Research Article

SERO-PREVALENCE OF BOVINE BRUCELLOSIS IN WEST BENGAL, INDIA: A 15 - YEARS STUDY

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ABSTRACT: Bovine brucellosis is an important zoonotic disease caused by *Brucella abortus*. The distribution of bovine brucellosis in West Bengal has not been reported so far. Here, a longitudinal epidemiological study was conducted from April 2002 to March 2016 to determine the distribution, pattern and trend of bovine brucellosis in different organized and unorganized farms of West Bengal, India. Bovine serum samples were examined for identification of brucellosis by Rose Bengal Plate Test and indirect ELISA. Results envisaged that the prevalence of brucellosis is 11.12 times higher in organized farm (average 6.6%) compared to rural areas (average 0.6%) with overall prevalence was 3.9%. The highest and lowest level of sero-positivity was observed in 2015 and 2011 with 9.8% and 0.5% respectively. In reference to the year 2002, trend of brucellosis was decreasing up to 2013 in a fluctuating manner (odd ratio ranging from 1.7 to 2.3); however, it again increased in 2014 and 2015 with final decrease in 2016. In organized farms, highest (12.6%) and lowest (0.6%) level of prevalence was recorded in 2015 and 2011 respectively. In rural areas, the highest and lowest level of prevalence was observed in 2010 and 2008 with 1.8% and 0.15% respectively. Chi square statistics revealed that location of cattle (χ^2 =713.8, df=1, p<0.001) and year of sample collection (χ^2 = 468.6, df=14, p<0.001) contributed significantly to increasing or decreasing sero-positivity. A univariate logistic regression analysis also revealed that location of the animals either in organized farm or in rural areas and year(s) of sampling have statistically significant (p<0.001) effect on individual animal level sero-positivity. The results suggested that brucellosis is endemic and moderately distributed in different regions of West Bengal including Burdwan, Nadia, Paschim Medinipur, Murshidabad and Hoogly districts.

Key words: Brucellosis, Cattle, Sero-prevalence, RBPT, iELISA, West Bengal.

INTRODUCTION

Bovine Brucellosis is a major zoonotic disease with worldwide distribution affecting both animals and human beings. In cattle and buffalo, the disease is mainly caused by a *Brucella abortus* serovars and is characterized by abortion, infertility, repeat breeding and reduced milk yield (BhanuRekha *et al.* 2013). In cattle, brucella infection has also been reported by *B. meletensis* and *B. suis* (Corbel *et al.* 1984).

Proper diagnosis at the correct time is essential to set up control program of the disease. The culture of the organism is the 'gold standard' test for definitive diagnosis of brucellosis. However, , disease confirmation by isolation of the pathogen is challenging in most of the cases because of low sensitivity and unconvincing results (Godfroid *et al.* 2010). In such scenario, serological testing and subsequent culling of infected animals are commonly employed in the cattle herd, especially in this country. Use of the at least two serological tests applied in succession is generally recommended for accurate diagnosis and maximum specificity (BhanuRekha *et al.* 2013). Rose Bengal Plate Test (RBPT) is used as a herd screening test. Subsequently, indirect ELISA is an important serological tool for diagnosis of the disease because of its sensitivity, specificity, rapidity, reproducibility and easy interpretation through colorimetric end point (Batra *et al.* 1989).

In India, brucellosis was first recognized in 1942 and now endemic in different states (Ramesh *et al.* 2013, Isloor *et al.* 1998). In spite of effective vaccination based control and preventive measures, the disease still remains a persistent problem in endemic areas (Smits and Kadri 2005). Due to the increase trade and rapid movement of livestock, incidence of brucellosis appears to have

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increased in recent times (Renukaradhya *et al.* 2002). Bovine brucellosis is more prevalent in adult, while young ones do not show symptoms till maturity.

Brucellosis is endemic in Northern and Southern part of India. Although a few information are available on sero-prevalence of brucellosis in West Bengal (Chatterjee *et al.* 1986, Saha *et al.* 2010), the long term study on sero-prevalence of brucellosis in West Bengal is not found.

The objective of the present study was to determine the long term study on sero-prevalence of bovine brucellosis in West bengal, India. Location wise risk factors are also analysed in terms of organised farms and rural areas for presence of bovine brucellosis.

MATERIALS AND METHODS
Collection of Sample

The longitudinal study was carried out on different breeds of cattle during April 2002 to March 2016 in two types of cattle rearing system *viz.*, organized sector and in rural areas. About, 10 ml blood was collected aseptically in an evacuated collection tube (BD Vacutainer System, Becton Dickinson, Franklin Lakes, NJ) from jugular vein of each selected cattle of different age groups from three different organized farms of West Bengal. Then, blood samples were kept for 3 hours at room temperature, followed by overnight refrigeration at 4°C. Finally, serum samples were preserved at -20°C before further study. Here, we have sampled total 31,428 serum including 13652 serum from rural areas and 17776 serum from organized farm for analyzing prevalence of brucellosis in West Bengal, India.

Rose Bengal Plate Test

At first 30 μl of rose Bengal colour antigen produced at antigen production unit, IAH & VB, Kolkata (name of manufacturer) was taken on a transparent plate. Then, 30 μl of thawed serum was charged on the colour antigen and mixed properly with toothpicks. The plate was kept undisturbed for 3-4 min and observed for agglutination or no-agglutination under the light.

Indirect ELISA (i-ELISA)

Indirect ELISA was performed by using a commercially available kit from IDEXX KIT, Switzerland following the manufacture's protocol. Finally, the absorbance was measured at 450 nm in ELISA reader (Sunrise Tecan). The result was analyzed by using X Check software.

Data Analysis

Data were analysed using SPSS (version16, Chicago, IL) program. The sero-prevalence rate over 13 years was determined by dividing the number of RBPT and iELISA-positive cattle by total number of cattle tested and confidence intervals were calculated at a 95% level. Odd ratio was used to measure the degree of association between risk factors such as location of animals *i.e.*, organized herds or rural areas and year-wise risk factors with brucellosis sero-prevalence. The difference between prevalence of organized farms and rural areas as well as year wise difference between prevalence of animals were tested by Chi-square test and univariate regression statistics.

RESULTS AND DISCUSSION

In this study, individual animal level sero-prevalence was determined. Out of total 31,428 sera samples examined from 2002 to 2016 in organized farms and rural areas of West Bengal, 1257 samples were sero-positive by STAT, RBPT and iELISA. Thus, overall animal level sero-prevalance of brucellosis was 3.9%. The highest and lowest rate of sero-prevalence was 9.8% and 0.5% in 2015 and 2011 respectively (Table 1). The trend of sero-prevalence of brucellosis from 2002 to 2016 showed a fluctuating manner. A significant decreasing trend of sero-prevalence from 2002 to 2005, followed by an increasing trend was observed during 2005 to 2009. Then further significant decreasing trend of sero-prevalence from 2009 to 2011 was seen with increasing tend from 2011 to 2015 and final decrease in 2016. A univariate analysis with the odd ratio and Chi-square value depicted the year wise trends in seroprevalence from 2002 to 2016 in reference to the year 2002 (Table 1).

Sero-prevalence of brucellosis in organized farms and rural areas were 6.6% and 0.6% respectively. In organized farms, the highest and lowest rates of prevalence were 12.6% and 0.6% in 2015 and 2011 respectively. In rural areas the highest and lowest rates of prevalence were 1.8% and 0.15% in 2010 and 2008 respectively. In organized farms, a significant fluctuating trend of sero-prevalence was noted from 2002 to 2016 ranging from 0.6 to 12.6% ($\chi^2 = 370.1$, df=14, p<0.001). Whereas, in rural areas, a significant decreasing trend of sero-prevalence was observed from 2002 to 2004, followed by almost similar pattern of sero-prevalence from 2004 to 2009 which further increased in 2010 to 2014. A significant decrease in sero-prevalence was seen from 2015 with final increase in the year 2016 ($\chi^2 = 73.19$, df=14, p<0.001). (Fig. 1). The location wise sero-prevalence of brucellosis in respect of organized
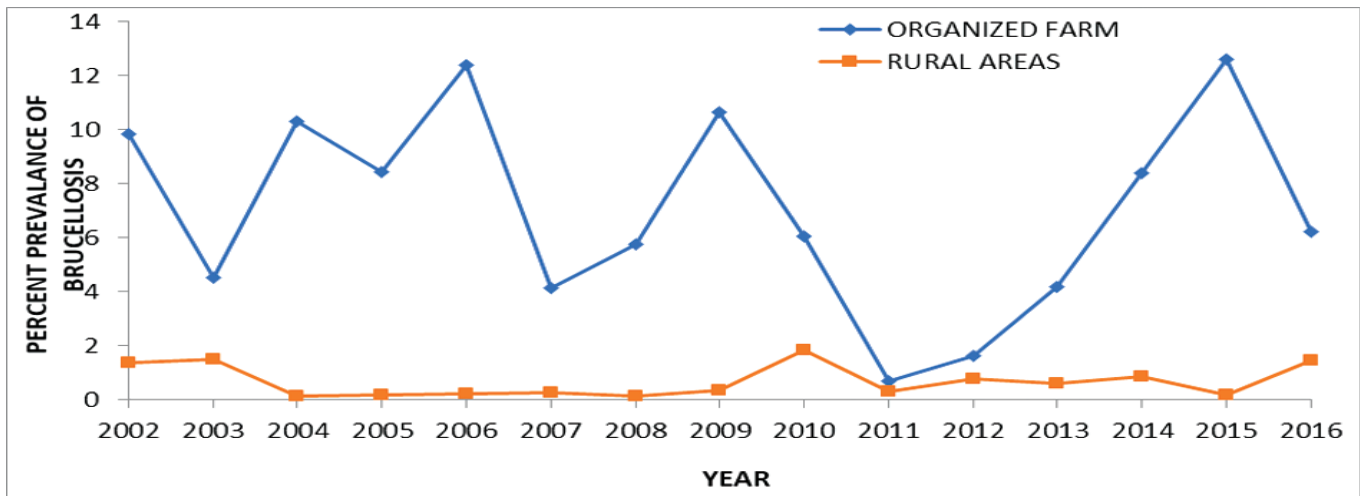
Fig. 1. Percent prevalence of bovine brucellosis in organized farm and rural areas of West Bengal during 2002 to 2016.

farms and rural areas was significantly different ($\chi^2 = 713.8$, df=1, p<0.001). The prevalence of brucellosis was 11.1 times higher in organized farms in comparison to rural areas (OR= 11.1, CI= 8.9-13.9, p<0.001) (Table 2).

Sero-prevalence of brucellosis in cattle varies widely from region to region across India (Mantur and Amarnath 2008). In an earlier study, Isloor *et al.* (1998)

reported that the overall prevalence of bovine brucellosis in India was 1.9%. However, long term serological studies in India reveal that the baseline sero-prevalence in cattle is about 5% (Renukaradhya *et al.* 2002). In our study, the overall individual cattle level sero-prevalence of brucellosis in West Bengal of 3.9% is lower than other states of India such as Uttar Pradesh (22.39%), Punjab (11.80%), Uttarakhand (8.57%) and Karnataka (45.80%) (Jagapur *et al.* 2013). In a previous study on sero-prevalence on bovine brucellosis, Chatterjee *et al.* (1986) found that prevalence of brucellosis varies from 0 to 13% with overall prevalence of 3.5% in rural West Bengal which corroborates with our findings. In Tamil Nadu, the prevalence of bovine brucellosis ranged from 3.3-11.4% (Senthil *et al.* 2013). The current national sero-prevalence of brucellosis in cattle is estimated at 13.5% with stable endemic equilibrium (Rahman 2013). Thus, taking long-term average during 2002 to 2016, the overall sero prevalence of brucellosis in organized farm and rural areas of West Bengal was lower than national estimates. However, it also portrays that, like other states, the disease is present as endemic form across the West Bengal.

STAT, RBPT and iELISA tests are used for screening of brucellosis in cattle sera. Out of 31,428 cattle tested, 1257 cattle were found to be sero-positive by all three tests.

Table 1. Year-wise trend in Sero-prevalence of bovine brucellosis from 2003 to 2016 in Reference to Year 2002.

| Variable (Year) | N | Number (%) Positive | Univariate Analysis | | | |
			OR	95% CI	Chi Sqare Value	P value
2002	952	57 (6.0)	Ref	Ref	Ref	Ref
2003	1804	49 (2.7)	2.3	1.5-3.4	18.03	p<0.001
2004	871	26 (3.0)	2.1	1.3-3.3	9.43	p=.002
2005	2839	43 (1.5)	4.1	2.7-6.2	55.53	p<0.001
2006	1871	66 (3.5)	1.7	1.2-2.5	9.16	p=.002
2007	1077	12 (1.1)	5.6	3.0-10.6	0.021	p=0.884
2008	1357	40 (2.9)	2.1	1.4-3.2	12.84	p<0.001
2009	2158	117 (5.4)	1.1	0.8-1.5	0.4	p=0.527
2010	3151	147 (4.6)	1.3	0.9-1.7	2.7	p=0.100
2011	2083	10 (0.5)	13.2	6.7-25.9	91.79	p<0.001
2012	1995	28 (1.4)	4.5	2.8-7.1	48.34	p<0.001
2013	2535	89 (3.5)	1.7	1.2-2.5	10.58	p=0.001
2014	2621	160 (6.1)	0.9	0.7-1.3	0.017	p=0.897
2015	2542	249 (9.8)	0.6	0.4 - 0.8	12.56	p<0.001
2016	3572	164 (4.6)	1.3	0.97-1.8	3.15	p=0.076

Table 2. Sero-prevalence of bovine brucellosis in two different management systems.

Variable (Year)	N	Number (%) Positive	Univariate Analysis			
			OR	95% CI	Chi Sqare Value	P value
RA	13652	86 (0.63)	Ref	Ref	Ref	Ref
OF	17776	1171 (6.6)	11.12	8.9-13.9	7.14	p<0.001

Additionally, 235 animals were shown to be sero-positive in RBPT without giving any reaction to iELISA depicting the false positive results of RBPT. There are many instances where RBPT has been shown to give false positive reaction (BhanuRekha *et al.* 2013, Mai *et al.* 2012). RBPT determines the IgM antibody titer in serum samples because of their appearance in early infection by *Brucella* and *Brucella*-like organisms. Thus, specificity of RBPT was not high compared to iELISA which determines *Brucella*-specific IgG antibody in serum samples and has high specificity (Corbel 1984). In view of this, we estimated the sero-prevalence of brucellosis as 3.9% considering those animals which are seropositive with both the RBPT and iELISA.

Individual animal level sero-positivity varied significantly with fluctuating trend over the 15 years of study from 2002 to 2016. Significant decreasing trend of prevalence (from 6 to 1.3%) was observed from 2002 to 2007 suggesting sero surveillance was achieved during this period mainly through culling of brucella infected animals. But further increasing trend of prevalence from 2007 to 2010 (1.3 to 4.6%) signifies that the culling activities were not successful and some infected animals might have survived the culling process which propagated the infection to other animals. Lowest level of prevalence (0.5%) was shown in 2011 suggesting successful preventive measure was taken in 2011 and preceding years. Still further gradual increase of positive reactor from 2011 to 2015 (from 0.5 to 9.8%) entails that some inherent infection was present mainly in organized farms which spread gradually to the healthy animals. But, in the year 2016 sharp decrease of positive reactor may signify proper culling of brucella infected animals may be practiced in a proper way, particularly in organized herd. Thus, like other states of India, bovine brucellosis is endemic in West Bengal.

The observed pattern of prevalence of brucellosis over the 15 years of study may depend on several factors. Here we determined sero-prevalence in two different rearing systems *viz.*, organized herd with intensive cattle rearing system and agro-pastoral farming system in rural areas.

It was observed that animals kept in organized herd were 11.1 times more likely to contract the brucella infection, in comparison to agro-pastoral animals in rural areas which agrees with the similar findings by others (Gill *et al.* 2000, Berhe *et al.* 2007, Dinka *et al.* 2009). The higher prevalence of brucellosis in organized herds may arise from large number of animals are maintained together that increases the risk of exposure, especially following abortion, through higher level of contact (Hellmann *et al.* 1984).

The two different types of farm management systems behaved differently to the observed pattern of brucellosis reactors over the entire period of study. The fluctuating trend of sero-prevalence ranging from 0.6 to 12.6% was observed in organized farm; whereas much lower prevalence with steady state ranging from 0.15 to 1.8% was found in animals from rural areas. In an earlier study on sero-prevalence of brucellosis in West Bengal, Saha *et al.* (2010) also found much lower prevalence with 0.25% in rural areas compared to organised herds with 12.02%. In organized farms, chronic brucella infection may be present because some apparently healthy but carrier cows are reproducing but transmit the infection to healthy animals. Also, in breeding bulls, brucellosis causes no impairment of libido or breeding capacity but the disease is present as subclinical form. Whereas, in agro-pastoral farming system particularly in rural areas, the chances of transmission of the disease from infected animal is very less. These factors contribute to the much higher prevalence of brucellosis in organized herds compared to agro-pastoral farming system in rural areas.

CONCLUSION

The present study showed that bovine brucellosis is endemic in West Bengal, an eastern state of India, with fluctuating trend of prevalence and is influenced by different management system of cattle rearing. Improved management system with routine sero-surveillance by using RBPT and iELISA, followed by culling of sero-positive animals could be good control strategy for the disease. However, implementation of vaccination against brucellosis under National Control Program on Brucellosis of India might be better effective in controlling the disease in near future.

ABBREVIATIONS USED

RBPT: Rose Bengal Plate Test; STAT: Standard Tube Agglutination Test; iELISA: Indirect Enzyme Linked Immune Sorbent Assay; CI: Confidence Interval; OR: Odd Ratio.

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