ORIGINAL ARTICLE

Human Brucellosis: Still an Unfamiliar and Misdiagnosed Disease in India

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Abstract:

Background: Human brucellosis is a disease with protean clinical manifestations. Despite many awareness programmes, it is still missed or wrongly diagnosed. This leads to chronic morbidity leading to misery and loss of working days. *Aim and Objectives:* To assess the microbiological, clinical and epidemiological aspects of human brucellosis. *Materials and Methods:* Patients with positive brucella screening test constituted the study material. A detailed laboratory, clinical, epidemiological study along with response to the treatment was analyzed. *Results*: Seroprevalence of brucellosis was found to be 1.75%. Brucellosis was clinically diagnosed in only 12.73% of cases. Fever, joint pain and low backache were the commonest symptoms. Close contact with animals and raw milk ingestion were the major sources of infection. Knowledge regarding brucellosis and its prevention was lacking in patients. Brucellosis was not considered as one of the differential diagnosis by the treating physicians. *Conclusion*: Brucellosis should be considered as one of the differential diagnosis in cases presenting with fever, low backache, arthritis and arthralgia. Laboratories should screen all the serum samples for brucella agglutinins by Rose Bengal Plate Test. Awareness regarding the prevention of brucellosis in the general population and regarding the existence of the disease among the doctors practicing in rural areas is needed.

Keywords: Human Brucellosis, Risk Factors, Rose Bengal Plate Test, Serum Agglutination Test, 2-Mercaptoethanol Test

Introduction:

Brucellosis, a zoonosis, has been present for millennia and has managed to elude eradication, even in most developed countries [1, 2]. Half a million new cases are reported worldwide each year, but according to the World Health Organization, these numbers are greatly under estimated and the true incidence of human brucellosis is far more than that reported [3, 4]. The disease continues to be of great health implication and economic loss in many countries. It has been included in the category of neglected endemic zoonoses by World Health Organization (WHO) [5]. Due to protean clinical manifestations and perception among physicians that brucellosis is rare in India it is not suspected and misdiagnosed, which results in low rate of reporting and failure to detect new cases [6-9]. Hence an attempt has been made to know the prevalence, clinical and epidemiological features and treatment aspect of human brucellosis.

Material and Methods:

The present cross sectional study was conducted from November 2008 to December 2013. In this study all the serum samples (non-repeat) received by the Microbiology laboratory of Shri B. M. Patil Medical College Hospital, Bijapur were screened by Rose Bengal Plate Test (RBPT). Patients with positive Brucella screening test were included for further study and those with negative result were excluded. The entire experimental protocol was approved by the Institutional Ethics Committee and utmost care was taken during the experimental procedure. Informed consent was taken from all the adults and from parents of pediatric age group before enrolment in the study. Detailed

information regarding patient's epidemiological data, previous treatment history and clinical investigations was collected. Five ml blood sample was collected from all the patients fulfilling the inclusion criteria. Serum was separated and subjected to Serum Agglutination Test (SAT) and 2-Mercaptoethanol Test (2-ME) to determine the titres. The diagnosis of brucellosis was made according to the CDC case classification criteria.

Case classification:

A clinically compatible case that was epidemiologically linked to a confirmed case or that had supportive serology finding (i.e. Brucella agglutination titre ⩾ 160 IU in one or more specimens obtained after onset of symptoms). Blood culture was performed in the patients with significant titres before starting anti-brucellar treatment. Repeat serological testing was performed after one week in all the patients with significant SAT but insignificant 2-ME titres. Follow-up of diagnosed brucellosis cases was done at the end of one month and after every 15 days thereafter till their 2-ME titres decreased to insignificant levels. Data was analyzed by Graph Pad Instat software.

Results:

A total of 12,054 serum samples (non-repeat) sent for various investigations were screened for brucella agglutinins, of which 218 showed positive test by RBPT. Significant SAT (⩾ 160) and 2-ME (⩾ 80) titres were seen in 212 and 198 individuals respectively (Table 1).

Acute presentation of brucellosis was seen in 86.79%, sub-acute in 8.01% and chronic in 4.71% patients. Fever was the major presenting symptom in 88.67% of cases. As described in the literature undulant fever pattern was noted in only three patients. Fever was sustained in 109 (57.97 %) individuals and evening rise of temperature was seen in 76 (40.42%) patients. Other common presentations were joint pain, backache, night sweats, weight loss, fatigue and headache (Table 2).

Table 2: Clinical symptoms in 212 patients with Brucellosis

Symptoms	No. of patients (%)
Fever	188 (88.67)
Joint pain	147(69.33)
Low backache	69 (32.54)
Fatigue	91 (42.92)
Headache	29 (13.67)
Pain in abdomen, nausea, vomiting	22 (10.37)
Night sweats	16 (7.54)
Loss of weight	16 (7.54)
CNS symptoms	14 (6.6)
Respiratory symptoms	07 (3.3)
Testicular involvement	04 (0.88)
Psychiatric manifestations	03 (1.41)
Chest pain	02 (0.94)
Ophthalmic involvement	02 (0.94)
Hearing loss	01(0.47)

Table 1: Break-up of SAT and 2-ME Test titres in 218 Rose Bengal Plate Test positive cases

Test	Titre IU/ml									Mean ± SD
	Nil	40	80	160	320	640	1280	2560	5120	
SAT	0	2	4	41	49	44	47	20	11	1002.20 ± 1178.9
2-ME	17	3	41	42	56	41	12	5	1	401.65 ± 557.09

Hepatomegaly and splenomegaly were the commonest signs observed (Table 3).

Table 3: Clinical Signs in 212 Patients with Brucellosis

Signs	No. of patients (%)
Hepatomegaly	52 (24.52)
Splenomegaly	36 (16.98)
Hepatosplenomegaly	19 (8.96)
Lymphadenopathy	09 (4.24)
Lymphadenopathy + hepatomegaly	08 (3.77)

Complications of brucellosis were seen in 40.27% (Fig. 1) and among them osteoarticular manifestations were the commonest. Out of 69 patients with osteoarticular complications, 56 had monoarticular involvement whereas in 13 it was polyarticular. Knee joint was the commonest joint affected. Neurobrucellosis was found in 6.6% of patients. Testicular involvement was noted in 4 patients. Semen sample of these patients revealed the anti-brucellar antibodies. Endocarditis was noted in one patient (Table 4).

Hematological investigations of these individuals showed anemia in 57/155 (36.77%) males and 31/57 (54.38%) females. Mean hemoglobin in males was 12.8±1.99 g/dl while it was 11.33±1.3 g/dl for females. Leukopenia (WBC <4300/mm3) was seen in 17 (8.01%), Erythrocyte sedimentation rate (ESR) was >20 in 26 (16.77%) patients.

Repeat serology performed after one week, in 14 patients with insignificant 2-ME titres showed rise in SAT as well as 2-ME titres in nine individuals thus confirming the diagnosis. In remaining five individuals no rise in SAT and 2-ME test titres was noted at the end of first week, then after every month for four months and hence were considered negative for brucellosis.

Table 4: Joints Involved in Osteoarticular Complications

Mono-articular		Poly-articular	
Joints involved	**No.**	**Joints involved**	**No.**
Knee	26	Knee + Hip	5
Sacroiliac	11	Knee + Ankle	2
Hip	09	Knee + Elbow	2
Lumbar	04	Knee + Sacroiliac	2
Wrist	04	Knee + Shoulder	1
Ankle	02	Knee + Wrist	1

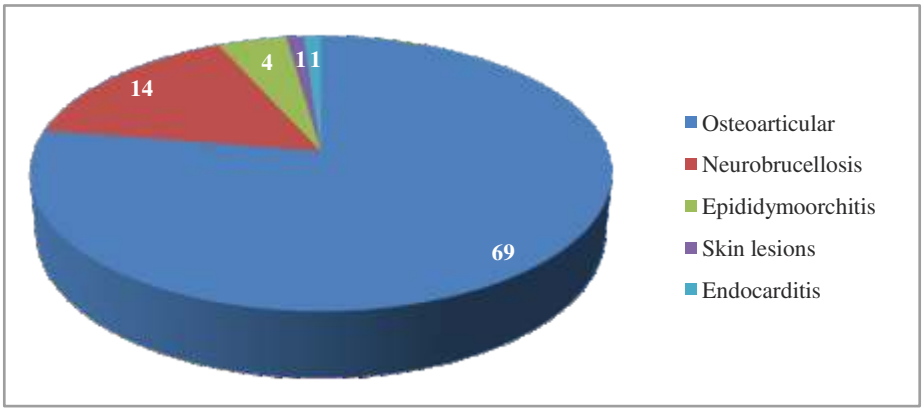

Fig. 1: Complications of Brucellosis

Blood culture could be performed in 172 patients and Brucellae could be isolated in 77 (44.76%) cases. One hundred and eighty four (86.79%) of our patients were treated by various doctors before the diagnosis of brucellosis was made. These patients were suspected to have enteric fever, malaria, arthritis, tuberculosis and tubercular meningitis and were treated for the same with no relief. The mean duration of the symptoms before the diagnosis of brucellosis could be made was 33.6 days (range 5 to 90 days).

Seasonal fluctuation in the number of cases was seen with two peaks one between March - May and the other between August-October (Fig. 2.).
No significant difference in the seropositivity was seen in different age groups, though the number of positive cases decreased after the age of 50 years (P=0.3). Thirty-nine cases (18.39%) were in the pediatric age group. The youngest patient in this study was 1.4 years and the eldest 70 years old. Male preponderance was seen in the study with male to female ratio of 3:1. Age wise there is no difference in males and females (Table 5).

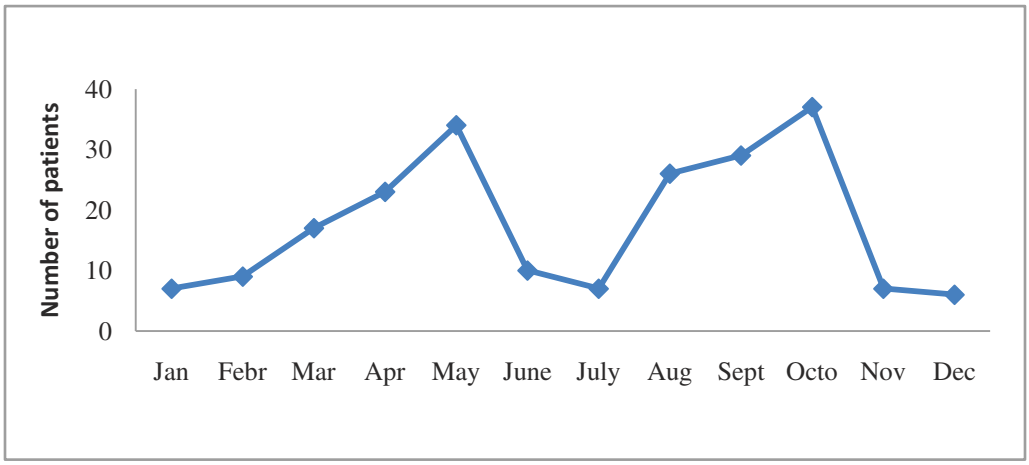

Fig. 2: Seasonal Distribution of 212 Patients

Table 5: Age and Sex distribution of 212 Brucellosis cases

Age group	No. of Males	No. of Females	Total No	Test Value	p Value
0 - 14 yrs	31	8	39	χ^2=7.351	0.2896
15 - 20 yrs	18	11	29		
21 - 30 yrs	45	13	58		
31 - 40 yrs	35	17	52		
41 - 50 yrs	13	3	16		
51 - 60 yrs	8	5	13		
⩾ 61yrs	5	0	5		
Total	155	57	212		
Mean ± S.D	28.52±13.85	27.81±14.6	28.00± 14. 38	t = 0.3261	0.7446

(χ^2= 7.351, p=0.2896)

Occupation wise distribution revealed significantly higher number of cases amongst either farmers (26.88%) or shepherds (25.47) P<0.0001 (Fig. 3).

Both animal exposure as well as raw milk ingestion was the major risk factors for brucellosis (Fig. 4).

All the patients with significant 2-ME titres were prescribed the standard anti-brucellar regimen consisting of rifampicin plus doxycycline for minimum of six to eight weeks [7]. For patients with neurobrucellosis along with the standard regimen streptomycin was added and the treatment was continued for six months. A case of brucellar endocarditis underwent surgical resection of the lesion and was treated with rifampicin, doxycycline, gentamycin and ceftriaxone for six months.

Response to the treatment with clinical recovery and decrease in 2-ME titres was seen in all the 86 patients who came for follow-up. Relapse was noted in two patients in this study. Five patients who had significant SAT but insignificant 2-ME titres did not show any rise on repeat serology and were not treated for brucellosis.

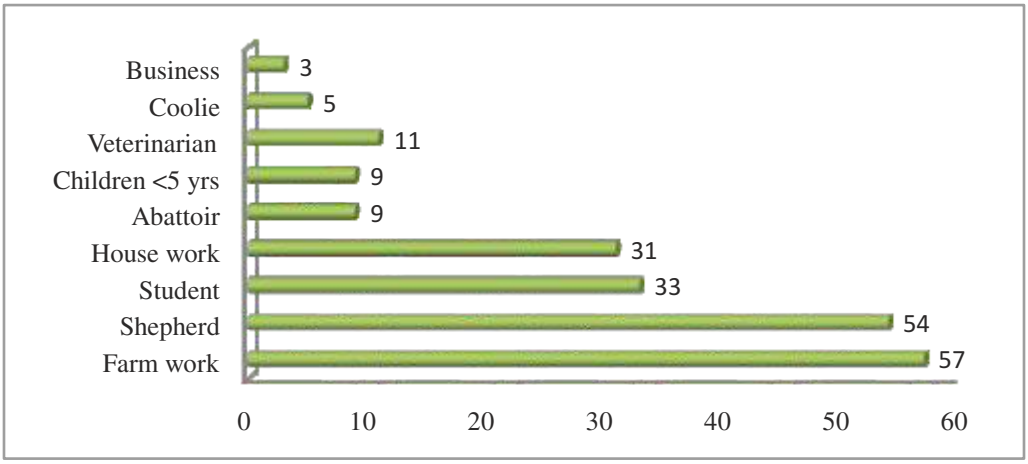

Fig. 3: Occupation wise Distribution of 212 Brucellosis Cases

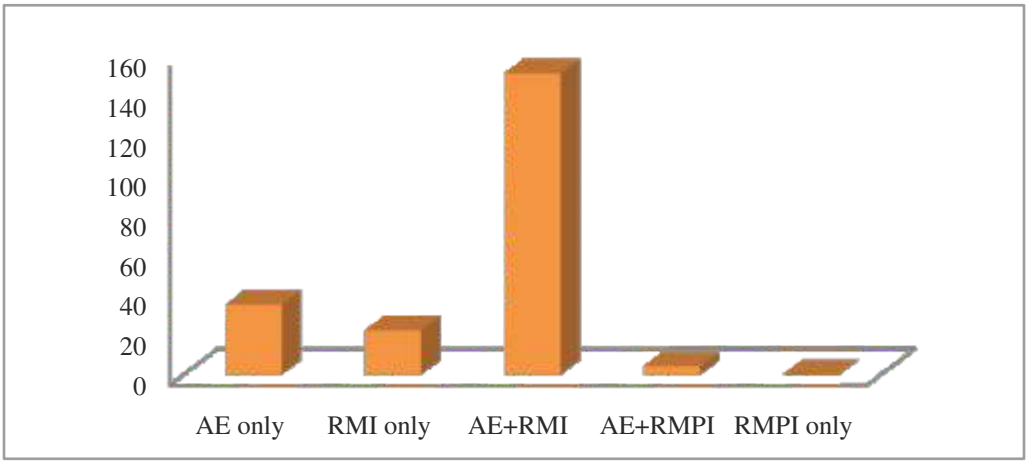

Fig. 4: Risk Factors for Brucellosis in 212 Patients

Discussion:

Wide variation in the prevalence of human brucellosis from 0.8 - 26.6% has been reported from India [8, 10-14]. Considering SAT titres ⩾ 160 IU as diagnostic, prevalence of brucellosis has been found to be 1.75 % in our study. This is in accordance with the study by Mantur *et al* and fairly high compared to the study of hospitalized patients at GMC, Srinagar indicating the endemicity of brucellosis in this area [8, 10]. Higher prevalence rate has been reported in other studies, the lower rate in this study might be attributed to screening of all the samples received [14, 17].

No correlation could be established between the SAT titres and the severity of symptoms or type of presentation. This could possibly be due to the difference in the age, prior antibiotic treatment and difference in the immune status of the patients.

Overall blood culture positivity in brucellosis ranges between 15-70 % [15]. In our study blood culture was positive in 44.76% patients.

Fever was the presenting symptom in most of the patients in our study, which is also reported by others [8, 16]. Along with fever, joint pain, low backache, fatigue, headache, pain abdomen, nausea and vomiting, night sweats were the common complaints noted. Hepatomegaly and splenomegaly were common observations which are in accordance with the study by Malik GM [16]. Hematological testing, such as white blood cell count and ESR were of little value as reported by Young EJ [15].

Osteoarticular complications were the commonest in the present study, which also have been reported by many workers [8,18,19]. Peripheral joints like knee sacroiliac and hip were the most frequently affected. Joints of the upper extremities were rarely involved, also mono-articular involvement was common than polyarticular. All these findings are comparable to the results of Mantur *et al* and Mousa *et al* [8, 19]. Erythema nodosum like lesions were noted in one patient which also has been reported by other authors [8, 20, 21].

Misdiagnosis of brucellosis as arthritis, enteric fever, malaria, tuberculosis, and tubercular meningitis reported in this study is in agreement with other studies [6-8].

Though brucellosis cases have been detected throughout the year, two peaks, one between March-May and the second between August-October were observed. This coincides with the peak period for parturition and abortions in the farm animals.

In our study, the young adults and children were commonly affected with male preponderance; these findings are in line with earlier studies [7, 8, 22].

Majority of brucellosis cases in this study were either shepherds or farmers and were from the rural areas; indicating that brucellosis is a disease of the rural population. Both animal exposure and raw milk ingestion have been the major associated risk factors. Except the veterinarians none of the patients had any knowledge of brucellosis. All the patients who had come for follow-up responded well to the standard drug regimen with decrease in 2-ME titres.

Five individuals had significant SAT and insignificant 2-ME titres and 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 described by Agasthya *et al*, Young EJ and Araj and Azzam [23-25]. Significant 2-ME titre is a better correlate of an active brucellosis requiring treatment, than a positive SAT titre as noted by Buchanan *et al* [26].

Conclusion:

Brucellosis is endemic in Karnataka, especially in rural areas. As clinical symptoms of brucellosis are inexplicit, if the laboratories screen all the serum samples for brucella agglutinins by RBPT, chances of missing brucellosis cases can be minimized. 2-ME test helps in differentiating

active and inactive brucellosis. Typical undulant fever pattern may not be seen hence brucellosis should be considered as a differential diagnosis in all the cases presenting with long standing fever. Awareness regarding the disease, risk factors and prevention in the general population and regarding the existence of the disease among the doctors practicing in rural areas is needed.

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