.52%)	74 (87.05%)
	Yes	42 (25%)	31 (36.47%)	11 (12.94%)
Co house with cattle at night	No	125 (73.5%)	55 (64.70%)	70 (82.35%)
	Yes	45 (26%)	30 (35.29%)	15(17.64%)
Contact with sick cattle	No	154 (90.6%)	72 (84.70%)	82 (96.47%)
	Yes	16 (9%)	13 (15.29%)	3 (3.52%)
Contact with coughing cattle	No	158 (92.9%)	75 (88.23%)	83 (97.64%)
	Yes	12 (7%)	10 (11.76%)	2 (2.35%)
Raw milk consumption	Both	28 (16.5%)	21 (24.70%)	7 (8.23%)
	Yes	33 (19%)	26 (30.58%)	7 (8.23%)
	No	109 (64.1%)	38 (44.70%)	71 (83.52%)
Have chronic disease	No	148 (90%)	69 (81.17%)	79 (92.94%)
	Yes	22 (10%)	16 (18.82%)	6 (7.05%)

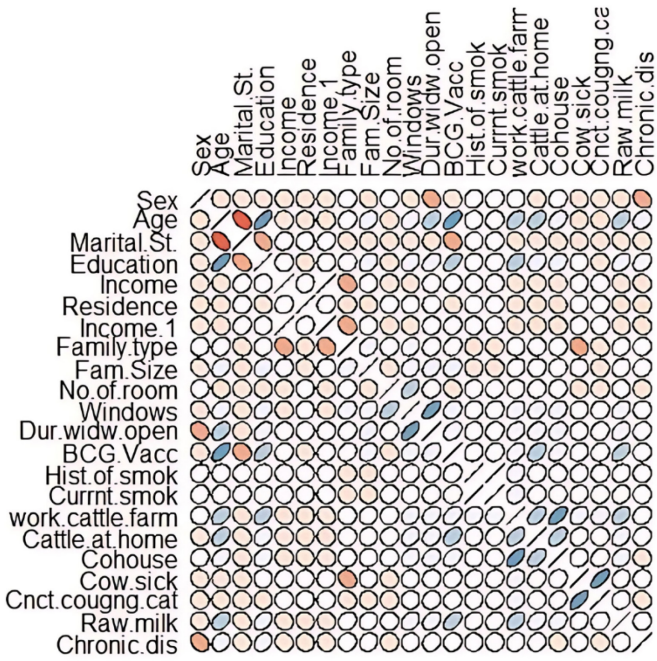
Fifty-nine (35%) participants were illiterate, 45 (26%) had primary education, 56 (32.9%) had secondary level education, 8 (4.7%) had intermediate level education and only 2 (1.2%) participants were graduates. Of 170 participants 136 (80%) were married and 34 (20%) were unmarried. The income of 134 (78.8%) participants was between 10,000-15,000 PKR. Most participants (n = 115, 67.6%) lived in a joint family and 37 (22%) participants were living in a big family i.e., 11 persons and more. Twenty-nine (17.1%) participants lived in houses with no windows, while 50% participants kept window open for the whole day. Of 170 participants, 128 (73.5%) had cattle at home and 91 (53.5%) worked at a cattle farm, only 45 (26%) cohoused with cattle at night (Table 1).

Risk factors of TB

Both cases (n = 85) and controls (n = 85) were interviewed. The collinearity between these variables was assessed and plotted

Variables, which showed correlation, were excluded from the analysis which included education, no of rooms, windows, duration of window open, current smoking, cattle at home, and contact with sick cattle. In a univariable analysis, 14 variables were screened, and 12 were associated with the case and control groups (Figure 3). Gender ($p < 0.0001$), age ($p = 0.001$), being married ($p = 0.14$), family type ($p = 0.005$), living in a big family ($p = 0.0003$), BCG vaccine ($p = 0.0001$), history of smoking ($p = 0.028$) family member had TB ($p < 0.0001$), working at cattle farm ($p = 0.00014$), co-housing with cattle at night ($p = 0.0143$), contact with coughing cattle ($p = 0.0371$), and consumption of raw milk ($p \leq 0.0001$) were selected for multivariable analysis based on p value less than 0.25 (Table 2).

Figure 3. Correlation analysis of risk factors of TB among cattle farmers.



Color intensity is proportional to the magnitude of the correlation. Education, number of rooms, windows, duration of window open, current smoking, cattle at home, and contact with sick cattle are correlated variables.

In the final multivariable analysis, three risk factors were shown to be significantly associated with TB in farmers, which included consumption of raw milk (OR: 7.74; 95%; CI: 1.96-30.70), living in a big family (OR: 6.21; 95%CI: 1.25-30.82) and working at cattle farm (OR: 4.23; 95% CI: 1.08-16.56). Gender was found to

Table 2. Univariable Analysis of risk factors associated with cases and controls.

Sr. No	Variable	Response	Total	Cases	Control	OR	95% CI	*p* value
1	Gender	Female	59	45	14	Ref	0.054-0.35	< 0.0001**
		Male	111	40	71	0.13		
2	Age	Number	170	85	85	1.03	1.01-1.04	0.001*
3	Being married	Married	136	21	13	Ref	0.57- 0.82	0.14
		Unmarried	34	64	72	1.72		
4	Family type	Nuclear	55	20	35	Ref	1.50-10.6	0.005**
		Joint	115	65	50	4		
5	Living in a big family	1-10	133	55	78	Ref	2.16-14.5	0.0003**
		11 and more	37	30	7	5.6		
6	BCG vaccine	Yes	124	48	76	Ref	2.22-11.26	0.0001**
		No	46	37	9	5		
7	History of smoking	No	143	66	77	Ref	1.11-7.18	0.028*
		Yes	27	19	8	2.8		
8	Work at cattle farm	No	91	32	59	Ref	2.15-10.9	0.00014**
		Yes	79	53	26	4.85		
9	Co-house with cattle at night	No	125	55	70	Ref	1.20-5.20	0.0143*
		Yes	45	30	15	2.5		
10	Raw milk consumption	No	109	38	71	Ref	2.75-15.35	< 0.0001**
		Yes	61	47	14	6.5		

*denotes significant and ** showed highly significant results.

be a protective factor with OR < 1 (OR: 0.06; 95% CI: 0.01-0.26) (Table 3).

Discussion

Tuberculosis caused by the *Mycobacterium tuberculosis complex* (MTBC) continues to be one of the most significant causes of death in humans as well as in animals [21]. It has been controlled in most developed countries but remained a global challenge to public health due to the large burden and cost of treatment in developing countries [22]. Data about TB transmission at the human and animal interface and associated risk factors is important to develop control strategies involving the One Health approach. In Pakistan, data about TB and associated risk factors in high-risk groups is very limited. We designed a retrospective matched case-control study to identify potential risk factors of TB in cattle farmers. In univariable analysis, being married was identified as a risk factor for tuberculosis among farmers. Farmers who were married were 2 times more likely to get tuberculosis than unmarried farmers. In rural Malawi, during contact investigations, 5% of adult TB cases disclosed a former TB-positive spouse. Through everyday intimate contact and nursing, wives are especially prone to have acute exposure to *M. tuberculosis*. If they accompany their partner to outpatient facilities or offer nursing care in the hospital ward, the spouse may also come into contact with other TB patients [23]. Similarly, an increase in age was a risk factor in univariable analysis in this study. However, in multivariable analysis, it was excluded due to selection criteria ($p < 0.05$). Natural aging of the lungs is linked to ongoing molecular and physiological changes that result in a low-grade pro-inflammatory and oxidative state that persists, diminished lung function, and altered immunological responses that make people more vulnerable to lung disease and respiratory infections [24]. One of the biggest risk factors for contracting TB is getting older. As a human age, a constant buildup of low-grade inflammation and an oxidative state (referred to as "inflammaging") worsens the homeostatic balance of stress responses and impairs intrinsic processes that support cell repair, regeneration, and immune surveillance [25,26]. Previously a study conducted in China found that TB incidence increased in age groups of 45 and above, which corroborates our findings [27]. Another important risk factor in univariable analysis was smoking. Smoking causes peri-bronchial inflammation, fibrosis, vascular thickening, and alveolar damage in the lower respiratory tract, among other histological alterations. As a result, the epithelium's function is altered, resulting in aberrant vascular and epithelial permeability, decreased ciliary activity, and impaired clearance of inhaled substances [28]. In current study people who had a history of smoking were 2.8 times more likely to develop tuberculosis than those who did not smoke. Various studies have reported similar findings [29,30].

Many studies have suggested that responses to illness differ in women and men. Different factors have been proposed to explain this gender gap which includes biological differences in disease and its presentation, access to health care especially in lower middle-income countries, and socio-cultural factors [31]. In current study, gender was a significant factor both in univariable and multivariable analysis and females were more likely of having TB compared to men ($p < 0.001$). In the past (from 1930-1950s), in industrialized countries, female notification rate of TB was higher than males. In other studies, men had been considered at higher risk of contracting TB. Gender differences may exist in terms of barriers to detection and successful treatment of TB [32]. Women once enrolled in health care facility may show more compliance and adherence to treatment than men [33]. In present study, more women were adherent to the treatment protocol than men.

Living in a crowded place has been reported as a potential risk factor for TB in literature. In the current study farmer living with 11 and more family members were 5 times more at risk of developing TB than compared to those who were living in less crowded place. People living in joint families were at more risk of TB than those living in nuclear families. Living in a crowded place may increase the likelihood of coming into contact with someone who was excreting (bacilli) the causative agent. Furthermore, a large family reduces household income, resulting in reduced nutritional consumption of household members. It is a sign of low socioeconomic status [34-37]. In present study, strong association was found between working at a cattle farm

Table 3. Multivariable Analysis of risk factors associated with cases and controls.

Sr No.	Variable	Odds Ratio	95% CI	*p* value
1	Gender	0.06	0.01 - 0.26	0.0001**
2	Raw milk consumption	7.7	1.95 - 30.68	0.003 **
3	Living in a big family	6.2	1.25 - 30.82	0.025 *
4	Working at cattle farm	4.2	1.08 - 16.56	0.038*

*denotes significant and ** showed highly significant results, CI are wide due to small sample size

and TB. Farmers who worked at cattle farms were 4.23 times more likely to develop tuberculosis than those who did not work at cattle farms. Farmers who had contact with sick and coughing cattle were at 5 times more risk of developing TB. Humans become infected by inhaling the infectious agent when there is close physical interaction between the owner and their cattle, particularly at night because some owners share shelters with their animals [8]. These findings are similar to the studies conducted by Fetene *et al* [38] and Mengistu *et al* [39], who found exposure to bovine tuberculosis and human tuberculosis disease have strong positive correlation. Furthermore, this could be explained by the lack of knowledge of community about the etiology and mode of transmission of zoonotic TB [40]. People can contract zoonotic TB after consumption of unpasteurized dairy products, handling the sick animals, and via occupational exposures [18]. In our study consumption of raw milk was associated with TB in farmers ($p < 0.001$) in multivariable analysis. In Pakistan, consumption of unpasteurized raw milk consumption is very common and awareness about zoonotic tuberculosis is also scarce. Occupationally exposed workers have limited knowledge of zoonotic tuberculosis and they did not use personal protective equipment while working with cattle in Pakistan [41].

The control of bovine TB may be difficult given that keeping cattle is connected to the cultural practices of many people in this country. The relevant difficulties could be addressed by using the One Health Approach involving healthcare professionals, anthropologists, social workers, and veterinarians. Infected cattle must be culled from the herd. The findings of the current study highlighted the importance of updating policy on TB control strategies at the human-animal interface to address risk factors, as well as coordinated efforts between the animal and human health sectors to monitor disease spread.

Our study has few limitations and one of them is that Gene Xpert only detects species level and does not differentiate *Mycobacterium tuberculosis* and *Mycobacterium bovis*. Secondly, by gathering sufficient data on the exposure status, an attempt was made to reduce the information bias in choosing controls; nonetheless, some recall bias might still exist. Thirdly, due to limited resources, the controls were not evaluated using laboratory procedures to determine their TB status; instead, they were recruited solely based on history.

Conclusions

The current study identifies potential risk factors of TB in cattle farmers. Gender, living in a big family, consumption of raw milk, and working at a cattle farm are risk factors for tuberculosis in farmers of Pakistan. The number of tuberculosis cases in Pakistan might be reduced by an efficient program to manage bovine TB in livestock populations.

Acknowledgements

The authors would like to thanks all the participants who provided data for this study and hospital staff for their cooperation.

Authors' Contributions

Conceptualization: CJ and MC. Data curation: CJ, RM, MSS, MA, KI, GU and MC. Methodology: CJ and MC. Project administration: HBR, SS, RM. Visualisation: CJ. Writing–original draft: CJ and MC. Writing–review and editing: HBR, MHM, JAK. All authors have read and agreed to the published version of the manuscript.

Funding

This research work was supported by the National Research Program for Universities, Higher Education Commission, Pakistan (Project No.20-13742/NRPU/R&D/HEC/2020). First author (Chanda Jabeen) was awarded with a PhD studentship by Higher Education Commission, Pakistan under Indigenous 5000 PhD phase II Fellowship Program (Grant number 520-139645-2MD6-1). There were no funds available for the publication of this manuscript.

Data Availability Statement

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

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Conflict of interests: No conflict of interests is declared.

Annex – Supplementary items

Supplementary Item 1: Questionnaire.

Id No:_____
Case:_____ Control:_____
Address:_____
Latitude:_____
Longitude:_____

1. **Gender**
   a. □ Male b. □ Female
2. **Age**
   a. □ 15-24 b. □ 25-34 c. □ 35-44 d. □ 45-54 e. □ 55 and above
3. **Marital Status**
   a. □ Unmarried b. □ Married
4. **Education**
   a. □ Illiterate b. □ Primary c. □ Secondary d. □ Intermediate e. □ Graduate
5. **Monthly Income (PKR)**
   a. □ 10,000-15,000 b. □ 16,000 and more
6. **Family type**
   a. □ Nuclear b. □ Joint
7. **What is your family size?**
   a. □ 1-10 b. □ 11 and more
8. **Number of windows in the house**
   a. □ No b. □ 1-3 c. □ 4 and more
9. **Duration the window in the house remain open in a day**
   a. □ 2-3 hours b. □ Half day c. □ Whole day d. □ Never
10. **Are you vaccinated against BCG?**
    a. □ Yes b. □ No c. □ Unknown
11. **Do you have history of smoking?**
    a. □ Yes b. □ No
12. **Do you currently smoke?**
    a. □ Yes b. □ No
13. **Do you work at the cattle farm?**
    a. □ Yes b. □ No
14. **Do you have cattle at home?**
    a. □ Yes b. □ No
15. **Do you co-house with livestock at night?**
    a. □ Yes b. □ No
16. **Does your cow often became sick, and have you handled with it when it is sick?**
    a. □ Yes b. □ No
17. **Did you have history of contact with the debilitated and coughing cattle around you?**
    a. □ Yes b. □ No
18. **Do you have a habit of drinking raw milk from cattle?**
    a. □ Yes b. □ No
19. **Do you have any chronic disease?**
    a. □ Yes b. □ No