Original Article

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.[3-5] 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.

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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

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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/L2 = [4 × 8.5 × 91.5]/1.52). 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.

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	02	01	00
	2-ME	00	00	00	00	00	02	01	00	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
	*P**	*n*†			*P**	*n*†		
Health problem								
Yes	118	30	*P*‡<0.0001	218.96	99	49	*P*‡<0.0001	3419.2
No	15	835			00	850		
Fever								
Yes	75	43	*P*‡<0.0001	33.461	75	43	*P*‡<0.0001	62.2
No	58	822			24	854		
Joint pain								
Yes	62	22	*P*‡<0.0001		62	22	*P*‡<0.0001	66.799
No	71	843			37	877		
Low backache								
Yes	75	43	*P*‡<0.0001	33.461	75	43	*P*‡<0.0001	62.2
No	58	822			24	854		
Fatigue								
Yes	73	80	*P*‡<0.0001	11.93	73	80	*P*‡<0.0001	28.74
No	60	785			26	819		
Weight loss								
Yes	15	128	*P*‡=0.240	0.702	15	128	*P*‡=0.763	1.076
No	123	737			84	771		
Headache								
Yes	14	128	*P*‡=0.229	0.677	14	128	*P*‡=0.561	0.992
No	119	737			85	771		
Sleep disturbance								
Yes	03	49	*P*‡=0.139	0.3843	03	49	*P*‡=0.4717	0.5421
No	130	816			96	850		

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

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.

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

Variable	2-ME titers		*P* value	OR	2-ME titers		*P* value	OR
	*P**	*n*†			*P**	*n*†		
Age								
0-14	27	102	*P*‡<0.0001	—	00	43	*P*§=0.180	
15-20	8	79			02	89		
21-30	18	121			01	228		
31-40	21	133			00	182		—
41-50	13	179			00	83		
51-60	10	153			00	72		
>60	02	132			00	35		
Sex								
Male	69	578	*P*§=0.319	1.277	03	526	*P*§=0.563	2.745
Female	30	321			00	206		
Educational status corrected up 2 above								
Illiterate	36	280	*P*‡=0.878	—	02	234	*P*‡=0.605	—
Primary	39	398			01	156		
Secondary	07	74			00	133		
HSC	06	57			00	207		
Graduate	06	42			00	02		
<5 years	05	48			—	—		
Occupation								
Farmers	34	393	*P*‡=0.002	—	—	—		
Shepherds	36	163			—	—		
<5 years	05	53			—	—		
Students	14	167			01	252	*P*‡=0.417	—
Household	10	123			00	224		
Others	00	00			02	259		

*Positive, †Negative, ‡By chi-square test, §By fisher's exact test, 2-ME: 2-Mercaptoethanol test, OR: Odds ratio, HSC: Higher secondary

Table 4: Association of significant 2-ME titers and risk factors

Variable		2-ME titers		*P* value	OR	2-ME titers		*P* value	OR
		P	*n*			*P*	*n*		
Type of animal									
Cattle only		09	198	P‡=0.0001		—	—	—	—
Goats only		17	182			—			
Sheep only		10	59			—			
Cattle+goat		36	332		—	—			
Goat+sheep		26	104			—			
Cattle+sheep		01	24			—			
Animal contact during				P§<0.0001					
Herding	Yes	91	567		6.66	—	—	—	—
	No	08	332			—			
Milking	Yes	91	567	P§<0.0001	6.66	—	—	—	—
	No	08	332			—			
Delivery	Yes	87	381	P§<0.0001	9.85	—	—	—	—
	No	12	518			—			
Raw milk ingestion (RMI)	Yes	76	554	P§=0.0007	2.25	03	289	P§=0.06	10.72
	No	23	345			00	443		

*Positive, †Negative, ‡By chi-square test, §By fisher's exact test, 2-ME: 2-Mercaptoethanol test, OR: Odds ratio

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 sub clinical 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.[20-23] Children and young adults were most commonly affected in this study. Our results are in line with earlier workers.[5,24] 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.[25-28] 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*.[17]

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.

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