



RESEARCH ARTICLE

Occurrence and Risk Factors Associated with *Mycobacterium tuberculosis* and *Mycobacterium bovis* in Milk Samples from North East of Pakistan

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ABSTRACT

Tuberculosis is a chronic, debilitating and highly contagious zoonotic disease threatening animals and public health. This study was designed to investigate the occurrence of *Mycobacterium tuberculosis* and *Mycobacterium bovis* and to identify the risk factors associated with the disease in milk samples of dairy animals. A total of 200 milk samples were collected from lactating animals and milk shops of five randomly selected union councils of district Kohat, Pakistan. The epidemiological and herd management data was collected on a pre-designed questionnaire. All milk samples were first screened by direct microscopy, followed by molecular detection of *M. tuberculosis* and *M. bovis* using PCR assay. Overall, PCR based prevalence of tuberculosis causing bacteria was 7.5%. The high prevalence rate of *M. bovis* was found in cattle (6.4%), followed by buffaloes (6.2%), while the prevalence of *M. tuberculosis* was higher in goats (6.3%). A higher prevalence of *M. bovis* (15%) was found in the Khushal Ghar area. Moreover, the prevalence was also high in milk samples collected from milk shops (7.4%). Additionally, animal herd size and hygienic measures significantly ($P < 0.05$) contributed to the prevalence rate. The prevalence rate in the age group of >8 years was found higher compared to other age groups. Furthermore, the high prevalence rate was found in animals having close contact with other animals in communal grazing and watering. This study concluded that some factors influence significantly in the prevalence of tuberculosis. Thus, actual managerial practices might be helpful in control and prevention of these pathogenic bacteria.

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INTRODUCTION

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is an infectious and chronic debilitating disease of humans, domestic animals as well as wild animals, which significantly prevails throughout the world (Ghumman *et al.*, 2013). *M. tuberculosis* is the principal microorganism related with human tuberculosis, whereas *M. bovis* is the most important pathogen causing bovine tuberculosis (BTB) and has great potential to infect human and other animals due to their broad range of host (Khan and Khan, 2007). This

disease has considerable economic and public health importance due to its occurrence in various animal species and their products, which are used for human consumption (Tschopp *et al.*, 2010). Recently, several developed countries of the world have decreased or eliminated BTB from their cattle population, but considerable pockets of infection are still present in the wildlife of Canada, the United Kingdoms, United States and New Zealand (OIE, 2017). Although, TB is prevalent throughout the globe, however, the disease is more frequently found in most countries of Africa, parts of Asia and the United States.

Pakistan is the fourth largest milk producing country in the world (Ashfaq *et al.*, 2015). Buffaloes and cattle are the major dairy animals for milk production in Pakistan. Several different studies reported the prevalence of TB in large and small ruminants in Pakistan. In 2001, a prevalence of TB in cattle and buffaloes had been reported 5.1% and 1.76%, respectively (Ifrahim, 2001). Similarly, in Lahore which is a metropolitan city of Pakistan, 6.91 and 8.64% prevalence of TB was noted in buffaloes and cattle, respectively. Whereas, the prevalence of isolated organism in the milk and nasal secretions of tuberculin positive buffaloes was 28.07 and 12.28%, respectively (Jalil *et al.*, 2003). In the year 2010, the prevalence of TB in buffaloes was reported to be 3% (Javed *et al.*, 2010). The prevalence of 7.6% was reported in cattle, with 100% herd prevalence at 11 experimental livestock stations of Punjab province of Pakistan (Javed *et al.*, 2011). Additionally, 11.3% prevalence was recorded in buffaloes at experimental livestock stations at Punjab with 86% herd prevalence (Javed *et al.*, 2012). The primary route of disease transmission into herds is the infected animals acquisition, herd size, poor husbandry and sanitary practices. Furthermore, animal herds with a higher tendency of animal's movement have a significant role in disease spreading (Belchior *et al.*, 2016).

The diagnosis of TB is usually carried out through the isolation of the pathogen from sputum, milk, faeces as well as from other body fluids (Shitaye *et al.*, 2006). Direct smear microscopy through fluorescent acid-fast staining technique and the Ziehl-Neelsen's (ZN) staining of clinical samples are the conventional methods used for diagnosis of TB (Shitaye *et al.*, 2006). Culturing on selective media provides a confirmatory diagnosis of *Mycobacterium*, but the main disadvantage is the slow growth of bacteria (Ramadan *et al.*, 2012). Rapid diagnosis of the *Mycobacterium* from clinical samples can be achieved by the amplification of the *Mycobacterial* DNA by Polymerase Chain reaction (PCR). PCR is a more accurate and reliable technique for rapid diagnosis and has sensitivity equal or greater than the culture method (Ramadan *et al.*, 2012).

In many developing countries, the investigation/evaluation of diseases and the strategies for its control program are based on the information and knowledge about prevalence and epidemiological factors of the disease (Belchior *et al.*, 2016). In many developing countries, few control measures are executed despite the high prevalence rate of TB and the potential risk to public health. It has been reported that 3% of all human tuberculosis cases are caused by *M. bovis*, through close contacts with livestock or consumption of raw milk and its products (Belchior *et al.*, 2016). Recently, we reported the prevalence of TB from tissue samples of ruminants collected from abattoir of the district, Kohat (Basit *et al.*, 2015).

There is always need for surveillance and screening of milk against TB and, to the best of our knowledge, no data are available regarding the prevalence of TB in milk and milk producing animals of district Kohat, Khyber Pakhtunkhwa. Therefore, the present study was designed to investigate the occurrence of *M. bovis* and *M. tuberculosis* in milk samples taken from cattle, buffaloes, and goats located in district Kohat. Moreover, the aim of

the current study also includes to determine different husbandry and managerial practices involved in the spread of this disease, which would be helpful in disease control measures.

MATERIALS AND METHODS

Ethical statement: The technical and ethical committee constituted by Faculty of Microbiology, Kohat University of Science and Technology, Pakistan and Veterinary Research Institute (VRI), Peshawar Pakistan approved the design and protocol of the current study.

Study area and sampling: The study was carried out in district Kohat of Khyber Pakhtunkhwa province, Pakistan, where five union councils (UC) were randomly selected which harbored a large animal population. Topographically, district Kohat lies between north latitude 32° 47' and 33° 53' and east longitude 70° 34' and 72° 17' with an area of 2,545 km² in the south of Peshawar, the capital of Khyber Pakhtunkhwa. In this study, a total of 200 milk samples were collected from lactating animals including cattle, buffaloes, and goats, and milk shops, which were screened for the detection *M. tuberculosis* and *M. bovis*.

The effects of different managerial practices in the herd on the prevalence of the disease were assessed through direct interviewing at the time of sample collection. Different husbandry practices were explored such as herds size, average milk yield, types of animal housings; hygienic conditions of herds, age and some other factors including communal grazing and contact with other animal herds. Equal numbers of samples were collected from each category, and all the information were collected on a predesigned questionnaire. Milk samples were also collected from randomly selected milk shops (Skider *et al.*, 2012).

Collection of samples: Sterile sample bottles (25ml) with screw-tight caps were used for the collection of milk samples. The bottles were then labeled and transported to Veterinary Research Institute, Peshawar in cold chain for further processing.

Ziehl-Neelsen's (ZN) Acid Fast Staining: Milk sample was subjected to high speed centrifugation, 12000 rpm for 3 min and then thin smear was prepared from the pellet, and the smear was stained with Ziehl-Neelsen's (ZN) acid fast staining and examined under a conventional microscope for the presence of acid fast bacilli (Quinn *et al.*, 1994).

DNA extraction and amplification: DNA was extracted from milk samples by using a commercially available DNA extraction kit according to the manufacturer's instructions (Fermatas Inc. Ltd USA). Purified genomic DNA isolated from milk samples was subjected to PCR by targeting the *pncA* gene sequence (Forward *pncA*; 5'-ATGCGGGCGTTGATCATCGTC-3', Reverse *pncA*; 5'-CGGTGTGCCGGAAGCGG-3') for *M. tuberculosis* and species specific JB21, 22 sequences (Forward *JB21*; 5'-TCGTCCGCTGATGCAAGTGC-3', Reverse *JB22*; 5'-CGTCCGCTGACCTCAAGAAG-3') for *M. bovis* as previously described (Kidane *et al.*, 2002). The reaction mixture for PCR contained 5µl of 10 × PCR buffer, 1µl of

the primer mix, 2µl of dNTP mix (5 mM each), 3µl of 25 mM MgCl₂, 1 unit of Taq DNA polymerase (Fermatas), 5µl of sample DNA in a total volume of 50µl. The reaction was performed in a thermocycler (MultiGene™ Labnet International Inc. USA). The cycling conditions were as: initial denaturation at 95°C for 4 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30 s, and primer extension at 72°C for 1 min, with a final extension at 72°C for 10 min. Electrophoresis analyzed the PCR products by using 1.2% agarose gel and photographed on UV photodocumentation system (MultiGene™ Lab Net International Inc. USA).

Statistical analysis: Data were analyzed by using SPSS 16.0 software. The prevalence rate was evaluated by using simple percentage values and frequencies, while the effect of different managerial practices was analyzed by using chi-square test. The statistical significance level was set at $P < 0.05$. The 95% CI was evaluated for *M. tuberculosis* and *M. bovis* according to animal species and sample types.

RESULTS

Prevalence of *M. tuberculosis* and *M. bovis*: A total of 200 milk samples were collected from cattle (62), buffaloes (64), goats (47) and milk shops (27), which were screened for tuberculosis through microscopy and further analyzed for *M. bovis* and *M. tuberculosis* by PCR assay. Out of these 200 samples, 13.5% (27/200) were found positive by ZN staining. The overall PCR based prevalence of *M. tuberculosis* and *M. bovis* in milk samples was determined to be 7.5% (Table 1). Fig. 1 shows the PCR amplified 185-bp and 500-bp DNA products of *M. tuberculosis* and *M. bovis*, respectively. Moreover, the prevalence of *M. bovis* in cattle was found to be 6.4% (95% CI=0.73-13.63) by PCR as shown in Table 2. However, the prevalence of *M. bovis* in buffaloes was 6.2% (95% CI=0.73-13.23), followed by *M. tuberculosis* 1.5% (95% CI=3.49-6.62), while in goats prevalence of *M. tuberculosis* was found to be 6.3% (95% CI=2.10-14.87), which was significantly higher than other animals (Fig. 2).

Among 200 milk samples, 40 samples were collected from each union council. The results showed a high

prevalence of *M. bovis* in Khushal Ghar area with an overall prevalence of 15% (6/40), followed by Usterzai area. Whereas, in Usterzai area the prevalence of *M. bovis* was higher as compared to *M. tuberculosis* with an overall 10% (4/40) prevalence of tuberculosis and there was a significant difference among the areas by PCR positive ($P=0.077$).

Furthermore, out of total 27 randomly collected milk samples from different milk shops, 25.9% (7/27) samples were positive by ZN staining, whereas PCR confirmed only 7.4% (2/27) milk samples for *M. bovis* (95% CI=5.01-19.82) (Table 2).

Effect of managerial practices: The effects of different managerial practices are shown in Table 3. In the present study, we examined the substantial impact of animal herd size on the prevalence of *M. tuberculosis* and *M. bovis*, with the highest prevalence in large herds (34%) than in medium herds (24.5%) and small herds (14.05%). The prevalence of TB was at highest ($P < 0.0064$) in high producing animals, i.e., >7 L/d, followed by 5-7 L/d and <5 L/d. Hygienic practices in the animal herds significantly contributed in the prevalence rate of tuberculosis. The animal herds with poor hygienic conditions found to be the highest prevalence rate (35.5%) of tuberculosis, whereas 18.5% and 13% cases were investigated in the herds with moderate to good hygienic conditions, respectively. Moreover, the high prevalence rate was recorded in animals with age greater than 8 years (43%) with statistically non-significant association among the age of different groups. Furthermore, 43.5% of the herds were in close interaction with other animal herds during grazing/watering, and 37.5% of the household animals grazed on common pasture. Furthermore, mostly the farmers (47.5%) were sharing their own living houses with cattle at night.

Table 1: Prevalence of tuberculosis causing bacteria by Ziehl-Neelsen's (ZN) acid fast staining and PCR

Result	ZN test positive (n)	Prevalence %	PCR positive (n)	Prevalence %	P-value
Positive	27	15.6*	15	7.5**	0.05
Negative	173	86.5	185	92.5	

*Prevalence rate by ZN test = 15.6%; **Prevalence rate by PCR = 7.5%.

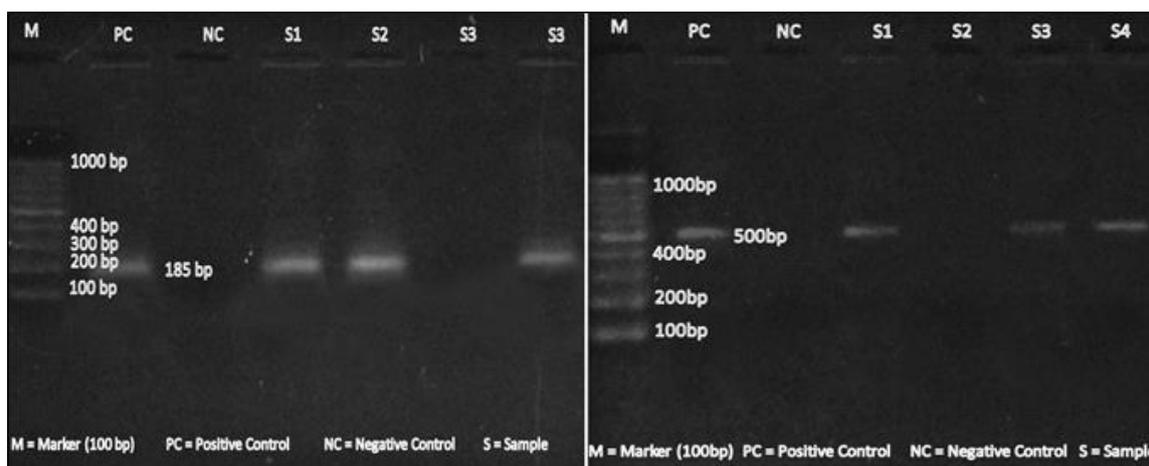


Fig. 1: PCR showing 185-bp and 500-bp DNA of *M. tuberculosis* and *M. bovis*, respectively. Agarose gel (1%) was used to run the PCR amplified product. Lane M, 1K DNA marker (Transgen, Beijing, China). Lane PC, positive control. Lane NC, negative control (PCR product without sample DNA). Lane S1-S4, different isolates of *M. tuberculosis* and *M. bovis*.

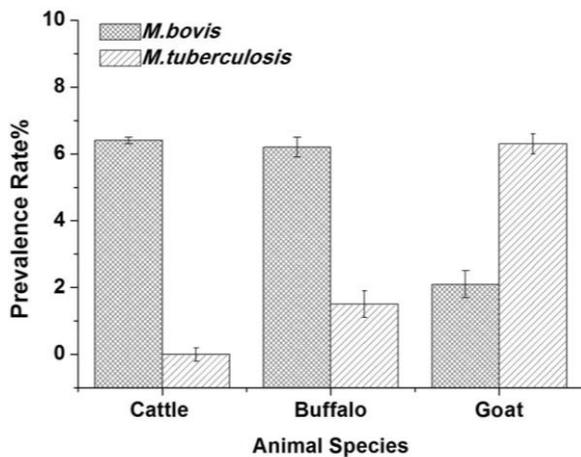


Fig. 2: Prevalence of *M. bovis* and *M. tuberculosis* in cattle, buffaloes and goats.

Table 2: Prevalence of *M. tuberculosis* and *M. bovis* on the basis of PCR

Species	<i>M. tuberculosis</i> (%) 95 % CI	<i>M. bovis</i> (%) 95 % CI
Cattle (n=62)	0	4 (6.4); (0.73 – 13.63)
Buffaloes (n=64)	1 (1.5); 3.49 – 6.62	4 (6.2); (0.73 – 13.23)
Goats (n=47)	1 (2.1); (4.59 – 8.85)	3 (6.3); (2.10 – 14.87)
Milk Shops (n=27)	0	2 (7.4); (5.01 – 19.82)
Total (n=200)	4 (2); (0.38 – 4.38)	11 (5.5); (2.06 – 8.94)

Table 3: Effects of managerial practices on the prevalence of *M. bovis* and *M. tuberculosis*

Parameters	Observations		%	p value
	Yes	No		
Herd Size				
Small herds	29	17	14.05	0.1020
Medium herds	49	20	24.5	
Large herds	68	17	34	
Milk Yield				
Low milk yielder (≤ 5)	31	19	15.5	0.0064
Medium milk yielder (6–10)	75	31	37.5	
High milk yielder (≥ 11)	19	25	9.5	
Hygienic Condition				
Poor	71	23	35.5	0.031
Moderate	37	21	18.5	
Good	26	22	13	
Age				
1-5 years	26	11	13	0.095
6-8 years	53	9	26.5	
>8 years	86	15	43	
Animal Shedding				
Outside shed	47	27	23.5	0.073
Indoor shed with people	95	31	47.5	
Other factors				
Contacts with other animal herds	87	17	43.5	0.319
Communal grazing	75	21	37.5	

DISCUSSION

Bovine tuberculosis is a chronic debilitating disease of humans and animals with high zoonotic potential, which could be transmitted between species through aerosol. The disease also exists in the list of zoonotic diseases maintained by OIE-listed diseases (OIE, 2017). The disease causing organism in milk is directly related to causing infection in humans (Arshad *et al.*, 2012). Different methods are available for the diagnosis of TB, but microscopy is more extensively used method along with molecular techniques (PCR) and culturing (OIE, 2009). This study was an attempt to investigate the prevalence of *M. bovis* and *M. tuberculosis* by PCR and further to determine the effects of different management

practices on the prevalence of these two organisms in milking dairy animals and milk shops. Firstly, the prevalence was examined in milk samples collected from Kohat district using PCR, and the results revealed an overall prevalence of 7.5% of TB caused by *M. bovis* and *M. tuberculosis*. High prevalence of *M. bovis* and *M. tuberculosis* was documented in milk samples of Khushal Ghar area (15%), followed by Usterzai area (10%). Similar studies were conducted by Khan *et al.* (2008) and Mumtaz *et al.* (2008), who reported a high prevalence rate of *M. bovis* (35%) and *M. tuberculosis* (29%) in milk using PCR. The application of PCR distinguished tuberculosis, but the prevalence rate varied from even farm to farm in the same region. Therefore, variation in prevalence rate in different areas was noticed. Moreover, the high prevalence rate of *M. bovis* and *M. tuberculosis* in the area might be due to some factors including a large number of animals were kept in the mixed farming system, poor hygienic conditions of the sheds and milking parlors, feeding practices which included household animals grazing on communal pasture. Moreover, the animal herds are significantly effected by the close contact with another infected animal during communal grazing and watering.

The present study also revealed the high prevalence of *M. tuberculosis* in goats. Tschoop *et al.* (2011) and Kassa *et al.* (2012) also reported high *M. tuberculosis* prevalence in goats. Many studies suggested that *M. tuberculosis* circulates among cattle and goat populations. Moreover, some authors suggested that humans might be infected through close contact with the infected animals. Therefore, it has been suggested a risk of infection to humans through consumption of raw milk. Moreover, the present study also revealed 7.4% prevalence of *M. bovis* in milk samples collected from milk shops of district Kohat. However, it is perceived that milk throughout the marketing chain, from farm to consumer, may harbor *M. bovis*, which could be a source of infection in those areas. However, it was observed that milk from different animals was brought to the local milk shops. It could be possible that the contamination at the milk shops may have another source, probably animal secretions. The source could be animal feed, milking person's hands or water. The water used for washing the udder could also be a source of contamination because no antiseptics are usually added to the water before washing the udder in countries like Pakistan (Bekele and Belay, 2011). Another critical and contributing factor for the spread of the disease to humans is especially through farmers, dairy workers associated with poorly ventilated houses. Furthermore, the individuals have close contact with the animals during feeding/milking, also lived near the carrier animals are more susceptible to the disease (Khan and Khan 2007; Ghumman *et al.*, 2013).

The present study also evaluated the risk factors associated with bovine tuberculosis and concluded that the large herd size (usually ≥ 10 animals) substantially involved in the high prevalence rate of disease. Moreover, the managerial practices are more difficult in larger herds as compared to smaller herd sizes and ultimately have more possibilities for transmission and spreading of infectious diseases (Ali *et al.*, 2014). A larger herd size of animals has a significantly positive impact of diseases on

animal health (Bilal *et al.*, 2004; Ali *et al.*, 2014). Similarly, milk yield of dairy animals showed significant effects on disease occurrence. The physiological stresses in milking animals adversely affect the immune system, which could lead to tuberculosis. The findings of several studies are in agreement with the present study that the increase of milk production is directly related to the high prevalence of disease (Hussain *et al.*, 2013; Gordon *et al.*, 2013).

Furthermore, proper hygienic measures are vital factors to prevent BTB, which reduce the pathogen survival chances. This study further reported the high prevalence rate of TB causing organisms in animal herds with poor hygienic conditions as compared to the animal farms in which proper hygienic conditions and measurements were adopted.

Conclusions: In conclusion, the milk samples were examined by microscopy and PCR to identify the *M. tuberculosis* and *M. bovis*. The 7.5% prevalence of *Mycobacterium* species in milk indicates that the raw milk is contaminated with these bacteria and its consumption might be a source of infection. It is recommended that dairy farmers and other related people should be properly guided about the high occurrence of tuberculosis in milk samples, and screening of tuberculosis should be adopted on routine basis in animals. Moreover, only pasteurized milk should be used for consumption. In addition, PCR proved to be a more reliable diagnostic tool for diagnosis of bovine tuberculosis.

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Authors contribution: AB, MS and SA designed and conceived the experiments. AB, MH, KR, IA and AUR performed the experiment. AB, MFH and TA analyzed the data and wrote the manuscript. SA and TA critically reviewed and revised the manuscript. All the authors read and approved the final manuscript.

REFERENCES

- Ali T, Rahman A, Qureshi MS, *et al.*, 2014. Effect of management practices and animal age on incidence of mastitis in Nili Ravi buffaloes. *Trop Anim Health Prod* 46:1279-85.
- Arshad M, Ibrahimi M, Ashraf M, *et al.*, 2012. Epidemiological studies on tuberculosis in buffalo population in villages around Faisalabad. *J Anim Plant* 22:246-9.
- Ashfaq M, Razaq A, Shamsheer-ul-Haq, *et al.*, 2015. Economic analysis of dairy animal diseases in Punjab: a case study of Faisalabad District. *J Anim Plant Sci* 25:1482-95.
- Basit A, Hussain M, Ayaz S, *et al.*, 2015. Isolation and identification of *Mycobacterium bovis* and *Mycobacterium tuberculosis* from animal tissues by conventional and molecular method. *Indian J Anim Res* 49:687-93.
- Bekele M and Belay I, 2011. Evaluation of routine meat inspection procedure to detect bovine tuberculosis suggestive lesions in Jimma Municipal Abattoir, South West Ethiopia. *Glob Vet* 6:172-9.
- Belchior AP, Lopes LB, Gonçalves VS, *et al.*, 2016. Prevalence and risk factors for bovine tuberculosis in Minas Gerais State, Brazil. *Trop Anim Health Prod* 48:373-8.
- Bilal MQ, Iqbal MU, Muhammad G, *et al.*, 2004. Factors affecting the prevalence of clinical mastitis in buffaloes around Faisalabad district (Pakistan). *Int J Agri Biol* 6:185-7.
- Ghumman MA, Manzoor AW, Naz S, *et al.*, 2013. Prevalence of tuberculosis in cattle and buffalo at various livestock farms in Punjab. *Int J Vet Med* 2013:242-7.
- Gordon PF, Van den Borne BH, Reist M, *et al.*, 2013. Questionnaire-based study to assess the association between management practices and mastitis within tie-stall and free-stall dairy housing systems in Switzerland. *BMC Vet Res* 9:200.
- Hussain R, Javed MT, Khan A, *et al.*, 2013. Risk factors associated with subclinical mastitis in water buffaloes in Pakistan. *Trop Anim Health Prod* 45:1723-9.
- Ibrahim M, 2001. Epidemiological studies on tuberculosis in cattle and buffalo population in villages around Faisalabad. M.Sc. (Hons.) Thesis Department of Veterinary Microbiology, University of Agriculture Faisalabad, Pakistan.
- Jalil H, Das P and Suleman A, 2003. Bovine tuberculosis in dairy animals at Lahore, threat to the public health. Metropolitan Corporation Lahore, Pakistan. <http://www.priory.com/vet/bovinetb.htm>.
- Javed MT, Ahmad L, Feliziani F, *et al.*, 2012. Analysis of some of the epidemiological risk factors affecting the prevalence of tuberculosis in buffalo at seven livestock farms in Punjab Pakistan. *Asian Biomed* 6:35-42.
- Javed MT, Irfan M, Ali I, *et al.*, 2011. Risk factors identified associated with tuberculosis in cattle at 11 livestock experiment stations of Punjab Pakistan. *Acta Tropica* 117:109-13.
- Javed MT, Latif SA, Farooq AF, *et al.*, 2010. Association of some of the possible risk factors with tuberculosis in water buffalo around two cities of Punjab Pakistan. *Acta Tropica* 115:242-7.
- Kassa GM, Abebe F, Worku Y, *et al.*, 2012. Tuberculosis in goats and sheep in Afar Pastoral Region of Ethiopia and isolation of *Mycobacterium tuberculosis* from goat. *Vet Med Int* 2012:869146.
- Khan IA and Khan A, 2007. Prevalence and risk factors of bovine tuberculosis in Nili Ravi buffaloes in the Punjab, Pakistan. *Ital J Anim* 8:17-20.
- Khan IA, Khan A, Mubarak A, *et al.*, 2008. Factors affecting prevalence of bovine tuberculosis in Nili Ram buffaloes. *Pak Vet J* 28:155-158.
- Kidane D, Olobo JO, Habte A, *et al.*, 2002. Identification of the causative organism of tuberculous lymphadenitis in Ethiopia by PCR. *J Clin Microbiol* 40:4230-4.
- Mumtaz N, Iqbal Z, Mahmood N, *et al.*, 2008. Reliability of PCR for detection of bovine tuberculosis in Pakistan. *Pak J Zool* 40:347-51.
- OIE, 2009. Bovine tuberculosis, in manual of diagnostic tests and vaccines for terrestrial animals. www.oie.int/en/international-standard-setting/terrestrial-manual/ access-online.
- OIE, 2017. OIE-listed Diseases, Infections and infestations. office international desepizootics, World Organization for Animal Health, Paris, France. <http://www.oie.int>.
- Quinn PJ, Carter ME and Markey BK, 1994. *Clinical Veterinary Microbiology* (Wolfe, London) pp:144-55.
- Ramadan HH, El-Gohary AHN and Mohamed AA, 2012. Detection of *Mycobacterium bovis* and *Mycobacterium tuberculosis* from clinical samples by conventional and molecular techniques in Egypt. *Glob Vet* 9:648-54.
- Shitaye JE, Getahun B, Alemayehu T, *et al.*, 2006. A prevalence study of bovine tuberculosis by using abattoir meat inspection and tuberculin skin testing data, histopathological and IS6110 PCR examination of tissues with tuberculous lesions in cattle in Ethiopia. *Vet Med (Praha)* 51:512.
- Skider S, Rahman AA, Faruque MR, *et al.*, 2012. Bovine brucellosis: an epidemiological study at Chittagong, Bangladesh. *Parity* 4:1-63.
- Tschopp R, Aseffa A, Schelling E, *et al.*, 2010. Bovine tuberculosis at the wildlife-livestock-human interface in Hamer Woreda, South Omo, Southern Ethiopia. *PLoS One* 5:e12205.
- Tschopp R, Bobosha K, Aseffa A, *et al.*, 2011. Bovine tuberculosis at a cattle-small ruminant-human interface in Meskan, Gurage region, Central Ethiopia. *BMC Infect Dis* 15:318.