Serological, Clinical, and Epidemiological Profile of Human Brucellosis in Rural India

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ABSTRACT

Background: Brucellosis is an important but neglected zoonotic disease in India. Due to frequent animal contact, high prevalence of this disease, though expected in rural population, has not been much studied. Aim: The study was carried out to determine serological, clinical, and epidemiological profile including associated risk factors for human brucellosis in rural India. Materials and Methods: In this cross-sectional study, serum samples from 1,733 individuals residing in rural areas were screened for the presence of anti-brucellar antibodies by Rose Bengal Plate test (RBPT), Serum Agglutination test (SAT), and 2-Mercaptoethanol test (2-ME). Clinical symptoms, epidemiological data including risk factors and knowledge about brucellosis were evaluated by personal interview using a structured questionnaire. Results: Of the 1,733 individuals, 998 had direct contact with animals, whereas 735 had no direct contact. The overall positivity rates by RBPT, SAT, and 2-ME test were 10.50% (182), 7.32% (127), and 5.88% (102), respectively. Clinical symptoms resembling brucellosis were seen in 151 (8.71%) subjects. Animal contact especially during milking, parturition-abortion was the major risk factor, followed by raw milk ingestion. None of the participant knew about brucellosis. Conclusion: Regular surveillance of the disease with awareness programs emphasizing prevention and control are needed.

Keywords: Human brucellosis, Rose Bengal Plate test, risk factors, serum agglutination test, 2-Mercaptoethanol test

Introduction

Brucellosis is the commonest zoonotic disease of worldwide distribution.¹,² In animals, it presents as a chronic infection that persists for life and Brucellae are shed in large numbers in milk, urine, and products of pregnancy. In humans, it is mainly seen in the individuals who come in contact with animals directly.³,⁴ It remains a significant threat to human health in India, especially in rural areas. The rural population is primarily engaged in agriculture for which cattle are used. To supplement the income, small ruminants especially goats and sheep are reared. These animals, most of the times, are kept in the yards of houses or even brought inside. Children adopt these animals as pets. Due to inordinate exposure to animals and their products and ignorance regarding zoonotic diseases, high prevalence of brucellosis, though expected, is not much studied in India.

The purpose of this study was to determine the prevalence of antibrucellar antibodies among the rural population, to study the association between the epidemiological data and seropositivity as well as to evaluate the clinical symptoms, risk factors, knowledge, attitude, and practice levels regarding brucellosis in rural population.

Materials and Methods

The present study was planned to be conducted in rural area, which was the catchment area of the Institute’s Hospital from where the brucellosis cases were being reported. This area belonged to three districts: Bijapur, Bagalkot, and Gulbarga. However, brucellosis burden...
in this area was not known. The villages from this catchment area were selected randomly, one by one, to fulfill the desired minimum sample size. It was planned to select the villages till the village wise cumulative total of studied subjects was less than the minimum sample size. The random selection of village was terminated when the cumulative total was equal or more than the desired minimum sample size.

General health check-up camps were conducted in these randomly selected villages. Brucellosis awareness program was one of the objective purposes behind organization of these camps. This cross-sectional health camp-based study was approved by the Institutional Ethical Committee.

The reported average prevalence rate of brucellosis in high risk population is found to be 8.5\%.(6,7) Considering this prevalence rate with 95% confidence level and 1.5\% allowable error minimum number of subjects (sample size) required to study was 1,383 \((n = 4pq/L^2 = [4 \times 8.5 \times 91.5]/1.5^2])\). To reach this targeted number of subjects, it required organization of health check-up camps in nine villages. The villages randomly selected in the study were Indi, Muddebihal, Sindagi, Basavan-Bagewadi from Bijapur district of population 38,217; 34,217; 37,213; 33,198, respectively; Badami, Jamakhandi, and Bilagi from Bagalkot district of population 30,943; 68,398; 17,792, respectively; and Aland and Afzalpur from Gulbarga district of population 27,088; 42,371, respectively. Thus, total number of subjects studied in health check-up camps in these nine villages was 1,733.

Subjects eligible to participate in the study were identified as per following inclusion-exclusion criteria.

**Inclusion criteria**
Individuals residing in the study villages for more than 1 year irrespective of symptoms of brucellosis.

**Exclusion criteria**
Individuals staying in the study villages for less than 1 year were excluded.

Consent from each study subject, eligible and willing to participate in the study, was obtained.

All the participants were interviewed with a pre-designed questionnaire regarding age, sex, occupation, contact with animal/animal products, type of animal, duration of contact, raw milk ingestion, knowledge regarding animal and human brucellosis as a disease, its transmission, clinical symptoms, prevention, etc.

The questionnaires were completed with the assistance of a trained person, in the local language. Depending on the nature of animal contact the individuals residing in rural areas were grouped into two groups as: Group I directly exposed (DE) and group II indirectly exposed (IE). Individuals in group I had regular direct contact with animals either at home or work place and those in group II had animals in the neighborhood, i.e., indirect contact.

About 3 ml of blood sample was collected from each individual with or without symptoms. Serological study was done using the Rose Bengal Plate test (RBPT), Serum Agglutination test (SAT), and 2-Mercaptoethanol test (2-ME). Antigens for RBPT and SAT tests were procured from Indian Veterinary Research Institute, Izatnagar, U.P. and Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore.

The tests were performed according to manufacturer’s guidelines. For 2-ME test, the dilution of serum was made in 0.85% saline containing 0.1M 2-ME in place of phenol saline.(8) Test results were noted after 20 ± 2 hours of incubation at 37°C in the water bath. For each serum, sample titers were noted after comparing the tubes in the test series with the antigen control tubes for degree of opacity of the supernatant fluid. The tests were considered positive if the SAT and 2-ME titers were ≥160 IU and ≥80 IU, respectively.

Repeat serological test was performed for the individuals with symptoms resembling brucellosis but negative tube tests titers and also in the asymptomatic individuals with positive tube tests. The results were evaluated for seroprevalence, clinical symptoms, and risk factors regarding brucellosis. The data was analyzed using GraphPad InStat designed by GraphPad Software Inc.

**Results**
Of the 1,733 subjects screened, 998 were grouped in group I and 735 in group II. Positive reaction by RBPT was noted in 179 individuals in group I and three in group II. Titers ranging from 40 to 5120 IU by SAT and from 40 to 2560 IU by 2-ME test were noted in 170 and 119 individuals, respectively, in group I. In group II, SAT and 2-ME titers ranged between 1280-2560 IU and 640-1280 IU, respectively [Table 1].

Significant titers by SAT (≥160) and 2-ME (≥80) test were noted in 133 (13.3\%) and 99 (9.9\%) individuals in group I and three (0.4\%) individuals in group II.

Fever, joint pain, low backache, fatigue, headache, sleep disturbance (insomnia and night sweats) were the reported symptoms. One hundred and forty-eight (14.82\%) individuals in group I had the clinical
symptoms of which 99 had significant SAT and 2-ME titers. In group II, only three individuals had symptoms and all of them had significant SAT as well as 2-ME test titers [Table 2].

All the three individuals of group II with significant SAT and 2-ME titers complained of fever, joint pain, low backache, fatigue, and headache. As the activity of brucellosis is indicated by presence of significant 2-ME titers, the results of 2-ME test were considered for further evaluation.(9)

Of the 1,733 subjects only 3 had heard about brucellosis as an animal disease, and no one was aware about its transmission to humans. As knowledge regarding human brucellosis was lacking, attitude toward preventive practices was also not observed.

Association between epidemiological factors and risk factors is given in Tables 3 and 4.

**Discussion**

Brucellosis in India was recognized early in the previous century and since then has been reported from almost all the states. The serological studies have reported prevalence rate of 5% in cattle, 3% in buffaloes, 8.23% in sheep, and 4.43% in goats.(10,11) A wide variation in the prevalence of human brucellosis ranging from 0.8 to 26.6% has been reported by various authors.(7,12-17) In the present study, seroprevalence documented was 13.3% and 9.9% by SAT and 2-ME tests, respectively, in group I and 0.4% in group II. Totally, 153 individuals complained of health problems like fatigue, headache, insomnia, joint pain, fever, low backache. Among the individuals who complained of symptoms 102 had significant SAT as well as 2-ME titers suggestive of an active brucellosis.

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**Table 1: SAT and 2-ME test titers in RBPT positive group I and II individuals**

| Group | Test | Nil | 40 | 80 | 160 | 320 | 640 | 1280 | 2560 | 5120 |
|-------|------|-----|----|----|-----|-----|-----|------|------|------|
| I     | SAT  | 09  | 17 | 20 | 29  | 39  | 26  | 28   | 04   | 07   |
|       | 2-ME | 60  | 20 | 09 | 29  | 32  | 16  | 08   | 05   | 00   |
| II    | SAT  | 00  | 00 | 00 | 00  | 00  | 00  | 00   | 02   | 01   |
|       | 2-ME | 00  | 00 | 00 | 00  | 00  | 00  | 02   | 01   | 00   |

SAT: Serum agglutination test, 2-ME: 2-Mercaptoethanol test, RBPT: Rose Bengal plate test.

**Table 2: Association of SAT and 2-ME test titers and clinical symptoms in group I**

| Variable       | SAT test | P value | OR | 2-ME test | P value | OR |
|----------------|----------|---------|----|-----------|---------|----|
| Health problem |          |         |    |           |         |    |
| Yes            | 118 30   | P<0.0001 | 218.96 99 49 P<0.0001 3419.2 | | |
| No             | 15 835   | 0.139   | 1.277 0.176 1.076 | | |
| Fever          |          |         |    |           |         |    |
| Yes            | 75 43    | P<0.0001 | 33.461 75 43 P<0.0001 62.2 | | |
| No             | 58 822   | 0.4717  | 0.229 0.763 1.076 | | |
| Joint pain     |          |         |    |           |         |    |
| Yes            | 62 22    | P<0.0001 | 62 22 P<0.0001 66.799 | | |
| No             | 71 843   | 0.417   | 0.802 0.771 | | |
| Low backache   |          |         |    |           |         |    |
| Yes            | 75 43    | P<0.0001 | 33.461 75 43 P<0.0001 62.2 | | |
| No             | 58 822   | 0.563   | 0.229 0.771 | | |
| Fatigue        |          |         |    |           |         |    |
| Yes            | 73 80    | P<0.0001 | 11.93 73 80 P<0.0001 28.74 | | |
| No             | 60 785   | 0.180   | 1.08 1.076 | | |
| Weight loss    |          |         |    |           |         |    |
| Yes            | 15 128   | P<0.0001 | 0.240 0.702 15 128 P<0.0001 1.076 | | |
| No             | 123 737  | 0.605   | 0.561 0.5421 | | |
| Headache       |          |         |    |           |         |    |
| Yes            | 14 128   | P<0.0001 | 0.229 0.677 14 128 P<0.0001 0.992 | | |
| No             | 119 737  | 0.002   | 0.561 0.5421 | | |
| Sleep disturbance |      |         |    |           |         |    |
| Yes            | 03 49    | P<0.0001 | 0.139 0.3843 03 49 P<0.0001 0.5421 | | |
| No             | 130 816  | 0.029   | 0.561 0.5421 | | |

*Positive, †Negative, §By chi-square test, ‡By fisher’s exact test, SAT: Serum agglutination test, 2-ME: 2-Mercaptoethanol test.

**Table 3: Association of significant 2-ME titers and epidemiological factors**

| Variable | 2-ME titers | P value | OR | 2-ME titers | P value | OR |
|----------|-------------|---------|----|-------------|---------|----|
| Age      |             |         |    |             |         |    |
| 0-14     | 27 102     | P<0.0001 | 00 43 P<0.0001 1.076 | | |
| 15-20    | 8 79       | 0.139   | 1.277 0.176 1.076 | | |
| 21-30    | 18 121     | 0.4717  | 0.229 0.763 1.076 | | |
| 31-40    | 21 133     | 0.180   | 0.802 0.771 | | |
| 41-50    | 13 179     | 0.605   | 1.08 1.076 | | |
| 51-60    | 10 153     | 0.605   | 0.561 0.5421 | | |
| >60      | 02 132     | 0.180   | 0.561 0.5421 | | |
| Sex      |             |         |    |             |         |    |
| Male     | 69 578     | P<0.0001 | 1.277 0.321 3.240 | | |
| Female   | 30 321     | 0.002   | 0.561 0.5421 | | |
| Educational status corrected up 2 above | | |
| Illiterate | 36 280     | 0.002   | 0.561 0.5421 | | |
| Primary   | 39 398     | 0.002   | 0.561 0.5421 | | |
| Secondary | 07 74      | 0.002   | 0.561 0.5421 | | |
| HSC       | 06 57      | 0.002   | 0.561 0.5421 | | |
| Graduate  | 06 42      | 0.002   | 0.561 0.5421 | | |
| <5 years  | 05 48      | 0.002   | 0.561 0.5421 | | |
| Occupation |          |         |    |             |         |    |
| Farmers   | 34 393     | P<0.0001 | 00 43 P<0.0001 1.076 | | |
| Shepherds | 36 163     | 0.002   | 0.561 0.5421 | | |
| <5 years  | 05 53      | 0.002   | 0.561 0.5421 | | |
| Students  | 14 167     | 0.002   | 0.561 0.5421 | | |
| Household | 10 123     | 0.002   | 0.561 0.5421 | | |
| Others    | 00 00      | 0.002   | 0.561 0.5421 | | |

*Positive, †Negative, §By chi-square test, ‡By fisher’s exact test, 2-ME: 2-Mercaptoethanol test, OR: Odds ratio, HSC: Higher secondary.
In the remaining 51 symptomatic subjects, 19 showed significant SAT but insignificant 2-ME titers and 32 showed insignificant titers both by SAT and 2-ME tests. Six individuals had no symptoms but had significant SAT titers. Repeat serological test for 51 symptomatic and 6 asymptomatic individuals did not show any increase in titers on twice, fortnightly follow-up. The significant SAT titers in these 25 individuals could be due to repeated exposure to antigenic stimuli or subclinical infection; consistent with the observations of other authors.\(^{[16,18,19]}\)

Fever, joint pain, low backache, fatigue, weight loss, headache, and sleep disturbance (insomnia and night sweats) were the reported symptoms. Noteworthy association \((P < 0.0001)\) could be established between fever, joint pain, low backache, and fatigue and significant tube test titers, whereas no association was found between weight loss, headache, and sleep disturbance.

Brucellosis was less frequent after the age of 60 years, in contrast to the findings of Ramos et al., Cetinkaya Z et al., Al-Sekait MA, and Nikokar et al. who have reported increase in prevalence of Brucella antibodies with age.\(^{[20,21]}\) Children and young adults were most commonly affected in this study. Our results are in line with earlier workers.\(^{[22,23]}\) Among the subjects with significant tube agglutination test titers, maximum (26.47%) cases were in the pediatric age-group; this might be due to the fact that all these children regularly played with goats and sheep. Mean age for males was found to be 27.05 ± 18.04 and for females 30.656 ± 15.55 years. Youngest patient in this study was 1.4 years and the eldest 74 years. Higher seropositivity in males than in females has been reported by earlier workers.\(^{[24,25]}\) However, in this study, no significant difference in seropositivity and gender was noted. Level of academic education also did not influence brucella seropositivity as reported by Yohannes et al.\(^{[26]}\)

Noteworthy difference was established between seroprevalence and the occupation in group I, with maximum positivity among shepherds. Raw milk ingestion, close contact with animals, exposure to their products during milking and parturition were the major risk factors and goat was the main animal to which these subjects were exposed.

None of the individuals in group II had come in contact with animals or animal products directly, but most of them had consumed raw milk regularly, hence for group II raw milk ingestion was the major risk factor.

Regarding the knowledge about brucellosis, of the 1,733 subjects, only 3 had heard about the disease but none knew about the modes of transmission or clinical manifestations.

**Conclusions**

Human brucellosis can be quite common in rural India. Close contact with goats and sheep, and raw milk ingestion are the major risk factors. It is also concluded that knowledge attitude and practice (KAP) levels regarding brucellosis are too poor and there is no association between academic education and KAP levels. Regular surveillance of the disease in animals is needed to control animal brucellosis. Efforts are needed to educate rural population, regarding the disease, modes of transmission, clinical symptoms, risk factors, and preventive measures to decrease the incidence of human brucellosis.

**References**

1. Corbel MJ. Brucellosis: An overview. Emerg Infect Dis 1997;3:213-21.
2. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet Infect Dis 2006;6:91-9.
3. Mantur BG, Amarnath SK, Shinde RS. Review of clinical and laboratory features of human brucellosis. Indian J Med Microbiol 2007;25:188-202.
4. Wallach JC, Samartino LE, Efron A, Baldi PC. Human infection by Brucella melitensis: An outbreak attributed to contact with infected goats. FEMS Immun Med Microbiol 1997;19:315-21.
5. Corbel MJ. Brucellosis in humans and animals. Geneva: World Health Organisation; 2006.
6. Mathur TN. Brucella strains isolated from cows, buffaloes, goats, sheep and human beings at Karnal: Their significance
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with regard to the epidemiology of brucellosis. Indian J Med Res 1964;52:31-40.

7. Punjarathinam R, Jhala CI. Brucellosis in Gujarat State. Indian J Pathol Microbiol 1986;29:53-60.

8. Atton GG, Jones LM, Pietz DE. Serological methods. Laboratory Techniques in Brucellosis. 2nd ed. Geneva: World Health Organization; 1973.

9. Buchanam TM, Faber LC. 2-Mercaptoethanol Brucella agglutination test: Usefulness for predicting recovery from brucellosis. J Clin Microbiol 1980;11:691-3.

10. Renukaradhya GJ, Isloor S, Rajasekhar M. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. Vet Microbiol 2002;90:183-95.

11. Rajeswari S, Shome BR, Deivanai M, Desai GS, Patil SS, Bhure SK, et al. Seroprevalence of brucellosis in small ruminants. Indian J Comp Microbiol Immunol Infect Dis 2006;27:13-5.

12. Kadri SM, Ruksana 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.

13. Sharma VD, Sethi MS, Yadav MP, Dube DC. Sero-epidemiologic investigations on brucellosis in the states of Uttar Pradesh (U.P.) and Delhi (India). Int J Zoonoses 1979;6:75-81.

14. Appannanavar SB, Sharma K, Verma S, Sharma M. Seroprevalence of Brucellosis: A 10-year experience at a tertiary care center in north India. Indian J Pathol Microbiol 2012;55:271-2.

15. Priyadarshini A, Sarangi LN, Palai TK, Panda HK, Mishra R, Behera PC. Brucellosis in cattle and occupationally exposed human beings: A Serosurvey in Odisha, India. J Pure Appl Microbiol 2013;7:3255-60.

16. Agasthya AS, Isloor S, Prabhudas K. Brucellosis in high risk group individuals. Indian J Med Microbiol 2007;25:28-31.

17. Yohannes M, Gill JP. Seroepidemiological survey of human brucellosis in and around Ludhiana, India. Emerg Health Threats J 2011;4:7361.

18. Young EJ. Serologic diagnosis of human brucellosis: Analysis of 214 cases by agglutination tests and review of the literature. Rev Infect Dis 1991;13:359-72.

19. Araj GF, Azzam RA. Seroprevalence of brucella antibodies among persons in high risk occupation in Lebanon. Epidemiol Infect 1996;117:281-8.

20. Ramos TR, Pinheiro Junior JW, Moura Sobrinho PA, Santana VL, Guerra NR, Demelo LE, et al. Epidemiological aspects of an infection by Brucella abortus in risk occupational groups in the microregion of Araguaina, Tocantins. Braz J Infect Dis 2008;2:133-8.

21. Cetinkaya Z, Aktepe OC, Ciftci IH, Demirel R. Seroprevalence of human brucellosis in a rural area of Western Anatolia, Turkey. J Health Popul Nutr 2005;23:137-41.

22. Al-Sekait MA. Survey of brucellosis antibodies in Saudi Arabia. Ann Saudi Med 1999;19:21-22.

23. Nikokar I, Hosseinpour M, Asmar M, Pirmohibatie S, Hakeimei F, Razavei MT. Seroprevalence of Brucellosis among high risk individuals in Guilan, Iran. J Res Med Sci 2011;16:1366-71.

24. Fallatah SM, Oduolujo AJ, Al-Dusari SN, Fakunle YM. Human brucellosis in Northern Saudi Arabia. Saudi Med J 2005;26:1562-6.

25. Mantur BG, Amarnath SK. Brucellosis in India - a review. J Biosci 2008;33:539-47.

26. Minas M, Minas A, Gourgulianis K, Stournara A. Epidemiological and clinical aspects of human brucellosis in Central Greece. Jpn J Infect Dis 2007;60:362-6.

27. Metri BC, Baragundi MC, Jyothi P, Lava R, Basavarajappa, Hanumanthappa AR, et al. Seroprevalence of Brucellosis in Davangere, Karnataka. J Clin Diagn Res 2011;5:41-4.

28. Bukharie HA. Clinical features, complications and treatment outcome of Brucella infection: Ten years’ experience in an endemic area. Trop J Pharm Res 2009;8:303-10.

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