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

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 vactainers 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 (Nuncpolysurp). 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 diversificaton 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.%| 10  | 0    | 0     | 20IU |         |
| NF121      | HF25%  | 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       |
| 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       |
| Sample   | Type (%) | Bioassay | Titer | Notes |
|----------|----------|----------|-------|-------|
| 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     | 20IU  |       |
| SNPP500  | HF75%    | 8        | 0     | 0     | 20IU  |       |
| SNPP507  | JR50%    | 8        | 1     | 1     | 1280IU| P     |
| SNPP517  | HF75%    | 8        | 0     | 0     | 40IU  |       |
| 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     | 0     | 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

References

Agasthya, A. S., Isloor, S., & Krishnamsetty, P. (2012). Seroprevalence study of human brucellosis by conventional tests and indigenous indirect enzyme-linked immunosorbent assay. The Scientific World Journal, Article ID 104239

Alton, G. G., Jones, L. M., Angus, R. D., & Verger, J. M. (1988). Techniques for the brucellosis laboratory. Institut National de la recherche Agronomique (INRA).

Atluri, V. L., Xavier, M. N., De Jong, M. F., Den Hartigh, A. B., & Tsolis, R. M. (2011). Interactions of the human pathogenic Brucella species with their hosts. Annual review of microbiology, 65, 523-541.

Cadmus S.I.B., Adesokan H.K. & Stack J., (2008). The use of the milk ring test and Rose Bengal test in brucellosis control and eradication in Nigeria. Journal of the South African Veterinary Association 79, 113–115.

Cadmus S.I.B., Alabi P.I., Adesokan H.K., Dale E.J. & Stack J.A., (2013). Serological investigation of bovine brucellosis in three cattle production systems in Yewa Division, south-western Nigeria, Journal of the South African Veterinary Association 84(1), Art. #217, 6 pages.

Islam, M.R.U., M.P. Gupta, G. Filia, P.K. Sidhu and T.A. Shafi et al., (2013). Sero-epidemiology of Brucellosis in organized cattle and buffaloes in Punjab (India). Adv. Anim. Vet. Sci., 1: 5-8.

Junaidu, A. U., Oboegbulem, S. I., &Salihu, M. D. (2011). Serological survey of Brucella antibodies in breeding herds. 1(1):60–65

Mantur, B.G. and Amaranth, S.K. (2008). Brucellosis in infection with brucellosis. None of the unvaccinated India-a review. J. Biosci., 33(4):539-547.

Paweska, J. T., Potts, A. D., Harris, H. J., Smith, S. J., Viljoen, G. J., Dungu, B.,&Prozesky, L. (2002). Validation of
an indirect enzyme-linked immunosorbent assay for the detection of antibody against *Brucella abortus* in cattle sera using an automated ELISA workstation. The Onderstepoort journal of veterinary research, 69(1), 61.

Renukaradhya GJ, Isloor S and Rajasekhar M (2002). Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. Veterinary Microbiology, 90(1–4): 183–195.

Sanogo M, Thys E, Achi YL, Fretin, D, Michel P, Abatih E, Berkvens D & Saegerman C (2013). Bayesian estimation of the true prevalence, sensitivity and specificity of the Rose Bengal and indirect ELISA tests in the diagnosis of bovine brucellosis. The Veterinary Journal, 195(1), 114-120.

Varshochi, M., Majidi, J., Amini, M., Ghabili, K., & Shoja, M. M. (2011). False positive seroreactivity to brucellosis in tuberculosis patients: a prevalence study. International journal of general medicine, 4, 207–210. doi:10.2147/IGM.S15120

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