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Prevalence and Risk Factors Associated with Bovine Tuberculosis in Cattle in Hyderabad and Tando Allahyar Districts, Sindh, Pakistan

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AAK designed the experiment. AL, KKM and SB performed the experiments. AL, SAL and FMK analyzed the experimental data. AL, IBC and JMS wrote this paper.

Key words
Bovine tuberculosis, Cattle, Tuberculin test, Rapid test, *Mycobacterium bovis*.

ABSTRACT

An investigation on the prevalence of bovine tuberculosis (BTB) in randomly selected cattle (n= 160) was carried out in Hyderabad and Tando Allahyar districts. The animals were first screened through single intradermal tuberculin test (SITT) and both positive and negative reactors of SITT were further investigated through rapid test. Attempts were also made to isolate the *Mycobacterium bovis* organism from the milk and nasal secretions of cattle using the Lowenstein-Jensen media. An overall prevalence of 34.38% was recorded by rapid test that was higher ($P< 0.001$) than SITT (3.13%) and culture test (2.50%). A somewhat higher prevalence was recorded in Hyderabad district (SITT 3.75%, Rapid 36%, culture 3.75%) as compared to Tando Allahyar district (SITT 2.5%, Rapid 32%, culture 1.25%). In Hyderabad district, rapid test showed a significantly higher ($P< 0.05$) prevalence in male than females. Similarly, a higher ($P< 0.05$) prevalence was observed for 5-8 years age than > 8 years, in non-pregnant animals than pregnant and in 2-4 and >4 parities than 1 parity. However, in Tando Allahyar district, rapid test declared a significantly higher prevalence ($P< 0.05$) in female than male cattle, in 5-8 years age than > 8 years, in non-pregnant than pregnant, having 2-4 liters/d milk production than more than 4 liters/d, and in late stage of lactation than early or mid-stage. The prevalence in Tando Allahyar district revealed by culture technique showed a significantly ($P< 0.05$) higher percentage in >4 parity than 1 parity or 2-4 parity, having 4-8 liters/d milk production than those having 2-4 or >8 liters/d, and in late stage of lactation than early or mid-stage. In brief, BTB is prevailing in both Hyderabad and Tando Allahyar districts; however it is relatively higher in Hyderabad district than Tando Allahyar district. Infected animals shed more *M. bovis* in nasal secretions (3/4; 75%) as compared to milk (1/4; 25%). Rapid test showed the highest prevalence as compared to other techniques hence could be regarded as the most sensitive technique for BTB.

INTRODUCTION

Bovine tuberculosis is an infectious and contagious disease caused by *Mycobacterium bovis,* which affects cattle, other domestic animals and certain wildlife species. It is characterized by production of nodular granuloma and debilitating disease. The disease can affect any body tissue particularly lungs, lymph nodes, liver, spleen, peritoneum and pleura (Prodinger *et al*., 2005). Bovine tuberculosis is a latent infection only a few animals may become severely affected with the disease within a few months. While, other animals may take many years to show clinical signs related to bovine tuberculosis (Carslake *et al*., 2011).

M. bovis, the causative agent of bovine tuberculosis mainly in cattle, is an acid fast, aerobic and slow growing bacterium. While the other important specie of the genus, *M. tuberculosis* and causes tuberculosis in humans. However,

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it can cause the infection in other mammals as well (Arshad *et al*., 2012). In many countries this pathogen remains the cause of major infection in cattle and other animals, hence distribution of this bacterium is recognized worldwide (Carslake *et al*., 2011). Bovine tuberculosis is a socio-economic disease and listed in OIE (Cousins *et al*., 2001).

The principle root of *M. bovis* transmission is inhalation, that becomes more vulnerable in case of close prolonged contact between infected and healthy animals (Neil *et al*., 1994). Infected animal may shed *M. bovis* in many ways: discharge lesions, milk, feces, urine and saliva (Neil *et al*., 1991). Vaccination and artificial insemination centers, dipping tanks, auction stations, market places and transportation are the gathering places are sites where transmission of infection could easily occur (Ayele *et al*., 2004; Menzies and Neil, 2000). *M. bovis* causes pulmonary tuberculosis in humans is identified in many countries; it is highly seen in the people working in the slaughter houses and those dealing with the milk. The elimination of transfer of tuberculosis can be achieved by complete pasteurization of milk before use as the milk is the main source of transmission of disease (O'Reilly *et al*., 1995).

Age of the animal is the main risk factor identified by various studies. Old age animals have more duration of exposure of disease as compare with younger animals. Animal expresses the signs of disease when it became adult even it might have got infection in early age. Breed is also identified as a risk factor in Africa through skin test. The predisposing factor for many diseases is immuno-suppressant identified through number of studies in both developed and undeveloped countries (De la Rau *et al*., 2006). An Italian scientist reported that the calf can get infection by consuming colostrum/milk of tuberculosis infected cattle (Zanini *et al*., 1998).

The present study is therefore planned to determine the prevalence of bovine tuberculosis in Hyderabad and Tando Allahyar districts. Prevalence was recorded through SITT, instant diagnostic kit (bTB) and isolation of *M. bovis* from milk, fecal and nasal discharge samples.

MATERIALS AND METHODS

A total of one hundred and sixty cattle from peri-urban and rural areas of Tando Allahyar and Hyderabad, (80 from each district) were randomly selected for single intradermal tuberculin test and sampling according to study. A questionnaire was designed to collection information regarding the sample and risk factors including breed, age, parity, milk production, pregnancy status (pregnant / non-pregnant), type of farming and lactation period.

Single intradermal tuberculin test (SITT)

The injection site was properly cleaned, clipped and skin was measured in a fold using Vernier caliper and marked with permanent marker. A dose of 0.1 ml mammalian PPD was injected obliquely intradermal in the skin. The skin-fold thickness of injection site was re-measured 72 hours post injection (Angus, 1978).

Rapid bovine tuberculosis (BTB) Ab test

Blood samples were analyzed for rapid BTB diagnosis using a commercial kit (Lilli dale Diagnostics, England). A 200 µl of the assay buffer and 20 µl was transferred into the provided centrifuge tube with the help of pipette and mixed well. About 100 µl of sample mixture drooped into the sample well, if T and C both the bands appeared within 5 min showed the positive result.

Bacteriological culture

Culture, homogenization and decontamination of blood, nasal discharge, milk and fecal samples was performed. A 10 ml of milk sample was centrifuged at 3000 rpm for 15 min, supernatant was discarded and sediment was suspended in 2 ml sterilized physiological saline solution, 4-N sodium hydroxide and 0.05% phenol red indicator, mixture was neutralized equally with sterilized 4-N hydrochloric acid solution and again centrifuge after incubation for 30 min. Similarly, other samples for culturing were treated with sodium hydroxide and N-acetyl as suggested by Malhi *et al*. (2018). All decontaminated samples were cultured on the *Mycobacterium* specific medium (Lowenstein Jensen) and were incubated at 37ºC for six to eight weeks (Memon *et al*., 2017). Growth after incubation was confirmed through nitrate reduction and niacin strip test for characterization and identification.

Statistical analysis

The data were analyzed using the statistical software 'SPSS' by chi-square tests for the relationship of different risk factors on the occurrence of tuberculosis in buffaloes where P value of ≤ 0.5 was considered statistically significant.

Table I.- The overall prevalence of bovine tuberculosis in cattle in Hyderabad and Tando Allahyar districts analyzed by different techniques.

Tests	Districts (Prevalence No. (%))		Overall prevalence No. (%)	P-Value
	Hyderabad	Tando Allahyar		
Tuberculin	03 (03.75)	02 (02.50)	05 (03.13)	0.6547
Rapid test	29 (36.00)	26 (32.00)	55 (34.38)	0.6858
Culture	03 (03.75)	01 (01.25)	04 (02.50)	0.3173
P-Value	< 0.0001	< 0.0001	< 0.0001	-

RESULTS

Overall prevalence of bovine tuberculosis in cattle

The overall prevalence of bovine tuberculosis 05 (03.13%) was determined in cattle through tuberculin test, while 55 (34.38%) and 04 (02.50%) on rapid and culture respectively of both districts (Table I). There was statistically non-significant difference (P> 0.05) between prevalence rates of both districts, however the difference between the analytical techniques was found highly significant for both districts (P> 0.01).

Table II.- Occurrence of bovine tuberculosis in cattle analyzed by SITT in Tando Allahyar and Hyderabad districts in relation to various factors.

Factors	Districts			
	Hyderabad		Tando Allahyar	
	Prevalence No. (%)	P-Value	Prevalence No. (%)	P-Value
Sex				
Male (n=20)	03 (15.00)	0.0833	0	0.1573
Female (n=60)	0		02 (03.33)	
Age (n=40 each)				
5-8 years	03 (07.50)	0.0833	01 (02.50)	1.0000
>8 years	0		01 (02.50)	
Breed (n=40 each)				
Local breed	0	0.0833	0	0.1573
Exotic breed	03 (07.50)		02 (05.00)	
Farming (n=40 each)				
Rural	0	0.0833	02 (05.00)	0.1573
Peri urban	03 (07.50)		0	
Pregnancy status (n=40 each)				
Non-pregnant	0	-	02 (05.00)	0.1573
Pregnant	0		0	
Parity (n=20 each)				
1 parity	0		0	0.2231
2-4 parity	0		01 (05.00)	
>4 parity	0		01 (05.00)	
Milk production (n=20 each)				
2-4 liters	0	-	01 (05.00)	0.2231
4-8 liters	0		01 (05.00)	
>8 liters	0		0	
Stage of lactation* **				
Early	0	-	0	0.2231
Mid	0		01 (05.00)	
Late	0		01 (05.00)	

Table III.- Occurrence of bovine tuberculosis in cattle analyzed by rapid test in Tando Allahyar and Hyderabad districts in relation to various factors.

Factors	Districts			
	Hyderabad		Tando Allahyar	
	Prevalence No. (%)	P-Value	Prevalence No. (%)	P-Value
Sex				
Male (n=20)	09 (45.00)	0.0411	05 (25.00)	0.0017
Female (n=60)	20 (33.33)		21 (35.00)	
Age (n=40 each)				
5-8 years	26 (65.00)	< 0.0001	19 (47.50)	0.0186
>8 years	03 (07.50)		07 (17.50)	
Breed (n=40 each)				
Local breed	14 (35.00)	0.8527	16 (40.00)	0.2393
Exotic breed	15 (37.50)		10 (25.00)	
Farming (n=40 each)				
Rural	14 (35.00)	0.8527	11 (27.50)	0.4328
Peri urban	15 (37.50)		15 (37.50)	
Pregnancy status (n=40 each)				
Non-pregnant	16 (53.30)	0.0073	18 (60.00)	0.0011
Pregnant	04 (13.33)		03 (10.00)	
Parity (n=20 each)				
1 parity	1 (05.00)	0.0211	04 (20.00)	0.2765
2-4 parity	10 (50.00)		10 (50.00)	
>4 parity	10 (50.00)		07 (35.00)	
Milk production (n=20 each)				
2-4 liters	10 (50.00)	0.2765	11 (55.00)	0.0183
4-8 liters	07 (35.00)		09 (45.00)	
>8 liters	03 (15.00)		01 (05.00)	
Stage of lactation (n=20 each)				
Early	04 (20.00)	0.2765	02 (10.00)	0.0498
Mid	09 (45.00)		08 (40.00)	
Late	07 (35.00)		11 (55.00)	

BTB in relation to various factors analyzed by tuberculin test

As shown in Table II, tuberculin test showed no effect (P > 0.05) of sex, age, breed, parity, type of farming, pregnancy status, milk production and stage of lactation on the occurrence of BTB in cattle of Hyderabad and Tando Allahyar district.

BTB in relation to various factors analyzed by rapid BTB Ab test

Rapid BTB Ab test showed a significant (P < 0.05) effect of sex, age, parity, pregnancy status, milk production and stage of lactation on the occurrence of bovine tuberculosis; however, breed and type of farming did not show their significant contribution (P > 0.05) in the occurrence of bovine tuberculosis in cattle of Hyderabad and Tando Allahyar district (Table III).

BTB in relation to various factors analyzed through conventional culture method

The statistical analysis showed a no significant association (P>0.05) between various risk factors including sex, age, breed, type of farming and pregnancy status with the occurrence of BTB in cattle of Hyderabad and Tando Allahyar district. Whereas, parity, milk production and stage of lactation showed significant association (P<0.05) with BTB occurrence in Tando Allahyar district (Table IV).

Table IV.- Occurrence of bovine tuberculosis in cattle analyzed by conventional culture method in Tando Allahyar and Hyderabad districts in relation to various factors.

Factors	Districts			
	Hyderabad		Tando Allahyar	
	Prevalence No. (%)	P-Value	Prevalence No. (%)	P-Value
Sex				
Male (n=20)	03 (15.00)	0.0833	0	0.3173
Female (n=60)	0		01 (01.67)	
Age (n=40 each)				
5-8 years	03 (07.50)	0.0833	0	0.3173
>8 years	0		01 (02.50)	
Breed (n=40 each)				
Local breed	0	0.0833	0	0.3173
Exotic breed	03 (07.50)		01 (02.50)	
Farming (n=40 each)				
Rural	0	0.0833	01 (02.50)	0.3173
Peri urban	03 (07.50)		0	
Pregnancy status (n=40 each)				
Non-pregnant	0	-	01 (03.33)	0.3173
Pregnant	0		0	
Parity (n=20 each)				
1 Parity	0	-	0	0.0025
2-4 Parity	0		0	
>4 Parity	0		01 (05.00)	
Milk production (n=20 each)				
2-4 liters	0	-	0	0.0025
4-8 liters	0		01 (05.00)	
>8 liters	0		0	
Stage of lactation (n=20 each)				
Early	0	-	0	0.0025
Mid	0		0	
Late	0		01 (05.00)	

DISCUSSION

Bovine tuberculosis is one of the most challenging endemic diseases currently facing government, the veterinary profession, and the farming industry in the United Kingdom, Ireland and in several other countries (Gumi *et al*., 2012). Jalil *et al*. (2003) stated that BTB is also present in endemic proportion in livestock in Pakistan. Infected animal may shed *Mycobacterium bovis* in milk and nasal secretion. Thus, humans are at risk to get infection by consuming unpasteurized milk, so the disease has significance importance in accounts of its transmission directly to human (Cousins *et al*., 2001). In cattle, prevalence of BTB was reported by different investigators from different countries that are varied as Sarker *et al*. (2015) recorded 2. 34% and Moyoni *et al*. (2014) determined 39.6% in Govuro. These variations could be due to the type of analyzing technique as observed in current study or might be due to the husbandry system, breed used in study and herd size *etc*. (Leghari *et al*., 2016). Moreover, various animal breeds/species have different susceptibility levels for microbial infections (Habib *et al*., 2015; Rajput *et al*., 2018).

In current study, highest occurrence of BTB was recorded by Rapid BTB Ab diagnostic test. The reason of that much prevalence on this test could be that rapid diagnostic test has 3 detector proteins namely MPB 70, 64 and 83 which are responsible to detect antibodies in the serum. The MPB 83 protein is antibody that appear in the circulation at very earlier stage when the animal have been infected very recently (Lillidale diagnostic of UK). This report was made in accordance with the study of Mondal *et al*. (2014), who did his work on 30 cattle and the prevalence of 30% was recorded through immunochromatographic assay (Antigen Rapid Bovine TB Ab test kit).

Findings from this study have indicated significant association between sexes in rapid BTB Ab test. The results are closer with previous studies where male cattle were observed more affected than female cattle as male cattle kept longer in a herd, exists more time in the same environment, hence more chances of contracting a disease than female cattle (Kazwala *et al*., 2001). Literatures have manifested that age is a principle risk factor of bovine tuberculosis, greater the age, more the chance to get infection. Rodwell *et al*. (2001) also reported high prevalence of BTB in old animals as observed in our study. Moreover, in the present study, exotic cross breeds indicated more prevalence than local breeds, which is close to the findings of earlier reports (Romha *et al*., 2014) in which the exotic cross breeds also showed high prevalence. The probable reason could be the fact that genetically improved cattle suffer more severely from poor housing and malnutrition and subsequently become more susceptible to infection (O'Reilly *et al*., 1995). Furthermore, our results also similar to Romha *et al*. (2014), who indicated that the non- pregnant cattle are more susceptible to BTB. Contrary to our findings, a study has reported an association between the pregnancy status and reaction to the infection (Khan *et al*., 2008).

CONCLUSION

This study concluded that BTB is prevalent in both the study areas *i.e.,* Hyderabad and Tando Allahyar. Sex, age, parity, pregnancy status, milk production and stage of lactation were regarded as risk factors for the occurrence of BTB in cattle.

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Statement of conflict of interest

The authors declare no conflict of interest.

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