



E-ISSN: 2320-7078  
P-ISSN: 2349-6800  
JEZS 2015; 3 (2): 15-19  
© 2015 JEZS  
Received: 17-02-2015  
Accepted: 02-03-2015

**Noorrahim**  
Department of Zoology, Hazara  
University Mansehra-21300,  
Pakistan.

**Mian Sayed Khan**  
Department of Zoology,  
University of Swabi, Pakistan

**Muhammad Shahid**  
Research Officer, Tuberculosis  
and Veterinary Public Health  
Section, Veterinary Research  
Institute, Peshawar Khyber  
Pakhtunkhwa.

**Alamgir shah**  
Department of Zoology, Hazara  
University Mansehra-21300,  
Pakistan.

**Muzafar Shah**  
Department of Zoology, Hazara  
University Mansehra-21300,  
Pakistan.

**Rafiullah**  
Department of Genetics, Hazara  
University, Mansehra-21300,  
Pakistan.

**Habib Ahmad**  
Department of Genetics, Hazara  
University, Mansehra-21300,  
Pakistan.

**Correspondence:**  
**Muzafar Shah**  
Department of Zoology, Hazara  
University Mansehra-21300,  
Pakistan.

## Prevalence of Tuberculosis in Livestock Population of District Charsadda by Tuberculin Skin Test (TST)

**Noorrahim, Mian Sayed Khan, Muhammad Shahid, Alamgir Shah, Muzafar Shah, Rafiullah, Habib Ahmad**

### Abstract

A total of 100 animals from each tehsil were screened for the prevalence of Tuberculosis (TB) in animal population of three tehsil of District Charsadda in which 4.33% (13/300) by TST, 61.54% (8/13) were +ve to avian tuberculin, 15.38% (2/13) to mammalian tuberculin while 23.08% (3/13) were reactive to both tuberculin showing a mixed infection. Cattles, buffaloes, sheep and goats were included in the study and a higher prevalence of TB was recorded in cattle (5.75%) as compared to buffaloes (4.44%) by TST. 4.28% 2-6 years of age and 5.88% in more than 6 years of age. 4.51% in female (2.94%) in male. 7.50% lactating females and 2.74% in non-lactating females. Present results show high prevalence of TB in animals of poor health status (8.13%) and poor management systems (5.95%) as compared to animals of satisfactory health condition (1.91%) and satisfactory management systems (3.06%).

**Keywords:** TST and ICT, Prevalence, Population, Cattle.

### 1. Introduction

Tuberculosis (TB) is one of the most frequent infectious, inflammatory and a chronic debilitating disease and is responsible for 2.9 million deaths [1] and 8 million new cases per year in the world [2]. It is one of the common infectious diseases of the developing world, resulting in high morbidity and mortality in these countries [3].

Tuberculosis is endemic in Pakistan with about 1.5 million people infected, and Pakistan ranks 6th among the 22 high-burden tuberculosis countries worldwide [4]. According to the World Health Organization (WHO), Pakistan accounts for 43% of the TB disease burden in the WHO Eastern Mediterranean Region. Every year, approximately 270,000 people in Pakistan develop TB, with nearly 80% occurring among young people aged between 15 and 49 years, although the TB cure rate in Pakistan is some 78% [4]. In spite of this cure rate, still 64,000 deaths occur due to TB each year in the country [5]. The disease is caused by the members of the *Mycobacterium* family that mainly affects the respiratory system. Three main types of TB and their causative agents are human TB, caused by *M. tuberculosis*, avian TB, caused by *M. avium* and bovine TB, caused by *M. bovis*. Human TB is rarely transferable to non-human species. Avian TB is typically restricted to birds. Bovine or cattle TB, presently known as zoonotic TB, is a highly infectious disease, caused by *M. bovis* having no geographical boundaries and infection occurs in diverse group of animals, which includes farm animals of economic importance, wildlife and humans [6, 7]. *M. bovis* is the main contributing mediator of human intestinal TB in those developing countries of the world where bovine milk had not often been pasteurized properly before use [8]. Although cattle are considered to be the true host of *M. bovis*, the disease has been reported in many domesticated and non-domesticated animals. Isolations have been made from buffaloes, sheep, goats, dogs, cats and several predatory felines including lions, tigers, leopards, and lynx [9]. The recognition of bovine TB in cattles and other susceptible animal species is often made on clinical history, necropsy findings, tuberculin skin test, biochemical tests [10], as well as by molecular techniques such as PCR [11, 12].

Tuberculin skin test (TST) has been referred to as the master key to the bovine TB control and eradication programmes in many countries of the world [13, 14]. But the main drawback of the TST is the poor specificity, since previous *M. bovis* BCG vaccination and environmental mycobacterial exposure can lead to false positive results [15, 16]. TST has also several operational drawbacks, including the need for a return visit and operator-dependent variability in placement and reading of the test. It is expected that most of the individuals never come back after the 2 to 3 days coming up period for further assessment and to have their test read [17, 18]. The T.B cure rate in Pakistan is some 78% still a lot of efforts need to be made to improve the detection rate (27%), which is still very low [4]. There is very little data about the

prevalence of tuberculosis in animal population in Pakistan and especially in the areas of Khyber Pakhtunkhwa (KP). Therefore, the present study was designed to investigate the prevalence of TB in the animal population of district Charsadda of KP Province.

## 2. Materials and methods

Charsadda is a district in the Khyber Pakhtunkhwa province of Pakistan. The district is administratively subdivided into three Tehsils namely Charsadda, Tangi and Shabqadar. Charsadda is located in the west of the KP and is bounded by Malakand District on the north, Mardan district on the east, Nowshera and Peshawar districts on the south and the Mohmand Agency of the Federally Administered Tribal Areas (FATA) on the west. The district covers an area of 996 square kilometers with total population of 14, 10000 [19].

### Sample collection and processing

During the present study blood samples were randomly collected from 300 animals from three Tehsils (100 from each) of District Charsadda. Four different species of animals i.e. cattle, buffalo, sheep and goat were included in the study.

### Tuberculin skin test (TST)

Two sites on the right side of the mid-neck region (12cm apart) of animal were disinfected with a suitable disinfectant, shaved and the skin thicknesses were measured with vernier calipers. One site was injected with an aliquot of 0.1ml avian tuberculin. Similarly 0.1ml mammalian tuberculin was injected into the second site. The injected area was left un-rubbed. After 48-72 hours, the skin thickness at the injection sites was measured. The interpretation of the result was such that when reactions were observed at both injection sites, the difference between the two reaction sizes was considered. Thus, an animal was classified as tuberculin positive if the increase in the skin thickness at the injection site for both tuberculin was at least 4mm or greater than this value.

**Table 1:** Distribution of number of collected samples during the present study

Species	Cattles	Buffaloes	Sheeps	Goats	Total
Charsadda	67	15	09	09	100
Tangi	61	17	10	12	100
Shabqadar	63	13	11	13	100
Total	191	45	30	34	300

**Table 3:** Prevalence of avian and mammalian tuberculosis in animal population of District Charsadda by avian and mammalian tuberculin

Tehsil	Total	TST total		+ve for avian		+ve for mammalian		Both avian and mammalian	
		+ve	%	+ve	%	+ve	%	+ve	%
Charsadda	100	03	03	02	66.67	01	33.33	00	00
Tangi	100	03	03	02	66.67	00	00	01	33.33
Shabqadar	100	07	07	04	57.14	01	14.28	02	28.57
Total	300	13	4.33	08	61.54	02	15.38	03	23.08

### Prevalence of tuberculosis in different species of animals in District Charsadda by TST

During the present study prevalence of TB in cattle, buffalo, goat and sheep was also investigated. As clear from Table IV, among the four different species of domestic animals, a higher prevalence of TB was recorded in cattle which was found to be 5.75% (11/191) by TST. A slight difference was recorded in the prevalence of TB in male and female cattle. In buffaloes, the prevalence was noted to be 4.44% (2/45) by TST. As the study was randomized, no male buffalo came under investigation during this study. Thirty four goats and 30 sheep

## 3. Results

During the present study the prevalence of tuberculosis was investigated in animal population of District Charsadda. For this purpose a total of 300 samples were randomly investigated for TB through TST.

### Overall and sex-wise prevalence of tuberculosis in animal population of District Charsadda by TST

The overall prevalence of tuberculosis in animal population of District Charsadda was found to be 4.33% (13/300) by TST. The prevalence was recorded higher by TST. It shows higher sensitivity and lower specificity of TST. The prevalence of TB was recorded higher in females (4.51%) by TST as compared to male animals in which the prevalence was found to be 2.94% by TST (Table II). In females, the prevalence of TB was found highest in Shabqadar Tehsil (6.97%) and lowest in Tangi Tehsil (3.29%). In males, the prevalence was recorded higher in Tehsil Shabqadar (7.14%) while no positive reactor was found in male animals of Tehsil Charsadda and Tangi.

**Table 2:** Overall and sex-wise prevalence of tuberculosis in animal population of District Charsadda by TST

Tehsil	Male			Female		
	Total	TST +ve	%	Total	TST +ve	%
Charsadda	11	00	00	89	03	3.37
Tangi	09	00	00	91	03	3.29
Shabqadar	14	01	7.14	86	06	6.97
Total	34	01	2.94	266	12	4.51

### Prevalence of avian and mammalian tuberculosis in animal population of District Charsadda by Avian and Mammalian Tuberculin

During the present study, both avian and mammalian tuberculin was used for the detection of TB in animals. Among 300 animals, 13 were found positive for TB by TST. Out of these 13 positive reactors, 61.54% (8/13) were positive to avian tuberculin, 15.38% (2/13) were reactive to mammalian tuberculin while 23.08% (3/13) of positive animals were reactive to both tuberculin showing a mixed infection. As clear from Table III, a higher number of animals (8/13) 61.54%, were found reactive as compared to those animals which were reactive to avian tuberculin (3/13) 23.08%. This showed a high reactivity to avian tuberculin as compared to mammalian tuberculin.

were also included in the study, but no positive reactor was found in goats and sheep by TST. In both buffalo and cattle, a higher prevalence was noted by TST.

### Association of different factors with the prevalence of tuberculosis in animals of District Charsadda by TST

Association of different factors like health status, age, sex etc. was also investigated with the prevalence of TB in animals of the selected area. Information was collected by filling a questioner. Association between these factors and prevalence has been presented below.

**Age**

All the animals were categorized in three age groups i.e.  $\leq 2$  years, above 2 to 6 years and of more than 6 years of age. A high association was found between age and the prevalence of the disease. As clear from Table V, The prevalence was recorded to be 4.28% in animals of above 2 up to 6 years of age and 5.88% in animals of more than 6 years while no positive animal was detected in animals of less than 2 years of age.

The disease was found more prevalent (4.51%) in female animals than in male animals (2.94%). It indicated high susceptibility of female animals as compared to male ones (Table V).

**Lactation**

Among 300 animals, 266 were females in which 120 were lactating while 146 were dry (Non-lactating). Prevalence of TB was found in 7.50% lactating females which was higher than in non-lactating females in which the prevalence was recorded in 2.74% females (Table V).

**Health status**

All the animals were categorized in three groups i.e. poor, satisfactory and good according to their health status. Animals of poor health showed high prevalence of TB which was found to be 8.13% as compared to animals of satisfactory health condition in which the disease was prevalent in 1.91% of animals. No positive reactor was recorded in animals having good health status (Table V).

**Table 4:** Prevalence of tuberculosis in different species of animal in District Charsadda by TST

Species	Sex	Total	Total TST +ve		+ve for avian		+ve for mammalian		Both	
			+ve	%	+ve	%	+ve	%	+ve	%
Cattle	Male	19	01	5.26	01	100	00	00	00	00
	Female	172	10	5.81	06	60	02	20	02	20
	Total	191	11	5.75	07	55.55	02	22.22	02	22.22
Buffalo	Male	00	00	00	00	00	00	00	00	00
	Female	45	02	4.44	01	50	00	00	01	50
	Total	45	02	4.44	01	50	00	00	01	50
Goat	Male	09	00	00	00	00	00	00	00	00
	Female	25	00	00	00	00	00	00	00	00
	Total	34	00	00	00	00	00	00	00	00
Sheep	Male	06	00	00	00	00	00	00	00	00
	Female	24	00	00	00	00	00	00	00	00
	Total	30	00	00	00	00	00	00	00	00
Total	Total	300	13	4.33	03	23.08	08	61.54	02	15.38

**Sex Management**

The disease was found more prevalent where the animals were overcrowded and sanitation and drainage system was very poor. TB was found to be prevalent in 5.95% animals in poor and 3.06% in satisfactory management systems while no positive animal for TB was recorded in good management systems (Table V).

**Symptoms**

Animals representing symptoms similar to TB were also recorded. The disease was found more prevalent (47.82%) in symptomatic animals as compared to low prevalence (0.72%) in non-symptomatic animals (Table V).

**Table 5:** Association of different factors with the prevalence of tuberculosis in animal of District Charsadda by TST

Parameter	Total	TST		
		+ve	%	
Age (years)	$\leq 2$	09	00	00
	Above 2 to 6	257	11	4.28
	> 6	34	02	5.88
	Total	300	13	4.33
Sex	Male	34	01	2.94
	Female	266	12	4.51
	Total	300	13	4.33
Health status	Poor	123	10	8.13
	Satisfactory	157	03	1.91
	Good	20	00	00
	Total	300	13	4.33
Lactation	Lactating	120	09	7.5
	Non-lactating	146	04	2.74
	Total	266	13	4.89
Management	Poor	168	10	5.95
	Satisfactory	98	03	3.06
	Good	34	00	00
	Total	300	13	4.33
TB Symptoms	With symptoms	23	11	47.82
	Without symptoms	277	02	0.72
	Total	300	13	4.33

#### 4. Discussion

In the present study, the overall prevalence of TB was found to be 4.33% (13/300) by TST. In a similar study<sup>[20]</sup>, investigated the prevalence of TB in 2668 bovines by single intra dermal tuberculin test. They found 0.69% (in Jersey cross breed) to 2.89% (in HF cross breeds) prevalence of the disease. A study conducted to generate epidemiological data on TB in cattle and humans in Enugu State, through a survey in abattoirs and hospital. In abattoirs, they found an overall mean prevalence of 1.4% in cattle. Our results are in similar line with the findings of the above authors. In our present study, the prevalence was recorded higher by TST which indicated high sensitivity and low specificity of TST<sup>[21]</sup>.

Out of 300 animals, 13 were found positive for TB by TST. Out of these 13 positive reactors, 61.54% (8/13) were positive to avian tuberculin, 15.38% (2/13) to mammalian tuberculin while 23.08% (3/13) of positive animals were reactive to both avian and mammalian tuberculin showing a mixed infection. The presence of *M. tuberculosis* in cattle was also reported by<sup>[22]</sup>, who documented three cases of TB caused by *M. tuberculosis* detected in 3 unrelated cattle farms. They also conducted an epidemiologic investigation on the cattle farms to determine the source of infection and in all cases, staff of the farms had active TB. Srivastava *et al.* (2008) also analyzed 768 specimens from 161 cattle of organized cattle farms in north India<sup>[23]</sup>. They also identified 40 *M. bovis* (74.08%) and 14 as *M. tuberculosis* (25.92%) out of 54 *M. tuberculosis* complex isolates. Similar results were also found<sup>[24]</sup>. In the present study a higher prevalence of TB was recorded in cattle (5.75%) as compared to buffaloes (4.44%) by TST while no positive reactor was found in sheep and goats. Sharma *et al.* (2011) also conducted a study and found high prevalence of TB in cattle than in buffaloes. They recorded 1.66 time higher risk of the disease in cattle as compared to buffaloes<sup>[25]</sup>.

During this study the prevalence was recorded to be 4.28% in animals of above 2 up to 6 years of age and 5.88% in animals of more than 6 years while no positive animal was detected in animals of less than 2 years of age. Salgado *et al.* (2009) also found TB infection most commonly among adult and female animals<sup>[26]</sup>. Our results are also in agreement with the findings of Khan *et al.* (2007), Phaniraja *et al.* (2010), Sharma *et al.* (2011) and Trangadia *et al.* (2013) who also recorded higher prevalence of the TB in old animals as compared to young animals. Prevalence was also recorded higher (4.51%) in female animals than in male animals (2.94%). Salgado *et al.* (2009) also found higher prevalence in females (5.9%) than males (3.1%). Similar results were recorded by Phaniraja *et al.* (2010), Nwanta *et al.* (2011), Arshad *et al.* (2012) and Trangadia *et al.* (2013).

Among 266 females, 120 were lactating while 146 were dry (Non-lactating). In the present study the prevalence of TB was found (9/120) 7.50% in lactating females which was higher than in non-lactating females (4/146) 2.74%. Rahman and Samad. (2008) also found the prevalence of TB in cattle and found a higher prevalence of 35.29% (6/17) in lactating cows as compared to 20.0% (1/5) in non-lactating cows. Arshad *et al.* (2012) had also reported similar results. A high prevalence of TB in animals of poor health status and poor management system was also observed during this study. Arshad *et al.* (2012) also found 2.8 times higher prevalence in animals with poor physical condition than the animals with good health conditions<sup>[27]</sup>. During this study the disease was found to be more prevalent (47.82%) in symptomatic animals as compared to non-symptomatic animals (0.72%). Eric-thys *et al.* (2011) also recorded high prevalence of TB in cattle having clinical signs of the disease<sup>[28]</sup>. Nisar *et al.* (2004) also found high

prevalence of TB in patients having TB symptoms than in non-symptomatic individuals<sup>[29]</sup>.

#### 5. References

1. Khan MA. TB: Need to revitalize its control programme in Pakistan. J Coll Physicians Surg. Pakistan 1996; 06:1-3.
2. Soini H, Musser JM. Molecular diagnosis of Mycobacteria. Clinical Chem 2001; 47:809-814.
3. Mazhar AU, Sarwar MSA, Mateen A. A survey of tuberculin test after BCG vaccination in children. J Coll Physicians Surg Pakistan 1995; 5:64-66.
4. WHO. Tuberculosis: Infection and transmission. Media centre, world health organization. Fact sheet No. 104, 2004.
5. Saeed, W, Ahmed J, Naseem A. Endorochial TB: clinical and diagnostic aspects. Pak Armed Forces Medical Journal 2002; 52:154-158.
6. Grange JM. *Mycobacterium bovis* infection in 11-32. Human beings Tuberculosis 2001; 81:71-77.
7. Pavlik I, Machackova M, Ayele WY, Lamka J, Parmova I, Melicharek I *et al.* Incidence of bovine tuberculosis in wild and domestic animals other than cattle in six Central European countries during 1990-1999. Veterinary Medicine Czh 2002; 47:122-131.
8. Bonsu OA, Laing E. Akanmori BD. Prevalence of tuberculosis in cattle in the Dangme-west district of Ghana, public health implications. Acta Tropica 2000; 76(1):09-14.
9. De-Lisle GW, Mackintosh CG, Bengis RG. *Mycobacterium bovis* free-living and captive wildlife, including farmed deer. Rev Sci Tech Off Int Epiz 2001; 20:86-111.
10. Kent PT, Kubica GP. Public Health Mycobacteriology: a guide for the level III laboratory. US Department of Health and Human Services, Public Health Service, Centers for Disease Control Atlanta., Georgia, USA, 1985, 200-207.
11. Boddingtonhaus B, Rogall T, Blocker H, Botteger EC. Detection of mycobacteria by amplification of rRNA. Journal of Clinical Microbiology 1990; 28:1751-1759.
12. Cousins DV, Wilton SD, Francis BR. Use of DNA amplification for the rapid identification of *Mycobacterium bovis*. Veterinary Microbiology 1991; 27:187-195.
13. Myers JA, Steele JH. Bovine tuberculosis control in man and animals. Warren H. Green, Inc., St Louis, Missouri, 1969, 403.
14. Weiss R. *Mycobacterium bovis* infections in Germany. In *Mycobacterium bovis* infection in animals and humans, Edn 2, (Theon, C. O., J. H. Steele. and M. J. Gilsdorf). Blackwell publishing, Ames, Iowa, 2006, 246-247.
15. Huebner RE, Schein MF, Bass JBJ. The tuberculin skin test. Clin Infect Dis 1993; 17:968-75.
16. Jasmer RM, Nahid P, Hopewell PC. Clinical practice: latent tuberculosis infection. N Engl J Med 2002; 347:1860-1866.
17. ATS. MMWR. Recommendations Report. Targeted tuberculin testing and treatment of latent tuberculosis infection. MMWR 2000; 49:01-51.
18. Lee E, Holzman RS. Evolution and current use of the tuberculin test. Clin Infect Dis 2002; 34:365-370.
19. Government of Khyber Pakhtunkhwa. District and Tehsil Wise Mid-Year Estimated Population of Khyber Pakhtunkhwa, By Urban/Rural 2005 TO 2009. Bureau of statistics, 2009.

20. Phaniraja KL, Jayaramu GM, Sanganal J, Kumar NGS. Incidence of Tuberculosis in and around Banglore. *Veterinary World* 2010; 3(4):161-164.
21. Nwanta JA, Umeononigwe CN, Abonyi GE, Onunkwo JI. Retrospective study of bovine and human tuberculosis in abattoirs and hospitals in Enugu State, Southeast Nigeria. *J Public Health Epidemiol* 2011; 3(7):329-336.
22. Romero B, Rodriguez S, Bezos J, Diaz R, Copano FM, Merediz I. Humans as source of *Mycobacterium tuberculosis* infection in cattle, Spain. *Emerg Infect Dis* 2011; 17(12).
23. Srivastava K, Chauhan D, Gupta P, Singh H, Sharma Vd, Yadav Vs *et al.* Isolation of *Mycobacterium Bovis* & *M. Tuberculosis* From Cattle Of Some Farms In North India Possible Relevance In Human Health. *Indian J Med Res* 2008; 128(1):26-31.
24. Mishra A, Singhal A, Chauhan DV, Katoch M, Srivastava SSK, Thakral SS, Sreenivas, Prasad KH. Direct Detection and Identification of *M. tuberculosis* and *M. bovis* in *Bovine* Samples by a Novel Nested PCR Assay: Correlation with Conventional Techniques. *J Clin Microbiol* 2005; 43(11):5670-5678.
25. Sharma S, Patil PK, Kumar H, Mahajan V, Filia G, Verma S *et al.* Bovine tuberculosis in intensive dairy operations of Punjab: longitudinal comparative study on prevalence and the associated risk factors. *Indian journal of comparative Microbiology, immunology and infectious diseases* 2011; 32:41-44.
26. Salgado M, Herthnek D, Bolske G, Leiva S, Kruze J. First isolation of *Mycobacterium avium subsp. Paratuberculosis* from wild Guanacos (*Lama guanicoe*) on Tierra del Fuego Island. *J Wildl Dis* 2009; 45(2):295-301.
27. Arshad M, Ifrahim M, Ashraf M, Rehman SU, Khan HA. Epidemiological studies on tuberculosis in buffalo population in villages around Faisalabad. *The Journal of Animal and Plant Sciences* 2012; 22(3):246-249.
28. Eric T, Emmanuel A, Djibo G, Issoufou A, Alhassane Y, Claude S. *Bovine Tuberculosis* Prevalence Survey on Cattle in the Rural Livestock System of Torodi (Niger). *PLoS ONE* 2011; 6(9):624-629.
29. Nisar, Ihsanullah M, Zia-Ullah, Muhammad J. Performance of ICT TB test in the detection of pulmonary and extra-pulmonary tuberculosis. *Journal of Ayub Medical College, Abbottabad* 2004; 16(2):55-56.