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Bovine Tuberculosis (bTB): Prevalence and Associated Risk Factors in Large Ruminants in the Central Zone of Khyber Pakhtunkhwa, Pakistan

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Authors' Contribution
AU, USK and SA designed and planned the study. MSQ, IK, IUJ and IK executed the study. RT, SN and NUK performed laboratory work and analyzed the data. MAK and MLS wrote the article.

Key words
Epidemiology, Large ruminants, Khyber Pakhtunkhwa, TB.

ABSTRACT

This cross-sectional study was conducted to determine the prevalence and associated risk factors of bovine tuberculosis (bTB) among large ruminants of the five districts (Peshawar, Nowshera, Charsadda, Mardan and Swabi) located in the central zone of Khyber Pakhtunkhwa (KPK), Pakistan. A total of 2400 large ruminants were sampled by convenience sampling approach and tested by comparative cervical intradermal tuberculin test (CCIT) for detection of *Mycobacterium bovis* infection. Data regarding socio-demographic status, risk factors and farming practices were gathered through a pre-form questionnaire. Results revealed that prevalence of bovine tuberculosis was 5.88%. Statistical analysis revealed significant association of age (OR= 3.267; CI = 1.686-6.331) and herd size (OR = 2.600; CI = 1.421-4.760) with CCIT positivity. Similarly, induction of new animals into the herd (OR= 2.661; CI = 1.758-4.028) and sheltering the animals at night (OR= 2.448; CI = 1.568-3.882) also showed association with a positive skin test. The herd owners and animal handlers had poor knowledge regarding signs, symptoms and the zoonotic nature of the infection. Three primary signs (persistent cough, weight loss and fever) were recognized by 4.7% of respondents including herd owners, herders and animal handlers. This study calls for instant measures for disease control among the animals and humans of the study area, and highlight the need for farmer education regarding bovine tuberculosis.

INTRODUCTION

Bovine tuberculosis (bTB) is an infectious zoonotic disease caused by *Mycobacterium bovis* (*M. bovis*), having a wide range of hosts (Okeke *et al*., 2016; Batool *et al*., 2017). In an area where bTB is endemic, uncontrolled or partially controlled, aerosol is the key route of transmission for the humans along with ingestion of contaminated water, meat and milk (Awah-Ndukum *et al*., 2016). Workers handling carcasses of infected animals get infection through skin and mucous membranes (de la Rua-Domenech, 2006; Michel *et al*., 2010). Bovine TB outbreaks trigger large economic losses to agricultural economies by curtailing international trade of animals and animal products, causing reduction in milk and meat production, compromising fertility, massive control and eradication strategies, and increased public health risks (Dejene *et al*., 2016). Although some countries managed to eradicate bTB through regular testing and removal of infected animals, the occurrence of bTB is higher in developing countries since the control and eradication policies are not sufficiently executed (Amanfu, 2006; de la Rua-Domenech, 2006). More than 125 countries reported prevalence of *M. bovis* infection in cattle herds to OIE, during 2005 to 2008 (Michel *et al*., 2010). Literature reported higher prevalence of disease with increasing age (Ameni *et al*., 2007), while at herd level, herd size and movement of animals were associated with the bTB (Kazwala *et al*., 2001). New animals must be intro-

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duced from non-endemic areas to minimize the risk of new infection in the herd (Reilly and Courtenay, 2007).

Endemicity of bovine TB among animals has been attempted to be controlled using test and slaughter strategy. For past decades, infected or carrier subjects are identified using the comparative cervical intradermal tuberculin test (CCIT), also termed as the tuberculin skin test (TST). This test is based on delayed-type hypersensitivity reaction, evoked by purified protein of *Mycobacterium tuberculosis* called tuberculin (Monaghan *et al*., 1994). Despite certain limitations, the importance of CCIT in control of TB can never be ignored. It is simple and does not demand extensive training and special laboratory aids for its procedure and interpretation of results (Mohamed, 2017).

Extensive struggle has been made around the globe to eradicate bTB because of its zoonotic and economic consequences. In developing countries, precise data on prevalence of infection is needed to improved surveillance programs and control of bTB (Moiane *et al*., 2014). Pakistan faces a serious dearth of epidemiological data epidemiology on bTB, rendering the surveillance and eradication targets of *M. bovis* infection, unaccomplished. National Tuberculosis Control Program, Pakistan (NTCP) doesn't address the transmission of bTB to humans, adding to the difficulty.

This study was designed and conducted to determine prevalence and associated risk factors of bTB caused by *M. bovis* in most populated and centrally located districts of Khyber Pakhtunkhwa in Pakistan.

MATERIALS AND METHODS

Study design and area

A cross-sectional epidemiological study was conducted to determine the prevalence of *M. bovis* and risk factors associated with presence of infection in large ruminants of urban and rural areas of central zone of Khyber Pakhtunkhwa, Pakistan. This study was carried out in the central zone (districts Peshawar, Nowshera, Charsadda, Mardan and Swabi) of Khyber Pakhtunkhwa, Pakistan. A total of 2400 asymptomatic large ruminants (1225 cattle and 1175 buffalo) were tested by convenience sampling approach (Tyrer and Heyman, 2016) using CCIT from January 2016 to December 2016.

Data collection

To identify the associated risk factors, semi-structured interviews were conducted after formal written consent from herd owners and livestock workers. A structured questionnaire was designed to gather animal and herd level information. Animal level data included information regarding location of the animal, species, age, gender and lactation status of the animal, while herd level data included information regarding herd size, procedure of animal procurement in the herd, breeding of animals, housing and feeding management practices and presence of various animal species in the vicinity of tested animals. Trained local government officials, conversant in local and the national language assisted with data collection. Information regarding contact of tested animals with various other species of the animals was gathered from herders. Presence of wild animal species in and around the grazing area and water source was described as contact with wild animals. The questionnaire template used, can be obtained from the corresponding author on request.

Comparative cervical intradermal tuberculin test

Comparative cervical intradermal tuberculin test was performed, briefly 0.1ml (25,000 IU) avian purified protein derivative (a-PPD) and 0.1ml (20,000 IU) bovine purified protein derivative (b-PPD) were injected at 12 cm distant points on left lateral side of neck. After 72 h of injecting a-PPD and b-PPD, the thickness of skin-fold at each injection site was measured using digital calipers. Animals were considered positive if the fold to the skin was thickened >4 mm at b-PPD injection site as compared to a-PPD injection site. While, if skin thickness at a-PPD injection site is 4mm higher than that at b-PPD injection site, the animal was considered a reactor.

Statistical analysis

The collected data was entered using the Microsoft Excel version 2013, and then exported to the Statistical Product and Service Solutions (SPSS) version 20.0 to establish associations between positive animals and the risk factors at 95% level of confidence. Summary statistics, Pearson's chi-square test and multivariable logistic regression were done to identify associations between selected risk factors and CCIT positivity. The prevalence was calculated by dividing the number of positive animals by the total number of tested animals. Variables with a p-value of <0.05 on Pearson's chi-square test (χ^2 test) were included in multivariable models. Finally, logistic regression was used to analyze the effect of potential risk factors.

RESULTS

In this study, 655 (27.29%) animals were from small scale farmers, reared for milk production at household level and 1745 (72.70%) animals were from commercial dairy herds. Out of 2400, 1225 (51.04%) were cattle and 1175 (48.96%) were buffaloes.

Table I.- Prevalence of bovine tuberculosis (bTB) in five districts of the Central Zone of Khyber Pakhtunkhwa, Pakistan.

District	Animals screened (N)	Tested positive		95% confidence level		Chi-square
		n	Overall prevalence (%) = (n/N x 100)	Lower	Upper	
Peshawar	480	31	6.46	4.26	8.66	*p*=0.669
Nowshera	480	23	4.79	2.88	6.70	
Charsadda	480	30	6.25	4.08	8.42	
Mardan	480	32	6.67	4.44	8.90	
Swabi	480	25	5.21	3.22	7.20	
Total tested	2400	141	5.88	3.78	7.98	

N, number of screened animals; n, number of infected animals for bTB.

Table II.- Bivariate frequency analysis of various parameters in positive and negative tuberculin reactors.

Parameters	Negative	Positive		Chi-square
		n	%	
Species				
Cattle	1146	79	6.45%	p=0.222
Buffalo	1113	62	5.28%	
Age (years)				
1-2	416	0	0.00%	p=0.000
2-3	504	18	3.45%	
3-4.5	779	32	3.95%	
5-≥8	560	91	13.98%	
Sex				
Male	260	10	3.70%	p=0.107
Female	1999	131	6.15%	
Lactation status				
Lactating	1500	108	6.72%	p=0.072
Non-lactating	504	24	4.55%	
Herd size				
1 to 10	495	5	1.00%	p=0.000
11 to 20	561	14	2.43%	
21 to 30	601	24	3.84%	
31 to 40	323	27	7.71%	
41 to 50	279	71	20.29%	
Animal source				
Raised at own farm	1481	51	3.33%	p=0.000
Purchased from market	778	90	10.37%	
Number of old animals in the herd				
0	474	32	6.32%	p=0.069
1	340	10	2.86%	
2	433	28	6.07%	
3	380	33	7.99%	
4	251	18	6.69%	
5 and more	381	20	4.99%	
Breeding of animals				
AI	1284	72	5.31%	p=0.387
Natural with Own bull	406	30	6.88%	
Natural with rented bull	569	39	6.41%	

Parameters	Negative	Positive		Chi-square
		n	%	
Presence of other animals with cattle and buffalo				
Goat	403	25	5.84%	p=0.132
Donkey	245	25	9.26%	
Poultry	412	19	4.41%	
None	349	20	5.42%	
Sheep	324	21	6.09%	
Horse	279	12	4.12%	
Mule	247	19	7.14%	
Animals kept at night				
Outdoor	188	4	2.08%	p=0.020
Indoor	2071	137	6.20%	
Ventilation status(if indoor at night)				
Ventilation	1113	101	8.32%	p=0.355
No Ventilation	478	36	7.00%	
Pet on farm				
Dog	563	36	6.01%	p=0.367
Backyard poultry	436	30	6.44%	
Other	498	37	6.92%	
None	762	38	4.75%	
Animals feeding through				
Only grazing	1181	61	4.91%	p=0.115
Only station feeding	282	21	6.93%	
Grazing plus station feeding	796	59	6.90%	
Grazing status				
Communal	636	44	6.47%	p=0.456
Non-communal	1271	76	5.64%	
Water trough				
Combined	1686	115	6.39%	p=0.065
Individual	573	26	4.34%	
Manure disposal				
Twice a day	189	16	7.80%	p=0.459
Daily	1927	117	5.72%	
Twice a week	143	8	5.30%	

Out of 2400 CCIT tested animals, 141 were positive for bovine tuberculosis yielding prevalence of 5.88% (95% CI; 5.68 - 6.08). No statistically significant difference was found (p = 0.669) among the different study districts (Table I). Results showed non-significant association among CCIT positivity and species of animals (p = 0.222), gender (p = 0.107), lactation status (p = 0.072), number of older animals within herd (p = 0.069), breeding practices (p = 0.387), presence of various livestock species in and around large ruminants (p = 0.132), ventilation status if kept indoor at night (p = 0.355), presence of pet animals on farm (p = 0.367), feeding practices (p = 0.115), grazing practices (p = 0.456), combined drinking water source (p = 0.065) and manure disposal (p = 0.459).

Table III.- Multivariable logistic regression analysis of various parameters.

Parameters	Odds ratio	95% CI		P value
		Lower	Upper	
Age				
1-2 years		Reference		
2-3 years	1.725	0.638	4.662	0.283
3-5 years	1.078	0.540	2.154	0.831
5 -≥8 years	3.267	1.686	6.331	0.000
Herd size				
1 to 10		Reference		
11 to 20	1.124	0.665	1.898	0.663
21 to 30	0.735	0.412	1.311	0.297
31 to 40	2.594	1.319	5.102	0.006
41 to 50	2.600	1.421	4.760	0.002
Animal source				
Raised at own Farm		Reference		
Purchased	2.661	1.758	4.028	0.000
Animals kept at night				
Outdoor		Reference		
Indoor	2.448	1.568	3.822	0.000

Bivariate analysis (Table II) and multivariable logistic regression analysis (Table III) revealed the significant association between CCIT positivity and age, animal source, herd size and animal housing during the night. Bovine TB was found to be more prevalent in older animals (≥5years) as compared to younger animals (OR = 3.267; 95% CI = 1.686-6.331; p = 0.000). Statistical analysis revealed larger herd size (41-50 animals) as a potential risk factor for CCIT positivity in large ruminants (OR = 2.600; 95% CI = 1.421-4.760; p = 0.002). Similarly, the rate of CCIT test positivity was higher in farms where animals were procured from live animal markets as compared to those farms where animals were privately raised (OR = 2.661; 95% CI = 1.758-4.028; p = 0.000). Results revealed higher odds of CCIT positivity in animals kept indoor during night (OR = 2.448; 95% CI = 1.568-3.822; p = 0.000).

Respondents were also interviewed to determine their knowledge about bovine tuberculosis. Around 33.68% herd owners mentioned about persistent cough (>3 weeks) as an indication of tuberculosis. Similarly, 75.26% herd owners believed weight loss a symptom of tuberculosis while 23.68% herd owners mentioned coughing up sputum or blood as an indication of tuberculosis. On the other hand, many respondents inaccurately linked some symptoms such as vomiting (7.37%) and diarrhea (10%) with TB. Livestock owners identified chest pain, fever and chills as signs and symptoms of tuberculosis (23.16%, 80% and 47.89%, respectively). Some animal handlers identified general weakness and loss of appetite (76.32% and 80.53%, respectively) as indications of TB. All three primary signs (persistent cough, weight loss and fever) of TB were mentioned only by 7.1% of respondents showing poor awareness among farmers and animal handlers (Fig. 1).

In this study, 190 livestock farmer were interviewed about the zoonosis of bTB. Almost 97.9% herd owners had some kind of knowledge regarding tuberculosis while 2.1% had not heard of the infection. Less than one third (30.1%) of the farmers were aware of the zoonotic nature of the disease. Unpasteurized milk was thought to be the source of infection by 37.4% of the dairy farmers. Similarly, 17.4% and 11.1% knew about the air and raw meat, respectively as the source of infection (Table IV).

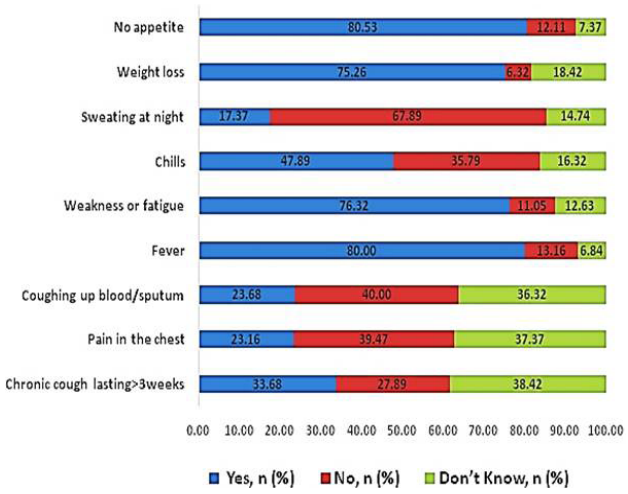

Fig. 1. Distribution of signs and symptoms of bTB known to the livestock farmers in the Central Zone of Khyber Pakhtunkhwa, Pakistan.

Table IV.- Knowledge of livestock farmers about zoonosis of bovine tuberculosis in the Central zone of Khyber Pakhtunkhwa, Pakistan.

Parameter	No. of farmers	Farmers (%)
Age (years)		
<20	16	8.4
20–30	25	13.2
31–40	35	18.4
41–50	43	22.6
>50	71	37.4
Education		
Illiterate	15	7.9
Middle	61	32.1
Secondary	85	44.7
Graduate	29	15.3
Socio-economic status		
Very Low	13	6.8
Low	54	28.4
Moderate	94	49.5
High	29	15.3
Farmers knowledge about zoonosis of Btb		
Have you heard of Tuberculosis?		
Yes	186	97.9
No	4	2.1
If yes, does TB spread from animals to human		
Yes	59	31.1
No	131	68.9
If TB spreads from animals to human, through which way of the following:		
Raw milk	71	37.4
Meat	21	11.1
Aerosol route	33	17.4
Urine	38	20.0
Feces of disease animals	27	14.2

DISCUSSION

Pakistan is a sub-tropical country and Central zone of Khyber Pakhtunkhwa (KP) is located in continental grasslands and has five administrative units/districts *i.e.;* district Peshawar, Nowshera, Charsadda, Mardan and district Swabi lying at the porous afghan border, with frequent movement of animals and humans across the border. Dearth of data on epidemiological dynamics of bTB in region can surge serious menace of infection among subjects in the central zone of KPK. Constant investigation of *M. bovis* infection and associated risk factors in large ruminants in the region is mandatory to design effective control measures. During the current investigation, the overall prevalence for *M. bovis* infection was 5.88% in large ruminants. Within Pakistan, several studies reported variation in prevalence *i.e.* 3% (Javed *et al*., 2010), 2.2% (Javed *et al*., 2009), 1.7% (Ifrahim, 2001), 9.6% (Mumtaz *et al*., 2008), 10.6% (Khan *et al*., 2008), 11.3% (Javed *et al*., 2012) and 12.72% (Khan and Khan, 2007). This variation in the prevalence of infection is attributed to differences in husbandry practices and higher numbers of crossbreds as well as exotic animals at the dairy farms (Memon *et al*., 2017). No significant difference was found in the prevalence among the different study regions because of similarity in animal husbandry and management practices.

Outcomes of multivariable statistical assessment revealed that 5 years or older animals are at higher odds of getting infection. Similar findings were reported by previous studies conducted in similar socio-economic settings (Javed *et al*., 2006, 2009, 2012; Khan and Khan, 2007; Moiane *et al*., 2014). Prevalence increased with the age of livestock animals because of the lengthier exposure to the etiological agent over time among older animals (Dejene *et al*., 2016). In endemic scenarios, older animals were exposed to infectious agent for longer periods of time, increasing the prevalence with increasing age (Mahmud *et al*., 2014). Cleaveland *et al*. (2007) proposed reactivation of latent infection of tuberculosis in older age to be the cause of greater proportions of human tuberculosis but this is yet to be confirmed in animals. Larger herd size was found to be significant risk factor associated with bTB, which might be because of presence of higher number of animals in larger herds (Proaño-Perez *et al*., 2009). Dairy farmers improve the herd size to increase farm yield which can lead to overcrowding causing increased animal-to-animal transmission (Ghebremariam *et al*., 2016). This study indicated that the positivity of bTB was meaningfully linked with buying and induction of new animals into the farm. These conclusions are in line with the findings of several studies as piloted in England (Gopal *et al*., 2006) and Uganda (Oloya *et al*., 2007; Kazoora *et al*., 2014), which can be attributed due to their interaction with various animals during their stay at different dairy farms or at live animal markets. The animals housed indoor during the night were found at higher risk of being CCIT positive as compared to those who were housed outdoor during night. These outcomes may be due to the aerosol route of transmission among closely tied animals. Hence, extra risk of close contact of susceptible animals might be the main cause behind greater ratio of CCIT positive animals when housed indoor. Our results are in line with the findings of Katale *et al*. (2012) who reported substandard ventilation as the cause of infection transmission.

The learning rate is relatively low in emerging nations including Pakistan (Mangesho *et al*., 2007; Proaño-Perez *et al*., 2009; McGeary, 2008). Different seminars, wakefulness promotions, learning sessions and use of

print and electronic media must be an important part of TB control programs.

CONCLUSIONS

The conclusions of current research provide evidence about the prevalence and possible risk factors linked with positivity of *M. bovis* infection in large ruminants (cattle and buffalo) in Central Zone of Khyber Pakhtunkhwa, which need to include *M. bovis* infection in NTCP.

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Statement of conflict of interest

The authors declare that there are no conflicts of interests regarding the publication of this article

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