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SEROPREVALENCE AND RISK FACTOR ASSESSMENT OF BOVINE TUBERCULOSIS IN CROSSBRED CATTLE OF CHATTOGRAM METROPOLITAN AREA, BANGLADESH

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ABSTRACT

This study was aimed to measure the seroprevalence and risk factors associated with bovine tuberculosis (bTB) in crossbred cattle in Chattogram Metropolitan area, Bangladesh. A total of 289 crossbred cattle were screened using ELISA and their epidemiological data were collected using a pre-set questionnaire. An overall 5.88% seroprevalence of bTB was found in crossbred cattle in this study. Seroprevalence of bTB in crossbred cattle was significantly associated with breed, coughing, age and parity as analyzed by the multivariate analysis of risk factors. The results of the present study prioritize the implementation of appropriate measures to control bTB in crossbred cattle in this region.

Key words: bovine tuberculosis, crossbred cattle, risk factors, seroprevalence

INTRODUCTION

Bovine tuberculosis (bTB) is a chronic contagious bacterial disease caused by Mycobacterium bovis. Tuberculosis in cattle can be diagnosed by 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 (Valente et al., 1997; Costello et al., 1998; Didugu et al., 2016). Among them, tuberculin test is the widely used technique for screening TB in cattle (Islam et al., 2007). However, traditional tests such as single intradermal test are time consuming, and molecular diagnostic tests such as PCR need technical expertise, whereas ELISA is simple, reliable and easy to perform (Didugu et al., 2016).

BTB is distributed all over the world including Bangladesh. In our previous study, the seroprevalence of bTB in Red Chittagong (RC) cattle, an indigenous breed of Bangladesh, was studied in some areas of Chattogram (previously Chittagong) district (Chakraborty et al., 2015). Pure temperate breed cattle such as Holstein Friesian (HF) (Vordermeier et al., 2011) and Jersey (Radostits et al., 2000) are presumed to be more susceptible to tuberculosis than the indigenous cattle. The government of Bangladesh has a cattle breeding policy to induce white revolution (i.e. producing more milk) all over the country by crossing European high yielding variety of cattle (especially HF and Jersey) with indigenous dairy cattle of Bangladesh. However, as mentioned earlier, these breeds are known to be highly susceptible to bTB and the prevalence of bTB in their crosses is yet to be explored. Therefore, it is crucial to know the prevalence of bTB in crossbred cattle. On this background, this study was conducted to determine the seroprevalence of bTB using ELISA and also to identify the associated risk factors of bTB in crossbred cattle reared in Chattogram metropolitan (CMP) area, Bangladesh.

MATERIALS AND METHODS

Area of study, cattle and their management, sample size calculation

This cross-sectional study was conducted in CMP area of Chattogram district, located around 300 km south-east from the capital city Dhaka in Bangladesh (Wikipedia, 2018). The area has a latitude and longitude of 22°22′0″N 91°48′0″E.

In this study, serum samples were collected from crossbred HF and Jersey cattle reared in well-managed intensive farming system. Randomly 18 dairy farms were chosen from the CMP area based on getting accessibility to the farm. Of them, six were large farms those bearing minimum 20 or more milk cows, seven were medium farm bearing not less than 10 cows and five were small farms bearing not more than 5 cows. The cows that were in or above two parity were selected for collecting blood samples. Crossbred cattle were defined as cattle which were mainly bred by crossing local cattle with HF (F×L) or Jersey cattle (J×L) as per record obtained from the farms.

The numbers of cattle were selected using following criteria by simple random sampling method. To determine the minimum sample size, an expected prevalence of 15% was taken into consideration based on the prevalence of bTB in indigenous cattle (11.38%) in two upazillas of Chattogram district (Chakraborty et al., 2015), as pure bred cattle are thought to be more susceptible to tuberculosis than the indigenous cattle (Radostits et al., 2000; Vordermeier et al., 2011). The desired minimum sample size for the study was calculated using following formula (Thursfield, 2005) with 95% confidence interval and 5% absolute precision: $n = 1.96^2 PE\ (1-PE)/d^2$
Where PE= expected prevalence; d= absolute precision; n= sample size.

The required sample size was 196 cattle. However, blood samples were collected from 300 available cattle from the farms of the study area and among them, 289 good quality sera were used for ELISA.

Seroprevalence study using ELISA

Internationally recognized guidelines for animal welfare were followed during handling of cattle for blood collection. Blood samples (5 ml) were collected from the jugular vein of the cattle with venoject blood collecting tubes (BD Vacutainer, NJ, USA) with no anti-coagulant. Blood sera were preserved at -20°C and they were brought to room temperature prior to ELISA procedures. 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 using ELISA in the laboratory of Chattogram Veterinary and Animal Sciences University (CVASU). The test was performed according to the manufacturer’s instructions as described previously (Chakraborty et al., 2015).

Data collection

During sample collection, all related epidemiological data were recorded in a pre-set questionnaire. Data on age, sex, breed, body weight, health status and parity of the animal were recorded. Age of the cattle was determined on the basis of owner’s information and also by dentition (Pace and Wakeman, 2003). Animals were categorized into two age groups as young (<3 years) and adult (> 3 years). Health status of cattle was measured by BCS (body condition score) by observing the condition of tail head and loin areas and was categorized as good (3-4), medium (2-3) and poor (1-2) (Nicholson and Butterworth, 1986).

Statistical analysis

All data were entered the Microsoft Excel 2010 spread-sheet program and then transferred to the computerized statistical package Minitab 18 for data management and analysis. General linear models (GLM) were used to assess the association between different risk factors and prevalence of bTB. A 95% confidence interval was used to determine whether there is significant difference between measured parameters (Thrusfield, 2005). A statistically significant association between variables was calculated at $p<0.05$.

RESULTS

In this study, seroprevalence of bTB was measured by ELISA in crossbred cattle of CMP area, Bangladesh. Table 1 illustrates the seroprevalence of bTB in different farms. The overall seroprevalence of bTB in crossbred cattle was 5.88% (17 out of 289 cattle) (Table 1).

The seroprevalence of bTB in crossbred cattle was varied according to the size of the farm as shown in Table 1. In large dairy farms category, 168 cattle were tested and 10 (5.95%) of them were found serologically positive to bTB. In this category, the cattle of Boss vita dairy farm showed highest seroprevalence (14.2%) of bTB whereas zero seroprevalence was found in Afsar and Mahsin dairy farm. In medium-sized dairy farms, seven (07) cattle (7.00%) were found positive among 100 cattle tested for bTB. Of which, the cattle of Super dairy farm and Kausaruzzaman dairy farm showed the highest (11.7%) and lowest (0.00%) seroprevalence of bTB, respectively. No cattle from small sized dairy farms showed positive reaction to bovine tuberculosis (Table 1).

Table 1: Farm-wise seroprevalence of bTB in crossbred cattle

Name of the dairy farm	No. of cattle tested	No. of bTB positive cattle	Farm-wise seroprevalence of bTB (%)	Total seroprevalence (%)
Large farms				
Afsar Dairy Farm	23	00	0.00	5.95
Noman Dairy Farm	20	02	10.0	
Boss Vita Dairy Farm	21	03	14.2	
Mahsin Dairy Farm	22	00	0.00	
Molla Dairy Farm	24	03	12.5	
Nahar Dairy Farm	58	02	3.45	
Medium farms				
Musa Dairy Farm	13	01	7.69	7.00
Kausaruzzaman Dairy Farm	17	00	0.00	
Noma Dairy Farm	12	01	8.33	
Aftab Dairy Farm	14	01	7.14	
Sagar Dairy Farm	16	01	6.25	
Chowdhury Dairy Farm	11	01	9.09	
Super Dairy Farm	17	02	11.7	
Small farms				
Maji Dairy Farm	04	00	0.00	0.00
CVASU Dairy Farm *	03	00	0.00	
Atik Dairy Farm	05	00	0.00	
Alam Dairy Farm	05	00	0.00	
Aslam Dairy Farm	04	00	0.00	
Total crossbred cattle	**289**	**17**		**5.88**

*CVASU- Chattogram Veterinary and Animal Sciences University

The results of univariate logistic regression of risk factors are presented in Table 2. Among the variables, history of coughing was significantly associated with seropositivity of bTB ($p<0.05$) in this analysis.

Table 2. Univariate analysis of risk factors for cattle positive for tuberculosis using General Linear Models (GLM)

Animal level risk factors	**Proportional % of sampled animals (No./Total)**	**OR**	**95% CI**	**p value**
Breed				
J × L cross	2.38 (2/84)	1	-	-
F × L cross	7.32 (15/205)	2.13	1.27; 3.44	0.13
Age				
Young (< 3 years)	3.38 (5/148)	1	-	-
Adult (> 3 years)	8.51 (12/141)	2.67	1.39; 5.23	0.14
BCS				
Good (> 3-4)	1.59 (1/63)	1	-	-
Medium (2-3)	4.49 (7/156)	2.46	0.63; 37.3	0.67
Poor (1-2)	12.8 (9/70)	5.18	1.51; 42.4	0.79
Coughing				
No	3.70 (6/162)	1	-	-
Yes	8.66 (11/127)	2.28	1.62; 4.22	0.03
Parity				
≤2-3 calves	5.37 (8/149)	1	-	-
≥4 calves	6.43 (9/140)	1.03	0.03; 31.7	0.18
Farm level risk factors				
Management				
Good	1.82 (1/55)	1	-	-
Moderate	4.41 (6/136)	2.25	0.36; 4.12	0.32
Poor	10.2 (10/98)	8.87	0.48; 3.43	0.27
Source of cattle				
Own cattle	5.77 (9/156)	1	-	-
Replacement stock	6.02 (8/133)	1.01	0.21; 43.6	0.87

OR= Odds ratio; CI= Confidence interval

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, breed, age and parity were also included in multivariate analysis. Results from multivariate analysis indicated that the risk factors (breed, age, parity and coughing) that were considered for this analysis showed significantly ($p<0.05$) positive association to bTB (Table3).

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

Variables	**Odds ratio**	**Z value**	**p value**
Breed			
J × L cross	1		
F × L cross	2.13	2.32	0.02
Age			
Young (< 3 years)	1		
Adult (> 3 years)	2.67	3.14	0.03
Coughing			
No	1		
Yes	6.7	2.6	0.00
Parity			
≤2-3 calves	1		
≥4 calves	2.3	-0.28	0.01

OR= Odds ratio; CI= Confidence interval

DISCUSSION

In this study, the crossbred cattle of Chattogram metropolitan area, Bangladesh were screened for measuring the seroprevalence of bTB and its associated risk factors. The prevalence of bTB may differ according to various factors such as locality, season of the year, type of diagnostic test used, health status of cattle and husbandry practices (Khan et al., 2008). Using ELISA, the overall seroprevalence of bTB was recorded 5.88% in this study which was lower than the prevalence of bTB in indigenous RC cattle (11.38%) as reported in our previous study (Chakraborty et al., 2015). A higher prevalence of bTB in exotic dairy breeds compared to indigenous cattle was reported (Acha and Szyfres, 2003), which is in agreement with the present study. Low prevalence of bTB in crossbred cattle than that of indigenous cattle may be attributed to improved farming practices, availability of timely veterinary services and better farmer awareness. However, a higher prevalence of bTB in crossbred cattle (3.22%) compared to non-descriptive cattle (2.08%) was also reported (Didugu et al., 2016).

Country-wise prevalence of bTB using ELISA also varied as 0.87% prevalence was observed in India (Didugu et al., 2016) and 2% in Ethiopia (Gumi et al., 2011) which are lower than the findings of this study, whereas higher values of prevalence compared to our study such as 13.82% in India (Prakash et al., 2015), 50% in Egypt and 51.92% in Iraq (Barak, 2012) were also reported.

Variability in seroprevalence of bTB was reported in other bovine species too such as 2.2 % in yaks (Han et al., 2013) which is lower compared to our findings and 10-11% (Khan et al., 2008; Lamichhaney, 2010) in buffaloes which is higher than the present study.

Pure HF cattle are susceptible to bTB (Vordermeier et al., 2011). Our study revealed that their crosses are also susceptible to bTB. Das et al. (2018) mentioned that HF crosses are more susceptible than Jersey crosses which is in agreement with our findings. In another study, HF crosses were also showed higher prevalence of bTB over Sindhi and Sahiwal crosses (Mahmud et al., 2014).

This study showed a significant variation on prevalence of bTB in different age groups of cattle such as age group >3 years showed a higher prevalence than that of other age group. Several previous reports (Phillips et al., 2002; Cleaveland et al., 2007; Tschopp et al., 2009; Biswas et al., 2017) claimed that aged cattle are more prone to tuberculosis infection due to diminution of immune status, a statement which may also be applicable here. Age has a direct relationship with parity (number of calves born) and it also showed significant association with bTB in this study.

A significant association was observed between coughing and bTB in the present study which is in agreement with other previous reports (Sauter and Morris, 1995; Chakraborty et al, 2015). This justifies the statement that aerosol is the main route of transmission of bTB in animals (Franck et al., 2005; Gumi et al., 2011).

In conclusion, our study revealed that bTB is prevalent in crossbred cattle in CMP area, Bangladesh and hence, proper control measures should be implemented to reduce the risk factors of bTB which in turn will help to diminish the economic losses and zoonotic spread incurred by bTB.

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