SEROPREVALENCE, ASSOCIATED RISK FACTORS AND ECONOMIC IMPORTANCE OF BOVINE TUBERCULOSIS IN RED CHITTAGONG CATTLE IN TWO SELECTED UPAZILLAS OF CHITTAGONG DISTRICT, BANGLADESH

Pankaj Chakraborty, Monoar Sayeed Pallab and MA Matin Prodhan
Department of Medicine and Surgery, Chittagong Veterinary and Animal Sciences University, Chittagong-4225, Bangladesh

Corresponding author:- pcb23m@yahoo.com

Bovine tuberculosis (bTB) is a chronic wasting disease and is a cause of morbidity and mortality in livestock, wildlife and humans all over the world including Bangladesh. Red Chittagong (RC) cattle are an economically important indigenous breed of Bangladesh. This study estimated the seroprevalence and risk factors associated with bTB and its effect on milk production in RC cattle. A total of 123 RC cattle from two selected Upazilla of Chittagong district were screened using ELISA and epidemiological and milk production data were collected using a pre-set questionnaire. The results of the study revealed an overall 11.38% seroprevalence of bTB in RC cattle. Seropositivity of bTB was found to be significantly associated with coughing, BCS and parity as revealed by the multivariate analysis of risk factors. There was also 17.83% reduction in milk production in bTB positive lactating cows compared to negative animals. To our knowledge, it is the first comprehensive study on bTB in RC cattle in Chittagong district and the results indicate that effective measures should be undertaken to control bTB in RC cattle.
Key word: Bovine tuberculosis, Red Chittagong cattle, seroprevalence, risk factors**.**

Bovine tuberculosis (bTB) is a highly contagious bacterial disease caused by *Mycobacterium bovis* which has a wide host range including domestic and wild animals and humans (O'Reilly and Daborn, 1995). It is a chronic contagious debilitating disease associated with progressive weakness or emaciation and tubercle (granuloma) formation, mainly confined to respiratory system (primarily in the lungs) and occasionally in other organs (Menzies and Neill, 2000). Cattle may serve as the main host for *M. bovis* worldwide (Gumi *et al.,* 2011), while many or most other species such as possums, pigs, cats, dogs, horses and sheep are considered to be spill-over hosts (Franck *et al.,* 2005). Tuberculosis (TB) can be transmitted from animals to humans (O'Reilly and Daborn, 1995). In humans, tuberculosis is by far the most common disease which continues to be a major cause of morbidity and mortality throughout the world (Khan *et al.,* 2008).

Tuberculosis is one of the most economically important diseases in dairy cattle. It affects over 50 million cattle world-wide resulting in economic losses of approximately US$3 billion per annum (Hermandez and Baca, 1998).The important economic losses are deprivation of the earning of foreign currency from exporting of animal and animal products and by products, imposing ban due to bTB (Barwineck and Taylor, 1996). The disease mainly spreads via aerosol in animals (Franck et al., 2005; Gumi et al., 2011). Other routes of infection such as ingestion of contaminated feeds, water and fomites have been identified (Gumi *et al.,* 2011). Various risk factors are responsible for the occurrence of this disease such as calving site, herd size, breed, presence of wild animals in proximity, mixed farming (dairy and beef) system, age and housing systems (Cetinkaya *et al.,* 1997). The disease is distributed all over the world including Bangladesh. It has been identified from 176 countries as one of the important bovine diseases causing great economic loss (Martin *et al*., 1994; Hines *et al*., 1995). The disease found more frequent in aged animals

and much more prevalent among dairy cattle than others. It was found that about 5% of tuberculus cow suffered from tuberculus metritis and 1-2% in mastitis (Hines *et al*., 1995). The case mortality of animals can reach up to 10-20%. The economic losses incur for bovine tuberculosis are condemnation of meat, milk and milk products in the market. TB in dairy cattle has been reported to cause a reduction of 17% milk production in Mexico (Anon, 1995) and 4% in a herd of USA (Hermandez and Baca, 1998). Thus, the TB is of paramount importance to cattle producers and public health authorities because of its economic and zoonotic implications (Hermandez and Baca, 1998). Bovine TB imposes serious public health and economic importance (Cosivi *et al.,* 1998) as it can interrupt international trade of animals and animal products (Ayele *et al.,* 2004). Though bTB has been controlled through 'a test-and slaughter policy' in developed countries, it still remains a major problem in most developing countries where surveillance and control activities are often insufficient or unavailable (Cosivi *et al.,* 1998) possibly due to lack of funds to carry on the whole process and provision of compensation for the slaughtered animals in these countries.

The disease can be diagnosed by using various tests including tuberculin test, serum immunoglobulin G test, histopathology, Enzyme Linked Immunosorbent Assay (ELISA), immunochromatographic assay (ICGA), Latex bead agglutination assay (LBAA) and PCR (Aranaz *et al.,* 1996; Jark *et al.,* 1997; Valente *et al.,* 1997; Costello *et al.,* 1998). Among them, tuberculin test is the widely used technique for the screening purpose (Islam et al., 2007) where purified protein derivatives (PPD) of Mycobacterium is used for single intradermal (SID) injection and the reaction read between 48 and 96 hours after injection and a positive reaction constitutes a diffuse swelling at the injection site (Radostits et al., 2000). However, there are many limitations of the SID tuberculin for the diagnosis of bTB such as delayed type hypersensitivity response, animal handling and false positive results (Koo *et al.,* 2005). To overcome the limitations of

tuberculin test, ELISA, ICGA and LBAA have been evaluated for serodiagnosis of bTB with high sensitivity and specificity (Koo *et al.,* 2005). Serological assays have shown promise as a diagnostic alternative to skin testing or culture testing for many of the animal species. Ritacco *et al.* (1990) recorded high specificity of ELISA (94.1%) for diagnosis of bTB. ELISAs measure antibody titers to *M. bovis*. By using ELISA, a total of 36.3% seroprevalence was observed with 45.7% seroprevalence in slaughtered cattle in sedentary herd in Southwestern Nigeria (Asiak *et al.,* 2007).

The indigenous zebu type cattle are thought to be much more resistant to tuberculosis than European *Bos Taurus* cattle of high yielding type, especially Holstein Friesian (Vordermeier *et al.*, 2011) and Jersey cattle of temperate breed (Radostits *et al.,* 2000). In Bangladesh, there has been a plan of government to produce more milk (white revolution) by introducing the European high yielding variety of cattle and their crosses through a nationwide artificial breeding program. In this plan, most of the indigenous dairy cattle are crossed with Holstein Friesian and Jersey breed of cattle which are known to be highly susceptible to bTB. Red Chittagong (RC) cattle are indigenous in Bangladesh developed and distributed mainly in the Chittagong district, Bangladesh. They are well known for their consistent calving interval and that's why, has a great economic importance to the farmers. However, most of them are maintained in poor hygienic management systems and thus bTB is a risk for these animals. Therefore, it is necessary to study the prevalence of bTB to know the real status of this disease in RC cattle. On this background, this study was carried out to explore the seroprevalence of bTB using ELISA, its economic importance and also to identify the associated risk factors of bTB in RC cattle in two selected Upazillas of Chittagong district, Bangladesh.

MATERIALS AND METHODS
Area of study
Several households rearing pure RC cattle were selected from six villages of Patia and Rangunia Upazilla, Chittagong. Pure RC

cattle were identified by observing the characteristic features of these cattle such as red hoof, red switch on tail and red eyelashes. RC cattle were reared in households at semi-intensive farming conditions.

Seroprevalence study

Specimen collection and storage

Blood samples (5 ml) were collected from the jugular vein of the cattle with venoject blood collecting tubes (BD Vacutainer, NJ, USA) containing no anti-coagulant. A total of 150 blood samples were collected and the samples were allowed to clot spontaneously to harvest serum. Of them, 123 good quality serum samples have been qualified for sandwich ELISA plate test. Blood sera were preserved at -20°C and they were brought to room temperature prior to performing ELISA.

Detection of *Mycobacterium bovis* antibody in serum using ELISA

The Anigen bTB Ab Test Kit (Animal Genetics Inc., 404-5, Woncheon-dong, Yeongtong-gu, Suwon-si, Kyonggi-do, Korea 443) was locally supplied by the Advance Animal Science Co. Ltd., Bangladesh. Serum samples were tested for ELISA in the laboratory of Bangladesh Livestock Research Institute (BLRI). The test was performed according to the manufacturer's instructions. Briefly, ELISA plates coated with the antigen were incubated with an equal mixture of serum and conjugate (1:100 dilution in the conjugate diluents) for 60 min at 37°C.

During the incubation, *M. bovis* antibodies present in test sample were bound to purified *M. bovis* antigen pre-coated in the well and conjugate. Following incubation, all unbound materials were removed by aspiration and washing before adding a substrate solution. The residual enzyme activity found in the well would thus be directly proportional to the conjugate concentration in specimens and was evidenced by incubating the solid-phase with a substrate solution. Figure 1 depicts ELISA with sera samples for the detection of bTB antigen. The reaction was stopped by addition of the stopping solution and colorimetric reading was performed by using a spectrophotometer at 450 nm with reference wavelength at 620 nm. Optical density (OD) values from two plates of serum samples are shown in Appendix. Sample to positive (S/P) values were calculated using following formula:

$$S/P = (Sample\ OD - Average\ OD\ of\ Negative\ control\ serum)/(Average\ OD\ of\ Positive\ control\ serum - Average\ OD\ of\ Negative\ control\ serum)$$

Data collection

All information relating the study objectives were recorded in a pre-set questionnaire. Data on animal ID, herd size, age, sex, breed, coughing history, body weight, health condition parity and milk production were recorded. Age of the cattle was determined on the basis of dentition and also by owner's information. Body condition scoring (BCS) was measured by observing the condition of
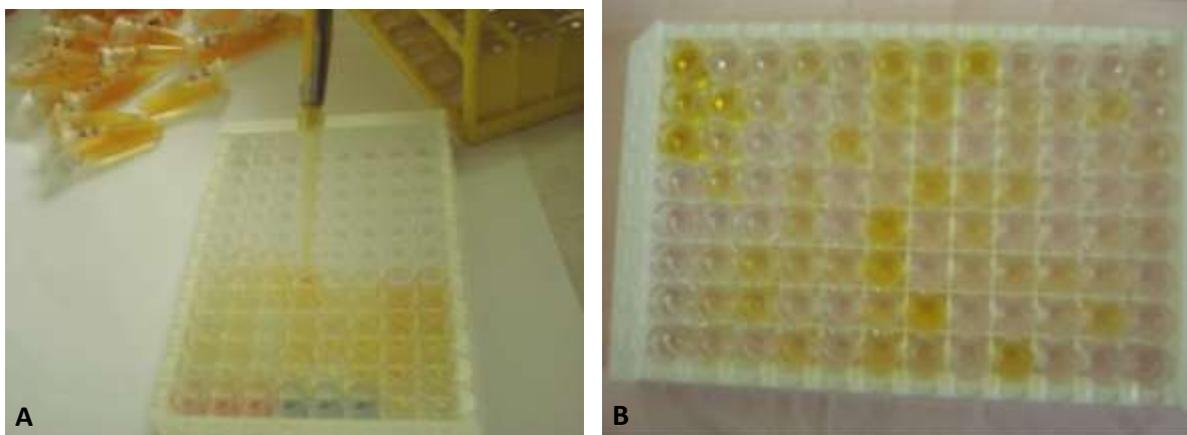

Fig 1: Performing ELISA with sera samples. (A) Bovine serum samples are added to each of the pre-coated bTB antigen well (B) Characteristic color development at the end of the test.

tail head and loin areas.

Tuberculosis and milk production

As described in Rahman and Samad (2008), to evaluate the effect of tuberculosis on milk production, daily milk production (liter/day) of each of all the milch RC cows were recorded for one week. Among 123 cattle, 15 were in dry period. The animals and their milk production values were then analyzed on the basis of two groups positive and negative to bTB.

Statistical analysis

All data were entered the Microsoft Excel 2010 spread-sheet program and then transferred to the computerized statistical package Minitab 16 for data management and analysis.

RESULTS

The seroprevalence of bTB was measured in RC cattle of Patia and Rangunia Upazilla of Chittagong district, Bangladesh, using ELISA. Table 1 represents the seroprevalence of bTB in illustrated form in different villages in two Upazillas. The overall seroprevalence of bTB in RC cattle was 11.38% (Table 1). A substantial number of RC cattle (14 out of 123 RC cattle) showed positive reaction to bTB with higher prevalence (15.34%) in Rangunia compared to Patia Upazilla (8.08%) (Table 1). The results of univariate logistic regression of risk factors are presented in Table 2. There was no significant association between seroprevalence of bTB and age as revealed by univariate analysis. Among the variables, only coughing was significantly associated

with bTB seropositivity ($p<0.05$). Variables with p-value ≤ 0.25 in the univariate analysis (Madsen *et al.,* 2013) were carried out for multivariate analysis (Table 3). Hence, other than coughing, BCS and parity were also included in multivariate analysis. Results from multivariate analysis indicated that, the risk factors (BCS, parity and coughing) which were considered into this study significantly ($p< 0.05$) contributed to positive reactivity to bTB (Table 3).

Effects of bTB on milk production

The comparison of the average milk production records of RC milch cows between the positive and negative groups to bTB showed that the average milk production was lower in bTB cows (1.38 liter/day) than the bTB negative cows (1.63 liter/ day) (Table 4), though the difference was not statistically significant ($p > 0.05$). It also accounts for more than 15% reduction in milk production in RC cattle.

DISCUSSION

In this study, seroprevalence of bTB, its economic importance and associated risk factors were estimated in RC cattle in the two selected upazillas of Chittagong district, Bangladesh. The overall seroprevalence of bTB was found 11.38% in this study which was higher than the figures reported by Pharo *et al*. (1981) and Samad and Rahman (1986) in Pabna (5.9%) and Mymensingh (3.05%) districts of Bangladesh, respectively, though in both cases the authors used Single intradermal tuberculin test. The prevalence of bTB may vary

Table 1: Seroprevalence of bTB in RC cattle in Patia and Rangunia Upazilla, Chittagong.

Villages in Upazilla	No. of animals tested	No. of bTB positive animals	Seroprevalence of bTB (%)	Upazilla wise seroprevalence of bTB (%)
Rangunia				
Sayed Bari	18	04	22.22	
Uttor Ghatchek	25	02	08.00	15.34
Dakkhin Ghatchek	19	03	15.79	
Patia				
Shikolbaha	20	02	10.00	
Char Patharghata	16	01	06.25	8.08
Char Lakshya	25	02	08.00	
Total	**123**	**14**	**11.38**	

Table 2: Univariate analysis of risk factors for seropositive RC cattle using General Linear models (GLM) (at 95% CI).

Variables	No. of cattle examined	No. of positive cases	Prevalence (%)	Z value	P value
Age					
Young (< 3 years)	57	6	10.53	-	
Adult (> 3 years)	66	8	12.12	0.38	0.43
BCS					
Good (>3-4)	27	2	7.41	-	-
Medium (2-3)	55	5	9.09	0.24	0.55
Poor (1-2)	41	7	17.07	2.53	0.19
Coughing					
No	77	3	3.90	-	-
Yes	46	11	23.91	2.4	0.04*
Parity					
≤2-3 calves	44	4	9.09	-	-
≥4 calves	79	10	12.67	-0.23	0.14

*= significant at p<0.05; CI= confidence interval

Table 3: Multivariate analysis of risk factors for seropositive RC cattle using GLM (at 95% CI).

Variables	Odds ratio	Z value	P value
BCS			
Good (>3-4)	1		
Medium (2-3)	1.03	0.35	
Poor (1-2)	4.4	3.12	0.02*
Parity			
≤2-3 calves	1		
≥4 calves	2.3	-0.28	0.01*
Coughing			
No	1		
Yes	6.7	2.6	0.00*

*= significant at p<0.05; CI= confidence interval

according to the region and season of the year (Khan *et al.,* 2008) and can also be different with the type of diagnostic test used, health status of cattle and various husbandry practices. Cleaveland *et al.* (2007) reported an overall 1.3% prevalence of bTB by using single comparative intradermal tuberculin test in the eastern zone of Tanzania. Similar result was also observed by Shirima *et al.* (2003). The prevalence in this study is also higher than the prevalence (2.0%) reported in southeast Ethiopia by Gumi *et al.* (2012).

The seroprevalence of bTB was measured in other bovine species and there was variability in those reports too. For example, Han *et al.* (2013) found a 2.2 % seroprevalence of bTB in yaks on the Qinghai–Tibet Plateau of China but Lamichhaney (2010) reported 11.71% prevalence of antibodies to *M. bovis* in wild buffaloes in eastern Nepal. Furthermore, Khan *et al.* (2008) reported 10.06 % prevalence of bTB in buffaloes at a livestock farm in Punjab, Pakistan. The seroprevalence of bTB may also vary in livestock production systems at different altitudes. Tschopp *et al.* (2010) found 2 % prevalence of bTB antibodies in cattle reared at 3,000 m above the sea level. However, a high percentage (37.17 %) of bTB antibodies has been detected in cattle reared in high lands of Cameroon (Awah-Ndukum *et al.,* 2012). In our report, a higher seroprevalence (15.34%) was observed in Rangunia Upazilla which is a hilly area

compared to that of Patia Upazilla (8.08%) which is a low land area.

Cleaveland *et al.* (2007) observed around 1% prevalence of bTB in cattle reared under pastoral sector which is in contrast to the present study where RC cattle showed 11.38% seroprevalence of bTB reared under family based semi-extensive production system. This could be because of poor hygienic practices by the owners as observed during sample collection. However, further studies will be required to clarify this.

Present study showed that seroprevalence of bTB in RC cattle was 11.38% (Table 1). Mahmud *et al.* (2014) also reported a 13.33% prevalence of bTB in local breed of Sirajgonj district, Bangladesh. A similar high prevalence (13.2%) of bTB was also reported in pastoral indigenous cattle in the southern highlands of Tanzania (Kazwala et al., 2001). The finding in this study differs from the findings of Rahman and Samad (2008) in which a 30% prevalence of bTB was reported in RC cattle. Though the method used by the authors (immunochromatographic assay) was quite different from that of ELISA. In this study, samples were collected from RC cattle reared scatteredly among various villages of Patia and Rangunia Upazilla, Chittagong. On the other hand, Rahman and Samad (2008) conducted their study in the RC cattle reared in intensive farming system of Bangladesh Agricultural University, Mymensingh. Due to the contagious nature of the disease, cattle reared in an intensive farming condition will be more affected with bTB which could probably be the reason for high prevalence of bTB in that report.

In the present study, coughing was found significantly associated with bTB (Tables 2 and 3) which is in agreement with Sauter and Morris (1995) and also match with the statement that aerosol is the main route of transmission of bTB in animals (Franck *et al.,* 2005; Gumi *et al.,* 2011). Findings from this study have indicated no significant association between age and seropositivity of bTB (Table 2). However, it has previously been reported that the older animals were within high risk of infection (Tschopp *et al.,* 2009). Likewise, Phillips *et al.* (2002) and Cleaveland *et al.* (2007)

suggested that older animals are more susceptible to tuberculosis. Kazwala et al., (2001) also found that older cattle were more affected by the disease than yearlings and calves. No significant association between age and bTB seropositivity could perhaps be due to the low sample size in this study, although another variable, parity, showed significant association with bTB in this study (Table 3). Parity (number of calves born) has a direct relationship with age in animals.

In our study, poor BCS has shown significant association with bTB seropositivity (Table 3). This is in contrast with the previous findings by Amen *et al.* (2002), Munyeme *et al.* (2009) and Katale *et al.* (2013) in which most of positive reactors cattle had good body condition as compared to negative reactors. Poor body condition of RC cattle could be attributed by improper supply of nutritious concentrate feed by the farmers and also lack of enough pasture lands in the study area as observed during sample collection period.

The economic importance of bTB was measured by comparing the milk production of bTB positive and negative RC cows. Previously, Rahman and Samad (2008) estimated that RC cows with bTB produced less milk than the cows negative to bTB and tuberculosis was associated with a 17.83% decrease in milk production. In our study, similar results were observed (15.34% reduction in milk production; Table 4) which was in agreement with the earlier report of 17% reduction of milk production due to bTB in Mexico (Anon, 1995). However, a lower (4%) percentage of milk production has also been reported in a herd infected with bTB in USA (Hermandez and Baca, 1998). There is a negative relationship between clinical mastitis and milk production (Miller *et al*., 1993; Shaw and Bean, 1995) which could also be same in case of bTB infection as Mdegella *et al.* (2004) and Durnez *et al.* (2009) found a prevalence of 14% and 19% of atypical mycobacteria in milk samples. Mycobacterium in milk samples could expose milk consumers at great risk of contracting milk-borne zoonotic infections. Consumption of unpasturalized milk was

Table 4. Comparison of milk production between positive and negative reactors to bTB in RC lactating cattle.

Reactions to bTB	Animal numbers	Milk production (liter/day)		Reduction of milk production (%)
		Range	Average	Difference	
Negative	95	0.75-2.50	1.63		
Positive	13	0.50-2.25	1.38	-0.25	15.34
Total	108				

observed as a common practice in most village communities in the study areas.

Taken together, this can be concluded from the present study that the prevalence of bTB in RC cattle in Chittagong district is quite alarming and therefore, proper measures should be implemented to control this disease in order to lessen the risk factors and also to cut the economic losses caused by bTB.

REFERENCES

1. Amen, G., Amenu, K. & Tibbo, M. (2002). Prevalence and risk factor assessment in cattle and cattle owners in Wuchale-Jida District, Central Ethiopia. International Journal of Applied Research in Veterinary Medicine. 1:17-26.

2. Anon. (1995). Campananacional contra la tuberculosis bovina (Mycobacterium bovis). Mexico, DF: Norma Official Mexicana. pp: 1-58.

3. Aranaz, A., Lie'bana, E., Mateos, A., Domi'nguez, L., Vidal, D., Domingo, M., Gonzolez, O., Rodriguez, F.E.F., Bunschoten, A.,Van Embden, J.D.A & Cousins, D. (1996). Spacer oligonucleotide typing of Mycobacterium bovis strains from cattle and other animals, a tool for studying epidemiology of tuberculosis. Journal of Clinical Microbiology 34:2734-40.

4. Asiak, I.E., Ohore, O.G., Emikpe, B.O., Abatan, O.O. & Ockiya, M.A. (2007). The use of ELISA in the detection of bovine tuberculosis in slaughtered trade cattle and sedentary herds in South West Nigeria. Journal of Animal and Veterinary Advances, 6: 883-86.

5. Awah-Ndukum, J., Kudi, A.C., Bah, G.S., Bradley, G., Tebug, S.F., Dickmu, P.L., Njakoi, H.N. & Agharih, W.N. (2012). Bovine tuberculosis in cattle in the highlands of Cameroon: seroprevalence estimates and rates of tuberculin skin test reactors at modified cutoffs. Veterinary Medicine International. 2012:798502.

6. Ayele, W.Y., Neill, S.D., Zinsstag, J., Weiss, M.G. & Pavlik, I. (2004). Bovine tuberculosis: an old disease but a new threat to Africa. The international Journal of Tuberculosis and Lung Disease. 8:924-37.

7. Barwineck, F. & Taylor, N.M. (1996). Bovine tuberculosis assessment of the socio-economic importance of tuberculosis in Turkey and possible strategies for control or eradication. Ankara Eschborn Deutsche Gesellschaft fur Technische Zusammenarbeit. pp: 33-45.

8. Cetinkaya, B., Erdogan, H.M. & Morgan, K.L. (1997). Risk factors for bovine paratuberculosis. The univariate analysis of risk factors for bovine paratuberculosis. Turk

Veterinerlik-ve-Hayvancilik dergisi. 21:297-302.

9. Cleaveland, S., Shaw, D.J., Mfinanga, S.G., Shirima, G., Kazwala, R.R., Eblate, E. & Sharp, M. (2007). Mycobacterium bovis in rural Tanzania: risk factors for infection in human and cattle populations. Tuberculosis. 87:30-43.

10. Cosivi, O., Grange, J.M., Daborn, C.J., Raviglione, M.C., Fujikura, T., Cousins, D., Robinson, R.A., Huchzermeyer, H.F., de Kantor, I. & Meslin, F.X. (1998). Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. Emerging Infectious Diseases. 4:59-70.

11. Costello, E., Doherty, M.L., Monaghan, M.L., Quigley, F.C. & O'Reilly, P.F. (1998). A study of cattle-to-cattle transmission of Mycobacterium bovis infection. The Veterinary Journal. 155:245-50.

12. Durnez, L., Sadiki, H., Katakweba, A., Machang'u, R., Kazwala, R., Leirs, H. & Portaels, F. (2009). The prevalence of Mycobacterium bovis infection and atypical mycobacterioses in cattle in and around Morogoro, Tanzania. Tropical Animal Health and Production. 41:1653-59.

13. Franck, B., Maria, L.B., Marie, F.T & Laurence, A.G. (2005). Zoonotic aspects of Mycobacterium bovis and Mycobacterium avium-intracellulare complex (MAC). Veterinary Research. 36:411-36.

14. Gumi, B., Schelling, E., Firdessa, R., Aseffa, A., Tschopp, R., Yamuah, L., Young, D. & Zinsstag, J. (2011). Prevalence of bovine tuberculosis in pastoral cattle herds in the Oromia region, southern Ethiopia. Tropical Animal Health and Production. 43:1081-87.

15. Gumi, B., Schelling, E., Firdessa, R., Erenso, G., Biffa, D., Aseffa, A., Tschopp, R., Yamuah, L., Young, D. & Zinsstag, J. (2012). Low prevalence of bovine tuberculosis in Somali pastoral livestock, southeast Ethiopia. Tropical Animal health and Production. 44: 1445-50.

16. Han, Z., Gao, J., Shahzad, M., Meng, X., Liu, M., Zhang, K., Zhang, D., Guo, A., Sizhu, S. & Li, J. (2013). Seroprevalence of bovine tuberculosis infection in yaks (Bos grunniens) on the Qinghai-Tibetan Plateau of China. Tropical Animal Health and Production. 45:1277-79.

17. Hermandez, J. & Baca, D. (1998). Effect of tuberculosis on milk production in dairy cows. Journal of the American Veterinary Medical Association. 213:851-54.

18. Hines, M.E., Kreeger, J.M. & Smith, P.K. (1995). Microbacterial infections of animals: Pathology and Pathogenesis. Laboratory Animal Science. 45:334-351.

19. Islam, M.M., Siddique, M.A.R., Haque, M.A., Baki, M.A., Majumder, S., Parrish, J.J. & Shamsuddin, M. (2007). Screening some major communicable diseases of AI bulls in Bangladesh. Livestock Research for Rural Development. 19:79.

20. Jark, U., Ringena, I., Franz, B., Gerlach, G.F. & Beyerbach, M. (1997). Development of an ELISA technique for serodiagnosis of bovine paratuberculosis. Veterinary Microbiology. 57:189-98.

21. Katale, B.Z., Mbugi, E.V., Karimuribo, E.D., Keyyu, J.D., Kendall, S., Kibiki, G.S., Godfrey-Faussett, P., Michel, A.L., Kazwala, R.R., van Helden, P. & Matee, M.I. (2013). Prevalence and risk factors for infection of bovine tuberculosis in indigenous cattle in the Serengeti ecosystem, Tanzania. BMC Veterinary Research. 9:267.

22. Kazwala, R.R., Kambarage, D.M., Daborn, C.J., Nyange, J., Jiwa, S.F. & Sharp, J.M. (2001). Risk factors associated with the occurrence of

bovine tuberculosis in cattle in the Southern Highlands of Tanzania. Veterinary Research Communications. 25:609-14.

23. Khan, I.A., Khan, A., Mubarak, A. & Ali, A. (2008). Factors affecting prevalence of bovine tuberculosis in Nili Ravi buffaloes. Pakistan Veterinary Journal. 28:155-58.

24. Koo, H.C., Park, Y.H., Ahn, J., Waters, W.R., Palmer, M.V., Hamilton, M.J., Barrington, G., Mosaad, A.A., Park, K.T., Jung, W.K., Hwang, I.Y., Cho, S.N., Shin, S.J. & Davis, W.C. (2005). Use of rMPB7D protein and ESAT-6 peptide as antigens for comparison of the Enzyme-linked immunosorbent, immunochromatographic and latex based agglutination assays for serodiagnosis of bovine tuberculosis. Journal of Clinical Microbiology. 43:4498-506.

25. Lamichhaney, S. (2010). Prevalence and Risk Factor Assessment of Bovine Tuberculosis: Impact of the disease in wildlife, domestic animals and human interface at Koshi Tappu Wildlife Reserve in Eastern Nepal. Lambert Academic Publishing, Saarbrücken, Germany.

26. Madsen, J.M., Zimmerrmann, G.N., Tiimmons, J. & Tablante, N.L. (2013). Avian influenza seroprevalence and biosecurity risk factors in Maryland backyard poultry: a cross-sectional study. PLoS One. 8:e56851.

27. Mahmud, M.A.A., Belal, S.M.S.H. & Shoshe, N.Z. (2014). Prevalence of bovine tuberculosis in cattle in the selected Upazilla of Sirajgonj district in Bangladesh. Bangladesh Journal of Veterinary Medicine. 12:141-45.

28. Martin, S.W., Dietrich, R.A., Genno, P., Heuschele, W.P., Jones, R.L., Koller, M., Lee, J.D., Lopez, H.C., Moon, H.W., Robinson, R.A., Smith, P.L. & Williams, G.W. (1994). Livestock disease eradication: Evaluation of the cooperative state federal bovine tuberculosis eradication program. National Academy of Sciences, Washington DC. pp: 71-97.

29. Mdegela, R.H., Kusiluka, L.J.M., Kapaga, A.M., Karimuribo, E.D., Turuka, F.M., Bundala, A., Kivaria, F., Kabula, B., Manjurano, A., Loken, T. & Kambarage, D.M. (2004). Prevalence and determinants of mastitis and milk-borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts in Eastern Tanzania. Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health. 51:123-28.

30. Menzies, F.D. & Neill, S.D. (2000). Cattle-to-cattle transmission of bovine tuberculosis. Veterinary Journal. 160:92-106.

31. Miller, G.Y., Bartlett, P.C., Lance, S.E., Anderson, J. & Heider, L.E. (1993). Cost of clinical mastitis and prevention in dairy herds. Journal of the American Veterinary Medical Association. 202:1230-36.

32. Munyeme, M., Muma, J.B., Samui, K.L., Skjerve, E., Nambota, A.M., Phiri, I.G.K., Rigouts, L. & Tryland, M. (2009). Prevalence of bovine tuberculosis and animal level risk factors for indigenous cattle under different grazing strategies in the livestock/ wildlife interface areas of Zambia. Tropical Animal health and Production. 41:345-52.

33. O'Reilly, L.M. & Daborn, C.J. (1995). The epidemiology of Mycobacterium bovis infections in animals and man, a review. Tubercle and Lung Disease. 76,1-16.

34. Pharo, H.J., Motalib, A., Routledge, S.F. & Alam, S. (1981). The prevalence of bovine tuberculosis in the Bangladesh Cattle Development Project.

Bangladesh Veterinary Journal. 15, 53-56.

35. Phillips, C.J., Foster, C.R., Morris, P. & Teverson, R. (2002). Genetic and management factors that influence the susceptibility of cattle to Mycobacterium bovis infection. Animal Health Research Reviews. 3:3-13.

36. Radostits, O.M., Gray, G.C., Blood, D.C. & Hinchelift, K.W. (2000). A text Book of Diseases of Cattle, Sheep, Pig, Goat and Horses. In: Veterinary Medicine. 9th ed. Harcourt Publisher Ltd., London. pp: 909-18.

37. Rahman, M.M. & Samad, M.A. (2008). Prevalence of bovine tuberculosis and its effects on milk production in Red Chittagong cattle. Bangladesh Journal of Veterinary Medicine. 6:175-78.

38. Rittacco, V., López, B., Barrera, L., Nader, A., Fliess, E. & de Kantor, I.N. (1990). Further evaluation of an indirect enzyme linked immunosorbent assay for the diagnosis of bovine tuberculosis. Zentralblatt für Veterinärmedizin. Reihe B. 37:19-27.

39. Samad, M.A. & Rahman, M.S. (1986). Incidence of bovine tuberculosis and its effect on certain blood indices in dairy cattle of Bangladesh. Indian Journal of Dairy Science. 39:3-6.

40. Sauter, C.M. & Morris, R.S. (1995). Dominance hierarchies in cattle and red deer (Cervus elaphus): their possible relationship to the transmission of bovine tuberculosis. New Zealand Veterinary Journal. 43:301-305.

41. Shaw, A.D. & Bean, A.L. (1995). The effect of mastitis upon milk production. Journal of Dairy Science. 18, 353-57.

42. Shirima, G.M., Kazwala, R.R. & Kambarage, D.M. (2003). Prevalence of bovine tuberculosis in cattle in different farming systems in the Eastern zone of Tanzania. Preventive Veterinary Medicine. 57:167-72.

43. Tschopp, R., Schelling, E., Hattendorf, J., Aseffa, A. & Zinsstag, J. (2009). Risk factors of bovine tuberculosis in cattle in rural livestock production systems of Ethiopia. Preventive Veterinary Medicine. 89, 205-11.

44. Tschopp, R., Aseffa, A., Schelling, E., Berg, S., Hailu, E., Gadisa, E., Habtamu, M., Argaw, K. & Zinsstag, J. (2010). Bovine tuberculosis at the wildlife-livestock-human interface in Hamer Woreda, South Omo, Southern Ethiopia. PLoS One. 17:5-8.

45. Valente, C., Cuteri, V., Giandomenico, R.Q., Gialletti, L. & Franciosini, M.P. (1997). Use of an experimental chicks model for paratuberculosis enteritis (Johne's disease). Veterinary Research. 28, 239-46.

46. Vordermeier, M., Ameni, G. & Glass, E.J. (2011). Cytokine responses of Holstein and Sahiwal zebu derived monocytes after mycobacterial infection. Tropical Animal Health and Production. 44, 651-55.