Asian Biomedicine Vol. 6 No. 1 February 2012; 35-42

DOI: 10.5372/1905-7415.0506.124

Original article

Analysis of some of the epidemiological risk factors affecting the prevalence of tuberculosis in buffalo at seven livestock farms in Punjab Pakistan

Muhammad Tariq Javed[a], Latif Ahmad[a], Francesco Feliziani[b], Paolo Pasquali[c], Masood Akhtar[d], Mahmood Usman[a], Muhammad Irfan[a], Giulio Severi[b], Monica Cagiola[b]

[a]Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad 38040, Pakistan, [b]Istituto Zooprofilattico Sperimentale del'Umbria e delle Marche, Perugia 06100, [c]Istituto Superiore di Sanit□, Rome 0016, Italy, [d]Department of Parasitology, Faculty of Veterinary Science, University of Agriculture, Fasialabad 38040, Pakistan

Background: Bovine tuberculosis is a disease of zoonotic importance. It is disease of high priority all over the world and needs to be investigated in each country.

Objective: This study was conducted to find out the prevalence of tuberculosis in buffaloes and associated risk factors.

Methods: The study was carried out at seven livestock experimental stations in Punjab, Pakistan. Buffaloes were studied by comparative cervical intradermal tuberculin test.

Result: The results of the study revealed an overall tuberculosis prevalence of 11.3% with 86% of farms having tuberculin positive animals. The frequency analysis revealed significant difference between different groups for age, calving, lactation length, average daily milk and live weight of the buffaloes. Data analysis by T-test also revealed significant difference between means for age, live weight, calving, lactation length and average daily milk between tuberculin positive and negative buffaloes. Bivariate and multivariate logistic analysis revealed significant association of age, live weight, calving, average daily milk and lactation length. Multivariate logistic regression analysis including all the variables in the model revealed significant association of tuberculosis with age, live weight, total milk produced, presence of cattle at the farm, lactation length, and total other animals at the farm.

Conclusion: The prevalence of tuberculosis in buffaloes increases with the increase in age, calving, live weight, milk production and presence of cattle at the farm, while the prevalence decreases with the increase in lactation length.

Keywords: Buffaloes, Pakistan, prevalence, tuberculin test, tuberculosis

Bovine Tuberculosis (BTb) is a zoonotically important disease that can infect wide variety of animal species and can be transmitted from animals to humans. The disease in animals is caused by *Mycobacterium bovis*, a member of the *M. tuberculosis* complex. The latter includes *M. tuberculosis, M. bovis, M. afri-canum* and *M. microti*. M. bovis has a wide host range and is found responsible for disease in free-living wildlife, captive wildlife including farmed deer, domestic livestock (cattle, goats, water buffalo, camels, alpacas, llamas, etc.), non-human primates and humans [1]. This disease has socio-economic and public health importance and is of great significance to international trade of animals and animal products [2]. This disease not only poses a threat to the economies of developing countries but also remains a problem in developed world [3]. It is a chronic contagious debilitating disease of animals associated with progressive weakness/ emaciation and tubercle (granuloma) formation, mainly confined to respiratory system (primarily in the lungs) and occasionally in other organs [4]. The infection to bovine can occur through the colostrum/milk to calves,

Correspondence to: Muhammad Tariq Javed, PhD, Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad 38040, Pakistan. E-mail: javedmt@gmail.com

ingestion of infected flies, droppings of birds, aerosol, contact with each other and other wildlife [5-9]. The causative agent of tuberculosis (Mycobacterium) can remain viable in the environment/soil for about two years [10]. Various risk factors responsible for the occurrence of disease include calving site, the size group of calves, the length of time calves kept in groups, the breed, the source of replacement, presence of wild animals and the region in which they are kept, presence of mixed (dairy and beef) production, herd size, age, housing systems and summer mountain pasture [11, 12]. Developed countries started an eradication program against the disease in the later nineteen hundreds and thus have been able to reduce its incidence to low levels but this is a very much neglected disease in the developing countries and is on the increase in the Asian, African and Latin American countries [13]. In the subcontinent, the prevalence of disease varies in different parts and during different months of the year [3, 14-21]. Recently, the prevalence of disease in cattle has been reported to be 7.6% in cattle at 11 Livestock Experiment Stations [22], 3% in buffaloes around two cities of Pakistan [23] and 0.9% in sheep and 0.4% in goats at Livestock Experiment Stations of Punjab Pakistan [24]. Prevalence rates of 39% in buffaloes in Nepal [25], 0.7% in buffaloes in Iran [26] and 7.6% in buffaloes in India [27] have been reported. The disease can be diagnosed by using various tests including tests like tuberculin, isolation of the organisms in suspected cases from saliva, milk, blood, urine and faeces, serum immunoglobulin G test, histopathology and PCR [28-31]. Recently, we have reported the risk factors for tuberculosis in sheep, goats, and cattle kept at Livestock Experiment Stations and at private farms around two cities of Pakistan and in buffaloes kept at private farms around two cities of Pakistan [22-24, 32, 33]. In this paper we analyzed the risk factors for occurrence of tuberculosis in buffaloes kept at seven Livestock Experiment Stations of Punjab Pakistan on the basis of positive tuberculin test in animals under study. Buffalo is the most important milch animal in Pakistan with its major population in Punjab [34] and is referred as black gold of the country. These farms are present in different locations of the Punjab province.

Methods

The study was carried out at seven experimental livestock stations of Punjab, Pakistan where buffaloes are being kept as dairy animals. These farms are far from each other in different regions of the Punjab Province. These farms have vast lands to grow fodder and other crops, and the animals are also taken for grazing in harvested fodder fields from about 7am to about 3pm. After grazing, animals are also stall fed after 3pm. Animals are milked before sunset and then taken other sheds. Each shed has a watering trough in a corner where water is available round the clock. The water in these troughs is stagnant but fresh water is added daily. The housing pattern semi closed. Each shed of semi close housing has a large open space in front covered with brick walls. Thus in winter animals enter in the shed and in summer they remain in the open space. Animals are kept unchained in these sheds. Animals are routinely vaccinated against FMD and HS. New animals are purchased almost every year at each farm, while the low producers and those having some untreatable diseases or having reproductive problems are sold. The stray dogs/cats wander in and around the sheds at these farms. Birds, including backyard poultry, sparrows, and crows also have free access to the feeding and watering areas. Flies are abundant in milking sheds or at the milk sale points at these farms. A total of 965 animals of more than two years of age were tested by single comparative cervical intradermal tuberculin (SCCIT) test using bovine (50.000 I.U./ml) and avian (25.000 I.U./ml) PPDs produced at the authorized laboratory at the Istituto Zooprofilattico Umbria e Marche, Italy. The protocols for the production of tuberculin, the execution of the test and its interpretation were carried out according to the criteria described in the OIE Manual of Standards for diagnostic tests and vaccines [2]. Data of all the tested animals were recorded including age, live weight, calving, lactation length, total milk produced, total animals at the farm, species of animals at the farm (i.e., cattle, buffalo, sheep, and goats), and total number of buffaloes. Data was entered into Excel sheet and was analyzed by using SAS statistical software version 9.1 [35]. The data analysis included frequency analysis, Mantel Haenszel chi-square, analysis of variance for comparison of means of various parameters between positive and negative animals (T-test) and logistic regression analysis. The bivariate logistic regression analysis was applied including each individual variable in the model and result of the tuberculin test as outcome variable. The multivariate logistic regression analyses including farm and individual variable along with farm, age, and

individual variable in the model and result as outcome variable were used to find association of various risk factors studied. The logistic analysis by including all variables in the model by backward elimination procedure was also used to infer the association of various risk factors with tuberculosis in buffaloes. The odds ratio and confidence intervals were also worked out. This study was formally approved by the donor agency and Faculty Scrutiny and Ethical Review Committee.

Results

The results of the study revealed an overall prevalence of 11.3% at seven Livestock Experiment Stations and varied from 0 to 18.8%, with 86% of farms having tuberculin positive animals. The frequency analysis carried out revealed non-significant differences between different groups for total milk produced during a lactation, total animals present at the farm including/excluding the buffaloes, presence of other animals at the farm including cattle, sheep and goat as can be seen in **Table 1**. Results also revealed non-significant difference in prevalence of tuberculosis at different farms. However, a significant difference was observed between different groups for age (P<0.0001), calving (P<0.0001), lactation length (P<0.0001), average daily milk (P<0.0001) and live weight of the buffaloes (P<0.0001) as can be seen in **Table 1**.

Table 1. Bivariate frequency analysis of different parameters in positive and negative reactor buffaloes to SCCIT test.

Parameters		Positive/Negative	Positive (%)	Stat Mantel Haenszel chi-square
Farms	1	14/151	8.5	P>0.832
	2	21/174	10.8	
	3	15/65	18.8	
	4	14/94	13	
	5	19/145	11.6	
	6	26/215	10.8	
	7	0/12	0	
	Total	109/856	11.3	95%CI = 9.3 to 13.6
Age groups (years)	<5	1/6+4	1.5	P<0.0001
	5-8	41/394	9.4	
	8.1-13	40/281	12.5	
	>13	27/117	18.8	
No. of calving	≤1	14/178	7.3	P<0.0001
	2-3	31/319	8.9	
	4-6	26/209	11.1	
	5-7	24/93	20.5	
	>7	14/57	19.7	
Lactation length (days)	<100	7/26	21.2	P<0.0001
	100-200	38/229	14.2	
	201-300	53/355	13	
	>300	11/246	4.3	
Milk produced (liters)	<1000	5/50	9.1	P>0.393
	1001-2000	39/381	9.3	
	2001-3000	57/323	15	
	>3000	8/102	7.3	
Milk produced/day groups (liters)	<5	2/19	9.5	P<0.0001
	5-10	51/705	6.8	
	10.1-15	52/124	29.6	
	>15	4/8	33.3	
No. of other animals groups	0	35/268	11.6	P>0.396
	1-100	15/77	16.3	
	101-500	19/145	11.6	
	501-1000	14/151	8.9	
	>1000	26/215	10.8	

Table 1. Bivariate frequency analysis of different parameters in positive and negative reactor buffaloes to SCCIT test. (Contin

Parameters		Positive/Negative	Positive (%)	Stat Mantel Haenszel chi-square
Live weight groups	≤400	0/70	0	P<0.0001
	401-450	11/116	8.7	
	451-500	22/233	8.6	
	501-550	28/217	11.4	
	551-600	34/165	17.1	
	>600	14/55	20.3	
Total animal groups	<100	0/12	0	P>0.280
	100-200	50/333	13.1	
	201-1000	19/145	11.6	
	>1000	40/366	9.9	
Other animals at the farm	Present	74/588	11.2	P>0.865
	Absent	35/268	11.6	Odds ratio = 0.96
Cattle at the farm	Present	55/431	11.3	P>0.982
	Absent	54/425	11.3	Odds ratio = 1.00
Goats at the farm	Present	40/366	9.9	P>0.227
	Absent	69/490	12.3	Odds ratio = 0.78
Sheep at the farm	Present	59/511	10.4	P>0.265
	Absent	50/345	12.7	Odds ratio = 0.80

Data analysis by T-test also revealed significant difference between means for age (P<0.0001), live weight (P<0.0001), calving (P<0.0001), lactation length (P<0.0001) and average daily milk (P<0.0001) between tuberculin positive and negative buffaloes. The other parameters including total milk produced during lactation and total animals at the farm including/ excluding buffaloes showed non-significant difference (**Table 2)**.

Table 2. Comparison of means (±sd) and 95% confidence limits of different parameters studied in positive and negative reactor buffaloes.

Parameters/Tuberculin Result	mean	sd	95% confidence limit		T-test
Age					
Positive	10.8	3.9	10.04	11.52	P>0.0001
Negative	8.9	3.6	8.68	9.15	
Live weight					
Positive	542.5	69	529.42	555.63	P>0.0001
Negative	505.5	69.9	500.83	510.21	
Calving (No.)					
Positive	4.5	2.6	4.01	5	P>0.0001
Negative	3.5	2.3	3.31	3.61	
Total milk produced					
Positive	2179.2	665.4	2052.92	2305.57	P>0.145
Negative	2067.8	762.3	2016.67	2118.95	
Lactation length					
Positive	218	66.6	205.31	230.6	P>0.0001
Negative	247.2	73.9	242.23	252.14	
Average daily milk production					
Positive	10.3	2.5	9.82	10.76	P>0.0001
Negative	8.4	2	8.27	8.54	
Total animals other than buffaloes					
Positive	590.4	651.7	466.67	714.13	P>0.4059
Negative	645.4	649.4	598.82	691.98	
Total Animals including buffaloes					
Positive	951.4	913.4	777.98	1124.82	P>0.1716
Negative	1083.2	951.3	1019.18	1146.82	

Bivariate logistic regression analysis including individual variable and multivariate analysis including farm and individual other variable revealed increase in prevalence of tuberculosis with the increase in age, live weight, calving and average daily milk, while the prevalence decreased with the increase in lactation length (**Table 3**). Multivariate logistic regression analyses including farm, age and individual other variable revealed significant increase in prevalence of tuberculosis with the increase in live weight and average daily milk, while the prevalence decreased with the increase in lactation length. Multivariate logistic regression analysis with backward elimination procedure including all variables in the model revealed significant association of tuberculosis with age, live weight, total milk produced, presence of cattle at the farm, lactation length, and total animals of other species at the farm. According to these results, the prevalence of tuberculosis increases with the increase in age, live weight, total milk produced and presence of cattle at the farm, while it decreases with the increase in lactation length and when higher numbers of animals of other species are present at the farm.

Discussion

Tuberculosis is an important disease, both in animals and humans. The disease from animals can be transmitted to humans mainly through infected milk or meat. This disease is widely distributed in the world in domestic and wild animals [36-39]. The advanced countries have programs to eradicate/control this disease in their animal population and they have reduced the infection to less than 1% at herd level. However, no serious effort is underway in most of the developing countries to eradicate/control this disease in their animals. To start a control or eradication program, knowledge about its prevalence in each country and the associated risk factors is mandatory. It is not known when Pakistan is going to get involved in the eradication or control strategies about tuberculosis in animals as Government will is required but it is always important to keep monitoring the disease situation from time to time in any country. Thus, we carried out studies to know the prevalence and risk factors associated with prevalence of tuberculosis in animals.

Table 3. Parameters showed significant association with tuberculosis in buffaloes in logistic regression analysis procedure.

Parameters	Odds ratio	95% confidence limits		P-value
		lower	upper	
Bivariate logistic regression analysis of each individual variable in the model				
Age	1.139	1.081	1.2	P>0.0001
Live weight	1.008	1.005	1.011	P>0.0001
Calving (No.)	1.188	1.099	1.284	P>0.0001
Lactation length	0.995	0.992	0.997	P>0.0001
Average daily milk production	1.388	1.269	1.581	P>0.0001
Multivariate logistic regression analysis including farm and individual variable in the model				
Age	1.149	1.088	1.213	P>0.0001
Live weight	1.008	1.005	1.011	P>0.0001
Calving (No.)	1.193	1.102	1.291	P>0.0001
Lactation length	0.995	0.992	0.997	P>0.0001
Average daily milk production	1.388	1.269	1.518	P>0.0001
Multivariate logistic regression analysis including farm, age and individual variable in the model				
Live weight	1.007	1.004	1.01	P>0.0001
Lactation length	0.993	0.99	0.996	P>0.0001
Average daily milk production	1.412	1.289	1.548	P>0.0001
Multivariate logistic regression analysis including all variables in the model				
Age	1.164	1.096	1.237	P>0.0001
Live weight	1.007	1.003	1.01	P>0.0001
Total milk produced	1.002	1.001	1.002	P>0.0001
Lactation length	0.98	0.975	0.985	P>0.0001
Presence of cattle at the farm	2.447	1.279	4.683	P>0.01
Total other animals at the farm	0.999	0.999	1	P>0.01

The prevalence of tuberculosis varied from 0% to 18.8%, with 86% of the farms that had infected animals. Greater than 10% prevalence of tuberculosis in animals is a serious issue. In the present study, five out of seven (71%) farms had higher than 10% prevalence. The only farm with 0% prevalence had only 12 buffaloes. If we remove that farm out of the herd-based analysis then 100% farms had infected animals and the prevalence in 83% of the farms was greater than 10%. It is speculated that this situation may get worse with the passage of time as the prevalence is on the increase from previous years. Earlier in Pakistan, a prevalence of about 7% is reported in buffaloes in year 1992 and 2003 at a slaughterhouse assessed by tuberculin testing [40, 41] and 2% to 9% in 2006 at two Livestock Experiment Stations of Punjab, Pakistan [32]. These data show that prevalence is increasing with the passage of time in Pakistan at well-established farms. This is very much expected under the circumstances of no test and slaughter policy in place. Considering a recent study in buffaloes at private setting which showed a herd prevalence of 14% [23] compared with 71% at Livestock Experiment Stations, it can be suggested that the prevalence is higher at old large farms. The frequency results revealed increase in prevalence in present study with the increase in age, calving, live weight and average daily milk, while the prevalence decreased with the increase in lactation length. Similarly, the mean age (10.8 vs. 8.9 years), live weight (543 vs. 506 kg), number of calving (5 vs. 4), and average daily milk (10.3 vs. 8.4 liter) was significantly higher in tuberculin positive than negative animals, while the mean of lactation length (218 vs. 247.2 days) was shorter in tuberculin positive than negative buffaloes. The shorter lactation length in tuberculin reactors than non-reactors is difficult to explain. One possibility is that lactation length is shortened due to infection in animals, but it is also possible that the disease and/or the positive tuberculin reaction occur in animals with shorter lactation length. Similar were the results of bivariate logistic analysis. Multivariate logistic regression analysis after controlling for farm and age revealed a significant increase in the prevalence of tuberculosis with the increase in live weight and average daily milk, while it decreased with the increase in lactation length. Earlier, it has been reported that susceptibility to *M. bovis* infection in cattle increases with age [42] and the prevalence is higher in adult cattle than heifers and bulls or calves [43]. It suggests that as the animal becomes older, the chance of it becoming infected increases. As the animals live for a longer period on the farm, they might have a greater possibility of contact with the infectious agent. The increase in milk production with age [32] together with the fact that they had given births many times, may suggest that these factors can lower the immune system of the animal and thus development of clinical disease. According to multivariate logistic regression analysis, the prevalence of tuberculosis increases with the increase in age, live weight, total milk produced and presence of cattle at the farm, while it decreases with the increase in lactation length and when higher number of animals of other species is present at the farm. These results revealed that the prevalence can increase by 2.5 times in the presence of cattle at the farm, while the risk of infection is lower when animals of other species are present at the farm. A recent study already indicated protective effect of sheep in the occurrence of tuberculosis. However in that study buffalo were not found to be associated with tuberculosis in cattle [22]. It has previously been suggested that a reduction in susceptibility to *M. bovis* can be achieved through management. Moreover, it has also been suggested that a considerable amount of further research is required [43]. Few other studies indicated importation of infected animals, incomplete depopulation of infected herds, movement of tuberculosis exposed animals between herds, and transmission from unidentified wildlife reservoirs as important epidemiological factors [44, 45].

Conclusion

It can be concluded from the present study that the prevalence of tuberculosis in buffaloes at these farms increases with the increase in age, calving, live weight, milk production and presence of cattle at the farm, while the prevalence decreases in buffaloes with the increase in lactation length.

Acknowledgments

Funding provided by Pakistan Science Foundation under project grant PSF/Res/P-AU/AGR (283) and bovine PPD donated by Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy, is highly acknowledged. The authors have no conflict of interest to report.

References

1. O'Reilly LM, Daborn CJ. The epidemiology of Mycobacterium bovis infections in animals and man: a review. Tubercle Lung Dis. 1995; 76 Suppl. 1:1-46.
2. OIE. Manual of Standards for Diagnostic Tests and Vaccines of World Organization for Animal Health, 4th edition. 2004.
3. Khan IA, Khan A, Mubarak A, Ali S. Factors affecting prevalence of bovine tuberculosis in Nili ravi buffaloes. Pakistan Vet J. 2008; 28:155-8.
4. Menzies FD, Neill SD. 2000. Cattle-to-cattle transmission of bovine tuberculosis. Vet J. 2000; 160: 92-106
5. Polyakov VA, Ishekenov MS, Kosenko VI. Flies-one of the links in the transmission mechanism of tuberculosis in animals. Problemy Veterinarnoi Sanitari Ekdojii. 1994; 93:84-92.
6. Sauter CM, Morris RS. Behavioural studies on the potential for direct transmission of tuberculosis from feral ferrets and possums to farmed livestock. New Zealand Vet J. 1995; 43:294-300.
7. Hejlicek K, Treml F. Epizootiology and pathogenesis of avian mycobacteriosis in the house sparrow (Passer domesticus) and tree sparrow (Passer montanus). Vet Med. 1993; 38:667-85.
8. Evangelista TBR, Anda JHD. Tuberculosis in dairy calves: risk of Mycobacterium spp. Exposure associated with management of colostrum and milk. Prevent Vet Med. 1996; 27:23-7.
9. Barlow ND, Kean JM, Hickling G, Livingstone PG, Robson AB. A simulation model for spread of bovine tuberculosis with in New Zealand cattle herds. Prevent Vet Med. 1997; 32:57-75.
10. Hutchings MR, Harris S. Effects of farm management practices on cattle grazing behavior and the potential for transmission of bovine tuberculosis from badgers to cattle. Vet J. 1997; 153:149-62.
11. Cetinkaya B, Erdogan HM, Morgan KL. Risk factors for bovine paratuberculosis. I. The univariate analysis of risk factors for bovine paratuberculosis. Turk Veterinerlik-ve-Hayvancilik dergisi. 1997; 21:297-302.
12. Marangon S, Martini M, Pozza MD, Neto F. A case-control study on bovine tuberculosis in the Veneto Region (Italy). Prev Vet Med. 1998; 34:87-95.
13. Bonsu OA, Laing E, Akanmori BD. Prevalence of tuberculosis in cattle in the Dangme-west district of Ghana, Public Health Implication. Acta Triop. 2000; 76:9-14.
14. Dhinakaran M, Nedunchelliyan S, Appajirao VN. Dhinakaran M Effects on certain economic traits in cattle due to tuberculosis. Indian Vet J. 1993; 70: 1143-5.
15. Aswathanarayana SR, Rao MS, Krishnappa G, Ramanatha KR, Raghavan R. Isolation of typical mycobacteria from milk of tuberculin test positive cows. Mysore J Agric Scie. 1998; 32:71-4.
16. Ifrahim M. Epidemiological studies on tuberculosis in cattle and buffalo population in villages around Faisalabad, [M.Sc. thesis]. Department of Veterinary Microbiology, University of Agriculture, Faisalabad; 2001.
17. Mukherjee F. Comparative prevalence of tuberculosis in two dairy herds in India. Rev Sci Tech. 2006; 25: 1125-30.
18. Niaz N, Siddiqi SH. Isolation and identification of mycobacteria from cattle slaughtered in Pakistan. Vet Rec. 1979; 104:478-80.
19. Rahman MM, Samad MA. Prevalence of Bovine Tuberculosis and its Effects on Milk Production in Red Chittagong Cattle. Bangl J Vet Med. 2008; 6:175-8.
20. Samad MA, Rahman MS. Incidence of bovine tuberculosis and its effect on certain blood indices in dairy cattle of Bangladesh. Indian J Dairy Sci. 1986; 39: 231-4.
21. Sharma AK, Vanamayya PR, Parihar NS. Tuberculosis in cattle: a retrospective study based on necropsy. Indian J Vet Path. 1985; 9:14-8.
22. Javed MT, Irfan M, Ali I, Farooqi AF, Wasiq M, Cagiola M. Risk factors identified associated with tuberculosis in cattle at 11 livestock experiment stations of Punjab Pakistan. Acta Trop. 2011; 117:109-13.
23. Javed MT, Shahid AL, Farooqi FA, Akhtar M, Cardenas GA, Wasiq M, Cagiola M. Risk factors associated with presence of positive reactions in the SCCIT test in water buffalo around two cities in Punjab, Pakistan. Acta Trop. 2010a; 115:242-7.
24. Javed MT, Munir A, Shahid M, Severi G, Irfan M, Aranaz A, Cagiola M. Percentage of reactor animals to single comparative cervical intradermal tuber-culin (SCCIT) in small ruminants in Punjab Pakistan. Acta Trop. 2010c; 113:88-91.
25. Chandra Hav, Yukio M, Dhakal M, Besnet B, Sato T, Nagai A, Kato M, Kozawa K, Yamamoto S, Kimura H. Isolation of Mycobacterium spp. from milking buffaloes and cattle in Nepal. J Vet Med Scie. 2007; 69:819-25.
26. Mosavari N, Jamshidian M, Feizabadi MM, Seyfiabad SMR, Mohammad TM, Arefpajoohi R, Tadayon K. Tuberculosis in buffalo: the first report on the phenotypic and genetic characteristics of the isolated organism in Western Azerbaijan, Iran. Archives of Razi

Institute. 2007; 62:75-82.
27. Joshi DV, Sharma DR, Sodhi SS, Dhillon SS. Survey of tuberculosis at a buffalo farm. Indian J Anim Hlth. 1976; 15:27-8.
28. Jark U, Ringena I, Franz B, Gerlach GF, Beyerbach M. Development of an ELISA technique for serodiagnosis of bovine paratuberculosis. Vet Microbiol. 1997; 57: 189-98.
29. Costello E, Egan JWA, Quigley FC, O'Reilly PF. Performance of the single intradermal comparative tuberculin test in identifying cattle with tuberculous lesions in Irish herds. Vet Record. 1997; 141:222-4.
30. Valente C, Cuteri V, Giandomenico RQ, Gialletti L, Franciosini MP. Use of an experimental chicks model for paratuberculosis enteritis (Johne's disease). Vet Res. 1997; 28:239-46.
31. Aranaz A, Lie′bana E, Mateos A, Domi′nguez L, Vidal D, Domingo M, Gonzolez O, Rodriguez FEF, Bunschoten A ., van Embden JDA, Cousins D. Spacer oligonucleotide typing of Mycobacterium bovis strains from cattle and other animals: a tool for studying epidemiology of tuberculosis. J Clin Microbiol. 1996; 34:2734-40.
32. Javed MT, Usman M, Irfan M, Cagiola M. A study on tuberculosis in buffaloes: some epidemiological aspects, along with haematological and serum protein changes. Vet Arh. 2006; 76:93-206.
33. Javed, MT, Ahmad L, Irfan M, Ali I, Khan A, Wasiq M, Farooq, FA, Latif MS, Cagiola M. Haematological and serumprotein values in tuberculin reac-tor and non-reactor water buffaloes, cattle, sheep and goats. Pak Vet J. 2010; 30:100-4.
34. Pakistan Livestock Census, 2006. Government of Pakistan, Statistics Division, Agricultural Census Organization.
35. SAS. SAS Statistical Software Version 9.1. SAS Institute Inc., Cary, NC, USA. 2003.
36. De Lisle GW, Mackintosh CG, Bengis RG. Mycobacterium bovis in free-living and captive wildlife, including farmed deer. Rev sci tech Off int Epiz. 2001; 20:86-111.
37. Wedlock DN, Skinner MA, de Lisle GW, Buddle BM. Control of Mycobacterium bovis infections and the risk to human populations. Microb Infect. 2002; 4: 471-80.
38. Griffin JFT, Mackintosh CG. Tuberculosis in deer: perceptions, problems and progress. Vet J. 2000; 160: 202-9.
39. Gormley E, Doyle MB, McGill K, Costello E, Good M, Collins JD. The effect of the tuberculin test and the consequences of a delay in blood culture on the sensitivity of a gamma-interferon assay for the detection of Mycobacterium bovis infection in cattle. Vet Immunol Immunopathol. 2004; 102:413-20.
40. Amin S, Khan MA, Hashmi HA, Khan MS, Ahmad I, Bhatti MA. Detection of buffalo tuberculosis by using short thermal test and isolation of causal organisms from lymph nodes. Buff J. 1992; 8:83-7.
41. Jalil H, Das P, Suleman A. Bovine tuberculosis in dairy animals at Lahore, threat to the public health. Metropolitan Corporation Lahore, Pakistan. 2003. Available from http://priory.com/vet/bovinetb.htm.
42. Cagiola M, Feliziani F, Severi G, Pasquali P, Rutili D. Analysis of possible factors affecting the specificity of the gamma-interferon test in tuberculosis–Free cattle herds. Clin Diagn Lab Immunol. 2004; 262:952-6.
43. Kazwala RR, Kambarage DM, Dabom CJ, Nyange J, Jiwa SF, Sharp JM. Risk factors associated with the occurrence of bovine tuberculosis in cattle in the Southern Highlands of Tanzania. Vet Res Commun. 2001; 25:609-14.
44. Phillips CJ, Foster CR, Morris PA, Teverson R. Genetic and management factors that in□uence the susceptibility of cattle to Mycobacterium bovis infection. Ani Health Res Rev. 2002; 3:3-13.
45. Neill SD Pollock, JM, Bryson DB, Hanna J. Pathogenesis of Mycobacterium bovis infection in cattle. Vet Microbiol. 1994; 40:41-52.
46. Szewzyk R, Svenson SB, Hoffner SE, Bolske G, Wahlstrom H, Englund L, Engvall A, Kallenius G. Molecular epidemiological studies of Mycobacterium bovis infections in humans and animals in Sweden. J Clin Microbiol. 1995; 33:3183-5.