ORIGINAL ARTICLE

Brucella infections in high-risk population and in patients hospitalized for fever: A serological study at Kolar, Karnataka

Dhanalaxmi Aniyappanavar, Subba Rama Prasad, Khaji Mohammed Tanveer, Srinivasa Rao[1]

Departments of Microbiology and [1]Medicine, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka, India

ABSTRACT

Background: Brucellosis, one of the world's major zoonoses, is endemic in many parts of India. It is an occupational hazard for veterinary employees, butchers, dairy personnel, and laboratory workers. There is no information available on human brucellosis from Kolar region of southern India. **Methods and Materials:** Serum samples from 154 adults, at high risk for brucellosis and residing in and around Kolar, Karnataka, India, were screened for antibodies to brucella by the Rose Bengal plate agglutination test and immunoglobulin (Ig)G Enzyme-linked immunosorbent assay (ELISA). Sera detected to be positive by these screening tests were further tested by standard tube agglutination test (SAT), SAT with 2 Mercaptoethanol, and IgM ELISA. In addition, serum samples from 100 hospitalized patients with prolonged fever were also subjected to the above tests to detect acute brucella infections among patients. **Results:** Among the 154 individuals at high-risk screened, brucella antibodies were detected in 15 (9.74%) of the subjects. The seropositivity rate was 30.76% among veterinarians, 14.28 among cattle businessmen, 9.67% among butchers, and 3.79% among animal owners. Seropositivity was associated with drawing blood and exposure to animals during parturition. Symptoms such as myalgia, low back ache, and joint pains were significantly more among seropositives. When the results of all the tests were taken together, 33.3% seropositives could be classified as past brucella infections and 66.6% as possible chronic brucella infections. Among the 100 hospitalized patients with prolonged fever acute brucellosis was diagnosed in one patient. **Conclusion:** Brucellosis is an important zoonosis in Kolar region. Among individuals at high risk many have serological profile of possible chronic brucellosis; such individuals may need treatment and follow-up.

Key words: Brucella IgG ELISA, Brucella IgM ELISA, brucella infections in high high-risk groups, brucella serology, past brucella infections, possible chronic brucellosis

Introduction

Brucellosis is one of the world's major zoonoses of public health importance and economic concern. It is caused by bacteria belonging to the genus *Brucella*, which are facultative intracellular, gram-negative coccobacilli.[1] Four species of Brucella are known to cause human infections: *Brucella melitensis* (*Br. melitensis*), *Brucella abortus* (*Br. abortus*), *Brucella suis* (*Br. suis*), and *Brucella canis* (*Br. canis*). Goats and sheep are reservoirs for *Br. melitensis*; cattle for *Br. abortus*, swine for *Br. suis*, and dogs for *Br. canis*. Recently, infections from *Brucella pinnipediae* and *Brucella cetaceae* acquired from marine animals have been reported.[2] Infections with brucella may either remain latent or manifest as acute, subacute, or chronic illness.[1]

Access this article online	
Quick Response Code:	Website: www.atmph.org
	DOI: 10.4103/1755-6783.133714

Most of the human infections are caused by *Br. melitensis* and *Br. abortus*.[1] The animal reservoirs of these organisms transmit infection to man when he consumes raw milk or eats insufficiently cooked meat. Brucellae also pose an occupational hazard for veterinary employees, butchers, dairy personnel, and laboratory workers who are often exposed to the infected animals or their tissues.[3,4]

In villages and small towns of India, people live in close contact with domestic animals and consume their products. This provides opportunities for transmission

Correspondence:

Dr. Subba Rama Prasad, Department of Microbiology, Sri Devaraj Urs Medical College, Tamaka, Kolar-563 101, Karnataka, India.

E-mail: subbaramaprasad@gmail.com

of brucellosis. Studies from various parts of India have shown that seropositivity rates for brucellosis ranges from 4.97% to 41.23% in populations at risk.[5-9] Brucellosis has been diagnosed in 1%-20% of patients with prolonged fever admitted to hospitals in different parts of the country.[7,10-13]

There are no studies on human brucellosis from Kolar region in Karnataka, Southern India, known for production of milk and sheep raring. In this study, we report the seropositivity for brucellosis among high-risk groups in the population residing in and around Kolar town. We also report brucellosis among the patients admitted with prolonged fever to R L Jalappa Hospital, Kolar.

Methods and Materials

Serum samples from 154 adults (146 males and 8 females) residing in and around Kolar, who were at risk of brucellosis, were tested for the presence of antibodies to brucella. The high-risk population tested included veterinary personnel, animal-owning farmers, cattle businessmen, and butchers. Initially, the sera from all the subjects were screened for brucella antibodies by two tests: Rose Bengal plate agglutination test (RBPT) using reagents obtained from Institute of Animal Health and Veterinary Biologicals (IAHVB, Bangalore) and immunoglobulin (Ig)G Enzyme-linked immunosorbent assay (ELISA) (Brucella IgG ELISA Kit; Novatec Diagnostica). To confirm and further categorize the serological reactions, the serapositive in the screening tests were subjected to three more tests: standard tube agglutination test (SAT) using reagents obtained from IAHVB, SAT with 2 mercaptoethanol (SAT with 2ME), and IgM ELISA (Brucella IgM ELISA Kit; Novatec Diagnostica). Tests were performed as per the standard procedure[14] and in accordance with the kit manufacturer's instructions. The absorbance values obtained in the IgG and IgM ELISA were converted to Nova Tech Units (NTUs) by multiplying the absorbance of the test sample by 10 and dividing the product by the mean absorbance of the cut off controls as per the manufacturer's instructions. The sera showing >11 NTU were taken as positive.[15] The serological profile thus obtained was categorized as suggestive of acute brucella infection, subacute brucella infection, chronic brucella infection, and past brucella infection, taking together the results of all the serological tests performed.[15,16]

To know the role of brucellosis among cases with prolonged fever, we performed the above mentioned serological tests on 100 patients who were admitted to R L Jalappa Hospital between November 2007 and May 2009. Patients with proven and obvious causes (like tuberculosis, malaria, etc) were excluded from the study. Among those tested, 56 were males and 44 were females; 14 were children (≤15 years) and 86 were adults (mean age: 33.98 years). A patient with a temperature of >38.3°C for 10 days or more was included in the study. Using a predesigned proforma, the name, age, sex, occupation, and details of exposure to risk factors for brucellosis were recorded both for subjects at risk and the patients with fever.

Results

The seropositivity for brucellosis in the high-risk population screened is presented in Table 1. Out of the 154 individuals screened, 13 (8.44%) were positive by RBPT and 15 (9.74%) were positive by IgG ELISA. The seropositivity among veterinary personnel was 30.76%, followed by cattle businessmen (14.28%), butchers (9.67%), and animal owners (3.79%).

The association of risk factors of brucellosis in 15 seropositives and 139 seronegatives among the high-risk population screened is shown in Table 2. The mean age of brucella antibody positives did not differ significantly (38 vs. 40 years). There were no seropositives among the eight females included in the study. An analysis of risk factors showed that only two activities: Drawing blood from cattle or sheep, and exposure to animals during parturition had high odds ratio with acceptable confidence Intervals and a significant *P* value ($P < 0.05$). Risk factors like handling cattle/sheep, washing the shed, cleaning or bathing animals, or consumption of raw milk/milk products or raw meat did not show a significant association with brucella infection.

The association of symptoms of brucellosis with seropositive and seronegative subjects is presented in Table 3. Among the symptoms that could be elicited, we found that myalgia, low back ache, and joint pains

Table 1: Seropositivity for brucellosis in subjects belonging to risk groups

Risk group	Number tested	Number positive	Number positive
		RBPT	IgG ELISA
Animal owners	79	3 (3.79)*	3 (3.79)
Butchers	31	2 (6.45)	3 (9.67)
Veterinary personnel	26	7 (26.92)	8 (30.76)
Dairy personnel	8	0	0
Cattle businessmen	7	1 (14.28)	1 (14.28)
Animal handlers	3	0	0
Total	154	13 (8.44)	15 (9.74)

*Figures in parentheses are percentages. ELISA = enzyme-linked immunosorbent assay, IgG = immunoglobulin G, RBPT = Rose Bengal plate agglutination test

Table 2: Association of risk factors of brucellosis in seropositives and seronegatives

Risk factor	Seropositives (*N* = 15)	Seronegatives (*N* = 139)	Odds ratio (95% CI)*	*P* value
Mean age (range)	38 (25-56)	40 (14-75)	—	—
Handling cattle/sheep	14 (93.33%)	128 (92.08%)	1.20 (0.14-10.02)	>0.05
Drawing blood from cattle/sheep	8 (53.33%)	32 (23.01%)	3.82 (1.28-11.34)	<0.05
Assisting in parturition of cattle/sheep	8 (53.33%)	39 (28.05%)	2.93 (0.99-8.62)	<0.05
Washing shed, cleaning, and bathing cattle/sheep	6 (40%)	99 (71.22%)	0.26 (0.09-0.80)	>0.05
Owning cattle/sheep	4 (26.66%)	98 (70.50%)	0.15 (0.04-0.50)	>0.05
Consumption of raw milk, milk products, or raw meat	2 (13.33%)	26 (18.70%)	0.66 (0.14-3.17)	>0.05

*: 95% Confidence interval

Table 3: Association of symptoms of brucellosis in seropositives and seronegatives

Risk factor	Seropositives (*N* = 15)	Seronegatives (*N* = 139)	Odds ratio (95% CI)*	*P* value
Fever	2 (13.33%)	09 (6.47%)	2.22 (0.43-11.39)	>0.05
Night sweats	2 (13.33%)	05 (3.59%)	4.1 (0.72-23.39)	>0.05
Myalgia	2 (13.33%)	03 (2.15%)	6.97 (1.06-45.59)	<0.05
Low backache	2 (13.33%)	03 (2.15%)	6.97 (1.06-45.59)	<0.05
Joint pains	5 (33.33%)	19 (13.66%)	3.15 (0.97-10.25)	<0.05

*95% Confidence interval

were significantly more common in seropositives (Odds ratio >1 and *P* value < 0.05).

All the 15 sera, positive by IgG ELISA were further tested by SAT, SAT with 2ME and IgM ELISA for brucella antibodies. Table 4 presents the results of these tests along with the serological profile, taking the results of all the tests together. The serapositive for IgG antibodies in the screening tests, showed SAT titers that ranged between 40 and 1280. Titers of 160 or more were found in 12 (80%) of the sera. The antibodies persisted even after treatment with 2 mercaptoethanol in all the sera and in addition, seven (46.67%) registered a fourfold drop in titers in the SAT with 2-mercaptoethanol (2ME).

In ELISA, IgG antibodies only, without IgM antibodies, were found in 5 (33.33%) of the above 15 samples tested. The IgG ELISA NTU values ranged in these sera between 17.58 and 47 (mean: 28.82). We could categorize the serological profile in these subjects who showed only IgG ELISA antibodies as brucella infections in the past. Sera from these patients did not record any change in the SAT titers after 2ME treatment. One of the subjects belonging to this serological profile, a veterinarian by profession, gave a history of being treated for brucellosis 20 years ago.

In ELISA, IgM antibodies in addition to IgG antibodies were found in 10 (66.67%) of the samples tested. The IgM ELISA NTU values ranged between 22.24 and 39.97 (mean: 20.95). In the IgG ELISA, the NTU values, in this group of sera, ranged between 17.57 and 102.97 (mean: 51.62). Based on these serological findings, we could categorize the serological profile in these 10 subjects, showing both IgG and IgM antibodies, as possible chronic brucella infections.[15] None of the sera from subjects in this category had IgM ELISA NTU values 3 times that of the IgG ELISA values and three of the sera had IgG ELISA NTU values 3 times above that of IgM ELISA NTU values. Six (60%) of the patients in this category complained of joint pains, low back ache, and excessive sweating of the palms — symptoms compatible with brucellosis.

Among the 100 patients with prolonged fever who were screened for brucellosis, sera from three patients were positive by RBPT. However, brucellosis could be confirmed only in one by the battery of tests mentioned above. The patient was a 38-year-old woman from a nearby village. Her serum showed a titer of 320 by SAT and was positive for IgG and IgM antibodies by ELISA. The patient complained of generalized weakness and had pain in the shoulder and in all other joints. Her leukocyte count was 2,700 cells/mm^3 and erythrocyte sedimentation rate was 70 mm/h. Peripheral smear for malaria and serological examination for dengue by ELISA were found to be negative. The patient recovered on treatment with doxycycline. The patient gave a history of owning cattle but no history of consumption of raw milk, milk products, or raw meat.

Discussion

We found that among 154 persons at high risk for brucellosis, 15 (9.7%) individuals had serological evidence of brucella infection. Veterinary personnel had a seropositivity rate of 30.76% followed by the cattle businessmen (14.28%), butchers (9.67%), and the animal owners (3.79%). Veterinary personnel and butchers involved in our study were from Kolar town and the cattle business men and animal owners were from the villages surrounding the town. Serological

Table 4: Results of the confirmatory tests and categorization of serological profile in subjects positive in the screening tests

Sample no	Occupation	SAT	2ME	IgG ELISA (NTU)*	IgM ELISA (NTU)	Clinical symptoms	Suggestive serological profile
Br/02/08	Veterinarian	160	160	Positive (17.58)	Negative (<11)	Nil	Brucella infection in the past
Br/10/08	Reserve Police officer; maintains dogs	160	160	Positive (20.51)	Negative (<11)	Occasional fever	Brucella infection in the past
Br/58/08	Farmer	80	80	Positive (29.77)	Negative (<11)	Nil	Brucella infection in the past
Br/92/09	Veterinarian	40	40	Positive (47.00)	Negative (<11)	Treated for brucella melitensis 10 yrs back	Brucella infection in the past
Br/128/09	Butcher	40	40	Positive (29.22)	Negative (<11)	H/o severe knee pain	Brucella infection in the past
Br/87/09	Veterinary assistant	640	160	Positive (102.97)	Positive (39.97)	knee pain, myalgia, low back ache.	Possible chronic brucella infection
Br/102/09	Veterinarian	1280	640	Positive (71.12)	Positive (23.71)	Nil	Possible chronic brucella infection
Br/121/09	Farmer/poultry worker	160	80	Positive (34.42)	Positive (33.47)	Nil	Possible chronic brucella infection
Br/127/09	Butcher	1280	160	Positive (71.98)	Positive (30.44)	Low back ache	Possible chronic brucella infection
Br/133/09	Butcher	640	160	Positive (73.50)	Positive (34.30)	Pain in knees and ankle joints, sweating of palms	Possible chronic brucella infection
Br/09/08	Veterinarian	640	80	Positive (67.62)	Positive (22.45)	Nil	Possible chronic brucella infection
Br/15/08	Veterinarian	640	160	Positive (21.55)	Positive (22.24)	Small joint pain, knee joint pain, not relieved by analgesic. Sweating of palms	Possible chronic brucella infection
Br/29/08	Veterinary driver	320	80	Positive (17.57)	Positive (28.74)	Occasional fever	Possible chronic brucella infection
Br/41/08	Cattle businessman	160	80	Positive (28.43)	Positive (29.37)	Nil	Possible chronic brucella infection
Br/08/08	Veterinary assistant	1280	320	Positive (27.04)	Positive (34.84)	H/O ankle and knee joint pain	Possible chronic brucella infection

ELISA = enzyme-linked immunosorbent assay, IgG = immunoglobulin G, IgM = immunoglobulin M, 2ME = 2-mercaptoethanol, NTUs* = Nova Tech Units, SAT = standard tube agglutination test

surveys conducted among high-risk populations earlier from India have also recorded a high positivity rate among veterinary personnel, butchers, and those in contact with domestic animals.[4-9] In the population studied, seropositivity for brucellosis was significantly associated with bleeding animals or conducting/assisting during their parturition. Transmission through aerosol generated during these procedures could explain this positive association. We could not find a positive association with consumption of raw milk, raw milk products, or raw meat, though these practices existed in the population. We think that occasional indulgence by the population with the above-risk factors might have resulted in a negative association. Contrary to the thinking that intimate contact with urine and dung of cattle may transmit brucellosis, we did not find any positive association between activities like washing the shed, cleaning, or bathing cattle or sheep, which provide ample opportunities for coming in contact with the urine and feces of these animals.[17]

The seropositives detected during serosurveys are usually classified as subclinical brucella infections.[18] We found that our seropositive subjects fell into two categories: Past infections and possible chronic infections. The subjects with past brucella infections had only IgG ELISA antibodies and lacked IgM ELISA antibodies. In addition, subjects with this serological profile had low SAT titers and lower NTU values in the IgG ELISA.

In contrast to the above serological profile, we found another group of subjects who had both IgG ELISA and IgM ELISA antibodies. They constituted 66.67% of the seropositive subjects. They had SAT titers (≥320) and most of them (70%) registered a four-fold or higher drop in SAT after 2ME treatment. The average IgG ELISA titers in this group was almost twice as much as the value in the past brucella infection group. The serological profile in these subjects is reminiscent of patients with chronic brucellosis.[15] A total of 60% of subjects falling under this group in our study had symptoms compatible with chronic brucellosis.

Patients with brucellosis are known to appear normal and complain of many nonspecific symptoms.[19] We think further studies to confirm the activity of brucellosis in such subjects with serological profile as above are required. Studies with western blotting have shown that active brucella infections are associated with persistence of antibodies to intracellular antigens of brucella.[20,21] Culture studies and detection of brucella deoxyribonucleic acid by polymerase chain reaction in the sera of subjects presenting with the serological profile of chronic brucellosis may also be useful in establishing the activity of brucellosis.

It has been shown that successful therapy of brucellosis results in fall and eventual disappearance of IgG class of antibodies to brucella.[15] We think it may be worthwhile treating these subjects with serological profile of chronic

brucellosis and follow them up for disappearance of IgG ELISA antibodies. As brucella infections in our study showed a high prevalence among professional groups such as veterinary workers, butchers, and villagers in contact with livestock, we reiterate that educating these groups of workers about the complications of the diseases and the practice of standard precautions, such as frequent hand washing, wearing masks, gloves, and protective clothing during work, may reduce the risk of brucella infections.[8]

Among 100 PUO patients studied by us, we could detect one patient with acute brucellosis. This emphasizes that all cases of PUO in Kolar region need to be screened for brucellosis.

Albeit the fewer women included in our study and smaller number of subjects screened in some of the professional groups, the data presented here show that brucellosis is an important zoonosis in the Kolar region. Further studies on isolation and identification of organism from animals and patients would throw more light on the disease.

References

1. Franco MP, Mulder M, Gilman RH, Smits HL. Human brucellosis. Lancet Infect Dis 2007;7:775-86.
2. Sohn AH, Probert WS, Glaser CA, Gupta N, Bollen AW, Wang JD, *et al*. Human neurobrucellosis with intracerebral granuloma caused by marine mammal Brucella spp. Emerg Infect Dis 2003;9:485-8.
3. Mantur BG, Biradar MS, Bidri RC, Mulimani MS, Veerappa, Kariholu PL, *et al*. Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. J Med Microbiol 2006;55:897-903.
4. Kumar P, Singh DK, Barbuddhe SB. Sero-prevalence of brucellosis among abattoir personnel of Delhi. J Commun Dis 1997;29:131-7.
5. Barbuddhe SB, Kumar P, Malika SV, Singh DK, Gupta LK. Seropositivity for intracellular bacterial infections among abattoir associated personnel. J Commun Dis 2000;32:295-9.
6. Thakur SD, Thapliyal DC. Seroprevalence of brucellosis in man. J Commun Dis 2002;34:106-9.
7. Umapathy BL, Nagamani M, Bhat P, Keshavamurthy BS. Seroepidemiological studies on human brucellosis in and around Bangalore. Indian J Comp Microbiol Immunol Infect Dis 1984;5:83-7.
8. Hemashettar BM, Patil CS. Brucellosis among practising veterinarians. Indian J Med Microbiol 1991;9:45-7.
9. Agasthya AS, Isloor S, Prabhudas K. Brucellosis in high risk group individuals. Indian J Med Microbiol 2007;25:28-31.
10. Handa R, Singh S, Singh N, Wali JP. Brucellosis in north India: Results of a prospective study. J Commun Dis 1998;30:85-7.
11. Kadri SM, Rukhsana A, Laharwal MA, Tanvir M. Seroprevalence of brucellosis in Kashmir (India) among patients with pyrexia of unknown origin. J Indian Med Assoc 2000;98:170-1.
12. Sen MR, Shukla BN, Goyal RK. Seroprevalence of brucellosis in and around Varanasi. J Commun Dis 2002;34:226-7.
13. Mantur BG, Akki AS, Mangalgi SS, Patil SV, Gobbur RH, Peerapur BV. Childhood brucellosis — a microbiological, epidemiological and clinical study. J Trop Pediatr 2004;50:153-7.
14. Alton GG, Jones LM, Pietz DE. Laboratory techniques in brucellosis. World Health Organ 1975;1-163.
15. Mantur B, Parande A, Amarnath S, Patil G, Walwekar R, Desai A, *et al*. ELISA versus conventional methods of diagnosing endemic brucellosis. Am J Trop Med Hyg 2010;83:314-8.
16. Pabuccuoglu O, Ecemis T, El S, Coskun A, Akcali S, Sanlidag T. Evaluation of serological tests for diagnosis of brucellosis. Jpn J Infect Dis 2011;64:272-6.
17. Corbel MJ. Brucellosis in animals and humans. Geneva: WHO Press; 2006. Chapter 3, Epidemiology; p.13-21.
18. Henderson RJ, Hill DM. Subclinical brucella infections in man. 1972;3:154-6.
19. Wright SG. Brucellosis. In: Cooki GC, Zumla A, editors. Manson's Tropical Diseases. 21st ed. London: Saunders; 2003. p. 1085-9.
20. Goldbaum FA, Leoni J, Wallach JC, Fossati CA. Characterization of an 18-kilodalton Brucella cytoplasmic protein which appears to be a serological marker of active infection of both human and bovine brucellosis. J Clin Microbiol 1993;31:2141-5.
21. Kwaasi AA, Al-Mohanna FA, Nakeeb SM, Roberts GT, Al-Thawadi S, Hassan AY, *et al*. Correlation of antigenic expression with progress in antibiotic therapy of acute human brucellosis. J Med Microbiol 2005;54:533-8.

Cite this article as: Aniyappanavar D, Prasad SR, Tanveer KM, Rao S. Brucella infections in high-risk population and in patients hospitalized for fever: A serological study at Kolar, Karnataka. Ann Trop Med Public Health 2013;6:549-53.

Source of Support: No, **Conflict of Interest:** None declared.