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"ASSESSMENT OF RISK FACTORS FOR HUMAN MYCOBACTERIUM BOVIS INFECTIONS IN RURAL COMMUNITIES IN CENTRAL GUJARAT, INDIA"

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Abstract:
Tuberculosis (TB) in humans is primarily caused by *Mycobacterium tuberculosis (M. tuberculosis)*, with millions of infections and hundreds of thousands of deaths worldwide. It creates a substantial economic burden on the community. Unlike *M. tuberculosis, Mycobacterium bovis* infects cattle and causes bovine TB, also known as zoonotic TB. People can contract zoonotic TB after consumption of unpasteurized dairy products, handling the sick animals, and via occupational exposures. The association between the zoonotic TB in humans and cattle is not well known in India. The study examined the associated risk factors, including milk consumption habits, animal handling practices, previous contact with case of tuberculosis and occupational exposures.
A cross-sectional study was conducted to investigate the risk factors associated with zoonotic tuberculosis in humans and its transmission to people living at the livestock–human interface. A questionnaire was administered to collect the required information. A total thousand number of sputum samples were collected. The sputum samples were screened for TB using the microscopy techniques and 100 samples were found positive. This was followed by mycobacterial speciation using molecular techniques. *M. bovis* was isolated from 4 (0.4%) TB positive sputum samples and both *Mycobacterium tuberculosis* and *Mycobacterium bovis* were detected from 6 (0.6%) indicating a mixed infection in the current study. Total 10 *Mycobacterium bovis* were isolated from the sputum samples.
Using logistic regression, owning an infected herd, being an animal handler (45%) and consumption of raw milk (61%) were recognized as highly significant risk factors associated with a history of TB in the current participants. The findings from this study have confirmed the potential for zoonotic TB transmission via both unpasteurized milk and aerosol thus, the role of *M. bovis* in human TB remains a concern for vulnerable communities.

Keywords: bovine tuberculosis (bTB); *Mycobacterium bovis*(*M. bovis*); risk factors; livestock–human interface; zoonotic TB

1. Introduction

Cattle serve as the primary host for *M. bovis*, but the pathogen also infects various other domestic and wildlife species.[1]*M. bovis* is part of the Mycobacterium tuberculosis complex (MTC), a group of closely related organisms responsible for tuberculosis (TB) in mammals, including humans.[2] While M. tuberculosis is the predominant cause of human TB, a portion of cases are attributed to *M. bovis*, known as zoonotic TB.[3-5]

The full impact of *M. bovis* on the human TB epidemic is not well understood, largely due to the absence of systematic bovine TB surveillance and the limited resources for identifying *M. bovis* in humans.[6-7] In 2016, it was estimated that there were 147,000 new cases of zoonotic TB globally, resulting in 12,500 deaths, with the majority of these cases occurring in Africa.[8] Müller et al. (2013) estimated that *M. bovis* could be responsible for up to 37.7% of all human TB cases in Africa based on the available data at that time.[6] Studies in several African countries have shown varying prevalence of *M. bovis* in TB patients, such as 7% in Uganda, 16% in Tanzania, and 17% in Ethiopia from lymph node biopsies or aspirates.[9-11] More recent investigations have reported lower prevalence of <1% in Zambia and <3% in Uganda from sputum samples of pulmonary TB patients.[12-13]

Zoonotic TB is primarily contracted through the consumption of unpasteurized milk and dairy products, less commonly through the ingestion of raw or improperly cooked infected meat, and via aerosols from infected animals during direct human–livestock contact.[5,14] This mode of transmission results in zoonotic TB more frequently presenting as extra-pulmonary TB (EPTB) (9.4%) compared to the pulmonary TB (2.1%).[15] Developed countries have largely eliminated the disease in cattle and humans through policies such as the 'test and slaughter policy,' compulsory pasteurization of milk, and abattoir surveillance.[6] In contrast, these measures are often inadequate or absent in developing countries, especially in India, where bovine TB remains widespread.

Several risk factors facilitate the transmission of *M. bovis* to humans, including demographic variables (e.g., family size, age), dietary habits, close contact with animals, socio-economic status, illiteracy regarding zoonotic TB, and traditional practices, exacerbated by the HIV/AIDS pandemic.[5,14,16,17]

In India, research on the zoonotic aspects of *M. bovis* is limited, with few comprehensive surveys assessing the public health impact of bovine TB. Studies by Shah et al. (2017)[18] and Prasad et al. (2018)[19] have highlighted the high incidence of *M. bovis* and *M. tuberculosis* in extrapulmonary samples from both humans and cattle. Similarly, Mittal et al. (2014)[20] emphasized the need for differential diagnosis of MTBC in humans and livestock, indicating a significant public health concern due to close human-animal interactions and the consumption of unpasteurized dairy products.

2. Methods

2.1. Study Site

The study was carried out in the 1000-bed tertiary care teaching hospital, Shree Krishna Hospital, in Karamsad, Gujarat. The genomic work was done at the Sat Kaival Hospital Pvt. Ltd. research and development facility in Lambhvel, Anand, Gujarat, India.

2.2. Study Design

A cross-sectional study was conducted between November 2017 and June 2018. Patients with clinical signs suggestive of pulmonary tuberculosis were included as the study participants.

These participants, who were either inpatients or outpatients, obtained medical care from the respiratory medicine department at Shree Krishna Hospital in Karamsad. Patients with

extrapulmonary tuberculosis, however, were not included in the research. Using a standardized proforma, each participant's complete demographic information as well as other pertinent data, like occupation, animal handling practices, and consumption of raw, unpasteurized milk, were collected.

2.2.1Sample Collection

The study included 1000 patients suspected of having pulmonary tuberculosis. Sputum samples were collected, ideally one early morning specimen and two spot specimens, in sterile, leak-proof containers. Patients were thoroughly instructed on the collection procedure. The collected specimens were then transported to the Microbiology laboratory for further processing.

2.2.2 Categories of the risk factors

The risk factors were categorized under socioeconomic status (occupations) and behavioural factors (previous TB history, raw milk consumption, animal handler or contact with animal).

2.2.3 Laboratory testing of sample

Initial microscopic examination of sputum smears was performed to identify Acid-fast bacilli, characteristic of Mycobacterium tuberculosis (MTB). Samples were decontaminated and were homogenized and liquefied using the Modified Petroff's method to release MTB cells from mucus. DNA was extracted from the concentrated samples using the QIAGEN DNA mini kit. PCR was performed using primers targeting the HupB gene, specific to MTB. The presence of a band of the expected size confirmed the presence of MTB DNA.

2.2.4. Ethics Statement

To ensure ethical conduct, this research was approved by the Institutional Ethics Committee of Shree Krishna Hospital (HREC clearance number HMPCMCE/HREC/14/SESSION-2/12). Prioritizing participant well-being, written informed consent was obtained from all individuals, respecting their autonomy and confidentiality. The researchers took meticulous steps to safeguard patient privacy throughout the study.

2.2.5. Statistical analysis

The data was collected in a pre – designed proforma and entered into an excel sheet. The statistical Package of Social Sciences (SPSS), IBM version 22, was used for the statistical analysis of data. Categorical measurement were summarized as the number and percentage, descriptive statistics were used for numerical variables wherever applicable.Chi-square tests were conducted to determine the significance of associations between demographic factors, clinical features, and TB.Logistic regression and multiple correspondence analysis were used to identify significant risk factors for zoonotic TB.

3. Results

Description of the study population – The prevalence (10%) of *M.bovis* among a samples of 100 individuals diagnosed with pulmonary tuberculosis in rural area of Anand, Gujarat was detected. Briefly, 90 out of 100 presented with only M. tuberculosis, and 4 also presented with *M.bovis* in addition to *M.tuberculosis* and 6 patient presented with mixed infection of both *M.tuberculosis* and *M.bovis* identified by PCR assay.

Demographically, as illustrated in Table I, this study analysed the association of various risk factors and clinical features with tuberculosis (TB) across different demographic groups. The data were stratified by age, sex, and specific clinical symptoms, and chi-square tests were conducted to determine the significance of associations. The age group of 18-40 years showed a significant association with TB risk factors ($p<0.004$), suggesting that individuals within this range who had previous contact with TB cases, were farmers, animal handlers, or consumed raw milk were at a

higher risk.Similarly, those over 40 years old also exhibited a significant association with the risk factors ($p<0.008$), indicating a heightened vulnerability in this age category.
The data revealed a highly significant association between sex (males) and risk of developing TB ($p<0.001$). This suggests that males who had previous TB contact, were farmers, animal handlers, or consumed raw milk were more likely to develop TB.In contrast, the association for females was not significant ($p=0.142$), indicating that these risk factors did not significantly affect the likelihood of TB among females in this study.

Furthermore**,** There was a strong and significant association between fever and TB ($\chi^2=19.43$, $df=3$, $p<0.001$), indicating that fever is a common symptom in TB cases. The presence of a cough was significantly associated with TB ($\chi^2=23.22$, $df=3$, $p<0.001$), highlighting it as a common clinical feature among TB patients.Weight loss also showed a significant association with TB ($\chi^2=20.71$, $df=3$, $p<0.001$), suggesting it is a critical symptom in TB diagnosis.Haemoptysissymptom was significantly associated with TB ($\chi^2=11.90$, $df=3$, $p=0.008$), though less common than fever, cough, and weight loss.

The demographic and clinical data provides valuable insights into the patterns of symptoms and risk factors associated with tuberculosis in our study population, offering crucial information for targeted public health interventions and further research.

Table I: Analysis of risk factors and clinical features inpulmonary tuberculosis patients (n=100)

Characteristics	Risk Factors						
	Previous contact with TB case (N=40)	Farmer (N=30)	Animal handler (N=45)	Raw milk consumption (N=61)	Chi-square	df	Significance level (p)
Age							
< 18	01	01	02	02	1.00	3	0.801
18-40	19	07	18	25	13.09	3	0.004
>40	20	22	25	34	11.83	3	0.008
Sex							
Male	30	22	35	46	17.91	3	<0.001
Female	10	08	10	15	5.45	3	0.142
Clinical features							
Fever	34	25	38	51	19.43	3	<0.001
Cough	34	29	43	59	23.22	3	<0.001
Weight loss	26	19	33	47	20.71	3	<0.001
Haemoptysis	13	9	17	25	11.90	3	0.008

Further details of the Mycobacterium species and risk factors in these patients are presented in Table II A significant 61% of the patients were involved in animal handling, while 40% had a history of contact with known TB cases. Additionally, lifestyle factors such as dietary habits were also notable; 45% of the patients reported consuming raw milk, and 30% were employed in farming.This study investigates the risk factors associated with different types of tuberculosis infections: *Mycobacterium tuberculosis*, *Mycobacterium bovis*, and mixed infections.

The current study demonstrates significant associations between various risk factors and different types of TB infections. Previous contact with TB cases, animal handling, raw milk consumption,

and farming are all significantly linked to the type of TB infection, with strong contingency coefficients indicating robust relationships.
These findings underscore the importance of targeted public health interventions and education for individuals involved in farming, animal handling, and raw milk consumption to reduce the risk of TB infections, particularly those caused by *Mycobacterium bovis* and mixed infections.

Table II: Analysis of Risk factors associated with Mycobacterium species

Risk Associated Factors	*Mycobacterium tuberculosis* (N=90)	*Mycobacterium bovis* (N=4)	Mixed (N=6)	Total (N=100)	Chi-Square	DF	p-value	Contingency Coefficient
Previous contact with TB cases	34 (38%)	2 (50%)	4 (67%)	40 (40%)	48.20	2	<0.001	0.739
Animal Handling/Contact with Animal	36 (40%)	4 (100%)	5 (83%)	45 (45%)	44.13	2	<0.001	0.704
Raw milk consumption	51 (57%)	4 (100%)	6 (100%)	61 (61%)	69.48	2	<0.001	0.730
Farmer	24 (27%)	2 (50%)	4 (67%)	30 (30%)	29.60	2	<0.001	0.705

Discussion:

The earliest recorded case of bovine pulmonary tuberculosis in a human, identified through bacteriological testing, was reported in 1909. This landmark discovery opened avenues for extensive research over the subsequent decades, revealing that *M. bovis* was the causative agent in 1-3% of human cases of pulmonary tuberculosis.[21]

In industrialized countries, animal TB control and elimination programs, alongside milk pasteurization, have significantly reduced the incidence of disease caused by *Mycobacterium bovis* in both cattle and humans.[22] Conversely, in developing countries, animal TB is widespread, control measures are inconsistently applied, and pasteurization is seldom practiced.[23,24] In India, there is a scarcity of data on the zoonotic aspects of*M. Bovis* and a lack of surveys assessing the public health concerns posed by bovine TB. Our study addresses this gap by examining the incidence of pulmonary tuberculosis caused by*M. Bovis* in a rural area of Central Gujarat and assessing the risk factors influencing disease occurrence.

The current study aimed to assess the risk factors for human *M. bovis* in a Tertiary Care Teaching Hospital in Gujarat, India, a region where tuberculosis remains a significant public health challenge. We collected 1000 sputum samples from patients suspected of having pulmonary tuberculosis and conducted tests for acid-fast bacilli. Of these, 100 samples were positive for pulmonary tuberculosis, resulting in a 10% positivity rate. This finding is consistent with a study from Peshawar, Pakistan[25], where a similar positivity rate highlighted the persistent prevalence of the disease in South Asia.

Our study identified that all significant risk factors were associated with male sex (73/100) (<0.001) and significantly associated with age group of 18 to 40 years, a disparity also noted by Tchatchouang et al. (2015)[26], who reported a higher infection rate in men (53.16%) compared to

women (46.84%). Similarly, Bapat et al. (2017)[27]found that the disease predominantly affected men between age group of 18 to 40 years. These observations suggest that occupational factors, potentially linked to increased exposure risks in professions predominantly held by men, may influence disease distribution.
Participants exhibiting clinical symptoms consistent with active TB, such as fever (87%), cough (96%), weight loss (73%), and haemoptysis (30%), were included in the current study. These symptoms are similar to those caused by*M. Tuberculosis* induced TB.[24,28]

In Melghat, India, a study reported higher percentages of participants with fever (39.7%), chills (34.4%), cough with expectoration (41.7%), and weight loss (27.1%), particularly among farmers, dairy workers, animal handlers, and residents of high TB endemic areas ($p < 0.0001$).[27]
Consumption of raw milk was highly prevalent among participants, with a 61% positivity rate strongly associated with mycobacterial species. Many participants preferred unpasteurized milk due to its richer taste, which may facilitate*M. Bovis* transmission from cattle to humans.[29] Michel et al.postulated that pastoralist and rural communities are at greater risk for bovine TB, but data for these populations is lacking.[1] Bapat et al. (2018) also found higher positivity (80%) among participants consuming raw milk.[27]

Statistical analysis revealed that residents of the high TB endemic region of Central India had the highest number of *M. bovis* infected cases (12.6%). This population, characterized by high crowding and TB prevalence, predominantly consumed meat from local abattoirs without proper inspection.[30]
The existing cultural practices of consuming raw meat and milk, coupled with low hygiene standards, may contribute to the spread of bovine TB. Hubbert and Hagstad suggested that ingesting inadequately cooked meat from infected cattle poses a significant zoonotic risk.[31]
Animal handlers had the second-highest positivity rate (45%), strongly associated with*M. bovis* (<0.001). Transmission mechanisms in this group likely include close contact during animal handling, cleaning barns, participating in necropsies, and living near animal cages. Michalak et al.reported a reverse zoonosis case whereM. tuberculosiswas transmitted from humans to elephants, underscoring the importance of TB diagnosis and prevention in all animals.[32]
Farmers from rural settings exhibited the third-highest positivity. These individuals frequently had close contact with animals during feeding and milking and often lived in close proximity, sometimes under the same roof. A cohort study in the UK suggested that agricultural workers might acquire bovine TB by inhaling cough spray from infected cattle, developing typical pulmonary TB.[33]

A systematic literature search on the occurrence of zoonotic TB by Müller et al. showed that there is lack of data for the World Health Organization region of Southeast Asia, including major cattle producing middle- and low-income countries (e.g., India, Bangladesh, Pakistan, Myanmar, and Indonesia). Recorded incidence rates for zoonotic TB in Europe, the United States, Australia, and New Zeal- and were consistently below 1/100,000 population/y. The incidence rates were not available for other countries.[6] Individual studies from various regions reported high proportions of zoonotic TB for specific population groups and settings. For example, in the Hispanic community in the United States, zoonotic TB appeared to be a considerable proportion of all TB cases and was associated with the consumption of unpasteurized cheese from Mexico. The highest median proportions for TB caused by *M. bovis* were observed in countries in Africa: Ethiopia, Nigeria, and Tanzania. However, the specific populations affected and risk factors of zoonotic TB in these settings remained largely elusive.[26] In keeping with the earlier reports, our present study also indicates pockets of zoonotic transmission of TB for specific population groups and settings.
In the Indian context, studies by Shah et al.and Prasad et al.have shown high incidence of *M. bovis* and *M. tuberculosis* in extrapulmonary samples of humans and cattle, respectively.[18,19] A similar study by Mittal et alhas demonstrated the importance of screening and differential diagnosis of MTBC in humans and livestock.[20]

Despite being an insightful study, our work suffers from the limitation of insufficient data on animal health. It was learned that worshipping of cattle has spiritual significance in some regions; therefore, withdrawing blood or performing any tests on their animals was not possible. The present study thus needs further evaluation in animal population to identify the main transmission drivers in these areas.

5. Conclusion

In conclusion, this study has documented the prevalence of neglected bovine TB in human population in rural area of Central Gujarat. Diagnosis and monitoring of this disease are essential, especially in developing countries such as India, where humans and animals have close association in routine life. This study would thus be valuable in guiding policy makers for further studies in bovine TB epidemiology.

In summary, this study successfully demonstrates the presence of pulmonary infections caused by *M. bovis* and *M. tuberculosis* in a tertiary care hospital in Anand, Gujarat. The results, confirmed via advanced molecular techniques, highlight the utility and necessity of PCR in differentiating *M. tuberculosis* and *M. bovis*. This distinction is crucial for appropriate disease management and the formulation of targeted public health strategies. Our findings contribute to the growing body of evidence on the prevalence and characterisation of tuberculosis-causing mycobacteria, reinforcing the need for continued research and surveillance in this critical area of public health.

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