Original Research

Seroprevalence of Brucellosis in Buffaloes of Malwa Region of Madhya Pradesh

Sachin Verma, G. P. Jatav*, Supriya Shukla, A. K. Jayraw[1], H. C. Chauhan[2] and Nidhi Shrivastava

Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Mhow, Madhya Pradesh, INDIA

[1]Department of Veterinary Parasitology
[2]Department of Veterinary Microbiology

***Corresponding author:** drgpjatavpath@gmail.com

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Abstract

Brucellosis is one of the five main notifiable bacterial diseases of zoonotic importance in the world. The most widely used serological tests for diagnosis of brucellosis in animals are Rose Bengal plate test, standard tube agglutination test and enzyme linked immunosorbent assay. The present study was undertaken to assess the seroprevalence of brucellosis in 150 buffaloes belonging to different regions of Malwa tract and nearby areas of Indore district by using Rose Bengal plate test (RBPT), indirect enzyme linked immunosorbent assay (i-ELISA), Milk ring test (MRT) and California mastitis test (CMT) for detecting anti-Brucellaantibodies. Sero-prevalence of brucellosis in buffaloes by using RBPT, i-ELISA, MRT and CMT was recorded as 13.33% (20/150), 00% (00/150), 38.88% (7/18) and 55.55% (10/18), respectively.

Key words: Brucellosis, CMT, ELISA, MRT, RBPT, Seroprevalence

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Introduction

Brucellosis in buffaloes is the best known as one of the main reproductive disease, capable of causing abortion storms in the breeding season during the last trimester of pregnancy, retention of the fetal membranes, still births and reduction in milk yield resulting in great economic losses (Refai, 2003). In ruminants, organism has a marked affinity for lymphoid and reproductive organs, since the organism replicates to a high numbers in the gravid uterus. In non-pregnant and persistently infected cows, the udder and supramammary lymph nodes are the most common sites for localization (Meador, 1989). It was

reported that after abortion up to 80% of infected cows develop chronic infection localized in mammary glands and supramammary lymph node, and marnmary gland infection may persist throughout the lifetime of the cow (Beytut*et al.*, 2009; Xavier*et al.*, 2009; Hamdy and Amin, 2002).Accurate diagnosis of brucellosis is essential for institution of control strategies, either disease as a whole or as species-specific. The most widely used serological tests for diagnosis of brucellosis in animals are Rose Bengal plate test (RBPT), standard tube agglutination test (STAT) and enzyme linked immunosorbentassay (ELISA).

Materials and Methods

The present study was conducted in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Mhow (M.P). Samples were collected from 150 buffaloes (both lactating and non-lactating) from Cantonment Board slaughter house, Mhow and nearby areas of Indore. Milk and blood without anticoagulant (for serum) were collected for the present investigation.

Serum samples and milk samples were examined by RBPT, i-ELISA and MRT, respectively for detecting anti-*Brucella* antibodies. The collected milk samples were also screened by California mastitis test (OIE, 2012). The reagents like, RBPT antigen and Milk Ring Test (MRT) / Abortus Bang ring test (ABR) - ABR-antigen were procured from the Institute of Animal Health and Veterinary Biologicals (IAH and VB), Hebbal, Bengaluru, Karnataka-560024. Whereas, the indirect-enzyme linked Immunosorbent assay - *Brucella* Antibody Test Kit, ELISA along with the user's manual was procured from National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru. While the California mastitis test (CMT) reagents were prepared according to the method described by Schalm *et al.* (1971).

Results and Discussion

The study was undertaken to assess the sero-prevalence of brucellosis in buffaloes belonging to different regions of Malwa tract and nearby areas of Indore district. The serum samples were collected from buffaloes before slaughter from Cantonment Board slaughter house, Mhow. For detection of anti-*Brucella* antibodies in serum and milk samples of buffaloes, the prescribed tests, *viz.* RBPT, i-ELISA (NIVEDI, Bengaluru) and MRT and CMT, respectively were used as per the standard protocol for detecting anti-*Brucella* antibodies and the findings of which are shown in Table 1, 2, 3 and 4 and Plate 1, 2 and 3.

Seroprevalence of Brucellosis in Buffaloes

Detection of Anti-*Brucella* Antibodies in Buffaloes by using RBPT

For this study a total of 150 serum samples were examined for the presence of anti-*Brucella* antibodies in buffaloes (both lactating and non-lactating) using the RBPT. Out of 150 serum samples, 20 (13.33 %) serum samples were found positive for anti-*Brucella* antibodies by Rose Bengal Plate Test (RBPT).

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Table 1: Prevalence of anti-*Brucella* antibodies in serum samples using the Rose Bengal Plate Test (RBPT)

S. No.	Particulars	Number of Animals	Prevalence (%)
1	Positive	20	13.33
2	Negative	130	86.67

Table 2: Prevalence of brucellosis by using the indirect enzyme linked immunosorbent assay (i-ELISA)

S. No.	Particulars	Number of Animals	Prevalence (%)
1	Positive	0	0
2	Negative	150	100

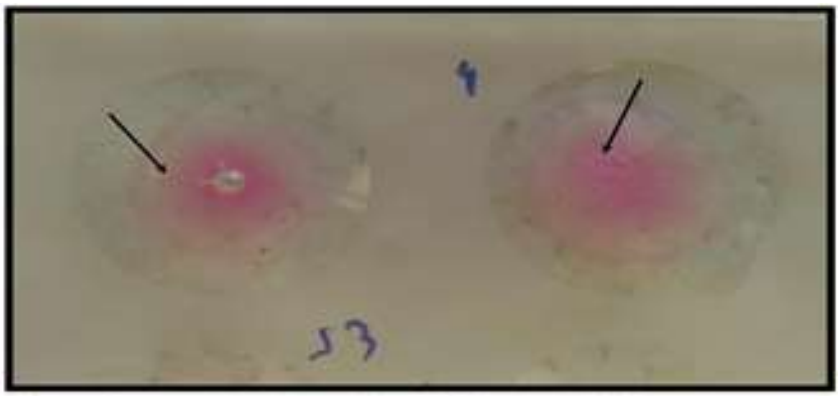

Plate 1a: Photograph showing positive RBPT for brucellosis(Arrows)

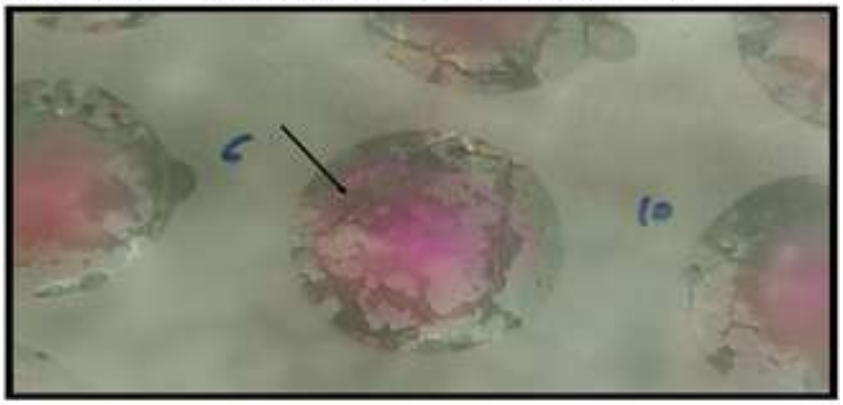

Plate 1b : Photograph showing negative RBPT for brucellosis(Arrow)

Plate 1: Photograph showing positive and negative RBPT for brucellosis

The findings of the present study are in close proximity with the findings of Lodhi *et al.* (1995) with 12.98 % seropositive cases, Sandhu*et al.* (2001) with 9.33% infection rate, Varasada (2003) with 16.80 % rate of infection, Nasir*et al.* (2004) with 15.38% buffaloes at government livestock farms, Bhattacharya *et al.* (2005) with 10.27 %, Dinka and Chala (2009) with 11.2 % infection in cattle, Saha *et al.* (2010) with 12.02% serum samples in organized herds in West Bengal, Karthik *et al.* (2014) with 16.49 % infection in cattle and Gogoi *et al.* (2017) with 12.69 % prevalence of brucellosis in bovines. The higher seroprevalence recorded in the present study is attributable to the fact that the present survey was carried out in slaughtered

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buffaloes which are being culled owing to their unproductivity, as culling of unproductive buffaloes is the routine practice of farmers of Malwa region of Madhya Pradesh.

Detection of Anti-*Brucella* Antibodies in Buffaloes by using i-ELISA

Findings of the present study are in contrast with the observations of Varasada (2003) with 20.00% infection, Munir*et al.* (2008) with 78% rate of infection, Grushina *et al.* (2010) with 100% positive serum samples in Kazakhstan, Kaleem *et al.* (2016) with 57.58 % infection and Gogoi *et al.* (2017) with 13.84% rate of infection when compared with findings of the present study which revealed all the animals were sero-negative by i-ELISA.

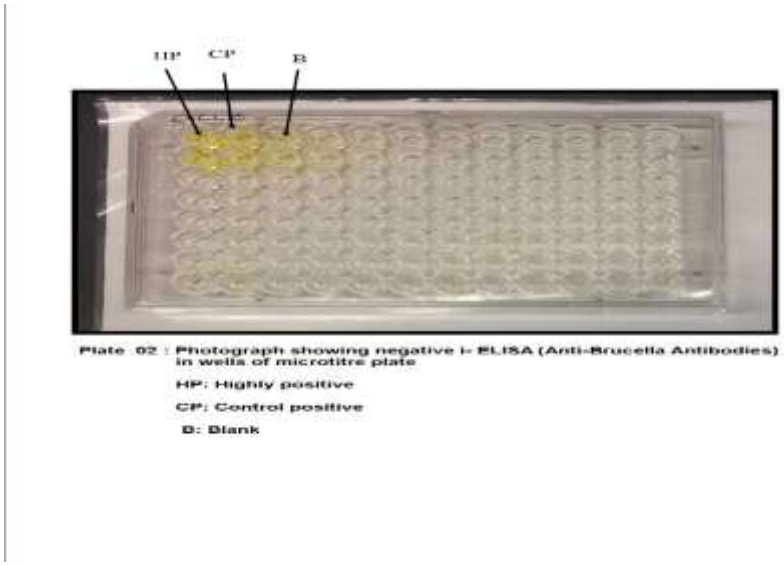

Plate 2: Photograph showing negative i- ELISA in walls of microtitre plate

Detection of Anti-*Brucella* Antibodies in Milk Samples of Buffaloes by MRT

Out of 150 buffaloes (18 lactating and 132 non-lactating), 18 milk samples were collected for detection of anti-*Brucella* antibodies by the MRT. Out of 18 milk samples, 07 samples were found positive for MRT with a prevalence of 38.88% (Table 3).

Table 3: Prevalence of brucellosis by using the Milk Ring Test (MRT)

S. No.	Particulars	Number of Animals	Prevalence (%)
1	Positive	7	38.88
2	Negative	11	61.55

The MRT is an agglutination test conducted on fresh milk collected from dairy animals, but it does not work on pasteurised or homogenized milk (Fleischhauer, 1937). This test detectsIgM and IgA antibodies

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bound to the fat globules which has wide acceptability as it is cost effective, easy to perform and can cover a large population in a short time (Cadmus *et al.*, 2008). Findings of this study are in consonance with the findings of Soomro *et al.* (2014) with 47.19% prevalence of brucellosis in aborted cattle and buffaloes in district of Hyderabad, Pakistan. Whereas the findings are in contrast with the findings of Shafee *et al.* (2011) with 4.6 and 1.7 % infection from cattle and buffaloes, Mohamand *et al.* (2014) with 18.35 % positive cases in dairy cow for anti-*Brucella* antibodies, Gogoi *et al.* (2017) with 10.53 % prevalence of brucellosis in bovines and Dalal *et al.* (2017) with 21.73% in pooled milk samples from Kaladera and Manpura Machedi.

Plate 03a: Photograph showing positive Milk Ring Test (MRT) for brucellosis

Plate 03b: Photograph showing negative Milk Ring Test (MRT) for brucellosis

Plate 3: Photograph showing positive and negative milk ring test for brucellosis

Detection of Mastitis by the California Mastitis Test

In the present study, 18 milk samples were also examined for mastitis by the CMT. Out of 18 milk samples, 10 cases were found positive for CMT with a prevalence of 55.55% (Table 4).

Table 4: Prevalence of mastitis by using the California mastitis test (CMT)

S. No.	Particulars	Number of Animals	Prevalence (%)
1	Positive	10	55.55
2	Negative	8	44.45

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Findings of the present study are in consonance with the findings of Mdegelaa *et al.* (2009) with 51.6 % infection in Tanzania and Gitau *et al.* (2014) with 56% cases of clinical mastitis.

Conclusion

Serological examination of 150 serum samples revealed 20 (13.33%) serum samples positive for anti-*Brucella* antibodies by RBPT. Whereas blood samples of all the animals (n=150) were found negative by i-ELISA. Overall 38.88% samples were recorded as positive by MRT and 55.55% cases were recorded as positive for mastitis by the Callifornia mastitis test that was more sensitive than the other for mastitis.

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