
ORIGINAL ARTICLE**Brucellosis: Seroprevalence, Knowledge, Attitude and Practice among Veterinarians**

Smita S. Mangalgi^{1*}, Annapurna G. Sajjan¹, Shivajirao T. Mohite²

¹Department of Microbiology, BLDEU's Shri B M Patil Medical College, Vijayapur - 586103(Karnataka) India, ²Department of Microbiology, Krishna Institute of Medical Sciences, Karad -415110 (Maharashtra) India

Abstract:

Background: Brucellosis is an important but ignored zoonotic disease in India, with high prevalence among livestock and humans. It is of particular concern among veterinarians as they come in contact with infected animals in their day to day work. *Aim and Objectives:* The present study was carried out to determine the prevalence of antibrucellar antibodies and assess the Knowledge, Attitude and Practice (KAP) levels with regards to brucellosis among the veterinarians. *Material and Methods:* The serum samples of 1084 veterinarians were evaluated using the Rose Bengal Plate agglutination Test (RBPT), Serum Agglutination Test (SAT) and 2-Mercaptoethanol Test (2-ME test). All the participants were interviewed with a pre-designed questionnaire. *Results:* Prevalence of antibrucellar antibodies among the veterinarians was 9.31% by RBPT. Clinical symptoms relating to brucellosis were seen in 4.33% individuals. Of the 1084 subjects screened for KAP, awareness was highest among the veterinary officers and students, while other veterinary workers were ignorant. Though most of the veterinary officers had adequate knowledge and positive attitude the regular preventive practices were not sound. *Conclusion:* Significantly higher seroprevalence of brucellosis was noted among veterinarians. High 2-ME titres were a better correlate of an active infection. Awareness regarding brucellosis among assisting staff was low. Training and health education programs to raise the KAP standard are necessary.

Keywords: Brucellosis, Knowledge, Attitude and Practice, Rose Bengal Plate agglutination Test, Serum Agglutination Test, 2-Mercaptoethanol Test

Introduction:

Brucellosis remains an important zoonotic disease worldwide, accounting for more than 500,000 cases annually [1]. It has been eradicated from many developed countries. However it remains an uncontrolled problem in regions of high endemicity such as the Mediterranean, Middle East, Africa, Latin America and parts of Asia [2, 3]. Presence of brucellosis in India was established in the year 1942 [4]. Since then it has been reported from almost all the states. However it remains neglected probably due to lack of awareness among population at risk and treating physicians. This study was undertaken to evaluate the serological and clinical aspects of brucellosis and to assess the KAP standards among the veterinarians.

Material and Methods:

Veterinarians from Satara and Sangali districts of Maharashtra and from Bijapur and Bagalkot districts of Karnataka were included in this cross-sectional study. The rationale behind the study was explained to the study subjects. Veterinarians and healthy individuals (controls) who gave their consent were included in the study and those who denied were excluded from the study. Blood samples were collected aseptically from 975 employees of Department of Animal Husbandry and Veterinary Services and 109 private veterinary practitioners. Seven hundred serum samples from

healthy subjects were included as control. The control group was similar to the study group with respect to age, sex and socioeconomic status but had no history of contact with animals or ingested raw milk, milk products. All the participants were interviewed with a pre-designed questionnaire consisting of education, knowledge about brucellosis as a disease, its routes of transmission, preventive measures to be followed, signs and symptoms etc. For veterinarians their designation, work experience in the field, past history of brucellosis, present clinical symptoms and practice of preventive measures were included in the questionnaire. Serological study was done using the Rose Bengal Plate agglutination Test (RBPT), Serum Agglutination Test (SAT) and 2-Mercaptoethanol Test (2-ME). Antigens for the tests were procured from Indian Veterinary Research Institute, Izatnagar, UP. The tests were performed according to the protocol supplied by the manufacturers [5, 6]. For 2-ME test the dilution of serum was made in 0.85% saline containing 0.1M 2-Mercaptoethanol in place of phenol saline [7]. Results of the agglutination test were noted after 20 ± 2 hours. The tubes in the test series were compared with the antigen control tubes and the degree of agglutination for each serum sample was noted. Analysis of the data was done using Graph Pad InStat software.

Results:

A total of 1784 blood samples including 1084 from veterinary staff and 700 from healthy individuals were screened for *Brucella* agglutinins by RBPT, SAT and 2ME test. The results are depicted in Table 1.

For veterinarians mean titre \pm SD for SAT and 2-ME test were found to be 323.96 ± 446.18 and 263.97 ± 26.27 respectively. Significant SAT titres (≥ 320 IU) were seen in 47 (46.53%) whereas 2-

Mercaptoethanol test showed significant titres (≥ 160 IU) in 40 (39.60%). None of the control sera showed significant titres by SAT or 2-ME test (Table 1). Of the 101 RBPT positive veterinarians, 47 had clinical manifestations resembling brucellosis and were taking some kind of treatment but none was being treated for brucellosis. Low grade fever, fatigue, joint pain and low backache were the commonest symptoms (Fig. 1). All these 47 symptomatic veterinarians had significant SAT titres but only 40 had significant 2-ME titres. These forty veterinarians were started on antibrucellar treatment with oral regimen consisting of 200 mg doxycycline along with 600-900 mg rifampicin daily. Repeat follow-up serological tests were performed and treatment was continued till their 2-ME titres declined to 80 IU.

Seven veterinarians who had significant SAT titres but insignificant 2-ME titres, with symptoms of backache/joint pain/fatigue were advised for fortnightly follow up. Repeat SAT and 2-ME test carried out in them did not show any rise on follow up and also the symptoms did not worsen, hence were considered negative for brucellosis.

Age of the veterinarians ranged from 20-60 years. The mean and SD of seropositive subjects was 39.84 ± 8.46 years. The highest prevalence (13.03%) was seen in the age group of 31-40 years followed by (9.32%) 41-50 years (Fig 2). Though significant difference was seen in the age group and seropositivity, no linear trend was observed.

Working category wise distribution of veterinarians and prevalence of antibodies to *Brucella* is given in Table 2. No relation could be established between the working category and prevalence of antibodies to *Brucella*.

Adequate knowledge regarding modes of transmission, clinical features of brucellosis and preventive measures to be followed was observed

in veterinary officers (86.62%) and students (83.78%) as compared to other staff. Most of the veterinary officers and students assumed that brucellosis can be prevented by using personal protective equipment. On the contrary other staff

did not feel so (Table 3). Despite the positive attitude, regular preventive practices were not followed even by officers and students (Table 4). None of the control group subjects had heard of a disease named brucellosis.

Table 1: Serological Test Results in Controls and Veterinarians

Group	Number of samples	RBPT positive	Significant SAT Titre (≥ 320 IU)	Significant 2-ME Titre (≥ 160 IU)
Control	700	31 (4.42%)	00	00
Veterinarians	1084	101 (9.31%)	47 (4.33%)	40 (3.69%)

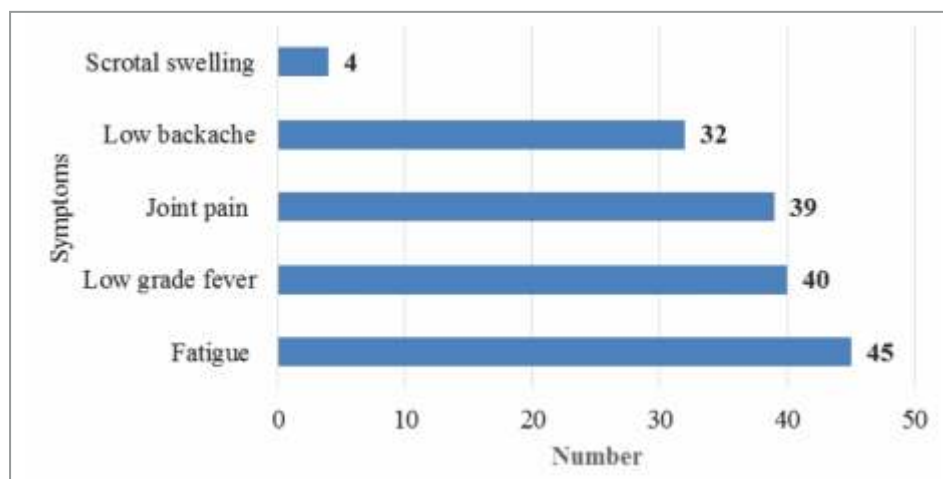


Fig. 1: Clinical symptoms in 47 veterinarians

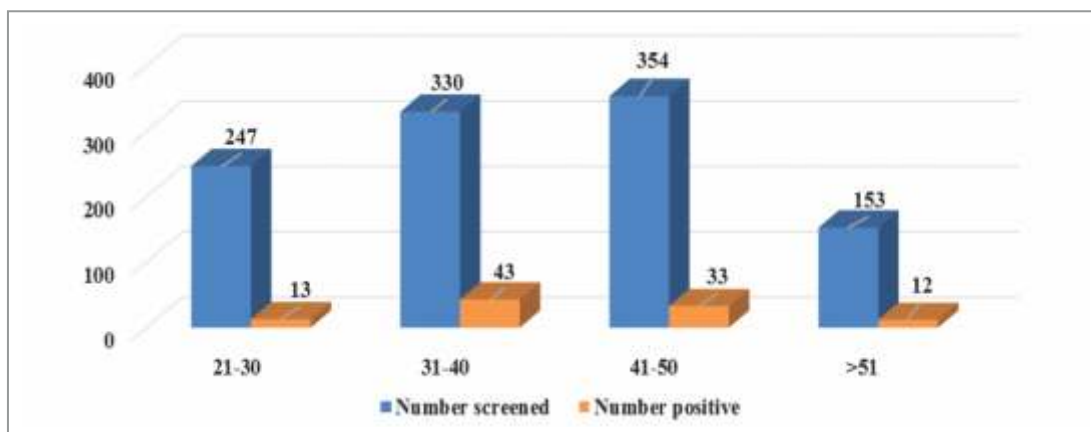


Fig. 2: Prevalence of Brucella Antibodies by RBPT among Different Age Groups

Table 2: RBPT, SAT and 2-ME Titres in Various Working Categories of Veterinarians

Category	Number screened	RBPT positive	Significant SAT titres	Significant 2-ME titres
Vet. doctors	385	35	13	10
Students	37	3	02	00
Inspectors	481	48	23	21
AI Technicians	14	0	00	0
Category D	167	15	09	09
Total	1084	101	47	40
χ^2 Test		P= 0.96	P= 0.78	P=0.59

No significant difference in seroprevalence was seen in various working categories of veterinarians
 RBPT - Rose Bengal Plate agglutination Test, SAT - Serum Agglutination Test, 2-ME - 2-Mercaptoethanol Test

Table 3: Knowledge and Attitude regarding Brucellosis among veterinarians

Category	Number screened	Knowledge Regarding			Attitude
		Modes of Transmission (%)	Clinical Features (%)	Preventive Measures (%)	
Vet. doctors	385	341(88.6)	349(90.6)	341(88.6)	303(78.7)
Students	37	31(83.8)	31(83.8)	31(83.8)	31(83.8)
Inspectors	481	151(31.4)	153(31.8)	203(42.2)	203(42.2)
AI Technicians	14	8(57.2)	2(14.3)	8(57.2)	7(50)
Group D	167	3(1.8)	00	3(1.8)	3(1.8)
χ^2 Test		P < 0.0001			

Significant difference in knowledge and attitude was observed in various working categories of veterinarians
 AI - Artificial Insemination

Table 4: Preventive Practices Followed by Veterinarians

Category	Number screened	Use of Personal Protective Equipment				Hand hygiene	Waste disposal
		Gowns	Gloves	Eye wear	Mask		
Vet. doctors	385	241(62.6)	04(1)	03(0.8)	00	385(100)	51(13.2)
Students	37	27(71.1)	03(8.1)	05(13.5)	00	37(100)	27(71.1)
Inspectors	481	00	08(1.7)	18(3.7)	00	481(100)	57(11.9)
AI Technicians	14	07(50)	14(100)	00	00	14(100)	06(42.9)
Group D	167	00	02(1.2)	19(11.4)	00	167(100)	17(10.2)
χ^2 Test		P < 0.0001				P = 1.0	P = 0.89

AI - Artificial Insemination

Discussion:

Brucellosis is endemic in India. A wide variation in prevalence of Brucella agglutinins in healthy individuals and among veterinarians has been reported. Nagarathna *et al.* and Vaishnavi *et al.* have reported prevalence rate of 1.1% and 16.8% in healthy population, while Rana *et al.* and Agasthya *et al.* have reported a rate of 27.7% and 9.06% among veterinarians [8-11]. In the present study prevalence of Brucella agglutinins was found to be 3.33% and 9.31% among the control group and veterinarians respectively. Compared to control group seroprevalence was significantly high among veterinarians, similar to the findings of Rana *et al.* [10].

Of the 47 symptomatic veterinarians, 40 had significant SAT as well as 2-ME titres. Remaining 7 individuals with significant SAT and insignificant 2-ME titres did not show any rise on follow up. The high SAT titres in these individuals could be due to repeated sub clinical infection/ exposure to antigenic stimuli as expressed by Agasthya *et al.*, Young EJ and Araj and Azzam. [11-13]. In our

study 2-ME Brucella agglutination titer of ≥ 160 IU was a better correlate of an active brucellosis requiring treatment, than a positive SAT titer of ≥ 320 IU alone in case of veterinarians, same has been noted by Buchanan *et al.* [14]. No relationship could be established between educational level, working category, age of the veterinarians and Brucella seropositivity. The knowledge regarding modes of transmission, symptoms, preventive measures to be followed and positive attitude was seen amongst veterinary officers and students whereas it was lacking in veterinary inspectors, Artificial Insemination (AI) technicians and Group D (class IV) workers. Most of the inspectors, AI technicians and group 'D' workers were unaware of transmission through inhalation and conjunctival routes. Majority of the veterinarians including officers did not use protective equipment regularly probably due to ignorance, unavailability and reluctance. In some areas, the protective equipment was available, but except gowns nothing was being used. All the staff including officers was reluctant

to use gloves and the reason given was - they were unable to get correct judgment/grip. This feeling can be overcome by making them use all the protective wear compulsorily right from the beginning of their career.

They did not use mask and eye wear as they did not consider inhalation and conjunctival routes to be important. Forty five veterinarians considered under using protective eye wear category (Table 5) actually wore spectacles. The physicians who were treating the symptomatic veterinarians also probably were unaware of the disease and did not consider brucellosis as one of the differential diagnosis, as described by Handa *et al.* [15] Hence programs to increase awareness regarding zoonotic diseases for physicians and KAP levels in veterinarians along with easy availability and compulsory use of the protective equipment are needed.

Since the clinical picture of brucellosis is not

distinctive, laboratory aid is must in the diagnosis [16-17]. Though isolation of Brucellae is considered the gold standard, many laboratories do not have the facilities for culture. In the absence of culture facilities, the diagnosis is done mainly by serological tests [18]. Commonly employed tests include RBPT and SAT. These tests detect both IgM and IgG antibodies. Demonstration of a rise in the antibody titre is diagnostic; however it is not always possible as the patients generally report late to the clinicians. Since high SAT titres are seen in treated cases and also in individuals with repeated subclinical exposures as in case of veterinarians, 2-ME test that detects specific IgG antibodies should be relied upon. Compulsory IgG Brucella antibody screening at least once a year is recommended.

Acknowledgement:

Authors are thankful to all the veterinary directors and officers who helped in arranging the camps.

References

1. Pappas G, Papadimitriou P, akritidis N, Christou L and Tsianos EV. The new global map of human brucellosis. *Lancet Infect Dis* 2006; 6(2):91-9.
2. Corbel MJ. Brucellosis: an overview. *Emerg Infect Dis* 1997; 3(2):213-21.
3. Refai M. Incidence and control of brucellosis in the Near East region. *Vet Microbiol* 2002; 90(1-4):81-110.
4. Renukaradhya GJ, Isloor S, Rajasekhar M. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. *Vet Microbiol* 2002; 90(1-4):183-95.
5. Rose Bengal Plate Test (RBPT) literature provided by Division of Biological Products, Indian Veterinary Research Institute, Izatnagar (UP).
6. Alton GG, Jones LM, and Pietz DE. Laboratory techniques in brucellosis, 2nd ed., 1975. p. 112. World Health Organization, Geneva.
7. Standard tube agglutination test (STAT) literature provided by Division of Biological Products, Indian Veterinary Research Institute, Izatnagar (UP).
8. Nagarathna S, Sharmada S, Veena Kumari HB, Arvind N, Sundar P, Sangeetha S. Seroprevalence of *Brucella* agglutinins: A pilot study. *Indian J Pathol Microbiol* 2009; 52(3):457-8.
9. Vaishnavi C, Kumar S. Investigation for background prevalence of *Brucella* agglutinins among blood donors. *Indian J Med Microbiol* 2007; 25(3):302-4.
10. Rana UV, Sehgal S, Bhardwaj M. A sero-epidemiological study of brucellosis among workers of veterinary hospitals and slaughter house of Union Territory of Delhi. *Int J Zoonoses* 1985; 12(1):74-9.
11. Agasthya AS, Isloor S, Prabhudas K. Brucellosis in high risk group individuals. *Indian J Med Microbiol* 2007; 25(1):28-31.

-
12. Young EJ. Serologic diagnosis of human brucellosis: analysis of 214 cases by agglutination tests and review of the literature. *Rev Infect Dis* 1991; 13(3):359-72.
 13. Araj GF, Azzam RA. Seroprevalence of brucella antibodies among persons in high risk occupation in Lebanon. *Epidemiol Infect* 1996; 117(2):281-8.
 14. Buchanan TM, Faber LC. 2-mercaptoethanol Brucella agglutination test: usefulness for predicting recovery from brucellosis. *J Clin Microbiol* 1980; 11(6):691-3.
 15. Handa R, Singh S, Singh N, Wali JP. Brucellosis in North India: results of a prospective study. *J Commun Dis* 1998; 30(2):85-7.
 16. Henk L. Smits & S. Manzoor Kadri. Brucellosis in India: a deceptive infectious disease. *Indian J Med Res* 2005; 122(5): 375-84.
 17. J. Corbel MJ, Beeching NJ. Harrison's Principles of Internal Medicine. In: Brucellosis. Fauci, Braunwald, Kasper, Hauser, Longo, Jameson, Loscalzo (eds). vol. 1. 17th Ed. New York: McGraw-Hill; 2008: 973-776.
 18. Franco MP, Mulder M, Gilman RH, Smits HL. Human brucellosis. *Lancet Infect Dis* 2007; 7(12):775-86.
-

***Author for Correspondence:** Mrs. Smita Mangalgi, Department of Microbiology, BLDEU's Shri B.M. Patil Medical College, Solapur Road, Bijapur - 586103, (Karnataka) India. Email: smitamangalgi@gmail.com, Cell: 09480263279