



## COMPARATIVE TRENDS OF BOVINE TUBERCULOSIS IN CATTLE AND BUFFALO POPULATION AROUND LAHORE, PAKISTAN

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Article Info

Received 24/05/2014; Revised 18/06/2014

Accepted 25/06/2014

### ABSTRACT

Bovine tuberculosis is a re-emerging zoonosis of socioeconomic importance. The present study was aimed to investigate the comparative prevalence of bovine tuberculosis in cattle and buffaloes at public and private livestock farms around Lahore district from year 2010-2012. A total of 1031 animals comprising 517 cattle (221 male; 296 female) and 514 buffaloes (218 male; 296 female) showing signs of emaciation and swollen lymph nodes were selected randomly. The selected animals were subjected to single intra-dermal tuberculin test (SICTT). The animals positive by SICTT were further confirmed by PCR for presence of *Mycobacterium bovis*. The results indicated that the overall incidence of tuberculosis was more in buffaloes than cattle (3.30% vs 2.12%). The study also revealed that the disease was more prevalent in females (buffaloes 4.05%; cattle 2.36%) than males (buffalo bulls 2.29%; cattle bulls 1.80%) in both species. We also determined the association of mastitis with T.B in female animals suffering from clinical mastitis. But the results indicated no co-relation of *Mycobacterium bovis* with mastitis. We concluded that in areas around Lahore the incidence of bovine tuberculosis is more in buffaloes than cattle with more percentage of female animals affected. While bovine T.B and mastitis are not interlinked.

**Keywords:** Bovine tuberculosis, Intradermal tuberculin test, Lahore, Mastitis.

### INTRODUCTION

Bovine tuberculosis is a chronic bacterial disease caused by *Mycobacterium bovis* (*M.bovis*). It is characterized by progressive emaciation and development of tubercles in various organs of body. The infected animals shed *M. bovis* in respiratory secretions, feces, milk and sometimes in excretions i.e. urine, semen and vaginal secretions. The disease is transmitted through aerosolic route, direct animal contact or through the consumption of un-pasteurized milk and dairy products [1]. Even though the mortality rate is negligible yet the economic losses are brought about in the form of poor carcass, low quality hides, loss in flesh and milk, prolonged morbidity and high treatment cost.

Although Pakistan is ranked at 4<sup>th</sup> position in the world in milk production with the major bovine population of 38.3 million cattle and 33.7 million buffaloes during the year 2012-13 yet the presence of bovine tuberculosis is a major constraint in production and marketing of the animals and their by products in our country. Another important aspect of bovine T.B is zoonosis that enhances its public health significance many folds. Although pasteurizing the milk eliminates the possibility of transmission but boiling and pasteurization of milk are not very common practices in Pakistan and we mainly depend on buffaloes and cow milk therefore we need to investigate the presence of bovine T.B in cattle and buffaloes which is responsible for 5-10% of human deaths due to bovine T.B. [2]. As most of previous studies



show endemicity of bovine T.B in Pakistan and most of the meat and milk for human consumption comes from affected bovine therefore we need to know the participation of cattle and buffaloes in the disease spread and a comparison of the incidence of T.B. in the cattle and buffalo is necessary around Lahore, Pakistan.

Previous studies also mentioned *Mycobacterium bovis* as an etiology for bovine mastitis [3]. Tuberculous mastitis occasionally has tubercle bacilli in the blood stream that can pass into the milk to be eliminated or to give rise to subclinical and clinical mastitis. One of the objectives of the present study was also to investigate whether the *Mycobacterium bovis* could be the reason of clinical mastitis in T.B infected animals.

## MATERIALS AND METHODS

A total of 1031 animals comprising 517 cattle and 514 buffaloes between age 6-8 years as recommended by (4) were subjected to T.B. testing by single intradermal comparative tuberculin test (SICTT) during the period of three years (July 2010- June 2012).

### **Study-1: Prevalence of bovine tuberculosis by single intra-dermal tuberculin test:**

The OIE recommended test for screening against T.B is single intradermal tuberculin test (SITT) with bovine tuberculin purified protein derivative (PPD). The bovine purified protein derivative (PPD) is a protein derivative extracted from *M. bovis*. The tuberculin used for the test was PPD of the mammalian and avian types. Both mammalian and avian tuberculin contained 2mg/mL and 0.5 mg/mL of PPD respectively. These PPD were prepared at Veterinary research Institute, Lahore.

Two areas about two inches apart on the left side in the middle third of the neck were selected and shaved so that they were parallel to the spine of scapula as per OIE recommendations. The fold of the skin at both the upper and lower shaved areas were measured with vernier caliper. Both avian and mammalian tuberculins were injected intradermally (0.1 mL) at upper and lower shaved areas respectively. A small bead was formed at the point of injection which was easily palpated with the help of finger tip. Seventy two hours post inoculation the fold of the skin over the site of the each injection was measured again. When the difference between the first reading (recorded before inoculation) and the second reading (72 hours post inoculation) was up to 2.9 mm, it was considered as negative, if it was between 3.0-3.9 mm it was considered as inconclusive and 4 mm or above as positive. Doubtful cases were retested after a lapse of at least eight weeks and right side of the neck was used during retesting according to OIE recommendations [5].

The animals that were tested by standard method of tuberculin test were confirmed for the presence of *Mycobacterium bovis* by PCR assay from milk sample

using primers set given below as previously described [6].

Forward: JB21 (TCGTCCGCTGATGCAAGTGC)

Reverse: JB22 (CGTCCGCTGACCTCAAGAAG)

DNA samples were extracted by using GENTRA PUREGENEDNA Purification Kit USA. The PCR conditions consisted of an initial denaturation (94°C for 4 minutes), 30 cycles of denaturation (94°C for 1 minute), annealing (55°C for 1 minute), Extension (73°C for 1 minute), and final elongation (74°C for 10 minutes).

### **Study -2: Link of bovine mastitis with presence of *Mycobacterium bovis***

California mastitis test (CMT) was performed only on the lactating animals (178 including 87 cattle and 91 buffaloes) showing signs of clinical mastitis. The results were scored and interpreted as trace 0, 1, 2 or 3 inflammatory response based on the viscosity of the gel formed by mixing the reagent with milk as described previously (7). Each milk sample positive for CMT was further centrifuged at 600× g for 15 minutes for the concentration of organisms. The cream and sediment layers were removed and examine microscopically by Zhiel Nelson's staining. These milk samples were also put to PCR assay for confirmation of T.B using same primers and conditions described above.

### **Statistical analysis:**

The prevalence of bovine TB was calculated by the following formula:

**Prevalence (%) =**

$$\frac{\text{Number of animals +ve through tuberculin tests}}{\text{Total number of animals tested}} \times 100$$

The *t* test was applied for comparative prevalence of cattle and buffaloes.

## RESULTS

### **Prevalence of bovine tuberculosis by single intra-dermal tuberculin test:**

The prevalence rate of bovine tuberculosis in buffalo and cattle population of different farms around Lahore district during the period of three years (July 2010- June 2012) is shown in Figure 1a, 1b, 1c. These figures indicate the year wise percentage prevalence of bovine T.B in cattle and buffaloes. Single intra-dermal comparative tuberculin test (SITT) was use to assess the comparative allergic reactions of the two tuberculins. Overall, 2.71% of the 1031 animal tested was positive for SITT. The positive reactors were subsequently confirmed by PCR from milk samples (Fig 2). The overall incidence of tuberculosis was more in buffaloes (3.30%) as compared to cattle (2.12%). The percentage of the disease was higher in females of both species (female cattle

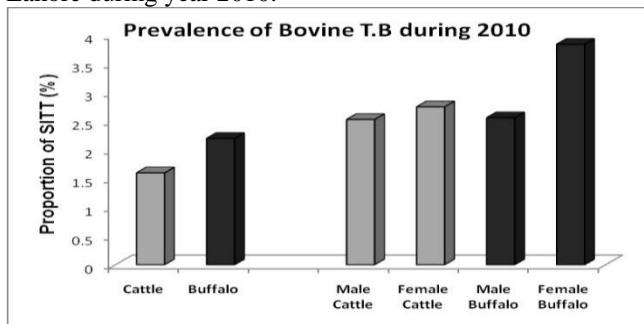


2.36%; female buffaloes 4.05%). While in comparison both the male cattle (1.80%) and male buffaloes (2.29%) had low incidence of bovine T.B (Fig 3). There was not a significant difference in the prevalence of 2010 and 2011 in both species but it suddenly raised in 2012 which is alarming for livestock holders. Link of bovine mastitis with presence of *Mycobacterium bovis*:

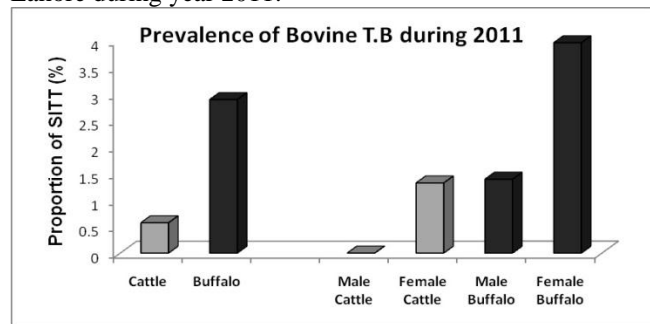
Another objective of the present study was also to determine the link of mastitis with *Mycobacterium bovis*. During the study, only the lactating animals (178 including 87 cattle and 91 buffaloes) showing signs of clinical mastitis were further tested by California mastitis

test (CMT) and 6.17% (total 11 animals including 4 cattle and 7 buffaloes) were found strongly positive for mastitis. However all of these CMT positive samples were negative for acid fast bacteria (AFB) by Ziehl Neelsen (ZN) staining. Further PCR analysis from milk samples of these animals revealed none of the positive sample and neither these animals were positive by SITT for T.B. As the milk samples did not reveal any AFB and non reactivity to tuberculin test indicated that we could presume that the udder of such animals could be infected by other inflammatory bacteria. Therefore, there was no significant relation between bovine T.B and mastitis.

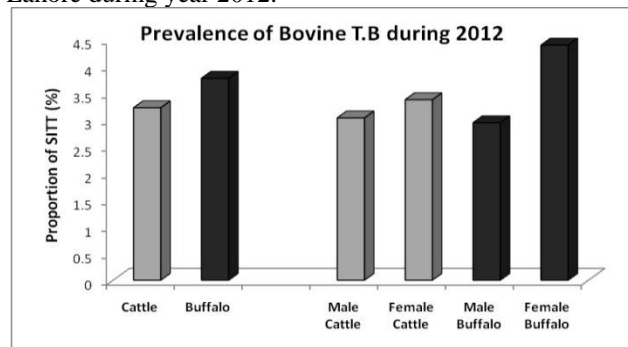
**Figure 1a:** Prevalence (%) of tuberculosis by Single intradermal tuberculin test (SITT) in bovine population around Lahore during year 2010.



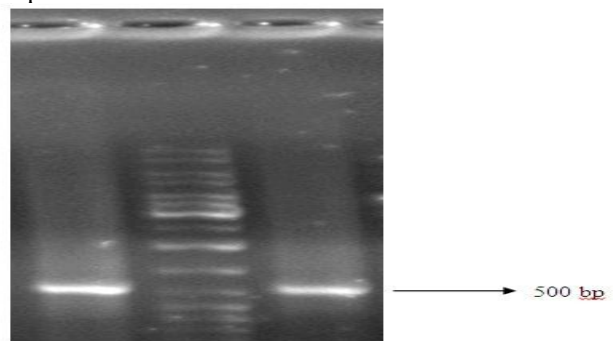
**Figure 1b:** Prevalence (%) of tuberculosis by Single intradermal tuberculin test (SITT) in bovine population around Lahore during year 2011.



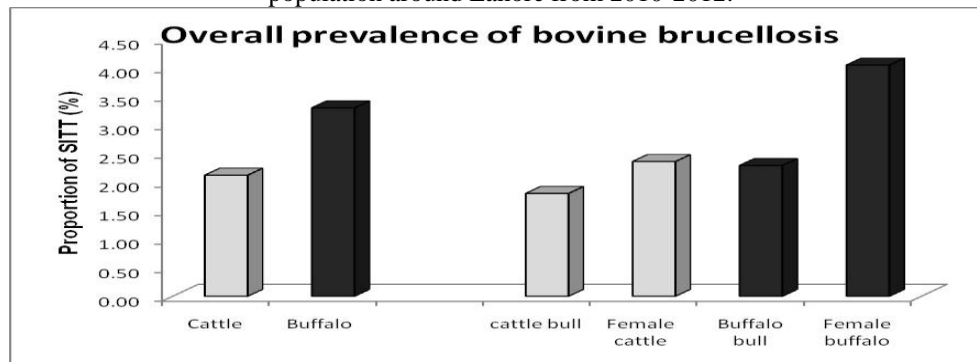
**Figure 1c:** Prevalence (%) of tuberculosis by Single intradermal tuberculin test (SITT) in bovine population around Lahore during year 2012.



**Fig 2:** Species-specific 500 bp fragment is well conserved in *M.bovis*L2= 50 bp DNA ladder, L1 and L3 = Positive samples



**Fig 3:** The species and gender wise prevalence (%) of tuberculosis by Single intra-dermal tuberculin test (SITT) in bovine population around Lahore from 2010-2012.



## DISCUSSION

*Mycobacterium bovis* (*M. bovis*) is widely distributed in Pakistan where the control measures are not applied at all or are applied sporadically, and the pasteurization is rarely practiced. As the main source of milk in our country is bovine therefore we need to know the status of *M. bovis* in cattle and buffaloes to assess the level of risk.

The overall incidence of tuberculosis in present study was more in buffaloes than in cattle. It is interesting to note that most of the previous studies in Pakistan are on cattle and the buffalo population has altogether been neglected which constitute a major portion of livestock in Pakistan and indeed is more infected. Few of the studies conducted on buffaloes were in other areas such as Faisalabad and Okara district [8]. The major population of buffalo is present in the province of Punjab, Pakistan. According to a report 94% of the cattle and more than 99% of the buffalo populations in Asia are either only partly controlled for bovine T.B or not controlled at all [9]. Though this statement came from last decade but still raise a question that why buffaloes are more infected than cattle in Asia. The increase incidence in buffaloes could be due to more susceptibility of tuberculosis in buffaloes as compare to cattle as buffaloes are more strong reactors of tuberculin than cattle. Genetic variation, lactation and environmental difference may also play a role in high prevalence of *M. bovis* in buffaloes. Large dairy herds, more milk production, , overcrowding of animals, sharing of pastures and territory with other infected animals, less veterinary supervision and industrialization could be other factors involved [10]. The close animal density in buffaloes as compare to cattle may also play a major factor in the transmission of *M. bovis*. As T.B transmission usually happens when animals are in close contact with each other. It primarily spread through the exchange of respiratory secretions between infected and uninfected animals as previously mentioned [11]. Animal crowding near watering ponds, dip tanks, markets, and corrals could be another reason for increased incidence of bovine T.B in buffaloes.

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Interestingly higher incidence of T.B was observed in female animals (both cattle and buffaloes) than male animals. This not only signifies the role of gender in disease resistance but may also be associated with the state of milking which lowers the immunity of lactating animals [12]. But this is not in line with previous findings of Arshad et al. [13] who explained that sex of the animal had not any association with the disease while theage, physical condition, milk yield and housing of the animals were linked with the disease.

In the present study we also determined the link of mastitis with bovine T.B. According to an estimation if only 1% of the animals have tuberculous mastitis it may affect all the calves fed on such milk. This fact highlights the importance of this dilemma therefore we also put an endeavor to investigate tuberculous mastitis but all the mastitis positive animals were found to be negative for T.B by SITT, Z.N staining and subsequent milk PCR. Therefore we may assume that the incidence of T.B in mastitis is not very common in screened population of bovines around Lahore. This is parallel to the studies of Al-Soub and Chako [14] who reported tuberculous mastitis as a rare disease.

Pakistan stands at second number with approximately 27.3 million buffalo population which is referred as black gold of country [15]. This contributes approximately 67% of the total milk production in the country. As there are more consumers of buffalo milk than cattle milk in Pakistan (human milk consumption for cow milk is 13,897 tons and for buffaloes is 24,370 tons) therefore this increased incidence first time reported by our study may produce awareness about bovine T.B in 30-35 million rural people who generate 30-40% of their income from raising of livestock and buffalo herds. Further anatomical and physiological research studies are required to explore the reason for increase incidence in buffaloes.

## ACKNOWLEDGEMENT

This research work was funded by Veterinary Research institute, Lahore.



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