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Sero-Prevalence of Bovine Tuberculosis in Cattle Population of Haryana State

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ABSTRACT

The present study was conducted to ascertain the sero-prevalence of bovine tuberculosis in cattle population of Haryana state. A total of 100 blood samples were collected from randomly selected cattle of a few gaushalas and tested with a commercially available kit to detect the presence of antibodies against Mycobacterium antigens in serum samples. Out of total 100 serum samples, 69 (69 %) samples were found positive for presence of antibodies against M. bovis (BTB) or/ and M. avium (NTM/ Avian TB). The results were compared using chi-square test. The comparison of results for antibodies against M. bovis was found statistically non-significant (p> 0.05) with respect to breed, age, location and gender. The results for antibodies against M. avium were found statistically non-significant (p> 0.05) with respect to breed. However, the results were statistically significant with respect to age, location and gender (p< 0.05). It was evident from the study that the sero-prevalence of bovine tuberculosis was considerably high in the selected cattle population. It could be predicted that cattle were infected with the organism and could be a potential source of infection for other animals as well humans also.

Key words: *Aerosol, Bovine tuberculosis, Sero-prevalence, Zoonotic*

INTRODUCTION

Bovine tuberculosis (BTB) is a chronic debilitating infectious disease of cattle caused by *Mycobacterium bovis* (*M. bovis)*, a member of *Mycobacterium tuberculosis* complex (MTC). Besides cattle, it also affects other domestic animals, wildlife, and humans with worldwide annual losses to agriculture of $3 billion[16,17]. *Mycobacterium bovis* infection spread to cattle primarily through infectious aerosol inhalation, but spread by ingestion of infected milk or ingestion of contaminated pasture or feed has also been reported. Cutaneous, congenital and genital infections have been rarely recorded[2]. This disease is widely distributed throughout the world and mainly affects animals with occasional human involvement.

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Bovine TB can have an impact on the national and international economy as it can have a considerable direct effect on milk and meat production and animal reproduction, affects on ecosystem and human health[15].

In developed countries, strict test and slaughter policy has resulted in the dramatic decline of BTB incidence. Although still present in some developing countries due to lack of resources to apply expensive test and slaughter schemes and sporadically applied control measures[5]. Therefore, there is a need to reorient the available diagnostic tests and policies on animal husbandry to achieve at least low BTB incidence in India.

Although most of the BTB control programs depend on detection of cellular immune response, but serological assays may be useful when cellular immunity based tests fail to detect the infected animals in advanced stages of disease. There is currently no single test which can identify all the infected animals at every stage of infection. A combination of approaches is likely to be needed to detect the actual burden of disease[9]. The disease control is aimed at early detection and removal of infected animals to prevent the spread of disease to other susceptible animals and humans[14]. This study was designed to know the sero-prevalence of BTB in few randomly selected cattle of Haryana state.

MATERIALS AND METHODS

Animal selection and sample collection

The study was approved by the institutional animal ethics committee. A total of 100 blood samples were collected from randomly selected cattle of a few gaushalas of Haryana, India. Calves of less than 1 year age, animals in advanced pregnant and 1 month post-partum animals were excluded in the study (Table 1). About 5ml of whole blood from the jugular vein of each cattle was collected in serum vials having clot activator. The blood sample was allowed to clot under a cool shade in order to obtain serum without centrifugation. The vials were labeled corresponding to the identity of the animal. The obtained serum was collected into 2 ml storage vials. The test was performed on the same day of sample collection. The left over serum samples were stored at -20^{0}C. The results were also compared using chi-square ($\chi 2$) test.

Table 1: Categories of animals from which samples were collected

Variables	No. of samples
Breed	
Cross-bred	40
Hariana	25
Sahiwal	35
Location	
‘A’ gaushala	30
‘B’ gaushala	40
‘C’ gaushala	30
Gender	
Male animals	30
Female animals	70
Age groups	
Heifer	23
Adult	77
Total samples collected	**100**

Rapid kit test (Immuno-chromatographic assay)

A commercially available kit (Bovine TB Ab Rapid Test Kit, Genomic Biotech, Hyderabad) was used to detect the presence of antibodies in serum. The test was performed as per the manufacturer's protocol. Briefly, the kit components were placed at room temperature (19-25^{0}C). Then 5 µl (1 drop) of serum sample was added into the sample well marked on the kit. Thereafter, 2 drops of diluent were added to the same well. The results were read after 20 min. A burgundy coloured band at 'C' always present for a valid test. In case of a positive sample, the band is present at 1 or/and 2 or/and 3 or/and 4 in addition to 'C' band. The band at 1 or/and 2 depicts the presence of antibodies against bovine specific M.TB. The 1 and 2 positions are specifically used for the identification of BTB. The band at 3 depicts the presence of antibodies against BTB. The band at 4 depicts the presence of antibodies against non-tuberculous *Mycobacteria* (NTM) or avian tuberculosis (*M. avium*).

RESULTS

A total of 100 serum samples collected from randomly selected 100 cattle of gaushalas of Haryana were subjected to antibody detection using commercially available Bovine TB Ab Rapid Test Kit. Out of total 100 serum samples, 69 (69 %) samples were found positive for presence of antibodies against *M. bovis* (BTB) or/ and *M. avium* (NTM/ Avian TB) (Table 2). Out of 69 antibody test positive samples, 4 (5.79 %) samples were positive for presence of antibodies only against *M. bovis*, 27 (39.13 %) samples were positive for presence of antibodies only against *M. avium* and 38 (55.07 %) samples were positive for presence of antibodies against *M. bovis* and *M. avium* both species. A total of 42 (60.86 %) samples were positive for having antibodies against *M. bovis* and a total of 65 (94.20 %) samples were found positive for having antibodies against *M. avium* (Fig. 1).

Table 2: Sero-prevalence of BTB estimated by antibody detection test

Test used	Number of samples positive (%)
Antibody detection test (either BTB or NTM/ Avian TB)	69/100 (69 %)
Antibody detection test (BTB)	4/100 (4 %)
Antibody detection test (NTM/ Avian TB)	27/100 (27 %)
Antibody detection test (BTB + NTM/ Avian TB)	38/100 (38 %)

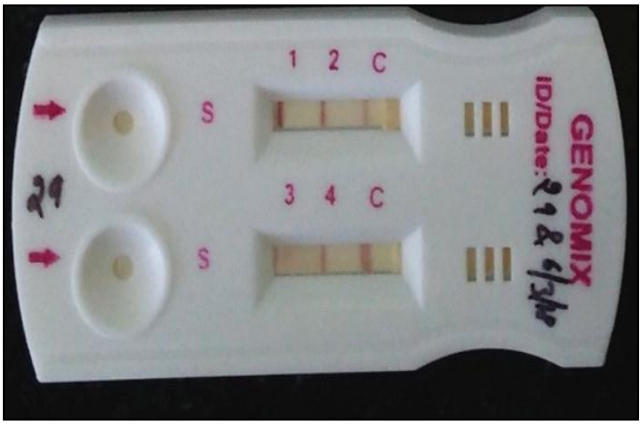

Fig. 1: Photograph showing positive results in Bovine TB Ab Rapid Test Kit
S = Sample well, C = Control area
1, 2, 3 = Antibodies present against BTB; 4 = Antibodies present against NTM or Avian TB

Out of 42/69 (60.86 %) samples positive for presence of antibodies only against *M. bovis* or antibodies against *M. bovis* and *M. avium* both species, 18/35 (51.43 %) were of Sahiwal cattle, 14/40 (35 %) were of crossbreds and 10/25 (40 %) were of Hariana cattle. A total of 22/40 (55 %) cattle of 'B' gaushala, 10/30 (33.33 %) cattle of 'C' gaushala, 10/30 (33.33 %) cattle of 'A' gaushala, 34/77 (44.16 %) adult cattle, 8/23 (34.78 %) heifers, 32/70 (45.71 %) female animals and 10/30 (33.33 %) male animals were found positive by antibody detection test. The results were compared using $\chi 2$ test with respect to breed, age, gender and location and it was found statistically non-significant ($p > 0.05$) (Table 3).

Table 3: Percent positivity of different categories of animals (antibodies against *M. bovis*)

Variables	Number of positive samples (%)	
Breed		
Cross-bred (40)	14 (35.00)	p= 0.346
Hariana (25)	10 (40.00)	
Sahiwal (35)	18 (51.43)	
Location		
A (30)	10 (33.33)	p= 0.099
B (40)	22 (55.00)	
C (30)	10 (33.33)	
Gender		
Male (30)	10 (33.33)	p= 0.250
Female (70)	32 (45.71)	
Age		
Heifer (23)	8 (34.78)	p= 0.424
Adult (77)	34 (44.16)	

Out of 65/69 (94.20 %) samples positive for presence of antibodies only against *M. avium* or antibodies against *M. avium* and *M. bovis* both species; 25/35 (71.43 %) were of Sahiwal cattle, 25/40 (62.5 %) were of crossbred cattle and 15/25 (60 %) were of Hariana cattle. A total of 23/30 (76.67 %) cattle of 'C' gaushala, 30/44 (75 %) cattle of 'B' gaushala and 12/30 (40 %) cattle of 'A' gaushala were found positive. A total of 46/77 (59.74 %) adult cattle, 19/23 (82.61 %) heifers, 53/70 (75.71 %) female animals and 12/30 (40.00 %) male animals were found positive. The results were compared using $\chi 2$ test and breed wise results were found statistically non-significant ($p > 0.05$) However, the results were statistically significant with respect to age, gender and location ($p < 0.05$) (Table 4).

Table 4: Percent positivity of different categories of animals (antibodies against *M. avium)*

Variables	Number of positive samples (%)	
Breed		
Cross-bred (40)	25 (62.50)	p= 0.600
Hariana (25)	15 (60.00)	
Sahiwal (35)	25 (71.43)	
Location		
A (30)	12 (40.00)	p= **0.003**
C (40)	30 (75.00)	
B (30)	23 (76.67)	
Gender		
Male (30)	12 (40.00)	p= **0.001**
Female (70)	53 (75.71)	
Age		
Heifer (23)	19 (82.61)	p= **0.044**
Adult (77)	46 (59.74)	

DISCUSSION

Out of total 100 serum samples, 69 (69 %) samples were found positive for presence of antibodies against *M. bovis* or/ and *M. avium*. Out of 69 % serologically positive animals, 14 (20.28 %) animals were also found positive by interferon gamma assay (IFN-γ assay) conducted during the present study. High sero-prevalence was found during the present study which agreed with Kalaf *et al.*[8], who reported 78.57 % cows positive to the antigen rapid bovine TB Ab test and most of the tuberculin positive animals were also positive to the antigen rapid bovine TB Ab test, due to the MP70 antigen present in the rapid test kit which is a major component of *M. bovis*. The present results also agreed with[7] who found that 62 % cows gave positive results in antigen rapid bovine TB Ab test and with[6] who reported that the antigen rapid bovine TB Ab test alone is not efficient in diagnosis of BTB. Other serological tests like ELISA must be used to validate these results. Awah-Ndukum *et al.*[1], reported that tuberculin skin test and lateral flow anti BTB antibody tests offered improved detection of BTB when used in parallel as compared to individual tests.

In contrast to our study, Danbirni *et al.*[6], reported that 2.8 % animals reacted to the tuberculin test but all were negative with the Quicking BTB Ab rapid test. In another study conducted by Mahmud *et al.*[11], the results indicate 7.78 % overall prevalence of BTB by Antigen Rapid BTB Ab test kit. Similar to the present study, females were found more susceptible in a study conducted by Mahmud *et al.*[11]. The higher prevalence in female animals suggests that they shed the infection for a long time and spreading the disease to other animals and humans. It is well known that indigenous animals are resistant to TB as compared to exotic animals. However, in the present study, more number of indigenous cattle were found serologically positive. The variation in the present results may be due to small sample size.

However, the specificity of these test kits could be affected by cross-reactions with members of *M. avium* complex[4]. Berm´udez *et al.*[3], reported more false positive results as observed by commercial multi-antigen lateral flow assay used in dairy cattle. Serological assays are relatively easy to perform and can be used to rapidly test a large number of samples[13]. The MPB70 and MPB83 proteins identified as early B-cell targets in *M. bovis* infection. These proteins could detect antibodies in experimentally infected cattle 7 (MPB70) to 18 (MPB83) weeks before a positive tuberculin response could be measured[10]. Selection of highly specific antigens could help to eliminate the cross-reactions between other species of myco-bacteria *e.g. M. avium paratuberculosis* and *M. kansassii*[18,13].

CONCLUSION

A single test could not detect the BTB infection at each stage so a combinational approach must be used. The animals should be regularly tested by using more sensitive and specific methods and infected/reactor animals should be segregated to prevent disease transmission to other susceptible animals. Awareness program should be undertaken to protect public health and to minimize the economic losses associated with BTB.

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