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Original article

Brucellosis: A cause for pyrexia of unknown origin

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ABSTRACT

Introduction: Human brucellosis is an important but ignored disease in India. Due to long standing fever and lack of typical signs and symptoms, patients with acute brucellosis are often tagged as the cases of Pyrexia of Unknown Origin (PUO). Generally these patients are investigated for diverse serological tests except for brucellosis. Aims: The present study was carried out to know the prevalence of brucellosis among the PUO cases and study the clinical and epidemiological aspects of brucellosis. Materials and methods: In this cross sectional study, 2379 PUO patients were investigated for the evidence of brucellosis. Results: Among 2379 cases, 114 patients were positive by RBPT. Significant titers could be demonstrated in 105 subjects by SAT and 2-ME tests. Brucellae could be isolated from 28 cases. Along with fever, joint pain and low backache were the commonest clinical symptoms. Stay in the rural area, animal exposure and raw milk ingestion were the major risk factors. Conclusions: Brucellosis accounted for 4.41 % of PUO cases. Serological tests are more sensitive when compared to blood culture. Efforts to create awareness regarding the existence of the disease among the physicians and preventive measures to be followed in rural population are needed.

1.Introduction:

Brucellosis is one of the world's major zoonotic diseases present since man domesticated animals. It has managed to escape elimination, even in most developed countries [1, 2]. It remains an uncontrolled problem in Mediterranean, Middle East, Africa, Latin America and parts of Asia [1, 3].

Due to protean clinical manifestations, absence of laboratory testing facilities and lack of perception about the disease among the clinicians, most of the times brucellosis is missed and indifferently diagnosed as pyrexia of unknown origin (PUO) [4].

2.Materials and Methods:

The present study was conducted from October 2008 to October 2011. Serum and blood samples collected from patients attending Shri B. M. Patil Medical College Hospital and private clinics in and around Bijapur, fulfilling the inclusion criteria were included in the study.

3.Inclusion Criteria

The patients who had a temperature greater than 38.3°C on several occasions accompanied by more than 3 weeks of illness and failure to reach a diagnosis, after 3 outpatient visits or 3 days in the hospital without elucidation of a cause or 1week of "intelligent and invasive" ambulatory investigations [5] were included in the study.

4.Exclusion Criteria:

Patients who did not fit in the PUO case definition were excluded from the study.

5.Collection of samples

The entire experimental protocol was approved by the institutional ethical committee and utmost care was taken during the experimental procedure. Informed consent was obtained from all the adults and from parents in pediatric age group before collecting the sample. About 3 ml of blood was collected for serological investigations. If serological tests showed significant titer, blood culture was performed. Demographic, occupational and clinical data were collected by questionnaire and personal interviews.

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The investigations were performed according to the guidelines stated by Alton G.G et al [6]. Serological follow-up was advised to all the patients with brucellosis undergoing treatment, till their 2-ME titers came down to insignificant levels.

6.Processing of sample:

Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and 2-Mercaptoethanol test (2-ME) were performed. The antigens for these tests were procured from The Division of Biological Products, Indian Veterinary Research Institute (I.V.R.I.); Izatnagar, Uttar Pradesh, India and the procedures were carried out according to the manufacturer's guidelines [7, 8].

For 2- ME test, 0.1M 2-Mercaptoethanol was used in place of phenol saline. A titer of 160 IU or more for SAT and 80 IU or more for 2-ME test were considered significant.

Blood samples for culture were collected in Castaneda's biphasic media, prepared using Brucella selective agar and broth with Brucella selective supplement (Hi-Media). The culture bottles were incubated at 37°C, under 10% CO2 for 45 days.

7.Statistical analysis:

Statistical analysis of the data was done using GrapPad InStat software.

8.Results

Of the 2379 samples from PUO cases screened for Brucella agglutinins, 114 showed positive reaction by RBPT. Titers ranging from 80 IU - 5120 IU by SAT and from 40 IU- 5120 IU by 2-ME test were noted in 111 cases. Significant titers were seen in 105 (4.41%) cases (Table1). Culture was found positive in 28 (24.56 %) individuals. All the isolates were identified as Brucella melitensis biotype 1. When compared to the serological tests the sensitivity of culture was 26.67% in our study (Table 2).

Along with fever, joint pain, low backache, fatigue, headache, pain abdomen, nausea, vomiting and night sweats were the commonly associated symptoms (Fig 1). Undulating fever pattern characteristic of brucellosis was seen only in one patient. Whereas evening rise of temperature was seen in 41.22% patients and sustained fever in 57.89 %. Complications of brucellosis were noted in 65 individuals (Fig 2). Osteoarticular complications involving peripheral joints were frequently noted. More than one joint were involved in 28.8% of cases.

Seasonal fluctuation in the number of cases was seen with two peaks one between March - May and the other between August - October (Fig 3). Mean age of the affected was 28.24 ± 17.38 years (range: 1.4 - 70 years) (Table 3). Majority of the patients were less than 30 years (59.64%) and children accounted for 28.07% of cases. The disease was less frequent after the age of 60 years. Male preponderance, with male to female ratio of 2:1 was seen in the study. About 50% of patients with brucellosis were either shepherds or farmers (Fig 4). Stay in the rural area, animal exposure and raw milk ingestion were the major risk factors (Fig 5). Except veterinarians none of the affected had heard about the disease. Response to the treatment with clinical recovery and decrease in 2-ME titres was seen in all the 51 patients at the end of 6 weeks who came for every fortnightly follow-up.

Table1. Break-up of The SAT and 2-ME test titres in 114 RBPT positive PUO cases

Titres	Nil	40	80	160	320	640	1280	2560	5120
SAT	03	00	06	15	25	23	28	07	07
2-ME	05	04	07	18	27	20	24	05	04

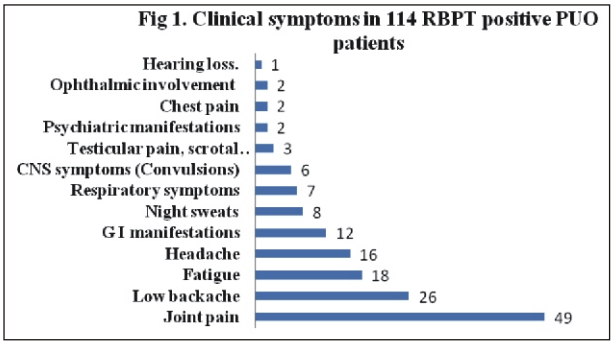
Table 2. Sensitivity and specificity of serological tests and culture

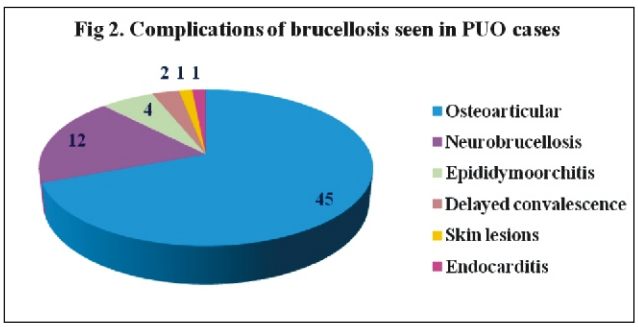
Serological test	Compared with	Sensitivity	Specificity
RBPT	SAT	100 %	99.6%
SAT	Culture	26.67%	100%
2-ME	Culture	26.67%	100%

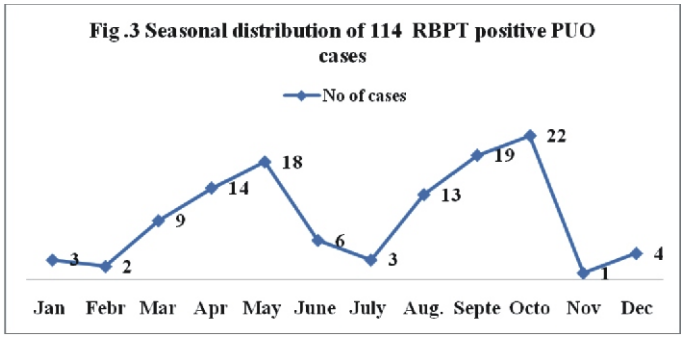
Table- 3 Age and sex wise distribution

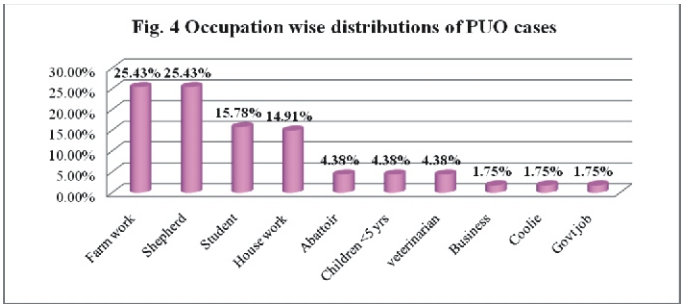
Age group	Total No	Male	Female
0 - 10 yrs	19	17	02
11 - 20 yrs	21	14	07
21 - 30 yrs	28	19	09
31 - 40 yrs	22	12	10
41 - 50 yrs	11	07	04
51 - 60 yrs	08	04	04
≥ 61yrs	05	05	NIL
Total	114	78	36
Mean ± S.D	28.157±17.327	27.050±18.043	30.657±15.547

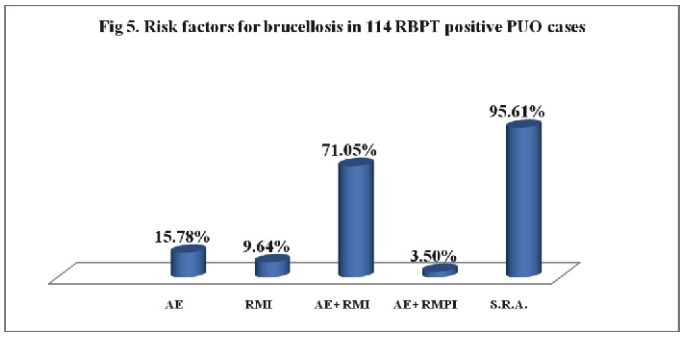
*Mean age of all the patients, ** Mean age of male patients, *** Mean age of female patients











AE- Animal Exposure, RMI- Raw milk ingestion only , AE+RMI - Animal exposure + Raw milk ingestion , AE+RMPI - Animal exposure + Raw meat/meat products ingestion , SRA - Stay in rural area.

9.Discussion:

Seroprevalence of brucellosis in PUO cases was found to be 4.79%, 4.41% and 4.41% by RBPT, SAT and 2-ME tests respectively. A wide variation in the number of brucellosis cases from 0.8% - 58.8% in patients with PUO has been reported [9- 12].

In the present study blood culture positivity was found to be 24.56%, which is low when compared to the results of other authors [13, 14]. The lower isolation rate may be attributed to the prior antibiotic treatment. Sixty percent of patients in the study had taken different antibiotics from many local doctors, before they were diagnosed as brucellosis cases. Among the treated cases, 35% were on anti tubercular drugs.

As brucellosis presents with non-specific symptoms, the patients are investigated for varied diseases before they are diagnosed as the cases of brucellosis. In this study patients were suspected to have and were investigated for malaria, enteric fever, tuberculosis and tubercular meningitis in 23.68, 19.29, 8.77 and 6.14% respectively. Brucellosis was not at all suspected in 94.84% of cases. The mean duration of the symptoms before the diagnosis of brucellosis could be made, was 33.6 days (range, 21 to 90 days).

Sixty five patients presented with complications. Osteoarticular complications involving knee, sacroiliac and hip joints were the commonest. These findings are comparable to the results of Mousa et al. [15]. Neurobrucellosis as a complication was found in 10.52% (12) patients.

No apparent relationship could be established between the magnitude of the titer, severity of the disease and culture positivity. A seasonal variation in brucellosis cases was noted. Majority of the patients were less than 30 years (59.64%) and 73.52% of them were males. Children accounted for 28.07% and the disease was less frequent after the age of 60 years. Ninety five percent of the brucellosis patients stayed in villages and around 50% of them were either shepherds or farmers. Major risk factors were stay in the rural area, animal contact and raw milk ingestion. Similar findings have been reported by Adel Shehata et al. from Kuwait and Kochar et al. from Bikaner, India [16, 17]. In our study only 4.38% of patients had some knowledge about brucellosis, which is contradictory to the report of 82.5% by Namanda et al [18] from Kenya.

10.Conclusions

Present study shows that brucellosis is most of the times indifferently diagnosed as PUO. Significant Brucella agglutinins could be detected in 4.41% of PUO cases. Serological tests are more sensitive when compared to blood culture. RBPT was the most sensitive and rapid screening test.

Fever, joint pain and low backache were the commonest symptoms and osteoarticular complications were frequent. Undulating fever pattern was rarely seen among the brucellosis cases in this study. Animal contact, raw milk ingestion and stay in the rural area, were the major risk factors. Awareness programmes are needed to impart knowledge regarding the existence of brucellosis among local doctors and about the disease, its routes of transmission and preventive measures in rural population.

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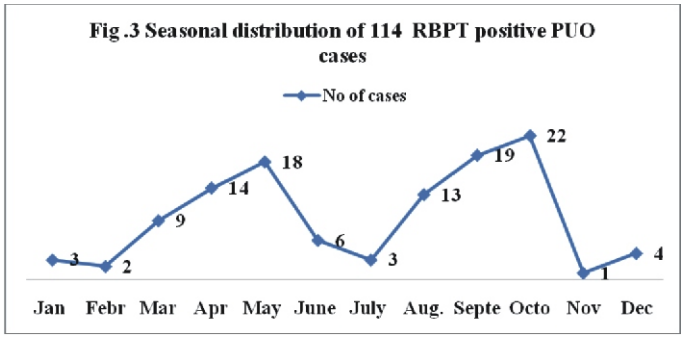

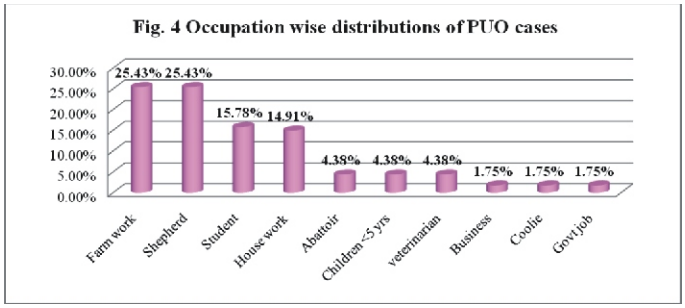

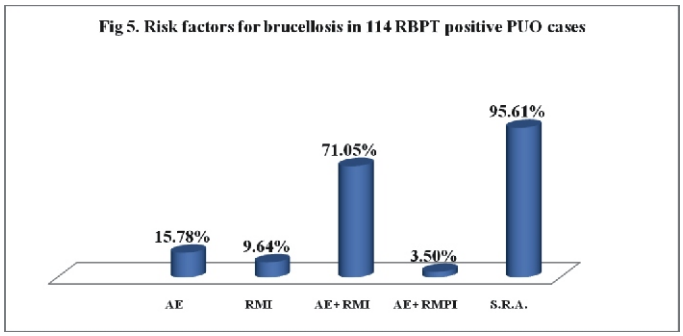

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