



RISK FACTORS ASSOCIATED WITH SEROPREVALENCE OF BOVINE BRUCELLOSIS

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ABSTRACT

Brucellosis is one of the most contagious bacterial zoonotic diseases, which causes severe economic impact to farmers by affecting reproductive performance of animals including abortion, retained foetal membranes and decreased milk production. Diagnosis of *Brucella* infected animals with associated risk factor assessment plays a crucial role in formulation of control measures in a better way. This study aimed at assessment of risk factors associated with seroprevalence of bovine brucellosis in Tamil Nadu. A total of 821 bovine serum samples were collected from various districts of Tamil Nadu and subjected to RBT, STAT and i-ELISA. The prevalence rate of bovine brucellosis was detected by RBT, STAT and i-ELISA was 4.02, 4.38 and 6.70% respectively. Based on general, clinical and epidemiological examinations the animals were divided into aborted, retained fetal membrane, other reproductive problems like anoestrus, repeat breeding and unknown history animals. The seroprevalence was high in aborted history animals, followed by other animals. This study concluded that, animals with history of abortion should be properly obtained to ensure of *Brucella* free herd, as high correlation was observed between prevalence and animals with aborted history

KEY WORDS: Brucellosis – Seroprevalence – Risk factor – Abortion.

INTRODUCTION

Brucellosis is one of the most contagious bacterial zoonotic diseases, which causes severe economic losses to the farmers by affecting reproductive health of the animals. Brucellosis in cattle mainly caused by *Brucella abortus* and characterized by abortion, retained fetal membrane, decreased milk production, orchitis and decreased sperm quality in animals which lead a severe problem in reproductive performance of animals (Radostits *et al.*, 2010). In general brucellosis was first detected in India at 1942 and now prevalence is observed in entire India. Diagnosis of brucellosis can be made directly by detecting antigen (Culture and polymerase chain reaction) and indirectly by detecting antibodies (Rose Bengal test, standard tube agglutination test, indirect enzymatic linked immunosorbent assay, fluorescent polarization assay, milk ring test and milk enzymatic linked immunosorbent assay; Poester *et al.*, 2010). However diagnosis is cumbersome due to the other demerits of other tests, hence there is no single test to diagnose brucellosis, combination of at least 2 or more test are needed to know the exact status of animals to be screened. Serological tests are having low specificity due to cross reacting antibodies with *Yersinia enterocolitica* O: 9 (MacMillan *et al.*, 1990b), vaccinated animals and failure in seroconversion of infected animals. Current diagnostic approach needs a minimum false positive and false negative test results which may infer a disease strategy and to understand epidemiology in a better way. Seroprevalence studies to determine the prevalence of brucellosis revealed

prevalence rate of 8.8 per cent in India and 9.3 per cent in bovines of Tamil Nadu (Renukaradhya *et al.*, 2002). The assessment of risk factors plays a crucial role effective systematic control of brucellosis. There are very limited literatures on risk factors assessment which may facilitate the control measures in a better way. Based on prevalence status of brucellosis status, the present study was aimed to study the risk factors associated with seroprevalence of bovine brucellosis from Tamil Nadu.

MATERIALS & METHODS

The present study was conducted in certain districts of Tamil Nadu, *viz.*, Erode, Salem, Kancheepuram, Tiruvallur, Tiruvannamalai, Viluppuram, Thiruvarur, Pudukkottai, Virudhunagar, Tirunelveli, Chennai to assess the status of *Brucella* infection. Sexually matured cattle were selected randomly from the study area with the history of animals with abortion, retained fetal membrane, repeat breeding, anestrus, infertility and unknown reproductive history of animals. Blood samples (3 ml) were collected from 821 cattle by jugular vein puncture in sterile test tubes (5 ml) and they were allowed to clot and then centrifuged at 2000 rpm for 15 minutes. Sera were separated and stored at - 20°C until further use.

RBT and STAT

Rose Bengal test antigen was obtained from Indian Veterinary Research Institute (I.V.R.I), Izatnagar. The antigen was stored at 4°C until use. The RBT was performed as per OIE, 2009 guidelines. Standard tube agglutination test

antigen was obtained from the Indian Veterinary Research Institute (I.V.R.I) Izatnagar. The antigen was stored at 4°C until use. The STAT was performed as per OIE, 2009 guidelines.

ELISA

The *Brucella* Antibody ELISA test kit was purchased from SVANOVIR, Sweden, and used for testing 821 serum samples according to manufacturer’s guidelines. The samples were run on Svanovir *Brucella*-Ab indirect ELISA kit and the optical densities (ODs) were determined in a microplate spectrometer (Bio rad) at 450-nm wavelength. Positive and negative control serum samples were included

in each test. Interpretation of the results was based on Per cent Positivity (PP) calculations; PP is calculated by (Test sample or negative control (OD) x 100) / (Positive control (OD)) and results were interpreted as positive for PP ≥ 60 and Negative for PP < 60 for the individual serum (10 µl) sample.

RESULTS & DISCUSSION

Prevalence of bovine brucellosis

The overall prevalence of bovine brucellosis in this study area were 6.70 per cent by i-ELISA followed by STAT (4.38 %) and RBT (4.02 %) (Table – 1).

TABLE 1: Prevalence of bovine brucellosis by various diagnostic tests

Prevalence by test	Sero-prevalence of bovine brucellosis		
	RBT positive	STAT positive	i-ELISA positive
Total no. of samples screened	821	821	821
Total no. of positive	33	36	55
Prevalence of brucellosis (%)	4.02	4.38	6.70

In this present study RBT showed the prevalence of 4.02 per cent for bovine brucellosis (33/821) which is in agreement with Nizeyimana *et al.*, 2013 (4.7 %) and Adamu *et al.*, 2016 (5.3 %) in cattle. This study was deviated from early workers and they found higher positivity by RBT *viz.*, Reddy *et al.*, 2014 (7.74 %) and Akhtar *et al.*, 2010 (26 %) whereas Khajuria *et al.*, 2014 found lower positivity (1.88 %) by RBT than the results of the present study. The variation in the positive percentage by RBT with different workers could be due to sampling methodology, false positive and negative reactions, various sampling places and different clinical conditions of animals.

In the present investigation STAT showed the prevalence of 4.38 per cent (36/821) in animals screened for brucellosis. These results were concurred with Adamu *et al.*, 2016 (3.9 %) and Samaha *et al.*, 2008 (4.73 %). Other researchers, Bhattacharya *et al.*, 2005 (8.05 %) and Nasir *et al.*, 2004

(18.53 %) in cattle documented higher prevalence whereas Amin *et al.*, 2005 (2 %) found lower positivity by STAT. On contradictory to present study, the higher percentage of prevalence was recorded by various researchers. This might be due to method of rearing, endemic area of sample collection and collection of samples from animals with the previous history of abortion (Nasir *et al.*, 2004).

In this present study i-ELISA showed the prevalence of brucellosis in 6.70% (55/821) of animals. Our results were agreed with Agarwal *et al.*, 2007 (8.4 %) and Bhattacharya *et al.*, 2005 (4.6 %). Findings of present study were deviated from Chand and Sharma, 2004 (26.50 %) and Patel, 2007 (29.00 %). The variation in the positive percentage by i-ELISA with different workers might be due to sampling size, demography, clinical conditions of animals, aborted history animals and vaccination status of animals (Patel, 2007).

TABLE 2: Clinical epidemiological data with prevalence of bovine brucellosis by various diagnostic tests

Previous reproductive history	Aborted animals	Retained foetal membranes	Other reproductive problems	Unknown history of animals	Total no. of positives
No. of samples screened	64	136	294	327	821
RBT	10 (15.62%)	9 (6.61%)	6 (2.04%)	8 (2.44%)	33 (4.02%)
STAT	11 (17.18%)	11 (8.08%)	4 (1.36%)	10 (3.05%)	36 (4.38%)
i-ELISA	19 (29.68%)	17 (12.50%)	7 (2.38%)	12 (3.67%)	55 (6.70%)

In this study the highest prevalence was recorded in aborted animals (i-ELISA - 29.68%) followed by Retained fetal membrane (RFM) (i-ELISA - 12.5%), unknown history animals (i-ELISA - 3.67) and other reproductive problems (i-ELISA - 2.38%) (Table 2).

This result were concurred with Bachh *et al.*, 1988 and Aulakh *et al.*, 2008 and concludes that brucellosis was found higher in animals with a history of abortion when compared to those animals with a history of returns to service. Two different studies conducted by Isloor *et al.*, 1998 in organized farms of Karnataka reported that high (17%) prevalence rate of brucellosis was observed with a history of abortion, retention of placenta and repeat breeding whereas

Dhand *et al.* (2005) in Punjab recorded higher prevalence of brucellosis in animals with a history of abortion (33.87%) than in those without such a history (11.63%). It can be inferred from the previous reports and the present study that brucellosis is confirmed to be the major etiological agent of abortion in farm animals worldwide.

Present investigation revealed that animals with unknown history have significant level of brucellosis, the reason behind this higher prevalence might be purchase of animals without proper awareness on brucellosis and most of the Indian farmers sold the animals with infertility problems which play a direct role in transmission of disease without knowing proper clinical history.

TABLE 3: Aborted history animals with prevalence of bovine brucellosis by various diagnostic tests

Previous reproductive history	Aborted time			Total No. of Positives
	III trimester	II trimester	I trimester	
No. of samples screened	31	17	16	64
RBT	7 (22.58%)	2 (11.76%)	1 (6.25%)	10 (15.62%)
STAT	8 (25.80%)	2 (11.76%)	1 (6.25%)	11 (17.18%)
i-ELISA	14 (45.16%)	4 (23.53%)	1 (6.25%)	19 (29.68%)

In this study different stages of abortion were analysed with brucellosis, among the three trimesters, high prevalence was recorded in animals with third trimester abortion (i-ELISA - 45.16%), followed by second trimester (i-ELISA - 23.53%) and first trimester aborted animals (i-ELISA - 6.25%) (Table 3). These findings almost coincide with Islam *et al.*, 2013b who reported that, the overall seroprevalence of brucellosis in third semester abortion due to brucellosis was high (57.14 %) than second (17.58%) and first (1.09%) trimester abortion animals.

Abortion would be high in last trimester animals due to the predilection site of brucellosis is the reproductive tract especially gravid uterus and allantoic factors including erythritol, the steroid hormones and uterine environment becomes conducive for the multiplication of bacteria which stimulates the growth of *Brucella* spp. (Gul and Khan, 2007).

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