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Investigation of Sero Prevalence of Brucella Outbreak in an Organised Dairy Farm

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ABSTRACT

Brucellosis is a zoonotic disease with epidemiological global effect. It has been found to affect the cattle industry worldwide and India is not an exception to it. The current sero survey was carried to illuminate the status of bovine brucellosis in an organised dairy farm from Nasik with past history of abortions. Total of 65 serum samples were collected from cattle and tested for the presence of brucella antibodies using Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT) and Indirect Enzyme Linked Immuno-Sorbent assay (I-ELISA). Out of 65 samples, 25 (38.4%) sera were positive by RBPT, and 19(29.2%) for STAT. Screening by Indirect ELISA revealed that 26 samples (40.0%) were positive for brucellosis. Overall, seroprevalence in the herd was found to be 23 (35.3%). The present study highlights the importance of study of seroprevalence in cattle population to eliminate the economic loss among farmers and raise awareness to avoid zoonoses caused by brucella infection.

Keywords

Brucellosis, ELISA, abortion, zoonoses, seroprevalence

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Introduction

Brucellosis is a potential zoonotic disease having world-wide distribution. It is caused by a Gram negative bacteria *Brucella abortus* in cattle, *B. melitensis* in goats and sheep and *B. suis* in swine characterised by abortions, infertility and reduced milk yield (Mantur *et al.*, 2007). Bovine brucellosis is an endemic

disease in most part of the states in India (Islam *et al.*, 2013) and the trend appears to be on the increase in recent times, perhaps due to trading and movement of livestock within the states. Brucella infection in endemic areas requires screening and rapid diagnosis to avoid high economic loss, morbidity. This may cause havoc to mankind if not kept under check (Atluri *et al.*, 2011). The serological assays which are mostly performed in farm

level include Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT) and Enzyme Linked Immuno-Sorbent assay (ELISA) (Yu and Nielsen, 2010; Priyadarshini *et al.*, 2013). Recently an indirect ELISA for milk antibody to *B. abortus* has been reported with high sensitivity and specificity (Nielsen *et al.*, 1996). Therefore, the present seroprevalence study was conducted in serum samples to illuminate the status of brucellosis outbreak in cattle.

Materials and Methods

Sample collection

The serum samples and whole blood samples (n = 65) were collected from cattle population of a dairy farm in Nashik. The serum samples were collected in vacutainers without anticoagulant. Sera were stored at -20°C until use. Most of the collected samples had a history of vaccination and showed symptoms of abortion or suspicion of *Brucella* occurrence.

Sample Tests

Rose Bengal Plate Test (RBPT) and Standard Tube Agglutination Tests (STAT) in all the serum samples (n=65) was carried out for detection of *B. abortus* antibodies according to the standard protocol of Alton *et al.*, (1988). Briefly, a suspension of *B. abortus* coloured with Rose Bengal stain is used as Rose Bengal antigen. (Agasthya *et al.*, 2012; Cadmus, Adesokan and Stack, 2008). For RB, 0.05 ml of serum was mixed with an equal volume of antigen on a test plate to produce a zone of agglutination was observed. The plate was rocked for about sometime. The mix was observed for agglutination and any visible reaction was considered positive in room temperature. No dilutions were performed for Rose Bengal test. All samples either positive for STAT or RBPT was then subjected further to ELISA.

Indirect ELISA was performed after standardisation in the 96 well plate (*Nuncpolysorp*). After antigen coating, blocking with 1% bovine serum albumin serum dilution @100 ul/ well was performed (Paweska *et al.*, 2000). Addition of conjugate was done, where 1ml of conjugate in 10 ml PBS @100ul/well was mixed. After incubation, substrate was added (OPD) and again the plate was incubated in the dark for 15min. The reaction was lastly stopped by addition of sulphuric acid (H₂SO₄) to it. Absorbance reading was taken at 492nm using the ELISA microtitre plate reader.

Assay of Samples

The appearance of agglutination within 1 minute was scored 2+ (++), while any agglutination between 1 and 4 min was scored 1+ (+). The absence of agglutination within 4 minutes of rocking was regarded to be negative. For STAT, the result was expressed in international units per ml of serum by doubling the serum titre showing 50% agglutination. The results showing 40 IU/ml or above was considered significant, and less than 40 IU/ml as negative. In ELISA, positive samples will show the colour change give yellow to orange colour.

Results and Discussion

The detailed test result is presented in Table 1 below. Prevalence estimate was calculated depending on specificity and sensitivity of the tests. The herd level seroprevalence was found to be 25 (38.4%), 26 (40.0%) and 19 (29.2%) for RBPT, I-ELISA and STAT respectively.

The above comparison has been shown in Figure 1. Sample showing positive for any two tests was considered to be positive for Brucellosis.

Overall, the individual test's prevalence was higher than the herd prevalence which was

observed as 23(35.3%). The cattle in age group of more than 5yrs had a higher prevalence rate than within 5 years. There was no such significant difference between the breeds in the study. However other findings have shown that breed was associated with brucellosis in cattle in Nigeria (Cadmus, Adesokan and Stack, 2008; Cadmus *et al.*, 2013; Junaidu *et al.*, 2011). It might be due to less diversification between the breeds in our study. ELISA test is considered to be highly specific and sensitive for the brucella

antibodies than any other serological tests (Varshochi *et al.*, 2011; Sanogo *et al.*, 2013).

Urgent need for control of brucellosis which may cause heavy economic losses to the organized farms and even affects the occupationally exposed individuals should be checked. An extensive epidemiological survey should be conducted state wise in order to adopt appropriate control and eradication measures for this crucial pathogen that possess public health concerns.

Table.1 Results of the tests conducted in the study

ANIMAL NO.	BREED	AGE	RBPT	ELISA	STAT	RESULTS
CH136	HF75%	13	0	0	40IU	
NF28	HF75%	12	1	1	640IU	P
NF78	HF25%	11	0	0	20IU	
NF41	HF37.5%	12	1	1	640IU	P
NF111	HF12.% GIR75%	10	0	0	20IU	
NF121	HF25% GIR75%	11	1	1	80IU	P
PF108	HF75%	11	0	0	20IU	
PF115	HF37.5%	10	0	0	20IU	
PF143	HF37.5%	10	0	0	20IU	
PF200	JR75%	9	0	0	40IU	
PF3	HF87.5%	10	1	1	20IU	P
PF4	HF37.5%	10	1	0	80IU	P
PF54	HF75%	10	0	0	40IU	
PF73	HF75%	11	1	1	320IU	P
PF82	HF75%	10	0	0	40IU	
PF88	HF75%	10	0	0	20IU	
PH71	HF37.5%	13	0	0	40IU	
SNPP036	HF37.5%	9	0	0	20IU	
SNPP126	HF37.5%	9	0	0	40IU	
SNPP174	HF75%	8	0	0	40IU	
SNPP228	JR75%	8	1	1	1280IU	P
SNPP255	HF75%	8	0	0	20IU	
SNPP259	HF25%	8	1	1	40IU	P
SNPP262	JR87.5%	8	0	0	40IU	
SNPP264	HF37.5%	8	1	1	320IU	P
SNPP275	HF37.5%	8	1	1	640IU	P
SNPP282	HF25%	8	1	1	1280IU	P

SNPP285	HF37.5%	8	1	1	320IU	P
SNPP367	HF68%	8	1	1	40IU	P
SNPP391	HF67.5%	7	1	1	640IU	P
SNPP402	HF75%	8	1	1	160IU	P
SNPP423	HF75%	8	0	0	20IU	
SNPP465	HF37.5%	8	0	0	40IU	
SNPP500	HF75%	8	0	0	40IU	
SNPP507	JR50%	8	1	1	1280IU	P
SNPP517	HF75%	8	0	0	40IU	
SNPP540	HF62.5%	8	1	1	80IU	P
SNPP557	HF75%	8	1	1	40IU	P
SNPP572	HF75%	7	0	0	20IU	
SNPP623	JR75%	7	0	0	40IU	
SNPP638	HF75%	7	1	1	640IU	P
SNPP639	HF12.5% GIR75%	7	0	0	40IU	
SNPP646	HF75%	7	0	0	40IU	
SNPP695	HF75%	7	0	0	40IU	
SNPP702	HF75%	7	0	0	20IU	
SNPP718	HF75%	7	0	0	40IU	
SNPP739	HF25%	7	0	0	20IU	
SNPP740	HF75%	7	0	0	20IU	
SNPP765	TP50%	7	0	1	640IU	P
SNPP781	HF62.5%	7	0	0	20IU	
SNPP827	HF69.5%	7	0	0	40IU	
SNPP927	HF12.5% GIR75%	7	0	0	40IU	
SNPP930	HF81.5%	6	0	0	20IU	
SNPP988	HF31%	6	0	0	40IU	
SNPP1028	HF67.5%	6	0	0	20IU	
SNPP1299	HF31.5% GIR50%	5	1	1	640IU	P
SNPP1335	HF62.5%	5	1	0	40IU	
SNPP1339	HF81.5%	5	1	1	1280IU	P
SNPP1597	HF62.5%	4	0	0	20IU	
SNPP1628	HF37.5%	4	0	0	40IU	
SNPP1678	HF62.5%	4	1	1	1280IU	P
SNPP1710	HF62.5%	4	0	0	20IU	
SNPP960EA0503	HF62.5%	4	1	1	640IU	P
SNPP8851		5	0	1	320IU	P
SNPP020		4	1	1	640IU	P

Fig.1 Comparison of Diagnostic Tests for Brucellosis

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