



Seroprevalence study of bubaline brucellosis in Kota Division of Rajasthan using various serodiagnostic tests

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Reproductive disease like brucellosis can cause severe losses to farmers. Various serological tests are being used for the diagnosis of brucellosis; conventional tests as RBPT and STAT have limitations of false positive and false negative results. Supplemental blood tests as HIT and 2-MET can differentiate specific and non-specific reactions found in bovine serum because nonspecific agglutinins can be inactivated when heated (Amerault *et al.* 1961) in HIT and reduced by 2-mercaptoethanol (McMahon 1983) in 2-MET. Immune precipitation methods like AGID test are used to demonstrate antibody in bovine sera which reacts with *Brucella* antigens other than the smooth lipopolysaccharide (Bruce and Jones 1958). ELISA was found more accurate than the conventional tests (Gall and Nielsen 2004) as it is a direct method of identification of specific antibody and able to detect all classes of antibody. Keeping in view these facts, the present study was under taken to (i) estimate the serum prevalence rate of bubaline brucellosis in Kota division, and (ii) compare the different serological tests in infected animals. Serum samples (436) from buffaloes and buffalo bulls from different regions of Kota division were collected for anti *Brucella* antibodies detection using serological tests, viz. RBPT (Davies 1971), STAT (WHO 1971), HIT, MET, AGID and AB ELISA. The RBPT antigen and *B. abortus* plain antigen for STAT (IVRI, Izatnagar) were used. Other tests performed were HIT (Amerault *et al.* 1961), MET (Nicoletti 1969), AGID (Stemshorn and Nielsen 1981), smooth lipopolysaccharide (S-LPS) based avidin biotin ELISA (AB ELISA kits supplied by AICRP on Animal Disease Monitoring and Surveillance, Bengaluru).

Brucellosis can be diagnosed by cultural or serodiagnostic tests, but serological detection is usually the method of choice for diagnosis and thereby control and eradication of bovine brucellosis as they are easier to perform and give rapid results. The seroprevalence of

brucellosis in animals of India was first reported by Polding (1942) and since then many workers have shown its existence in various parts of the country and thus it is expected to find the presence of antibodies in buffaloes of Kota region somewhat in the same range as found in other areas of the country by other researchers. This survey is probably the first attempt to assess the seroprevalence among buffaloes in Kota division of Rajasthan.

Out of 436 sera samples of buffaloes tested for brucellosis, 165 (37.84%) were found positive by AB ELISA. Similar findings were reported by others (Nasir *et al.* 2004, Jagapur *et al.* 2013) in buffaloes in three states of India.

Higher seroprevalence was also shown by Mahato *et al.* (2004) in cows in Asom. Relatively lower seroprevalence was also reported by Wadhwa (2007) in Bikaner region of Rajasthan and by Brahmabhatt *et al.* (2009) in Central Gujarat.

In the present study, the seroprevalence of brucellosis in females and males was detected as 38.46 and 30.30%, respectively. The apparently high seroprevalence figure in female animals compared to males in this study agrees with other works; 38.24% in females and 22.50% in males by Mittal *et al.* (2005). Lower seropositivity in males may be due to the fact that generally males are not vaccinated for brucellosis.

Similar results for RBPT were recorded by Nasir *et al.*

Table 1. Overall seroprevalence of brucellosis by different diagnostic tests

Total number of animals tested	Name of test	Number of seropositive animals	Per cent seroprevalence recorded
436	RBPT	153	35.09
436	STAT	129	29.59
436	HIT	114	26.15
436	2-MET	110	25.22
436	AGID	61	13.99
63	MRT	20	31.75
436	AB ELISA	165	37.84

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(2004) finding 35.40% buffaloes positive by RBPT. Lower seroprevalence values were recorded by Chakravarty *et al.* in 2007 (29.07%). A higher seropositive sera in STAT were found as 33.72% by Chakravarty *et al.* (2007). Lower seroprevalence was recorded in bovines by Sharma *et al.* (2007), (18.07%) in Punjab.

Higher seroprevalence (33.96%) was recorded by Shringi (1999) in cattle by HIT. In MET, Shringi (1999) found higher seropositivity in cattle (31.32%).

The AGID test was found to be the least sensitive in the current study. Stemshorn and Neilsen (1981) also reported that AGPT is less sensitive than other serological tests and of limited value for general diagnostic use.

In the present investigation, the relative sensitivity value was highest with RBPT followed by MRT, STAT, HIT, 2-MET and lowest with AGID considering AB-ELISA as Gold standard test. The relative specificity values were highest with AGID followed by 2-MET, HIT, STAT, MRT and lowest with RBPT.

The study revealed presence of *Brucella* antibody in serum of buffaloes of Kota division. The results also indicated that for serological diagnosis of brucellosis in buffaloes AB ELISA is better than RBPT and STAT and most suitable as a screening test because chances of non detection of an infected animal in ELISA are minimum. AGID was found the least sensitive and most specific test in our study.

SUMMARY

The study was undertaken to investigate the seroprevalence of brucellosis in buffaloes of Sangod, Mandana, Bundi, Talera, Chhabra, Jhalrapatan and Dag regions of Kota division of Rajasthan. Blood samples (436) were collected for detection of *Brucella* antibody using Rose Bengal plate test (RBPT), standard tube agglutination test (STAT), heat inactivation test (HIT), 2-mercaptoethanol test (2-MET), agar gel immunodiffusion test (AGID) and avidin biotin enzyme linked immunosorbent assay (AB ELISA). Overall seroprevalence was recorded as 37.84% by AB ELISA, 35.09% by RBPT, 29.59% by STAT, 26.15% in HIT, 25.23% in 2-MET and 13.99% in AGID. AB ELISA was found as the most sensitive whereas AGID was the least sensitive test. Higher number of females (38.46%) showed seropositivity as compared to males (30.30%). The results of the study indicated (a) relative suitability of ELISA as a screening test, and (b) endemacy of infection in villages of Kota division, Rajasthan.

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REFERENCES

Amerault T E, Manthei C A, Goode E R and Lambert G. 1961. A

heat inactivation test for differentiating specific and nonspecific agglutination reactions for bovine brucellosis. *American Journal of Veterinary Research* **22**: 564–69.

Brahmbhatt M N, Varasada R N, Bhong C D and Nayak R N. 2009. Seroprevalence of *Brucella* spp. in the buffaloes in the Central Gujarat region of India. *Buffalo Bulletin* **28** (2): 73–75.

Bruce W and Jones L M. 1958. The use of gel diffusion precipitin plates in the study of *Brucella*. *Bulletin of World Health Organisation* **19**: 187–96.

Chakravarty A, Barman N N and Sarma S. 2007. Seroprevalence of bovine brucellosis in different age groups of cattle. *Indian Veterinary Journal* **84**: 873–74.

Davies G. 1971. The Rose Bengal test. *Veterinary Record* **88**: 447–49.

Gall D and Nielsen K. 2004. Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. *Revue scientifique et technique Office International des Epizooties* **23**: 989–1002.

Jagapur R V, Rathore R, Karthik K and Somavansh R. 2013. Seroprevalence studies of bovine brucellosis using indirect enzyme linked immunosorbent assay (i-ELISA) at organized and unorganized farms in three different states of India. *Veterinary World* **6**(8): 550–53.

Mahato G, Sharma K and Mahanta P N. 2004. Comparative evaluation of serological tests for detection of brucellosis in bovine. *Indian Journal of Veterinary Medicine* **24**: 46.

McMahon K J. 1983. Comparison of the 2-mercaptoethanol and dithiothreitol tests for determining *Brucella* immunoglobulin G agglutinating antibody in bovine serum. *Canadian Journal of Comparative Medicine* **47**: 370–72.

Mittal V, Kumar M and Ambwani T. 2005. Seroepidemiological pattern of brucellosis among livestock of district Udhamsinghnagar in Uttaranchal. *Indian Journal of Veterinary Medicine* **25**: 28–32.

Nasir A A, Parveen Z, Shah M A and Rashid M. 2004. Seroprevalence of brucellosis in animals at government and private livestock farms in Punjab. *Pakistan Veterinary Journal* **24**(3): 144–46.

Nicoletti P. 1969. Further evaluations of serological test procedures used to diagnosis brucellosis. *American Journal of Veterinary Research* **30**: 1811–16.

Polding J B. 1942. Brucellosis in India. *Indian Journal of Veterinary Science* **13**: 27.

Sharma S, Mahajan A V, Kaur K, Verma S, Meenakshi and Kumar H. 2007. Screening of dairy farms for brucellosis and paratuberculosis. *Indian Veterinary Journal* **84**: 315–16.

Shringi B N. 1999. 'Sero-reactivity of *Brucella abortus* antigens for the diagnosis of brucellosis.' M.V.Sc. thesis submitted to the Rajasthan Agricultural University, Bikaner, Rajasthan.

Stemshorn B and Nielsen K. 1981. The bovine immune response to *Brucella abortus*. 4. Studies with a double immunodiffusion test for antibody against antigen A2. *Canadian Journal of Comparative Medicine* **45**: 147–53.

Wadhwa A. 2007. 'Survey of brucellosis, IBR, PPR and gastrointestinal helminthosis in cattle and buffaloes in and around Bikaner.' M.V.Sc. thesis, Rajasthan Agri. Univ., Bikaner, Rajasthan

WHO. 1971. *Technical Report Series Joint FAO/WHO Expert Committee on Brucellosis*. 5th Report No. 464.