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Prevalence of bovine tuberculosis in buffaloes in Hyderabad and Tando Allahyar districts of Sindh, Pakistan

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ABSTRACT

An investigation in buffaloes was carried out in Hyderabad and Tando Allahyar districts, Sindh province, Pakistan to determine the prevalence of bovine tuberculosis. The buffaloes (n=120) were first screened through single intradermal tuberculin test (SITT), then their sera were used for enzyme-linked immunosorbent assay (ELISA). Attempts were further made to isolate the *Mycobacterium bovis* organism from the milk samples using traditional culture test. Overall prevalence of 4.16%, 8.33% and 2.5% was recorded by SITT, ELISA and culture test respectively. A somewhat higher prevalence was recorded in Tando Allahyar district (SITT 6.66%; ELISA 10%; culture test 5%) as compared to Hyderabad district (SITT 1.66%; ELISA 6.66%; culture test 0%). Statistical analysis did not show any association (P> 0.05) of herd size, sex, age, milk yield and farming type with the prevalence of the disease. Whereas, SITT showed a significant (P< 0.05) association of sex, age and milk yield with the prevalence of the disease in Tando Allayer district. The results of present study revealed that bovine tuberculosis is present in apparently healthy buffalo herds of Hyderabad and Tando Allayer districts. Moreover, infected animals shed the *M. bovis* pathogen in milk that could be a potential hazard to public health.

Key words: Bovine Tuberculosis, Buffaloes, ELISA, Milk culture, Prevalence, SITT.

INTRODUCTION

Bovine tuberculosis (TB) is caused by bacilli *Mycobacterium bovis* (*M. bovis*), family *Mycobacteriaceae* and genus mycobacterium; it is zoonotic disease which affect domestic and wildlife animals (Etter *et al*., 2006). Bovine TB is commonly noticed, through clinical presence of nodular granulomas. It can cause harmful effects to any body tissue, but lesions are often formed in the lymph nodes (especially in head and/or thorax), lungs, intestine, spleen, liver, pleura and peritoneum (Prodinger *et al.,* 2005). The major pathogen, *M. bovis* is a slow growing, intracellular aerobic bacterium that is the causative agent of bovine TB in domestic animals while, *M. tuberculosis* cause TB in humans (Arshad *et al.,* 2012). Infected animals may shed *M. bovis* through feces, milk, discharge lesions, saliva and urine, thus could be a potential source of transmission (Firdessa *et al.,* 2012).

The tuberculin intradermal skin test is the field technique for diagnosis of bovine TB. Intradermal inoculation of bovine tuberculin purified protein derivative (PPD) produce response by swelling (delayed hypersensitivity response) at the site of injection within 72 hours, by using bovine tuberculin alone (Etter *et al*., 2006). Besides intradermal test, other techniques like serological assays, molecular techniques and *M. bovis* organism isolation are used for diagnosis of bovine TB (Hassanain *et al*., 2009).

Animal reservoirs of *M. bovis* pose a serious public health threat. In developing countries, raw milk is consumed traditionally by the peoples of rural areas that may cause the transmission of *M. bovis* organism and results the human gastrointestinal TB (Firdessa *et al.,*2012). Prevalence of bovine TB in buffaloes of Pakistan has been reported from 0.51-12.72% (Arshad *et al.,* 2012). Keeping in view the endemicity of bovine TB in the country and its potential health hazard, it is advisable to take continued efforts to recognize the epidemiology of disease. Moreover, control of bovine tuberculosis infection in buffaloes of Pakistan practically becomes unrealistic. Most of the work is confined to the prevalence of tuberculosis in cow and detected by tuberculin tests (Leghari *et al.,* 2016). The present study was designed to investigate not only the prevalence of the disease but also the epidemiological factors of disease in buffaloes. However, there is comparatively less information on the sources of *M. bovis* in buffaloes, thus this data is needed to provide baseline information for the planning of innovative bovine TB control strategies both in animals and humans.

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MATERIALS AND METHODS

Ethics statement

The aim of this study was to determine the prevalence of bovine TB in buffaloes due to scanty information and lack of awareness, especially in the areas of Sindh province of Pakistan. The study design was reviewed and approved by the Directorate of Advanced Studies, SAU Tandojam.

Study area and animal population

A cross-sectional study was carried out during the year 2014-2015 to record the prevalence of bovine TB in buffaloes in rural and peri-urban farming of Hyderabad and Tando Allahyar districts, where approximately 309,163 and 139,000 buffalo population were raised, according to Livestock Census of Pakistan (Anonymous, 2014).

Single intradermal tuberculin test (SITT)

SITT was carried out in randomly selected 120 buffaloes (60 from each district). The mammalian PPD was procured from Veterinary Research Institute, Ghazi Road, Lahore, Pakistan and used according to manufacturer's instructions. In brief, thickness of the skin-fold was measured using digital Vernier' Caliper, then mammalian tuberculin (0.1 ml) was injected intradermally. Skin-fold thickness was re-measured 72 h post injection and results were interpreted according to OIE manual (OIE, 2009).

Enzyme-linked immunosorbent assay (ELISA)

About 7 ml blood was collected from jugular vein of buffaloes and used to separate serum via centrifugation method. The ELISA was performed according to the manufacturer's instruction (BIONOTE, Suwan, Korea). Briefly, 50 µl each of controls (positive and negative) and serum samples were dispensed in respective wells, 50 µl of *M. bovis* antigen – HRP (Horseradish Peroxidase) was added to each well, then microplate was sealed with adhesive plate sealing paper and placed on orbital plate shaker in an incubator at 37°C for 60 minutes. Wells were washed 6 times each with 350 µl of 1:9 (10× washing solution: deionized water) washing solution and liquid was aspirated completely by tapping upside down on the absorbent tissue paper. One hundred microliters of substrate solution was added to each well and plate was incubated for 15 minutes at room temperature. The absorbance was read with bichromatic spectrophotometer (Helsinki, Finland) at 450 nm.

Culture of milk samples

Milk samples were collected under aseptic conditions and prepared for culturing onto the Lowenstein Jensen medium (Neill *et al* 1988). Thick inoculums of sediments were smeared on the surface of medium slope and the cultured tubes were incubated at 37ºC for 6-8 weeks. The growth obtained after incubation was subjected to nitrate reduction and niacin strip test for further identification and characterization (Buxton and Fraser., 1971).

Statistical analysis

On completion of the study, the data obtained were stated in absolute values and percentages using the Microsoft office 2013 software package. Association between the factors (herd size, sex, age, milk yield and type of farming) and prevalence rate was measured by the Chi-square test.

RESULTS AND DISCUSSION

Overall prevalence of bovine TB in buffaloes

The overall prevalence of bovine TB in buffaloes from Hyderabad and Tando Allahyar was recorded 5/120 (4.16%) and 10/120 (8.33%) by SITT and ELISA respectively. Of them, 3/120 (2.5%) milk samples from Tando Allahyar were positive on bacterial culture for *M. bovis* (Fig 1).

The district-wise prevalence of bovine TB in buffaloes recorded by SITT was 1/60 (1.66%) and 4/60 (6.66%), and by ELISA 4/60 (6.66%) and 6/60 (10%) in Hyderabad and Tando Allahyar respectively. During bacterial culture of milk samples, prevalence was recorded 3/60 (5%) in Tando Allahyar (Fig 2).

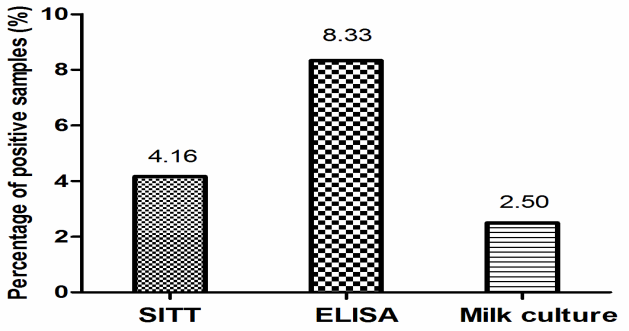

Fig 1: The overall prevalence rate (%) of bovine tuberculosis inbuffaloes from Hyderabad and Tando Allahyar districts. A total of 120 buffaloes/samples from Hyderabad and Tando Allahyar districts were tested by SITT, ELISA and bacterial culture. A relative higher prevalence was determined by ELISA, compared with by SITT and culture test, however, no significant difference was shown among different techniques.

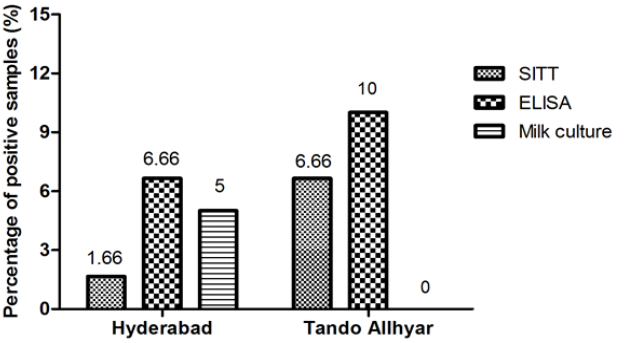

Fig 2: The respective prevalence rate (%) of bovine tuberculosis in buffaloes from Hyderabad and Tando Allahyar districts. Each 60 buffaloes/samples from either Hyderabad or Tando Allahyar districts were tested by SITT, ELISA and bacterial culture. The highest prevalence rate was recorded by ELISA, followed by SITT and lowest in culture test in both districts. No significant difference was shown among different techniques.

Prevalence of bovine TB in relation to various factors

The prevalence of bovine TB in relation to various factors including herd size (small: 5-15 buffaloes; large: 25-35 buffaloes), sex, age (3-5 years; >5 years), milk production (3-5 liters; >5 liters) and type of farming (rural, peri-urban) were analyzed and results were presented in Table 1. In Hyderabad, a higher prevalence was recorded in large herd (SITT: 1.67%; ELISA: 5%) as compared to small herd (SITT: 0%; ELISA: 1.67%). Similar trend was observed in Tando Allahyar for both large herd (SITT: 5%; ELISA: 8.33%; culture: 5%) and small herd (SITT: 1.67%; ELISA: 1.67%; culture: 0%). However, no significant difference was found between herd sizes for all techniques (P> 0.05).

In district Hyderabad, the prevalence was 0% and 1.67% in male, and 1.67% and 5% in female on SITT and ELISA respectively. Whereas, in Tando Allahyar, the prevalence was 0% in male and 6.67% in female on SITT, 1.67% in male and 8.33% in female on ELISA and 5% in female on culture test was positive. A relative higher prevalence of bovine TB was determined in female buffaloes through ELISA (P > 0.05), SITT (P < 0.05) and culture test (P > 0.05) in Tando Allahyar than Hyderabad (Table 1).

The prevalence in 3-5 years age group was 0% on SITT, 1.67% on ELISA and 0% through culture test in Hyderabad; while, in >5 years age was 1.67% on SITT, 5% on ELISA test and 0% was through culture test. The prevalence in 3-5 years age was 0% on SITT, 1.67% on ELISA and culture test in Tando Allahyar; while, in >5 years was 6.67% on SITT, 8.33% on ELISA and 3.33% through culture test was recorded. A relative higher (P < 0.05) prevalence of bovine TB was recorded in >5 years as compared to 3-5 years buffaloes for SITT in Tando Allahyar district (Table 1).

In Hyderabad, the prevalence of 0% was recorded on SITT and culture test, and 1.67% on ELISA in buffaloes having 3-5 liters of milk production, whereas, in Tando Allahyar, the TB prevalence was 0% on SITT, 1.67% on ELISA and 0% through milk culture. The buffaloes having >5 liters production, the TB prevalence was 1.67% on SITT, 5% on ELISA and 0% through culture in Hyderabad; and in Tando Allahyar it was 6.67% on SITT, 8.33% on ELISA test and 5% through culture. A significant higher prevalence rate (P < 0.05) was recorded by SITT in Tando Allahyar district (Table 1).

The prevalence in rural farming was 1.67% on SITT, 5% on ELISA and 0% through culture test in Hyderabad, while, in Tando Allahyar, it was 5% on tuberculin test, 8.33% on ELISA and 5% through isolation. In peri-urban farming 0% on SITT, 1.67% on ELISA and 0% through culture test was recorded in Hyderabad, while in Tando Allahyar 1.67% on SITT, 1.67% on ELISA test and 0% through culture test was found (Table 1).

The study was conducted to record the seroprevalence of bovine TB in buffaloes in district Hyderabad and Tando Allahyar using ELISA. The animals were first screened through SITT and both positive and negative reactors of SITT were used for determination of seroprevalence of *M. bovis*. Results demonstrated an overall 4.16% prevalence of bovine TB through SITT. Some other workers from Pakistan have reported 2.47% to 12.72% prevalence of TB in buffaloes by SITT (Arshad *et al*., 2012; Khan *et al*., 2007). However, another study from Egypt reported the 4.35% prevalence of bovine TB using single intradermal tuberculin technique (Hassanain *et al*., 2009). The ELISA showed 8.33% seroprevalence of TB in buffaloes in our study. Whereas, Hassanain *et al*. (2009) reported the 50% seroprevalence of bovine TB by ELISA, and Awah-Ndukum and coworkers reported 37.17% level of circulatory anti-bovine TB antibodies in Cameroon (Awah-Ndukum *et al.,* 2012). These differences might be due to difference in geographical locations, as evidences have suggested that

Table 1: Prevalence of bovine tuberculosis in relation to various risk factors.

Risk factor		Hyderabad			Tando Allahyar		
		SITT	ELISA	Culture test	SITT	ELISA	Culture test
Herd Size	Large herd(n=25-35)	1/60 (1.67)	3/60 (5)	0/60(0)	3/60(5)	5/60(8.33)	3/60(5)
	Small herd(n=5-15)	0/60(0)	1/60(1.67)	0/60(0)	1/60 (1.67)	1/60 (1.67)	0/60 (0)
	P-Value*	0.3173	0.3173	—	0.3173	0.1025	0.0833
Sex	Male	0/60 (0)	1/60 (1.67)	—	0/60 (0)	1/60 (1.67)	—
	Female	1/60 (1.67)	3/60 (5)	0/60 (0)	4/60 (6.67)	5/60 (8.33)	3/60 (5)
	P-Value*	0.3173	0.3173	—	0.0455	0.1025	—
Age	3-5 years	0/60 (0)	1/60 (1.67)	0/60 (0)	0/60 (0)	1/60 (1.67)	1/60 (1.67)
	>5 years	1/60 (1.67)	3/60 (5)	0/60 (0)	4/60 (6.67)	5/60 (8.33)	2/60 (3.33)
	P-Value*	0.3173	0.3173	0	0.0455	0.1025	0.5637
Milk Production	3-5 Liters	0/60 (0)	1/60 (1.67)	0/60 (0)	1/60 (1.67)	3/60 (5)	0/60 (0)
	>5 Liters	0/60 (0)	1/60 (1.67)	0/60 (0)	4/60 (6.67)	5/60 (8.33)	3/60 (5)
	P-Value*	0.3173	0.3173	0	0.0455	0.1025	0.0833
Type of farming	Rural farming	1/60 (1.67)	3/60 (5)	0/60 (0)	3/60 (5)	5/60 (8.33)	3/60 (5)
	Peri- urban farming	0/60 (0)	1/60 (1.67)	0/60 (0)	1/60 (1.67)	1/60 (1.67)	0/60 (0)
	P-Value*	0.3173	0.3173	0	0.3173	0.1025	0.0833

bovine TB could be varied from region to region and even from farm to farm in the same region depending upon the management conditions of the farm like sanitation, animal density and nutrition etc. (Khan *et al.,* 2008). Moreover, it has been estimated that different species/breeds have different natural susceptibility levels for bacterial pathogens (Habib *et al.,* 2015; Rajput *et al.,* 2018).

Paratuberculosis, or Johne's disease (JD), is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) which have similar characteristics as bovine TB (Rajib and Goswami, 2013). Vaccination is regarded as the only cost effective method for controlling JD in bovine population (Begum *et al.,* 2017). There are many reports of paratuberculosis from Pakistan. Sikandar *et al*., (2011) reported in buffaloes 12.5% confirmed cases for paratuberculosis in 134 suspected samples. The ability of MAP to survive at pasteurization temperature in milk and its potential role in the etiology of a human's chronic granulomatous ileocolitis (Crohn's disease) is of serious concern (Naser *et al*., 2000). These circumstances also exist for *M. bovis* whose names imply separate species, although by genetic criteria the organisms are not different species, but rather subtypes. Literature showed that milk, faeces, and nasal discharges etc. are the routes of excretion of the *M. bovis* pathogen (Sgarioni *et al*., 2014). In present study, 2.5% milk samples showed growth on Lowenstein Jensen medium. Similar level of TB prevalence was declared by Qamar and Azhar, (2013) in their study from Lahore city; and also in our earlier study conducted in Tando Allahyar and Hyderabad in cattle (Leghari *et al.,* 2016). These findings and results of current study suggested the apparently healthy animals as major reservoir for transmission of infection to other livestock animals.

Various risk factors associated with bovine TB were analyzed during present study through a questionnaire survey during collection of sample in district Hyderabad and Tando Allahyar. In both districts, a higher prevalence (but statistically non-significant) was recorded in large herd and females as compared to small herd and males. This finding was observed very close to results of (Arshad *et al.,* 2012). It is evidently concluded from these results and also from literature that females are more susceptible to chronic infections than the males (Hussin *et al.*, 2016). These findings were also closer with previous studies conducted in northern regions of Tanzania and elsewhere (Rogan and Gladen*.,* 1978).

Recent literatures have reported high prevalence of bovine TB in older animals (Rodwell *et al*., 2001). Susceptibility of getting infection of mycobacteria increases with age (Cagiola *et al*., 2004) and adult cattle and buffaloes are more affected than calf (Kazwala *et al*., 2001). Our research has also indicated that the younger buffaloes (3-5 years) had less prevalence of disease than older animals (>5 years). The possible explanation for this could be that higher yield and elder age developed the state of immunocompromised due to long term production stress (Khan *et al*., 2008). A higher prevalence rate of bovine TB was recorded in rural farming comparing with peri-urban farming, however, these differences were observed statistically non-significant (P>0.05) for both districts regardless of analytical method. Similar finding was declared by Khan *et al* (2008) in their study.

CONCLUSION

We concluded that bovine TB is present in apparently healthy buffalo herds of Hyderabad and Tando Allahyar districts of Sindh province, Pakistan. Female buffaloes were at higher risk as compared to male, and aged animals (>5 years) were also at higher risk of *M. bovis* infection. Infected animals shed the *M. bovis* pathogen in the milk that could be a potential hazard to public health.

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