PREVALENCE OF BOVINE TUBERCULOSIS IN BUFFALOES IN HYDERABAD AND TANDO ALLAHYAR DISTRICTS OF SINDH PROVINCE, PAKISTAN

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ABSTRACT

Bovine tuberculosis commonly called TB, is caused by bacilli *Mycobacterium bovis* (*M. bovis*), a potential zoonotic pathogen that seems to cause direct economic losses other than single microbial agent on livestock and indirect impact on human health. An investigation of bovine tuberculosis in buffaloes was carried out in Hyderabad and Tando Allahyar districts to determine the prevalence of bovine tuberculosis. The buffaloes (n=120), were first screened through single intradermal tuberculin test (SITT) and both positive and negative reactors of SITT were used for enzyme linked immunosorbent assay (ELISA). Attempts were further made to isolate the *M. 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%). The statistical analysis did not showed any association (P>0.05) of herd size, sex, age, milk yield and type of farming with the disease, regardless of district, whereas, sex, age and milk yield showed significant (P<0.05) association with the disease through SITT in Tando Allayer district. This study concluded 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.

Keywords: *Bubalus bubalis*, buffalo, seroprevalence, hyderabad, Tando Allahyar, bovine tuberculosis

INTRODUCTION

The economy of a country rely on the healthy and sound livestock. There are two types of riverine buffaloes in Pakistan i.e., Nili-ravi and Kundi. Approximately 79% contribution of Nilli-ravi buffaloes of the total buffalo population is present in the Pakistan. Pakistan exist at 2nd

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position having 27.3 million buffaloes before china with 23 million and after India 98 million (Khan *et al*., 2007) and contribute approximately 67% of the total milk produced in the country (Afzal *et al*., 2007). Approximately 30 to 35 million rural people are engaged in the raising of livestock. The economy of the country is directly linked with the livestock and the families get 30% to 40% of the income from these animals directly or indirectly. Buffaloes are at higher risk (usually exotic cross breed) and is easily exposed to various diseases that affect the health of animal and their production is reduced by various infectious diseases, like brucellosis and tuberculosis (Gul and Khan, 2007).

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 tuberculosis in domestic animals while, *M. tuberculosis* cause tuberculosis in humans. However, it can jump the species barrier and cause tuberculosis in other mammals (Grange *et al*., 1996).

Respiratory aerosols are the main source of *M. bovis* transmission that gets more chances in the case of close contact between infected and non-infected animals (Neil *et al*., 1994). Infected animal may shed *M. bovis* through feces, milk, discharge lesions, saliva and urine (Neil *et al*., 1991).

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 h, by using bovine tuberculin alone (Angus, 1978). Besides intradermal test, other techniques like serological assays, molecular techniques and culturing of *M. bovis* organism are used for diagnosis of bovine TB (Hassanain *et al*., 2009).

Animal reservoirs of *M. bovis* poses 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 which results the human gastrointestinal tuberculosis (Firdessa *et al*., 2012). Prevalence of bovine TB in buffaloes of Pakistan has been reported from 0.51% to 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 continues efforts to recognize the epidemiology of disease. Because diagnosis is vital for the control of disease. Present study is therefore, planned to investigate the prevalence of bovine TB in buffaloes of Hyderabad and Tando Allahyar districts of Sindh province, Pakistan using single intradermal tuberculin test (SITT), enzyme linked immunosorbent assay (ELISA) and culture test.

MATERIALS AND METHODS

Ethics statement

The aim of this study was to determine the prevalence of bovine TB in buffaloes due to scanty information, unpublished data and lack of awareness of bovine TB especially in buffaloes owners particularly in the areas of Sindh province

of Pakistan. The study design was reviewed and approved by the institutional animal care and use committee.

Study area and animal population

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

SITT, ELISA and bacteriological culturing of mycobacterium *Bovis*

Single intradermal tuberculin test (SITT)

Single intradermal tuberculin tests (SITT) were carried out in randomly selected 120 buffaloes (60 from each district). The mammalian PPD (purified protein derivative of tubercle bacilli) was procured from Veterinary Research Institute, Ghazi Road, Lahore and used according to manufacturer's instructions. Injection site was clipped and properly cleaned with a swab of 70% alcohol and left to dry for a while. The skin-fold thickness of the skin was measured using digital Vernier' Caliper and injection site was encircled with permanent marker. A 0.1 ml mammalian tuberculin was injected intradermally as pre inoculation reading. The skin-fold thickness of injection site was re-measured 72 h post injection and reading was noted as post inoculation as described in the manual of OIE (2009).

Interpretation of results: An increase in the thickness of skin up to 2.9 mm Negative, An increase in thickness of skin up to 3 to 3.9 mm Doubtful, An increase in thickness of skin up to 4 mm or more Positive, Inflammation and edema regardless of measurement Positive.

ELISA (enzyme linked Immuno-sorbent assay)

In present study, ELISA was performed as a confirmatory analysis for the SITT positive animals. AniGen BTB Ab ELISA 2.0 kit (BIONOTE, Suwan, Korea) used in this study. For this purpose, about 7 ml blood was collected from jugular vein of buffaloes, from both positive and negative reactors of SITT using disposable sterile syringe. The blood containing syringes were kept at ambient temperature in slanting position at room temperature for two hours. Then the clotted blood containing syringes were kept in refrigerator at 4^{o}C for overnight to get maximum serum yield and following day serum was separated by centrifugation method and stored at -20^{o}C until tested.

The ELISA test protocol and interpretation of results were performed according to the manufacturer's instruction. Briefly; 50 µl each of controls (positive and negative) and serum samples were dispensed in respective wells, 50 µl of *M. bovis* antigen -HRP (Horse Redish 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^{o}C for 60 minutes. Wells were washed 6 times each with 350 µl of 1:9 (10x washing solution: deionized water) washing solution and liquid was aspirated completely by tapping upside down on the absorbent tissue paper. 100 µl of substrate solution was added to each well and plate was incubated for 15 minutes at room temperature (23^{o}C to 25^{o}C). The absorbance was read with bichromatic spectrophotometer (Helsinki, Finland) at 450 nm with reference wave length of 600 nm. SP values were calculated as:

$$SP = \frac{\text{(sample of OD – average OD of negative control)}}{\text{Average OD of positive control-average OD of negative control}}$$

Results were interpreted as:
Positive: S/P of sample > 0.5
Negative: S/P of sample < 0.5

Culture and direct microscopy of milk samples

Ten ml of milk was collected under aseptic conditions from each animal in sterile universal bottles and was stored at 4°C until used. Prior to microscopy, culture, homogenization and decontamination of milk samples was performed as described by Neil and coworkers (1988). In brief, 10 ml of milk was transferred into test tubes and centrifuged at 3000 rpm for 15 minutes. Supernatant was discarded, sediment suspended in sterilized physiological saline solution and this suspension was added with equal volume of 4N Sodium dihydro oxide solution. A drop of 0.05% phenol red indicator was added and mixture was incubated for 30 minutes at 37°C. The mixture was neutralized with sterilized 4N Hydrochloric acid solution and centrifuged at 3000 rpm for 15 minutes. Supernatant was discarded and sediment was used for direct microscopy and culture.

Bacteriological culture

All the samples were cultured on the *Mycobacterium* specific medium (Lowenstein Jensen) as described previously Buxton *et al.* (1971). Briefly; thick inoculum of sediments were smeared on the surface of medium slope and the cultured tubes were incubated at 37°C for six to eight weeks. The growth obtained after incubation was subjected to nitrate reduction and niacin strip test for further identification and characterization as described earlier Neil *et al.* (1988).

Direct microscopy

From sediment of each sample two smears were prepared, dried, fixed over flame and stained with acid fast stain (Ziehl Neelsen stain). The stained slides were examined under oil- immersion lens for the *M. bovis,* as described previously Neil *et al.* (1988).

Statistical analysis

On completion of the study, the data obtained, were stated in absolute values and percentages. For the determination of percentages and calculations, the Microsoft Office 2013 software package were used. Association between the factors (herd size, sex, age, milk yield and type of farming) and disease was measured by the Chi-square test (Thrusfield, 1995).

RESULTS AND DISCUSSION

Overall prevalence of bovine tuberculosis in buffaloes determined by various techniques

The results of overall prevalence of bovine TB in buffaloes showed that, 5/120 (4.16%) buffaloes in SITT and 10/120 (8.33%) buffaloes in ELISA were found positive for bovine TB from both districts Hyderabad and Tando Allahyar. Whereas, 3/120 (2.5%) milk samples were found positive on bacteriological culture for *M. bovis* from district Tando Allahyar. Relatively a higher prevalence was determined through ELISA assay that followed by SITT and culture test (Figure 1).

The district-wise prevalence of bovine TB in buffaloes recorded by SITT was 1/60 (1.66%) and 4/60 (6.66%) in district Hyderabad and Tando Allahyar respectively, while, on ELISA 4/60 (6.66%) in Hyderabad and 6/60 (10%) in Tando Allahyar district was observed. The bacteriological

culture of the milk samples revealed the growth and prevalence was recorded 3/60 (5%) in Tando Allahyar. Generally the highest prevalence was recorded through ELISA followed by SITT and lowest in culture test in both Tando Allahyar and Hyderabad districts (Figure 2).

The study 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 and Khan, 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 Awah-Ndukum *et al*. (2012) reported 37.17% level of circulatory anti-bovine TB antibodies in Cameroon. These differences might be due to difference in geographical locations, as evidences have suggested that 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, animals density, nutrition etc., Khan *et al*., 2008. Moreover, it has been estimated that different species/breeds have different natural susceptibility levels for bacterial pathogens (Mangi *et al*., 2015).

ELISA test is a most sensitive test in terms of accuracy and there is no or least chances of false results in ELISA. In present study was used this diagnostic technique in addition to tuberculin and culture tests. It was observed that prevalence on this test was higher than rest of techniques. The ELISA detect the antibody proteins that appeared in the blood at very earlier stage of infection. This report was made in accordance with a previous study Silva *et al.* (1999) sgariwho also recorded a very high specificity of ELISA test for diagnosis of bovine TB. The authors concluded that ELISA is a most appropriate tool for the detection of bovine TB antibodies in non-endemic areas.

Literature showed that milk, faeces, nasal discharges etc., are the route of excretion of the *M. bovis* pathogen (Srivastava *et al*., 2006; Sgarioni *et al*., 2014; Hassnain *et al*., 2009; Jalil *et al*., 2003). In present study, milk samples were collected to isolate *M. bovis*, as, Jalil *et al.* (2003) reported that milk is the main route of acid fast bacteria shedding. Overall 2.5% milk sample showed growth on Lowenstein Jensen medium after decontamination of samples. Similar level of TB prevalence was declared by Qamar and Azhar (2013) in their study from Lahore city. Kleeberg and coworkers (1984) indicated that one cow with tuberculosis can excrete enough viable mycobacterium to contaminate the milk of up to 100 cows when milk pooling and bulk transportation is used. The same study also declared *M. bovis* presence in milk products such as yogurt and cheese made from non-pasteurized milk 14 days after processing and in butter as long as 100 days after processing. These findings and results of current study suggested the apparently healthy animals as major reservoir for transmission of infection to other livestock animals. Moreover, untreated milk and milk products suggested as a potential major health hazard to consumers particularly in developing countries where more than 50% of milk consumed without heat treatment Qamar and Azhar (2013).

Prevalence of bovine tuberculosis in large and small herds

The prevalence of bovine TB in large (25 to 35 buffaloes) and small herd (5 to 15 buffaloes) was also determined by ELISA and culture test and

results were presented in Table 1. In Hyderabad district, a higher prevalence was recorded in large herd (SITT 3.33% and ELISA 10%) as compared to small herd (SITT 0% and ELISA 3.33%). Similar trend was observed in Tando Allahyar district for both large (SITT 10%; ELISA 16.66% and culture test 10%) and small herd (SITT 3.33%; ELISA 3.33% and culture test 0%). However, the difference between herd size for all techniques was found statistically nonsignificant (P>0.05).

Various risk factors associated with bovine TB including herd size, age, sex, type of farming, and `milk production were analyzed during present study through a questionnaire survey during collection of sample in district Hyderabad and Tando Allahyar. The tuberculin test was performed on both large and small herds of buffaloes randomly in the study area to compare the effect of herd size (large herd 25 to 35 and small herd 5 to 15 buffaloes) on the prevalence of bovine TB in buffaloes. In both districts, a higher prevalence (but statistically nonsignificant) was recorded in large herd as compared to small herd. This finding was observed very close to results of Arshad *et al.* (2012).

Sex wise prevalence of bovine tuberculosis in buffaloes

The prevalence of bovine TB in buffalo in district Hyderabad was 0% in male and 1.66% in female on SITT, on ELISA 1.66% in male and 5% in female and on culture test 0% in female. Whereas, in district Tando Allahyar the prevalence of bovine TB in buffalo on SITT was 0% in male and 6.66% in female, while on ELISA it was 1.66% in male and 8.33% in female and on culture test was 5% in female (Table 2).

A relatively a higher prevalence of bovine TB was determined in female buffaloes through ELISA test, SITT and culture test in Tando Allahyar district in comparison to Hyderabad district. However, the statistical analysis showed a significant difference (P<0.05) for only SITT in Tando Allahyar district (Table 2).

All the techniques applied during research indicated a higher prevalence of bovine TB in females as compared to males. 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). However, our findings from this study have indicated a non-significant association for bovine TB between sexes. The findings are closer with previous studies conducted in northern regions of Tanzania and elsewhere, which reported similar n of bovine TB disease between male and female buffaloes (Rogan *et al*., 1978). Another study have reported same association between sexes using intradermal skin test (Kazwala *et al*., 2001; Cadmus *et al*., 2010; Inangolet *et al*., 2008). However, contrary to this finding an earlier study demonstrated that male were more affected than female particularly castrates that kept longer for their use as draught oxen (Kazwala *et al*. (2001).

Prevalence of bovine tuberculosis in buffaloes in relation to age and milk production

The prevalence of bovine TB in different age groups (3 to 5 and >5 years) of buffaloes from both districts was summarized in Table 3. The prevalence in 3 to 5 years age group of buffaloes was 0% on SITT, 3.33% on ELISA and 0% through culture test in Hyderabad district; while, in >5 years age was 3.33% on SITT, 10% on ELISA test and 0% was through culture test. The prevalence in 3 to 5 years age group of buffaloes was 0% on SITT, 3.33% on ELISA and culture test in Tando Allahyar; while, in more than 5 years was 13.33%

on SITT, 16.66% on ELISA test and 6.6% through culture test was recorded (Table 3). Relatively a higher prevalence of bovine TB was recorded in older buffaloes (>5 years) as compared to young buffaloes (3 to 5 years) regardless of analytical technique, however, the significant difference ($P<0.05$) was recorded only for SITT in Tando Allahyar district (Table 3).

In Hyderabad district, the prevalence of 0% was recorded on SITT in buffaloes having 3 to 5 liters of milk, whereas 3.33% on ELISA and 0% through culture was observed. However, for the buffaloes having >5 liters, the TB prevalence was 3.33% on SITT, 10% on ELISA test and 0% was through culture. Similarly, in Tando Allahyar district, the TB prevalence in buffaloes having 3 to 5 liters of milk was 0% on SITT, 3.33% on ELISA test and 0% through milk culture; while, for the group of buffaloes having more than 5 liters was 13.33% on SITT, 16.66% on ELISA test and 10% through culture (Table 4). A moderately higher prevalence of bovine TB was recorded in high milk producing buffaloes (>5 liters) as compared to low producers (3 to 5 liters) regardless of analytical technique, however, the significant difference ($P<0.05$) was recorded only for SITT in Tando Allahyar district (Table 4).

Evidences have suggested that age and milk production are the principle risk factors for *M. bovis* infection, greater the age and/or yield, more is the chance to get infection. Recent literature have reported high prevalence of bovine tuberculosis in older animals (Amin, *et al.* (1992); Rodwell *et al.* (2000). 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 have also indicated that the younger buffaloes (age group having 3 to 5 years) had less prevalence of disease than older animals (>5 years old). These findings are very close to the results of earlier research those declared a many times higher prevalence in animals those have more than 5 years of age and/or milk production higher than 5 liters (Khan and Khan, (2007); Khan *et al.* (2008). It has been speculated that in young age animals have immunocompetent thus can easily aggravate the intensity of pathogens (Soomro *et al*., 2014). Moreover the authors showed a significant association between age/ milk production with susceptibility of bovine TB. The possible explanation for this could be that high yield and old age are the key factors which aggravate the disease load and animals with these factors could be immunocompromised due to long term production stress (Khan *et al*., 2008). Similar type of findings were declared by Khan and coworkers in their study (Khan *et al*., 2008).

Prevalence of bovine tuberculosis in buffaloes in relation to type of farming

The prevalence of TB in buffaloes of peri-urban and rural types of farming was compared and results were presented in Table 5. The prevalence in rural farming of Hyderabad district was 3.33% on SITT, 10% on ELISA and 0% through culture test, while, in peri-urban farming 0% on SITT, 3.33% on ELISA and 0% through culture test was recorded. In Tando Allahyar district the TB prevalence in rural farming was 10% on tuberculin test, 16.66% on ELISA test and 10% through isolation; and in peri- urban farming 3.33% on SITT, 3.33% on ELISA test and 0% through culture test was found (Table 5).

A higher prevalence of bovine TB was recorded in rural farming comparing with peri-urban farming, however, these differences was observed statistically non-significant ($P>0.05$) for

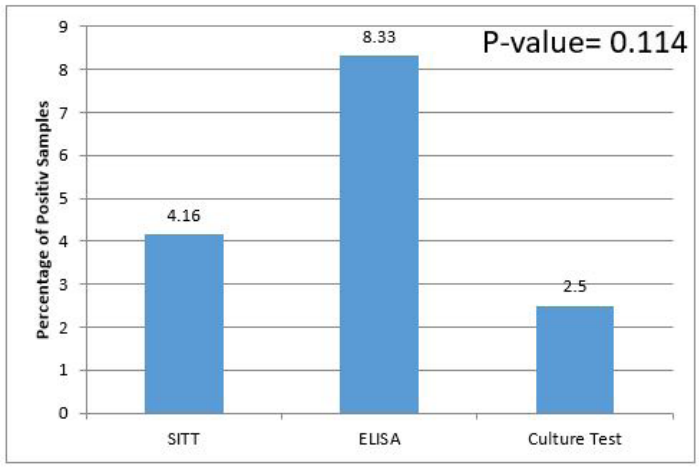

Figure 1. The overall prevalence (%) of bovine tuberculosis in buffaloes in Hyderabad and Tando Allahyar district by various techniques.

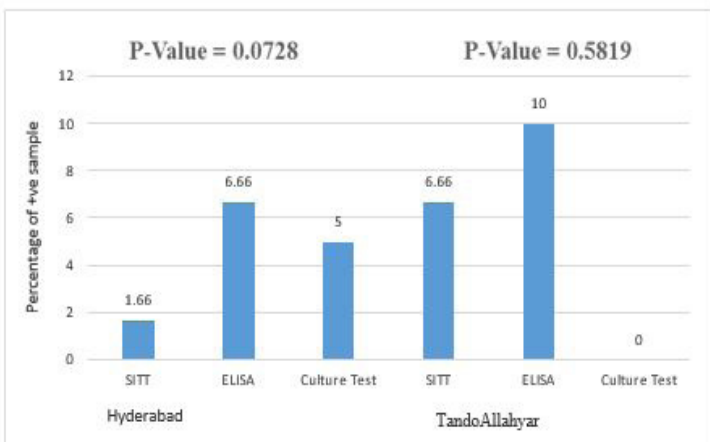

Figure 2. The prevalence (%) of bovine tuberculosis in buffaloes in Hyderabad and Tando Allahyar district.

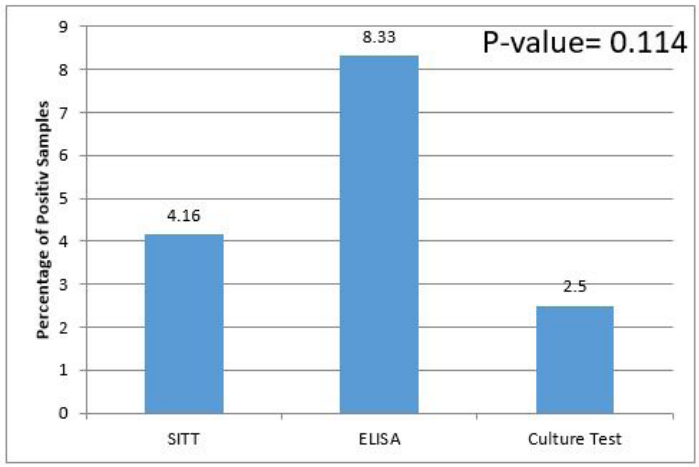

Figure 1. The overall prevalence (%) of bovine tuberculosis in buffaloes in Hyderabad and Tando Allahyar district by various techniques.

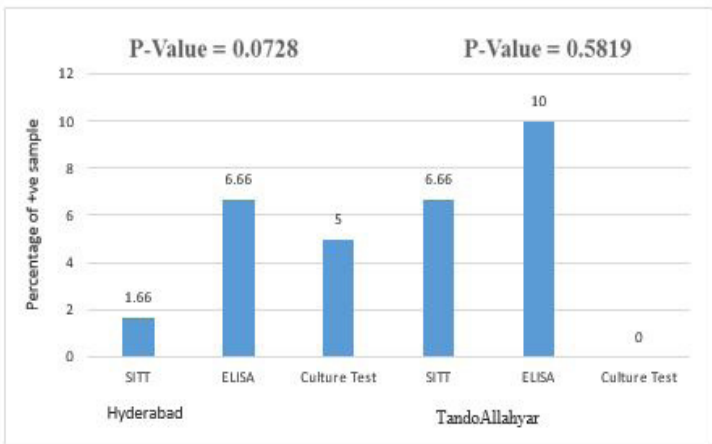

Figure 2. The prevalence (%) of bovine tuberculosis in buffaloes in Hyderabad and Tando Allahyar district.

both districts regardless of analytical method (Table 5). Intensive livestock farming and close contact between animals at water points favor the spread of *M. bovis* infection. Rural farmings usually have a poor sanitary conditions which results the more appropriate environment for spread of bacterial infections (Mshelia *et al*., 2016).

This study concluded that bovine tuberculosis is present in apparently healthy buffaloes herds of Hyderabad and Tando Allahyar districts. Female buffaloes are at high risk as compared to male, and aged animals (>5 years) are also at high 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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Table 1. Prevalence of bovine tuberculosis in large and small herd.

Tests*	Herd size		P-value**
	Large herd (n = 25-35)	Small herd (n = 5-15)	
	Positive No. (%)	Positive No. (%)	
Hyderabad			
SITT	1 (3.33)	0 (0)	0.3173
ELISA	3 (10)	1 (3.33)	0.3173
Culture test	0 (0)	0 (0)	Nil
Tando Allahyar			
SITT	3 (10)	1 (3.33)	0.3173
ELISA	5 (16.66)	1 (3.33)	0.1025
Culture test	3 (10)	0 (0)	0.0833

n = 60 for each test/ district; SITT = Single Intradermal Tuberculin test;
ELISA= Enzyme Linked Immunosorbent Assay
**considered statistically significant when $P<0.05$

Table 2. Sex wise prevalence of bovine tuberculosis in buffaloes in Hyderabad and Tando Allahyar districts.

Tests*	Sex of buffaloes			P-value**
	Male	Female		
	Positive No. (%)	Positive No. (%)		
Hyderabad				
SITT	0 (0)	1 (1.66)		0.3173
ELISA	1 (1.66)	3 (5)		0.3173
Culture test	--	0 (0)		--
Tando Allahyar				
SITT	0 (0)	4	(6.66)	0.0455
ELISA	1 (1.66)	5	(8.33)	0.1025
Culture test	--	3	(5)	--

*n = 60 (male and female) for each district; SITT = Single Intradermal Tuberculin test;
ELISA = Enzyme Linked Immunosorbent Assay
**considered statistically significant when P<0.05

Table 3. Age wise prevalence of bovine tuberculosis in buffaloes in Hyderabad and Tando Allahyar districts.

Tests*	Age of buffaloes		P-value**
	3-5 Years	> 5 Years	
	Positive No. (%)	Positive No. (%)	
Hyderabad			
SITT	0 (0)	1 (3.33)	0.3173
ELISA	1 (3.33)	3 (10)	0.3173
Culture test	0 (0)	0 (0)	Nil
Tando Allahyar			
SITT	0 (0)	4 (13.33)	0.0455
ELISA	1 3.33)	5 (16.66)	0.1025
Culture test	1 (3.33)	2 (6.6)	0.5637

*n = 60 for each test/ district; SITT = Single Intradermal Tuberculin test;
ELISA = Enzyme Linked Immunosorbent Assay
**considered statistically significant when P<0.05

Table 4. Prevalence of bovine tuberculosis in buffaloes in Hyderabad and Tando Allahyar districts in relation to milk production.

Test*	Milk production		P-value**
	Positive No. (%)	Positive No. (%)	
SITT	0 (0)	1 (3.33)	0.3173
ELISA	1 (3.33)	3 (10)	0.3173
Culture test	0 (0)	0 (0)	Nil
Tando Allahyar			
SITT	0 (0)	4 (13.33)	0.0455
ELISA	1 (3.33)	5 (16.66)	0.1025
Culture test	0 (0)	3 (10)	0.0833

*n = 60 for each test/ district; SITT = Single Intradermal Tuberculin test;
ELISA = Enzyme Linked Immunosorbent Assay
**considered statistically significant when P<0.05

Table 5. Prevalence of bovine tuberculosis in buffaloes in rural and Peri- urban farming.

Test*	Type of farming		P-value**
	Rural farming	Peri- urban farming	
	Positive No. (%)	Positive No. (%)	
SITT	1 (3.33)	0 (0)	0.3173
ELISA	3 (10)	1 (3.33)	0.3173
Culture test	0 (0)	0 (0)	Nil
Tando Allahyar			
SITT	3 (10)	1 (3.33)	0.0455
ELISA	5 (16.66)	1 (3.33)	0.1025
Culture test	3 (10)	0 (0)	0.0833

*n = 60 for each test/ district; SITT = Single Intradermal Tuberculin test;
ELISA = Enzyme Linked Immunosorbent Assay
**considered statistically significant when P<0.05

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