Reverse component of Bovine tuberculosis: Knowledge as a confounding factor in disease spread.

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Abstract

Bovine tuberculosis(bTB) is a chronic progressive granulomatous zoonotic disease in cattle with economic and public health importance, especially in developing countries like Pakistan. It is caused by Mycobacterium bovis. Mycobacterium tuberculosis complex (MTBC) is a group of Mycobacteria that are genetically related and are known to cause bTB in cattle as well as in humans. bTB has a zoonotic and a reverse component that contributes to spread of infection from cattle to human and from human to cattle. Pakistan being the world's top 5 milk producing countries, harbours this pathogen in milk and lack of knowledge and traditional practices of consumption of milk spread the disease. We have recruited 126 individuals working in close proximity to cattle and buffaloes. Demographic characteristics of these people along with knowledge and practices of cattle handling and milk consumption were recorded. Consumption of raw milk (71.4%), close proximity to cattle and buffaloes(animals at home=30.2% & people working at farms=28.6%) and poor knowledge of bovine tuberculosis(72.2%) are the contributing factors to the high toll of the disease. The zoonotic aspect of the disease must be addressed so that disease can be restrained.

Key words

Bovine Tuberculosis, Public health, Knowledge, Consumption, Proximity

Introduction

The *Mycobacterium tuberculosis* complex (MTBC) is a group of *Mycobacteria* that comprises of *Mycobacterium tuberculosis (M. tuberculosis),* Mycobacterium africanum *(M. africanum), Mycobacterium bovis (M. bovis), Mycobacterium canettii (M. canettii),* Mycobacterium microti *(M. microti), Mycobacterium pinnipedii (M. pinnipedii)* and *Mycobacterium caprae (M. caparae) all of which are genetically related(*Forrelland et al.,2013). Additionally, two novel species (*Mycobacterium orygis* and *Mycobacterium mungi*) are referred as MTBC besides the seven common species (Pfyffer, 2015).

Transmission of M. bovis from infected animals to other animals of herd takes place via direct contact through contaminated utensils (Chang et al.,2023). Physical contact with their discharge (Blood and body fluids) is also contagious (Poza et al.,2024). <10 viable bacilli are sufficient to cause infection through aerosols. The infected animal may expel tubercle bacilli in small droplets of secretions which shrink to 5 µm in diameter. This small droplet may remain suspended indefinitely in the air and infect upon release (Dean et al.,2005). Ingestion of infected food and water also cause infection specially in calves where 10 mg of bovine tubercle bacilli can cause alimentary infection whereas 0.01 mg of bovine tubercle bacilli can cause pulmonary infection (Johnson et al.,2007). Bite of infected ticks are a source of spread of infection to deer, cattle, guinea pigs and rabbits. Artificial insemination of infected bull may also transmit the disease (Teppawar et al.,2008).

Direct exposure of infected farm workers and urine contamination of food and water supplies by workers having renal tuberculosis may result in human to animal transmission (Peto et al.,2009). Dissemination of tuberculosis in humans takes place via airborne droplets carrying viable bacilli in 1-5 µm range and other aerosol generating activities including sneezing, talking and singing from the infected person. Bovine tuberculosis acquisition through the milk and dairy products and consumption of infected meat from infected cattle has greatly increased the toll of bovine tuberculosis in humans as well (Khan et al.,2019)

Suppressed immune system is an important risk factor for M.bovis infection in humans(Vu et al.,2024). This contributes to the inter-personal transmission of disease. A list of factors contributing to compromised immune systems are diabetes, cancers, drug users, organ transplant recipients, malnourished individuals and comorbid conditions. Since humans acquire M.bovis infection mainly through ingestion of unpasteurized milk and dairy products, counselling of immune-compromised individuals about zoonotic TB and risk factors for the acquisition of M. bovis infection should be made compulsory at the healthcare facilities (Choudhary et al.,2024).

In some communities in Pakistan, raw cattle milk consumption is considered healthy and a staple diet of some families and societies(Fareed et al.,2024). The traditional practice of boiling milk before consumption as practiced in urban population since time immemorial should be revived and propagated by increasing awareness in rural communities(Nayak et al.,2024). Moreover, there is no gender predilection to tuberculosis transmission and spread. Both genders are equally susceptible to disease contraction and spread. However, the females residing in houses are found to be the carriers of latent infections and may contribute to disease spread in children(Trevisi et al.,2024). Similarly, the population density at a particular given space may also contribute to disease spread and transmission of infection from household contacts(Pinto et al.,2024). Every day exposure such as visit to workplace and occasional trips to crowded places for males is an added factor towards acquiring infection more from the outside sources and contribute to spread of the disease to the domestic contacts(Dolphyn et al.,2022). This implies that social contact pattern is likely to quantify exposure to putative sites of transmission and spread of infection to indoor contacts(Vamsi et al.,2023).

Material and methods

The study was carried out in Faisalabad, the third largest city of Pakistan and informally called the Manchester of Pakistan, with a population of over 3.7 million. It is situated in the north-east; lying between the plains of Ravi and Chenab River. The dairy industry of the city contributes 9.4 MT of milk production annually. The government and private dairy farms were randomly selected for screening purpose for the detection of M. tuberculosis and M. bovis.
Before proceeding to the formal research process, an approval was taken from Institutional Biosafety Committee (IBC) of University of Agriculture, Faisalabad (UAF/1021,July 2021) and Ethical review committee of Public Health Department of Tertiary care hospital, Faisalabad (MTH/ 2021/92/16-03-2021).

A total of 210 (n) samples were collected from human and bovine suspects and processed in tertiary care Allied hospital and Al-Shifa Research and Diagnostic center, Faisalabad. Later, some part of the processing was done in institute of Microbiology, University of Agriculture, Faisalabad.

Occupational groups at risk were divided into three major groups in accordance to the type of activity, closeness to livestock and duration of exposure as high, medium and low-risk sets. High-exposure group refers to workers in close-proximity such as abattoir workers, milkers and veterinary personnel. Medium-risk group comprises of herders, tractor operators, feeders, household contacts living in cowsheds. Low-risk group included individuals involved in commercial activities and cowshed owners having no direct contact with livestock. The People working in slaughter houses, aged between 20 -60 years, experiencing non-specific symptoms; cough, shortness of breath, unexplained weight loss, fever and fatigue were included and people who were labelled as low risk group, Age < 20 years or > 60 years and those not willing to participate in the study were excluded from the study.

Sampling began with random selection of persons working in close-proximity to animals. A verbal invitation to participate provided informed consent and preliminary agreement for participation after being given an explanation of all field examination and testing was clarified. A pre-designed questionnaire following the literature review of all the potential risk factors was used.

After consent, tuberculin skin test(TST) was performed in which 0.1 ml purified protein derivative(PPD) was injected intradermally at the inner surface of the forearm. Tuberculin skin test (TST) is a conventional in vivo skin test to determine the individual's immune response in terms of delayed hypersensitivity reaction-IV against mycobacterial antigens. Commercially available Eplisol and Amson PPD were injected in a total of 367 subjects (241 bovine and 126 human) and results were recorded. Later the positive TST were recruited in the study.

Thick purulent portion of sputum from individuals with positive Tuberculin skin test was taken by using sterile wire-loops on a clean glass slide and spread over an area of 1-2 cm. The smear was air-dried for 15-20 minutes and then passing the slide over flame for three to five times for 4-5 seconds each. Carbol Fuschin was poured on the smear and heated until fumes produced. Slides were allowed to stand for 5 minutes and slide was washed with distilled water. 3% HCL was poured on the smear for 20-30 seconds. Methylene blue dye was flooded onto the smear and slide was washed and dried.

Direct microscopy was done first at 4 x, 10 x and then 40 x followed by 100 x (oil immersion lens) for direct visualization of Acid fast bacilli (AFB). Later on, these positive sputum samples were inoculated on Lowenstein Jensen media (Lj) for up to 6 weeks for observation of growth of Mycobacteria.

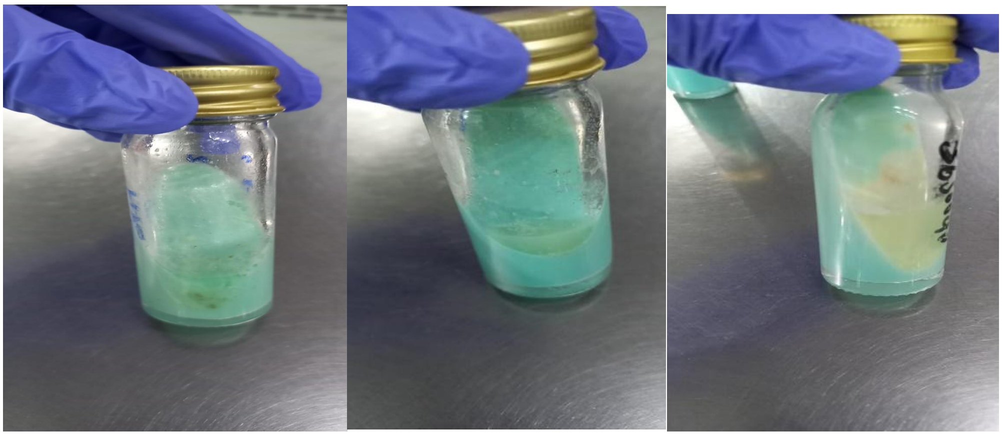

Figure 1: Lowenstein Jensen media observed at 2,4 and 6 weeks' time

During the current study, various herds located in different regions of Punjab, were visited for the collection of samples from suspected animals of tuberculosis. Moreover, data was collected to check the risk factors associated with disease spread among herds. A total of 551 animals were examined in 06 different localities with a total of 19 herds. Out of these, 241 animals with significant lesions on the body were inoculated with purified protein derivative(PPD) to observe the delayed hypersensitivity reaction in terms of wheal formation through tuberculin skin test TST. Out of these, 102 animals showed desired thickness Later, 102 milk samples were collected from these infected animals.

Area of sampling	**Herds visited**	**Animals examined**	**Tuberculin skin test**	**Milk samples**
1.	3	94	21	18
2.	4	102	22	16
3.	2	75	18	13
4.	4	111	27	22
5.	1	45	17	07
6.	5	124	46	26
Total	19	551	151	102

Table 1: Number of animals examined and samples collected from different herds

Out of the total 102 milk samples collected from different herds, 09 samples showed acid fast bacilli after Zn staning and direct microscopy.

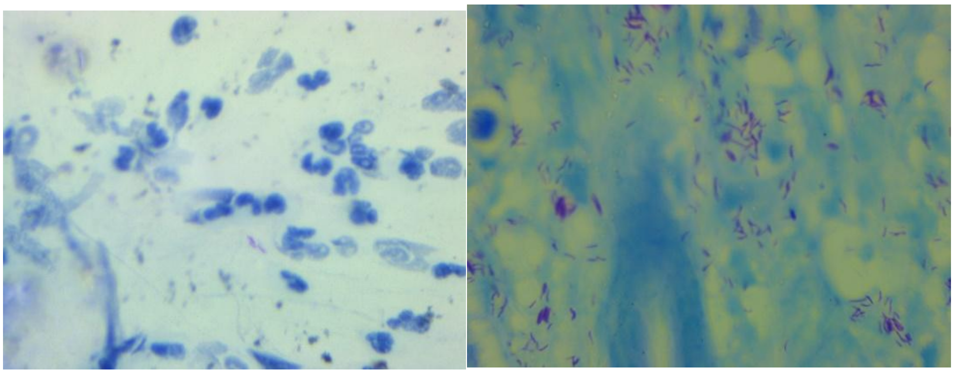

Figure 2 Observation of Acid-fast bacilli under 40x and 100x in milk samples

Results

Out of the total 126 patients examined, 77(61.1%) were males and 49(38.9%) females. The patients were divided in to four age groups i.e. 20-30 years, 31-40 years, 41-50 years and 51-60 years. Table 2 illustrates that the maximum number of study population is from ages 31-40 years preceded by 41-50 years, i.e. 46 and 38 respectively.

Gender	**Age in Years**				
	20-30	**31-40**	**41-50**	**51-60**	**Total**
Male	16	21	28	12	**77**
Female	5	25	10	9	**49**
Total	**21**	**46**	**38**	**21**	**126**

Table 2: Age distribution of individuals according to Gender

Regarding the practices of consumption of milk in the study population, 71.4%(90/126) of the population consumed raw milk, 26.2%(33/126) boiled it before consuming directly or adding it to tea and only 2.4%(3/126) consumed pasteurised milk enclosed in tetra-packages from the farms and shops.

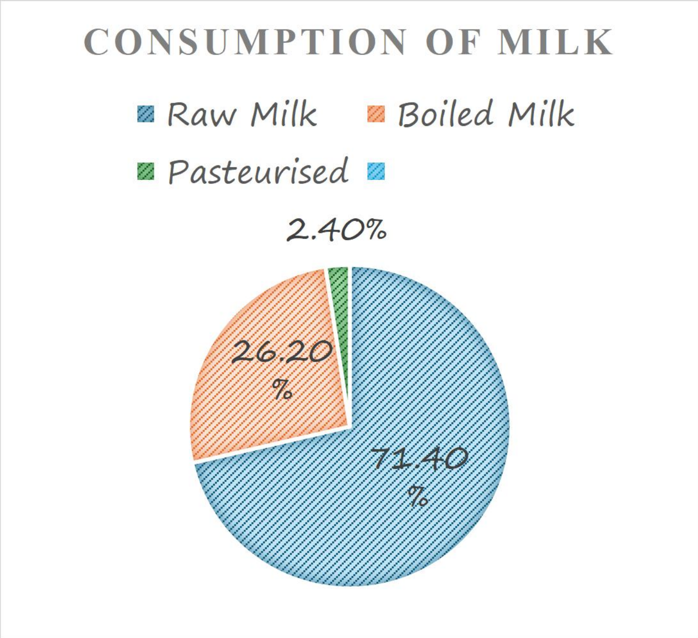

Figure 3: Percentage of Consumption of milk practices among study individuals

However, the consumption of raw milk was mostly seen in rural areas as compared to boiled or pasteurised milk. Consumption of raw milk in urban areas in 33.3% whereas in rural areas it was 66.7%. 13.5% of the urban and 12.7% of the rural residents consumed boiled milk whereas1.6 % of the urban and o.8% of the rural population consumed pasteurized milk.

Residence	Consumption of Milk			
	Raw	Boiled	Pasteurized	Total
urban	23	17	2	42
%	18.3	13.5	1.6	33.3
Rural	67	16	1	84
%	53.2	12.7	0.8	66.7
Total	**90**	**33**	**3**	**126**
%	**71.4**	**26.2**	**2.4**	**100**

Table 3: Comparison of area of residence to the consumption of milk practices

The knowledge of bovine tuberculosis that can be spread with drinking unpasteurised milk or working with infected animals was very low 27.8% (35/126) as compared to people with no knowledge of bovine tuberculosis72.2%(91/126).

Knowledge of bovine tuberculosis		
Yes	35	27.8%
No	91	72.2%
Total	**126**	**100**

Table 4: knowledge of bovine tuberculosis among study population

Comparison of the knowledge of bovine tuberculosis shows that 72.2%(91/126)people had no knowledge with maximum percentage of males 46.0%(58/77) as compared to 15.1%(19/77) who knew about bovine tuberculosis.

Knowledge of bovine tuberculosis	Gender		Total
	Male	**Female**	
Yes	19	16	35
%	15.1	12.7	27.8
No	58	33	91
%	46.0	26.2	72.2
Total	77	49	126
%	**61.1**	**38.9**	**100**

Table 5: Comparison of gender to the knowledge of bovine tuberculosis in study population

Comparison of knowledge of drinking unpasteurised milk can result in bovine tuberculosis to the drinking practices in compared. 65.7% of the study population consuming raw milk had knowledge of bovine tuberculosis but it had no effect on this practice.73.6% of the people had no knowledge that drinking raw milk may spread the bovine tuberculosis. 28.6% of people consuming boiled milk had knowledge of bovine tuberculosis and 25.3% of people had no knowledge of bovine tuberculosis. Moreover, only 5.7% of people consuming pasteurised milk had knowledge of bovine tuberculosis and 1.1% of people consuming pasteurised milk had no knowledge of bovine tuberculosis.

Consumption of	Knowledge of bovine tuberculosis	Total

milk	Yes	No	
Raw	23	67	**80**
%	65.7	73.6	**71.4**
Boiled	10	23	**33**
%	28.6	25.3	**26.2**
Pasteurised	2	1	**3**
%	5.7	1.1	**2.4**
Total	**35**	**91**	**126**

Table 6: Comparison of knowledge of bovine tuberculosis to the consumption of milk practices

Contact of people with animals at home in the study population is 30.2%(38/126) as compared to people working at farms which was 28.6%(36/126). Out of these only 10.3% people who had cows at home had knowledge of bovine tuberculosis and 4.8% working at farms had knowledge of bovine tuberculosis. 27%(34/126) people had cows in the neighbourhood and 14.3%(18/126) had no contact with cattle at all.

Knowledge of bovine tuberculosis	Contact with cattle				
	At home	Neighbourhood	Working at farm	No contact	Total
Yes	13	11	6	5	**35**
%	10.3	8.7	4.8	4.0	**27.8**
No	25	23	30	13	**91**
%	19.8	18.3	23.8	10.3	**72.2**
Total	**38**	**34**	**36**	**18**	**126**
%	**30.2**	**27.0**	**28.6**	**14.3**	**100**

Table 7: Comparison of knowledge of bonie tuberculosis to individual's contact with animals

Discussion

Bovine tuberculosis in middle- and low-income countries is a serious health concern. Substantial economic losses due to productivity of infected animals is affecting humans. However, health concers in terms of being infected from bovine sources is still a challenge. Poor knowledge and practices of milk consumption cause health concerns in both genders. Our study revealed 61.1%

males and 38.9% females out of the total sampling population of 126 individuals, which are in consistent with the study done by Salari et al.,2023 showing 76.2% males and 23.8% females. However. Another study showed that females were more prone to develop extra- tuberculosis as compared to males in an overall prevalence of 20.9% with a rising trend from 22.6% in 2016 to 27.9% in 2021(Rolo *et al.*, 2023). A cross-sectional community survey conducted in East Africa yields old age burden of the disease accounting 23000 cases in age of more than 50 years in a period of 5 years (Obeague & Onuoha, 2023). Deteriorating immunity with increasing age is one factor that contributes to high burden of tuberculosis. Prevalence per 100,000 population showed increasing trend with age in twelve surveys in Africa however, the absolute number of cases was significantly higher in individuals age 35-44 years (Law & Floyd, 2020). These findings are consistent with the results showing higher trend in ages 31-40 years irrespective of gender.

Kemal et al.,2019 conducted a cross-sectional study in selected areas of eastern Ethiopia with 54.17% urban inhabitants to find out the prevalence of bovine tuberculosis and associated risk factors for transmission of disease. The overall prevalence turned out to be 20.3% (n=64), with 50% of these showed preference for consuming raw unpasteurised milk as compared to our study in which 71.4% of the population showed preference towards consuming raw milk .7% of the population in his study had direct contact with the animals whereas 30.2% of the population in our study had animals at home and 28.6% were working at farms.

In another study, by Sibhat et al.,2017, a review of 56 cross sectional studies and meta-analysis was done to decree the prevalence of bTB in humans and risk factors associated with spread. The prevalence of bTB was 5.8% reflecting lack of awareness, production systems and breeds of cattle. In our study, 72.2% had no knowledge of bovine tuberculosis. Out of these, 46% of the individuals were males who were working in close proximity with no knowledge of bovine source of transmission whereas, 26.2% of the females had no knowledge. A study conducted in West Africa, Nigeria by Cadmus et al.,2019, a total of 1693 isolates were studied and molecularly categorised to decipher the species of mycobacterium infecting humans and cattle. 1131 represented M tuberculosis out of which 1.3% (15/1131) isolates were taken from cattle, 286 reported as M bovis out of which 8.0% (23/286) isolates were from humans that clearly depicts the ongoing transmission between animals and humans in both directions. Our study showed 27% of the population had bovine source of tuberculosis in the neighbourhood whereas 14.3% of the individuals had no contact. Similar results were seen in study by Mulatu & Mohammad,2023 which showed 24.0% prevalence in people which has no contact with animals and 76% in population who were in direct contact with animals.

Transmission dynamics and control challenges in spread of M. bovis and M tuberculosis infection were highlighted in a study by Rohma et al.,2018. The study elaborated the life style of communities, close contact of rural dwellers with domestic animals and habits of consuming raw milk products as the main factors responsible for high prevalence of zoonotic tuberculosis however, the incidence of mTB in bovine animals suggested the circulation of different strains in both directions. A similar study was carried out in district Kohat of Khyber Pakhtunkhwa, North East of Pakistan, to find out the prevalence in milk samples and to identify the potential risk factor associated with transmission of disease through dairy animals. It also highlighted the husbandry and managemental practices involved in the spread of this disease. Out of the total of 200 milk samples (n=200) collected from cattle(n=62), buffalos (n=64), goats(n=47) and milk shops(n=27). Similar results were seen in our study in which consumption of raw milk in 71.4% of population showed traces of bacterial infection. PCR based prevalence of bTB in cattle was found to be 6.4%, buffalos 6.2%, goats 6.3% and random samples collected from milk shops showed 7.4% with total prevalence of 5.5.

In Pakistan, a study aimed to know the frequency of tuberculosis infection in cattle and buffaloes(n=265), was conducted in Punjab by Aslam et al.,2019, and the whys and wherefores of economic loses in dairy industry were assessed. The sensitivity and specificity of isolation and identification techniques were ruled out using culture characteristics, staining techniques (Zn staining procedure) and PCR. The overall prevalence turned out to be 10.56% with 11.04% in buffaloes and 9.76% in cattle with 100% herd infectivity. This is in accordance with our study in which 9 samples from infected cows showed AFB on direct microscopy.

In

a study in India by Duffy et al.,2020, molecular epidemiological survey was done to obtain estimates of human prevalence of animal associated members of Mycobacterium tuberculosis complex MTBC on 940 positive mycobacteria growth indicator tube MGIT cultures. A PCR based genome sequencing was done to sub-speciate the cultures. Isolates identified as MTBC other than M. tuberculosis or as inconclusive on PCR were subjected to whole genome sequencing (WGS) and compared phylogenetically with culture isolates. Interestingly, out of the total (n=940) 548 pulmonary and 392 extrapulmonary, wild-type M bovis was not identified at all. However, the identification of M tuberculosis in cattle reinforced the need for better molecular isolation, characterization and investigation in countries with endemic bovine tuberculosis.

Kwaghe et al.,2023 in his study claimed Africa and Asia as the highest burdened areas globally. His study encompassed 160 bovine and 229 human subjects with symptomatic evidence of tuberculosis. Out of the total(n=229) the prevalence of bTB was 65.4% which is a significantly high. The risk factors disproportionately affect people in resource-poor settings and laboratory diagnosis of TB is limited to smear microscopy in humans thus limiting the estimation of role of M.bovis in human infection.

Differences in pathogenesis of both species of tuberculosis i.e., mTB and bTB were highlighted by Garcia et al.,2020 in a study determining the non-replicative state during latent phase. According to study conducted in Argentina, hypoxia induced gene expression in mTB pushes the mycobacterial cell into dormant phase. However, bTB lacks this mechanism and shows acute infection in cattle only although human and bovine tuberculosis are clinically similar. Moreover, contrary to this, Capparelli et al.,2013 was able to recover and conserve the non-replicative M. bovis from milk samples and lung aspirates from non-treated cattle. Similarly, in our study, we were able to directly obtain AFB from 09 samples out of the total 102.

Elaborating the challenges and concerns of global zoonotic tuberculosis, Popelka et al.,2017 proposed that the true incidence remains uncertain because of absence of surveillance data from most high burden countries and the proportion of scientific attention and resources allocated are insignificant. In view of WHO's end TB strategy endorsed by health authorities to achieve a world free of tuberculosis by 2035 calls for TB stakeholders to accurately and aggressively diagnose and treat Btb in humans.

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