Journal of Bioresource Management

Volume 11 | Issue 3 Article 4

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Recommended Citation

Khan, A., Ali, A., Aimen, U. e., Ullah, W., Khan, I., Ullah, Q., Hussain, B., & Rahman, M. (2024). Estimation of Bovine Tuberculosis Prevalence, Associated Risk Factors, and Public Health Significance in District Dera Ismail Khan, *Journal of Bioresource Management, 11* (3).
ISSN: 2309-3854 online
(Received: Nov 3, 2023; Accepted: Jul 8, 2024; Published: Sep 30, 2024)

Estimation of Bovine Tuberculosis Prevalence, Associated Risk Factors, and Public Health Significance in District Dera Ismail Khan

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ESTIMATION OF BOVINE TUBERCULOSIS PREVALENCE, ASSOCIATED RISK FACTORS, AND PUBLIC HEALTH SIGNIFICANCE IN DISTRICT DERA ISMAIL KHAN

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ABSTRACT

Bovine tuberculosis (bTB) is zoonotic disease of global concern, affecting both animal and human populations. To estimate the prevalence of bovine TB in District Dera Ismail Khan and identify associated risk factors. A total of 138 cattle from different herds were included in the survey, and diagnostic tests, including the tuberculin skin test and Ziehl Neelsen stain, were employed. The results revealed a high prevalence of bovine TB, with an estimated prevalence of 10.87 % in the study area. Prevalence analysis based on Tehsil revealed varying rates: 6.66 % in Dera Ismail Khan, 11.11 % in Kulachi, and 14.81 % in Daraban and Paroa. Paharpur had a prevalence of 7.40 %. The overall district prevalence was 10.87 %. Age-wise prevalence showed no significant difference, with 10 % in calves (<1 year) and 9.33 % in 1-3-year-olds. Prevalence in cattle over 3 years was 13.95 %. Sex-wise prevalence showed no significant difference between male (10 %) and female (12.38 %) cows ($p > 0.05$). Seasonal prevalence indicated 10 % in spring, 6.06 % in summer, 11.11 % in autumn, and 27.27 % in winter (highest prevalence) ($p < 0.05$). Overcrowding of animals and inadequate biosecurity measures were significantly associated with higher prevalence rates ($p < 0.05$). Herd size and presence of co-infections showed no significant correlation with bovine TB prevalence ($p > 0.05$). This study found a high prevalence of bTB in District Dera Ismail Khan and identified overcrowding of animals and inadequate biosecurity measures as significant risk factors for disease transmission.

Keywords: Bovine tuberculosis, mycobacterium, prevalence, public health, zoonosis.

INTRODUCTION

Bovine tuberculosis is highly prevalent and economically significant transmissible disease affecting cattle and poses a potential risk to human health (Borham et al., 2022). Caused by *Mycobacterium bovis*, bovine TB is primarily transmitted through respiratory droplets, making it a zoonotic disease with implications for both animal agriculture and public health (Palmer et al., 2012). District Dera Ismail Khan, located in Khyber Pakhtunkhwa, Pakistan, is known for its substantial livestock population and has identified bovine TB as a major problem affecting cattle in the region (Rizwan et al., 2022; Humblet et al., 2009).

Accurate estimation of bovine TB prevalence is essential for understanding the disease burden and implementing effective control measures. Estimating prevalence in District Dera Ismail Khan requires systematic surveillance and diagnostic testing of cattle herds (Islam et al., 2021). Methods such as tuberculin skin

tests, serological assays, and post-mortem examinations can be employed to determine the extent of infection and provide valuable insights into disease dynamics. These prevalence estimates inform targeted interventions and enable monitoring of control efforts (Islam et al., 2020).

Several risk factors contribute to spread and maintenance of bTB in the District. Overcrowding of animals, poor animal husbandry practices, inadequate biosecurity measures, and existence of wildlife reservoirs significantly escalated the disease transmission risks within and between herds (Skuce et al., 2012; Mekonnen et al., 2019). Additionally, the consumption of unpasteurized milk and dairy products from infected cows posed direct public health menaces, as humans can become infected through consuming contaminated animal products or close contact (de Klerk and Robinson, 2022). The public health significance of bovine TB should not be underestimated. Zoonotic transmission can lead to serious illness in humans, including pulmonary and extrapulmonary TB forms (Luciano et al., 2020).

To combat bovine TB, a comprehensive One Health approach is crucial. This approach integrates veterinary and human health interventions, focusing on effective surveillance programs, improved biosecurity measures, enhanced animal husbandry practices, and the promotion of milk pasteurization. By addressing these issues, stakeholders can work together to reduce prevalence of bTB, improve animal welfare, and safeguard public health in the district (Thoen et al., 2016; Devi et al., 2021).

The aims of the study were to investigate bovine tuberculosis with a comparative intradermal tuberculin test, demonstrated by Ziehl Neelsen staining, and to determine possible risk factors.

MATERIAL AND METHODS

Study Area

The study was conducted in District Dera Ismail Khan, located in the Khyber Pakhtunkhwa province of Pakistan (Figure 1). The district was selected due to its substantial livestock population, for determining the prevalence of bTB. Cross-sectional epidemiological investigation determined prevalence of bTB and risk factors accompanying it. Research was performed at Veterinary Research and Disease Investigation Center, Dera Ismail Khan surveying different farms of cows in five tehsils of the district including tehsil Dera Ismail Khan, Kulachi, Daraban, Paroa and Pahapur.

Sample Size and Selection

Sample size was calculated keeping 10 % expected prevalence of the disease, bovine tuberculosis at 95 % confidence interval (Khan et al., 2017).

$n= 1.96^2 \times P_{exp} (1-P_{exp})/ d^2$

$n= 1.96^2 \times (0.01) (1-0.01)/ 0.05^2$

$n= 138$

Where n is the sample size, P_{exp} is the expected prevalence and d is the absolute precision value.

Sample of 138 was calculated from the above-mentioned formula and the random sampling protocol was followed in the following study areas (Figure 2).

Study Duration

The investigation was conducted during the calendar year 2022-23.

Data Collection

Data collection involved a combination of field visits, interviews, and diagnostic testing. The following steps were undertaken:

Figure 1: Map of District Dera Ismail Khan

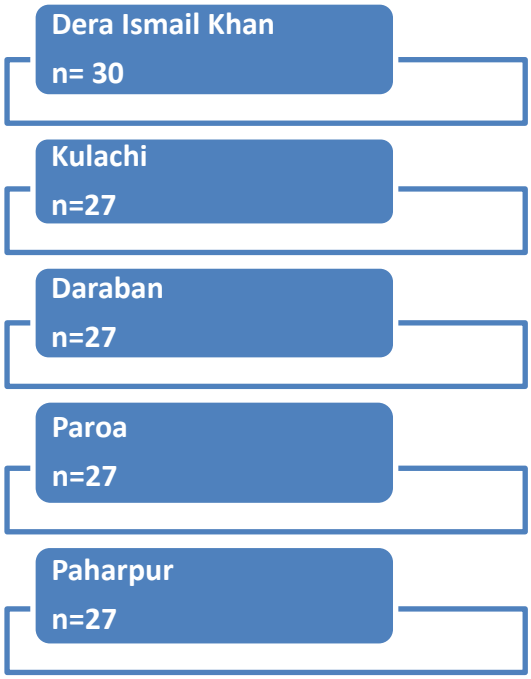

Figure 2: Allocation of sample size in different Tehsils of the study area (n=138)

Herd Surveys

Field visits were conducted to each selected herd. Information on herd size, management practices, and potential risk factors was recorded using structured questionnaires. The data collection team was trained to ensure standardized data collection procedures.

In Tehsil Dera Ismail Khan, 30 were sampled from the total of 2,239 cattle distributed across 51 herds. Another settlement, Kulachi, contained 1,802 cattle distributed across 38 herds. With 31 livestock herds and 1,320 cattle, Daraban also had 27 cattle sample, similarly, Paroa, had substantially larger cattle population of 3,400 from 29 herds. Paharpur, which had the greatest cattle population of 6,300 animals in 63 herds. Across all settlement, 138 cattle were sampled out of 15,061 cattle distributed among 212 herds (Table 1).

Field visits and interviews were essential in our investigation of the prevalence and risk factors of bovine tuberculosis in the study area. In order to guarantee a representative sample of the bovine population, field visits were conducted by conducting systematic visits to randomly selected herds within the district. In order to mitigate selection bias, livestock were examined and cattle were selected according to a predetermined random sampling protocol during these visits.

A structured questionnaire was employed to collect detailed information on livestock management practices, biosecurity measures and other potential risk factors during interviews. Respondents were chosen to offer a comprehensive perspective on farming practices throughout the district, including a variety of herd sizes and management methods. Interviewers were subjected to rigorous training to ensure that they adhered to standardised data collection procedures in order to reduce observer bias and guarantee the consistency of the information collected.

Several strategies were implemented to further mitigate potential biases. By anonymizing responses, transparency was promoted without concern for potential consequences, thereby reducing reporting bias. Furthermore, the study design incorporated measures to ensure the consistency and accuracy of the data by conducting a cross-verification of the information provided and revisiting a subset of the farms. This method contributed to the improvement of the reliability of our findings and the reduction of the probability of misreporting.

Diagnostic Testing

Diagnostic tests were performed on selected animals within each herd. The tuberculin skin test, a commonly used method for bovine TB diagnosis, was employed. The skin test involved intradermal injection of purified protein derivative tuberculin and subsequent measurement of the skin reaction after a

specified time at > 4mm (cut-off value). The positive animals were further demonstrated by Ziehl Neelsen stain for acid-fast bacteria.

Table 1: Sampling Details from Five Settlements in Dera Ismail Khan Region

Settlement Name	Total Herds	Total Cattle Population	Sampled Cattle
Dera Ismail Khan	51	2239	30
Kulachi	38	1802	27
Daraban	31	1320	27
Paroa	29	3400	27
Paharpur	63	6300	27
Average	**212**	**15061**	**138**

Comparative Cervical Intradermal Tuberculin Test (CCIT)

A CCIT test was conducted in the following manner. First, 0.1ml (25000 IU) of a-PPD and 0.1ml (20000 IU) of b-PPD were injected intradermally at points 12 cm apart on the left lateral side of the neck. After 72 hours, the thickness of the skin fold at each injection site was measured using Vernier calipers. Animals were classified as positive if skin-fold thickness at the b-PPD injection site was 4 mm greater than a-PPD. If skin thickness at a-PPD injection site was 4 mm higher than b-PPD injection site, animal was considered reactor [14].

Ziehl Neelsen Stain for Acid Fast Bacteria (AFB)

To demonstrate the positive skin test, AFB method was employed as follows. Smears were prepared from milk or fecal samples after centrifugation. The resulting slides were air-dried and heat-fixed. Carbol Fuchsin stain was then flooded onto the smears and heated on a spirit lamp for 3-5 minutes, ensuring the stain remained moist. In case any evaporation occurred, additional stain was added to the smears. After cooling, slides were rinsed with tap water for 30 seconds. To decolorize the slides, acid alcohol was added drop by drop until the smear appeared light pink. Following this, slides were rinsed for 5 seconds with tap water. Counter stain (methylene blue) was applied for 2 minutes. Subsequently, slides were rinsed with tap water for 30 seconds and allowed to air dry and examined under 100X. In results interpretation, acid-fast organisms appeared stained in red, while background and other microorganisms would appear as blue or brown. This method enabled the identification and demonstration of presence of AFB, providing valuable information in the diagnosis of bovine tuberculosis [15].

Controlling Environmental and Management Factors

We meticulously assessed and controlled for a variety of environmental and management factors that could potentially affect the transmission and detection of the disease. These factors were deemed essential for the precise evaluation of the risk factors associated with bovine tuberculosis and comprehension of its epidemiology in the region.

i. ***Environmental Factors*:** We documented seasonal fluctuations, as environmental conditions can have a substantial impact on the transmission of infectious diseases such as bovine tuberculosis. In order to investigate the correlations between the prevalence rates

observed during various seasons and temperature, humidity, and rainfall data were collected.

ii. ***Management Factors:*** A substantial amount of data was gathered regarding herd management practices, which encompassed:
   a) ***Herd size:*** The herd's size was recorded due to its impact on the density of animals and the potential for disease transmission.
   b) ***Housing conditions:*** Data was collected regarding the extent to which cattle were grazed, housed in confined spaces or a combination of both.
   c) ***Biosecurity measures:*** The assessment included the evaluation of practices such as the use of protective gear by handlers, regular disinfection protocols and the quarantine of new animals.
   d) ***Animal movement:*** We monitored the movement of animals within and between populations, as this can be a significant factor in the transmission of disease.

Validation and Reliability of the used Tests

The diagnostic procedures for bovine tuberculosis, specifically the tuberculin skin test and Ziehl Neelsen stain, were implemented in this study to determine the prevalence of the disease. These methods are widely recognized for their utility in diagnosing *Mycobacterium bovis* infection in cattle. The tuberculin skin test is appreciated for its specificity and sensitivity, although it can be influenced by various factors such as infection stage and concurrent diseases, which might affect its accuracy. The Ziehl Neelsen stain, used to identify acid-fast bacteria in tissue samples, is a critical tool in confirming the presence of the bacteria post-tuberculin test. While these are established methods and provide a stronger foundation for the results presented.

Statistical Analysis

The collected data were compiled and entered into SPSS 24.0 for analysis. Chi-square tests were employed extensively to investigate the correlation between categorical variables, including presence of bovine tuberculosis and factors such as livestock size, management practices and biosecurity measures. This test was selected due to its exceptional efficacy in identifying statistically significant associations between categorical variables in extensive datasets. ANOVA (Analysis of Variance) was employed to discern differences in means among multiple groups for continuous data, such as the measurements of skin reactions in the tuberculin skin test. This was pertinent for evaluating the differences in infection rates among various age categories and tehsils within the district.

Ethical Considerations

The study followed ethical guidelines for animal research and was approved by the Directorate of Livestock and Dairy Development (Research), Khyber Pakhtunkhwa, Peshawar. Informed consent was obtained from herd owners before data collection, and animal welfare was prioritized throughout the study.

RESULTS

There were 15 positive tests for bovine TB out of a total sample size of 138 cattle. This indicated that these 15 cattle were infected with the bacterium *Mycobacterium bovis.* Prevalence of bTB in District Dera Ismail Khan was calculated to be 10.87 % based on these findings (Table 2). However, they provided valuable insight into the disease

burden and can guide targeted control strategies and interventions to mitigate the prevalence of bovine TB in district.

Number of positive and negative samples for two diagnostic tests: Comparative Cervical Intradermal Tuberculin Test (CCIT) and Ziehl Neelsen Stain for AFB was statistically assessed. 15 samples tested positive for bovine TB on CCIT test, while 123 samples tested negative. The milk and fecal samples were collected from these infected cows for demonstration of AFB and again these 15 samples tested positive for bovine TB using Ziehl Neelsen Stain, while 123 samples tested negative ($p > 0.05$). The p-value quantified the statistical significance of relationship between diagnostic tests and existence of bovine TB (Table 3).

In District Dera Ismail Khan, prevalence of bovine TB was examined by Tehsil, and evaluations were recorded in Tehsil-specific format. Two of thirty samples tested in Tehsil Dera Ismail Khan were positive for bovine TB, which gave a prevalence of 6.66 percent ($p > 0.05$). This indicated that prevalence of bovine tuberculosis in Tehsil Dera Ismail Khan did not differ substantially from other Tehsils in the district. Three of twenty-seven samples proved positive for bovine tuberculosis in Tehsil Kulachi, yielding a prevalence of 11.11 percent. Tehsil Daraban and Tehsil Paroa each had four out of twenty-seven positive samples for bovine tuberculosis. This equates to a prevalence of 14.81 percent in both Tehsils. Two of the twenty-seven samples tested positive for bovine tuberculosis in Tehsil Paharpur, with a prevalence of 7.40 % ($p > 0.05$). Taking into account the total number of samples tested across all Tehsils (138 samples), there were 15 positive samples for bovine tuberculosis, resulting in a district prevalence of 10.87 %. Notably, the p-values associated with the prevalence in each Tehsil are provided to evaluate the statistical significance of any observed differences. In this instance, all p-values exceed the conventional threshold of 0.05, indicating that the results are insignificant. Therefore, the prevalence of bovine tuberculosis in each Tehsil did not differ significantly (Table 4).

Age-wise prevalence indicated that two out of twenty-two tested samples were positive for bovine TB in age cohort of less than one year, resulting in a prevalence of 10 % ($p > 0.05$). It indicated bearing non-significant variations in prevalence of bTB between this age group and other age groups. Among calves aged 1 to 3 years, prevalence recorded was 9.33 %, as 7 of 82 tested samples were positive for the disease. Six of the 49 samples tested positive in the group of animals over 3 years, resulting in a prevalence of 13.95 percent ($p > 0.05$). There were 15 positive samples for bovine tuberculosis out of 138 total samples tested across all age categories, resulting overall prevalence of 10.87 % in district. Thus prevalence of bovine TB did not differ significantly across age groups (Table 5). Sex-wise prevalence of bTB indicated that two out of twenty analyzed samples of male cattle were positive for bovine tuberculosis, resulting in a prevalence of 10 %. Out of 118 samples tested from female cattle, 13 samples were positive for bovine tuberculosis, resulting in a prevalence of 12.38 % ($p > 0.05$). In this instance, p-value exceeded the standard threshold of 0.05, indicating that the result is not statistically significant (Table 6).

Season-wise prevalence of bTB showed that four out of forty samples tested positive for bovine tuberculosis in spring, for a prevalence rate of 10 %. Two of the 33 samples tested positive during summer, resulting in prevalence of 6.06 %. In autumn, 11.11 % of the 54 samples analyzed were positive for bovine tuberculosis, with 6 positive samples out of 54. In the winter, 3 of 11 analyzed samples were positive for bovine tuberculosis, resulting in a prevalence of 27.27 %. This is the highest observed prevalence across all seasons ($p < 0.05$).

The provided p-values indicated the statistical significance of seasonal prevalence differences. However, incidence in other seasons was not significant ($p > 0.05$). This data would help to comprehend the factors contributing to the seasonal variation in prevalence and to develop targeted control strategies based on seasonal patterns (Table 7).

The correlation between various risk factors and bTB prevalence in the study animals was examined in terms of overpopulation of animals, poor animal husbandry practices, insufficient biosecurity measures, existence of wildlife reservoirs, and consuming unpasteurized milk as risk factors. Out of 70 samples tested for the risk factor "overcrowding of animals," 8 samples were positive for bovine tuberculosis, resulting in a prevalence of 11.42 percent ($p < 0.05$). This association is statistically significant, as its p-value is 0.032 indicating that animal congestion was significantly linked to higher prevalence of bTB. Out of 78 samples tested for risk factor "poor animal husbandry practices," six samples were positive for bovine tuberculosis, resulting in a prevalence of 7.69 % ($p < 0.05$). Out of 78 samples tested for the risk factor "inadequate biosecurity measures," 10 samples were positive for bovine tuberculosis, resulting in a prevalence of 12.82 percent. This frequency had the p-value of 0.011, indicating a statistically significant association ($p < 0.05$). This indicates that inadequate biosecurity measures were substantially linked to the higher prevalence of bovine TB. Out of sixty samples tested for the risk factor "presence of wildlife reservoirs," five samples were positive for bovine tuberculosis, resulting in a prevalence of 8.33 %. Their analysis indicated non-statistically significant relationship between presence of wildlife reservoirs and prevalence of bTB ($p > 0.05$). Out of 71 samples tested for the risk factor "consumption of unpasteurized milk," 4 samples were positive for bovine tuberculosis, resulting in a prevalence of 5.63 % ($p > 0.05$). This prevalence had the p-value of 0.215 that indicated non-significant results ($p > 0.05$). These findings indicated that among examined risk factors, animal congestion and inadequate biosecurity measures are statistically associated with a higher prevalence of bovine tuberculosis (Table 8).

Herd-based prevalence of bTB was recorded and the analysis classified it into three herds, small (1–10), medium (11–50), and large (> 50 animals). Out of the 34 samples tested in small herd category (1-10), four samples were positive, resulting in a prevalence of 11.76 percent ($p > 0.05$). Out of 57 samples tested for bovine tuberculosis in category of medium herd size (11-50 animals), six samples were positive, with 10.52 percent prevalence. Out of 47 samples tested for bovine tuberculosis in large herds (> 50), 5 samples were positive, resulting in a prevalence of 10.63 % (Table 9).

Table 9 presented bTB prevalence in bovine TB in District Dera Ismail Khan based on the presence of co-infections in cattle. This study examined co-infections classified as "None," "Mastitis," and "Foot-and-Mouth." For the "None" category, which represents cattle without co-infections, 10.41 % of 96 tested samples were positive, yielding a prevalence of 10 positive samples ($p > 0.05$). This indicates there is no statistically significant relationship between the absence of co-infections and the prevalence of bovine tuberculosis in sample. In the "Mastitis" category, 12.50 % of the 24 samples tested were positive for bovine tuberculosis, with 3 positive samples out of 24. Two of eighteen analyzed samples for "Foot-and-Mouth" were positive for bovine tuberculosis, resulting in a prevalence of 11.11 percent.

Table 2: Prevalence of bTB in Dera Ismail Khan

Total Sample Size	Number of Positive Samples	Number of Positive Samples	Prevalence (%)
138	15	123	10.87

Table 3: Results of Diagnostic Tests for bTB

No. of samples (n=138)	Diagnostic Test			
	Comparative Cervical Intradermal Tuberculin Test	Ziehl Neelsen Stain for Acid-Fast Bacteria	$\chi 2$	p-value
Number of Positive Samples	15	15		
Number of Negative Samples	123	123	0.037	0.8475 (Non-significant)

Table 4: Distribution of Bovine Tuberculosis Prevalence by Tehsil

Tehsil	Number of Positive Samples	Total Samples Tested	Prevalence (%)	p-value
Tehsil Dera Ismail Khan	02	30	6.66	0.5578 (Non-significant)
Tehsil Kulachi	03	27	11.11	
Tehsil Daraban	04	27	14.81	
Tehsil Paroa	04	27	14.81	
Tehsil Paharpur	02	27	7.40	
Total	15	138	10.87	

Table 5: Prevalence of Bovine Tuberculosis by Age Group

Age Group (Years)	Number of Positive Samples	Number of Negative Samples	Prevalence (%)	p-value
< 1	2	20	10.0	0.0754 (Non-significant)
1-3	7	75	9.33	
> 3	6	43	13.95	
Total	15	138	10.87	

Table 6: Sex-Based Prevalence of Bovine Tuberculosis

Sex	Number of Positive Samples	Number of Negative Samples	Total number of samples	Prevalence (%)	$\chi 2$	p-value
Male	02	18	20	10.00	0.0642	0.8000 (Non-significant)
Female	13	105	118	12.38		
Total	15	123	138	10.87		

Table 7: Season-Based Prevalence of Bovine Tuberculosis

Season	Number of Positive Samples	Number of Negative Samples	Total number of samples	Prevalence (%)	p-value
Spring	4	36	40	10.00	**0.00001*** (Significant)
Summer	2	31	33	6.06	
Autumn	6	48	54	11.11	
Winter	3	8	11	27.27	
Total	15	123	138	10.87	

**indicated that this value is significant at p < 0.05*

Table 8: Correlation of Risk Factors and Bovine Tuberculosis Prevalence

Risk Factor	Positive Samples	Negative Samples	Total number of samples	Prevalence (%)	p-value
Overcrowding of Animals	8	62	70	11.42	**0.032***
Poor Animal Husbandry	6	72	78	7.69	0.157
Inadequate Biosecurity Measures	10	68	78	12.82	**0.011***
Presence of Wildlife Reservoirs	5	55	60	8.33	0.089
Consumption of Unpasteurized Milk	4	67	71	5.63	0.215

**indicated that the value is significant at p < 0.05*

Table 9: Prevalence of Bovine Tuberculosis by Herd Size

Herd Size	Positive Samples	Negative Samples	Total samples	Prevalence (%)	p-value
Small (1-10)	4	30	34	11.76	0.5317 (Non-significant)
Medium (11-50)	6	51	57	10.52	
Large (> 50)	5	42	47	10.63	
Total	15	123	138	10.87	

Table 10: Prevalence of Bovine Tuberculosis by Co-Infections

Co-Infections	Positive Samples	Negative Samples	Total samples	Prevalence (%)	p-value
None	10	86	96	10.41	0.7619 (Non-significant)
Mastitis	3	21	24	12.50	
Foot-and-Mouth	2	16	18	11.11	
Total	15	123	138	10.87	

Table 11: Prevalence of Bovine Tuberculosis by Management Practices

Management Practices	Positive Samples	Negative Samples	Total samples	Prevalence (%)	p-value
Grazing	5	36	41	12.19	
Confined Housing	7	58	65	10.76	0.2611 (Non-significant)
Mixed (Grazing + Housing)	3	29	32	9.37	
Total	15	123	138	10.87	

These co-infections do not appear to be significant factors influencing the prevalence of bovine TB in this study, as the prevalence percentages are relatively similar across co-infection categories (Table 10).

Prevalence of bTB based on various cattle management practices was also recorded on the approved questionnaire. This study considered the management practices "Grazing," "Confined Housing," and "Mixed (Grazing + Housing)." 5 out of 41 samples tested positive for bovine TB in the "Grazing" category, with a prevalence of 12.19 %. This prevalence has a p-value of 0.2611, which indicated a non-significant result. In "Confined Housing" category, seven out of sixty-five tested samples were positive for bovine tuberculosis, with a prevalence of 10.76 %. The prevalence of bovine tuberculosis in the "Mixed (Grazing + Housing)" category was 9.37 %, with three positive samples out of 32 tested. According to these results, there is no statistically significant relationship between specific management practices (grazing, confined lodging, or mixed) and bTB prevalence for sample (Table 11).

DISCUSSION

We estimated prevalence of bTB in District Dera Ismail Khan, identified associated risk factors, and emphasized the public health significance of the disease by creating awareness among the farmers' community regarding disease control and prevention at their farms. The findings of this research informed targeted control strategies, contributed to understanding of infection dynamics, and delivered insights for operative management of bTB in both animal and human populations.

The survey determined prevalence of bTB in Dera Ismail Khan, to identify associated risk factors, and to highlight the disease's public health significance. The study's findings contributed to the comprehension of disease dynamics and offered insights into effective management of bovine TB in both animal and human populations. Estimated prevalence of bTB was 10.87 % in District Dera Ismail Khan based on diagnostic test results. This frequency indicated a substantial burden of bovine tuberculosis in the region. It is essential for implementing effective control measures and monitoring the efficacy of control efforts to have an accurate estimation of prevalence.

Utilizing CCIT and Ziehl Neelsen stain for AFB, prevalence of bovine TB was demosntarted (Ameni et al., 2018; Hussey and Zayaltz, 2016; Agbalaya et al., 2020). Additionally, prevalence of bTB in various tehsils of District Dera Ismail Khan was investigated. Although there were differences in prevalence between tehsils, they were not statistically significant. This indicated that the prevalence of bovine TB did not vary significantly between the examined tehsils. Understanding the distribution of bovine tuberculosis within the district can improve the effectiveness of control strategies (Belete et al., 2021). Analysis of prevalence by age showed that there was no significant difference in prevalence of bTB between age groups. Findings

suggested that bTB equally affects animals of all ages and gender.

Analysis of seasonal prevalence revealed that bTB in winter was significantly higher than in other seasons. This seasonal variation in prevalence may be a result of environmental or management factors. Understanding the factors contributing to seasonal variation can aid in the development of targeted, seasonal-based control strategies (Gong et al., 2021; Dergal et al., 2023).

Various risk factors associated with prevalence of bTB were also examined. Animal overcrowding and insufficient biosecurity were found significant with higher prevalence of bTB. Risk factors emphasized significance of proper animal management and biosecurity measures in preventing the disease's spread. Poor animal husbandry practices, presence of wildlife reservoirs, and consumption of raw milk were not statistically associated with bTB (Humblet et al., 2009).

Prevalence of BTB was investigated and identified associated risk factors using CCIT test on 174 herds comprising 2,754 dairy cattle in cities of Gondar, Hawassa, and Mekelle. At cutoff > 4 mm, herd prevalence was 22.4 % and animal prevalence was 5.2 %. Mean prevalence within herds of positive farms at the > 4 mm cutoff was 22.7 %. Analysis revealed that herd size, farm hygiene, feeding condition, and biosecurity were significantly associated with BTB status, whereas new cattle introductions were only marginally significant and farm age, housing condition, farmers' educational status, and animal health care practices were not. Findings of this research were consistent with our investigation regarding prevalence and risk factors associated with bTB (Mekonnen et al., 2019). In Ethiopia, bTB was a neglected disease that had a significant impact on the economy and subsistence of farmers. Postmortem examinations were utilized to identify tuberculous lesions, whereas smear microscopy was utilized to identify AFB. BTB-suspicious tuberculous lesions were detected on 45 out of 497 inspected slaughtered cattle (9.1 %, 95 % CI: 0.0668 – 0.1193). A greater proportion of macroscopic lesions were found in the lymph nodes of the lungs, specifically the mediastinal (14, 31.1 %) and bronchial (10, 22.2 %) lymph nodes, followed by the mesenteric (9, 20 %) lymph nodes. Histopathology and smear microscopy revealed that only two (4.4 %) of 45 tuberculous lesions were AFB-positive (Belete et al., 2021).

The significance of bovine tuberculosis to public health should not be understated. Zoonotic transmission of the disease can result in severe human illness, including pulmonary and extrapulmonary forms of tuberculosis. The emergence of *Mycobacterium bovis* strains with drug resistance complicates disease management and control efforts. For effective control of bovine tuberculosis, a comprehensive One Health approach that integrates veterinary and human health interventions is essential (Kasir et al., 2023).

This study provided insightful information regarding the prevalence, risk factors, and public health significance of bTB. The findings can guide targeted disease control strategies, enhance disease management, and increase agricultural community awareness of disease control and prevention. Implementing effective surveillance programs, enhancing biosecurity measures, and promoting milk pasteurization are essential interventions for reducing bTB in district and protecting public health. A cross-sectional study significantly supported our findings, conducted on dairy cattle randomly selected from smallholder farms in districts of Arba Minch Zuria and Chencha. CIDT was used to diagnose bTB in dairy animals. 221 dairy cattle received CIDT treatment. A questionnaire was administered to 110 smallholders to assess the risk factors responsible for the

occurrence of bTB in the human population. The prevalence of bTB at the animal level was 8.2 % in the study sites. (OR=1.34) Dairy cattle older than 4 years were 34 % more likely to be infected with bTB than those younger than 4 years. However, housing, body condition, herd size, and parity were not substantially associated with the occurrence of bovine tuberculosis. According to an evaluation of bTB awareness, 29.7 % of participants were aware that cattle could be infected, and 13.4 % of respondents believed the disease can travel from animals to humans and vice versa. In addition, 66.1 % of participants regularly consumed raw milk and soured milk products. Moreover, the respondent's perspective on zoonoses and the consequences of bTB was precarious (Tora et al., 2022).

Another study reported that the prevalence of TB in slaughtered animals was 0.83 percent overall, with the following distribution by species: 2.73 percent in cattle, 0.001 percent in sheep, and 0 percent in goats. During the study period, there was a significant correlation ($R = 0.82$; $p < 0.01$) between the incidence of tuberculosis and the number of cattle slaughtered. Between 2018 and 2020, fluctuations in monthly TB prevalence ranged from 2 % to 24.8 %, but there were no statistically significant correlations between infection and the age or gender of the animals, except for 2020 when a significantly higher ($p = 0.017$) proportion of TB cases were recorded in female cattle than in male cattle. The monthly average weight of seized livers and lungs varied substantially ($p < 0.05$) between 150 kg and 350 kg (Dergal et al., 2023).

The global status of bTB as a zoonosis remains extremely alarming. Principal risk factors for bovine tuberculosis at three levels: animal, herd, and region or country. Animal health professionals and scientists directly involved in the control and prevention of bTB in cattle were given recommendations. The establishment of Millennium Development Goals for bTB was proposed to enhance the global control and eradication of the disease (Ameni et al., 2018). Another similar study found that zoonotic tuberculosis is strongly linked to poverty and inadequate hygiene, with low- and middle-income countries bearing the brunt of the disease's burden. In developing countries, BTB is increasingly recognized as a growing public health threat. However, the absence of effective surveillance programs in many of these nations prevents accurate estimation of the true disease burden. In addition, the emergence of drug-resistant strains that compromise the efficacy of current treatment regimens threatens the control of bTB. Following PRISMA guidelines, a total of 90 studies conducted in the MENA region were chosen based on their findings that the prevalence of bTB among humans and cattle varied substantially based on population size and country (Kasir et al., 2023).

The present investigation revealed that bTB is an animal health problem affecting dairy cattle in Pakistan and for mitigating public health hazards and risk factors associated, it is necessary to increase public awareness, test for bovine tuberculosis, and implement a segregation strategy (Firdessa et al., 2012).

Public Health Implications

The public health implications of the findings from this investigation are substantial, as the disease is zoonotic. The significant risk to the cattle population and humans, particularly those in close contact with infected animals or who ingest animal products such as unpasteurized milk, is underscored by the high prevalence rate of 10.87 %.

Initially, the study underscored the necessity of immediate and robust public health interventions to regulate the transmission of bTB. Enhanced surveillance and rigorous testing programmes for cattle have the potential to

reduce the transmission of the disease to humans by facilitating the detection and management of cases more effectively. The identification of overcrowding and insufficient biosecurity measures as significant risk factors implies that the incidence of bTB could be significantly reduced by the implementation of more stringent regulations and the enhancement of farm management practices.

In addition, it is imperative to implement public health education campaigns to increase awareness among the general public and farmers regarding the hazards associated with the consumption of unpasteurized dairy products and the management of livestock. Educating the public on the symptoms of bovine and human tuberculosis and the significance of early medical intervention could be instrumental in the early detection and prevention of it.

Furthermore, the results could be used to advocate for the integration of One Health strategies, which could inform policy. This strategy would entail the integration of the veterinary health, public health and environmental sectors in order to implement a more comprehensive response to bTB. The cycle of infection may be perpetuated by the monitoring and control of wildlife reservoirs, which can also harbour *Mycobacterium bovis*. Therefore, such policies may involve coordinated efforts to monitor and control these locations.

Ultimately, the public health implications of this study underscored the importance of multifaceted strategies that encompassed policy changes, enhanced farm practices and public education in order to effectively manage and reduce the prevalence of bovine tuberculosis, thereby safeguarding the health of both animals and humans.

CONCLUSION

The estimated prevalence of bovine TB in District Dera Ismail Khan was 10.87 % according to our study. Using diagnostic assays such as the tuberculin skin test and Ziehl Neelsen stain, we were able to demonstrate *Mycobacterium bovis* in infected cattle, thereby validating our findings. In addition, our analysis identified animal overcrowding and inadequate biosecurity as significant risk factors for bovine tuberculosis. These results emphasized the urgent need for targeted control strategies and interventions to reduce the prevalence of bovine tuberculosis, as well as the significance of implementing proper animal husbandry practices and enhancing biosecurity measures to protect both animal and human populations from this infectious disease.

CONFLICT OF INTEREST

None.

ACKNOWLEDGMENT

The research was funded by Government of Khyber Pakhtunkhwa, Pakistan, vide Grant No. L&DD/Dev/2022-23/3702)

AUTHORS CONTRIBUTION

Arsalan Khan, Waseem Ullah and Qamar Ullah, conducted the investigation and also Arsalan Khan wrote the original draft. Akhtar Ali, Umme Aimen and Imtiaz Khan conceptualized the study and conducted the statistical analysis. Baqir Hussain and Muneeb Ur Rahman significantly helped in the revisions and formatting of this manuscript.

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