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Brucellosis an under Diagnosed Cause of Pyrexia

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Human Brucellosis is an important but a commonly neglected disease in India. This zoonotic disease involves all livestock systems .An increased demand for dairy products accompanied with changing and intensified farming practices has raised the concern for intensified transmission of this infection to the humans. The main aim of the study was to assess the seroprevalence of brucellosis in pyrexic patients and to compare different serological methods for detection of Brucellosis. This study was conducted in the tertiary care hospital of Uttarakhand, over a period of 12 months using ELISA, Rose Bengal Card Test and Standard Agglutination Test. Out of 60 pyrexic patients, 20% were positive by ELISA, 18.3% were positive by SAT and 16.6% were positive by RBPT. None of the healthy controls were found to be positive for antibodies to Brucella. All patients showed good response to treatment with Doxycycline and Streptomycin. Timely laboratory diagnosis of brucellosis prevents the initiation of empirical antitubercular treatment in patients as clinical features resemble that of tuberculosis. Alertness of medical staff and awareness of risk groups is required so as to recognize and adopt appropriate preventive measures and to control the disease. RBPT can be used as a useful screening tool.

Key words: Brucellosis, zoonotic disease, seroprevalence, RBPT, SAT, ELISA.

Brucellosis is one of the commonest bacterial zoonotic diseases that continue to be of public health and economic concern in many parts of the world. The reported incidence of human brucellosis worldwide in endemic areas varies widely, from <0.01 to >200 per lakh population. The true incidence however, is unknown and it has been estimated that it may be 25 times higher than the reported incidence due to misdiagnosis and underreporting[1]. The Mediterranean basin, the Arabian Gulf, the Indian subcontinent and parts of Mexico as well as Central and South America, are especially endemic for human brucellosis[2].

Six species are recognized within the genus Brucella: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis,* and *B. neotomae*. In recent times, two new species have been added to this genus, *B. cetaceae* and *B. pinnipediae*[3]. *B. melitensis* and occasionally *B. abortus* and *B. suis* are responsible for the disease in India[4].The variable symptoms and occurrence of subclinical and atypical infections makes the clinical diagnosis of human brucellosis particularly difficult. In human beings it is rarely fatal, but can lead to severe debilitation and disability. Brucellosis is amenable to treatment with the antibiotics now available, and so it is highly important that the proper diagnosis be made early.

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Bacteriological and serological examination is usually essential for confirmation of the diagnosis.

Goals and Objectives

- To study the seroprevalence of Brucellosis in pyrexic patients in a tertiary care hospital of Uttarakhand
- To compare different serological methods for detection of Brucellosis

MATERIALS AND METHODS

The study was conducted in the department of Microbiology at Himalayan Institute of Hospital Trust (HIHT), Swami Ram Nagar, Dehradun over a period of 12 months. The study group comprised of 60 patients attending the general medicine OPD/IPD with complaints of fever and other associated symptoms. Twenty healthy controls of both sexes were selected randomly after matching for age and sex. Serum samples from all patients with fever and control subjects were collected. All serum samples were evaluated for detecting antibodies to Brucella by Standard Agglutination Test (SAT; Fig. 1), Rose Bengal Plate Test (RBPT; Fig. 2) and ELISA (IgG & IgM) techniques.

We used the *B.abortus* antigen for SAT and RBPT procured from Division of Biological Products, Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh.Commercially available ELISA kits from Novatec, Immundiagnostica Gmbh, Technologic & Waldpark Germany were used for detection of IgM and IgG antibodies.

Detailed case history of every patient was taken with special emphasis on animal contact, ingestion of raw milk/raw cheese and liver meat.

RESULTS AND DISCUSSION

Brucellosis is an important but a commonly neglected disease in India. Only a few recent studies have addressed the prevalence and importance of brucellosis as a human disease in India. This is partly explained by the absence of proper laboratory facilities, lack of awareness of endemicity, under-reporting as well as poor co-operation and exchange of information between veterinary and health services. The nonexistence of regional data contributes towards the perpetuation of this zoonosis in the country while it has been eradicated from most developed countries.

Though blood culture remains the most definitive modality to diagnose brucellosis, it has some major disadvantages. Brucella is a highly infectious organism and its isolation can be carried out only in specialized laboratories with proper biohazard safety containment facilities due to risk of occupational exposure to laboratory workers[5]. Furthermore prolonged incubation periods (up to 8 weeks), requirement of CO_2 incubator, poor positivity rates of up to 20% and technical expertise required for its identification doesn't make it a practical or an investigation of choice[6] . Antigen detection based tests have not yet been validated[7]. Polymerase chain reaction (PCR) has been explored for the rapid detection and confirmation of *Brucella* and is a useful tool for differentiating *Brucella* spp. However it is expensive and requires technical expertise. In light of this, serological tests remain the mainstay for the diagnosis of brucellosis[8]. Hence in our study serological tests were used for the diagnosis of brucellosis.

Table 1. Showing comparison between different serological tests used for detecting Antibodies to Brucella

S. No.	SAT	RBPT	ELISA (IgM)	ELISA (IgG)
1.	-	+	+	-
2.	-	+	-	+
3.	+	+	+	-
4.	+	-	+	-
5.	+	+	+	-
6.	-	+	+	-
7.	+	+	+	+
8.	+	-	-	+
9.	-	+	-	-
10.	-	-	-	+
11.	-	+	-	-
12.	+	+	+	-
13.	-	-	+	-
14.	+	-	-	-
15.	+	-	-	-
16.	+	-	-	-
17.	-	+	-	-
18.	-	-	+	-
19.	+	-	-	-
20.	+	-	-	-
Total	11 (18.3%)	10 (16.6%)	09 (15%)	04 (6.6%)

ELISA was carried out along with conventional serological tests i.e. SAT and RBPT. Twenty percent of pyrexic patients were found positive by ELISA. It is a more sensitive and specific test as compared to SAT and is also reported to be the most sensitive test for the diagnosis of neurobrucellosis. Furthermore ELISA helps in identifying IgM and IgG antibodies individually which helps to diagnose acute and chronic cases unlike the other conventional methods[9,10].

SAT and RBPT were positive in 18.3% and 16.6% pyrexic patients respectively. SAT remains the most popular worldwide diagnostic tool. It measures the total quantity of agglutinating antibodies (IgM and IgG), and the quantity of specific IgG is determined by 2-mercaptoethanol (2ME). SAT titres above 1:160 are considered diagnostic along with a compatible clinical presentation. Some studies have shown persistence of various levels of SAT antibodies in many clinically cured patients[11]. This emphasizes the over diagnosis and diagnostic challenges faced in an area where typhoid, malaria, tuberculosis and rheumatoid arthritis clinically mimic human brucellosis, thereby exposing/denying patient's

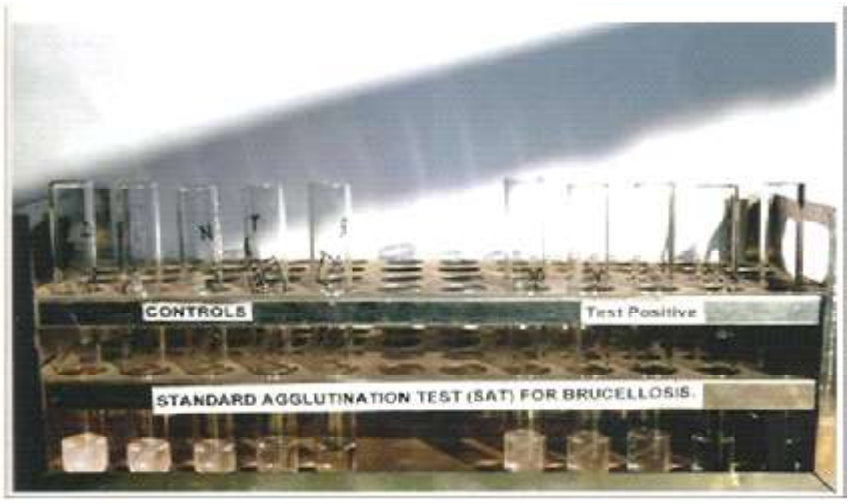

Fig. 1. Showing SAT

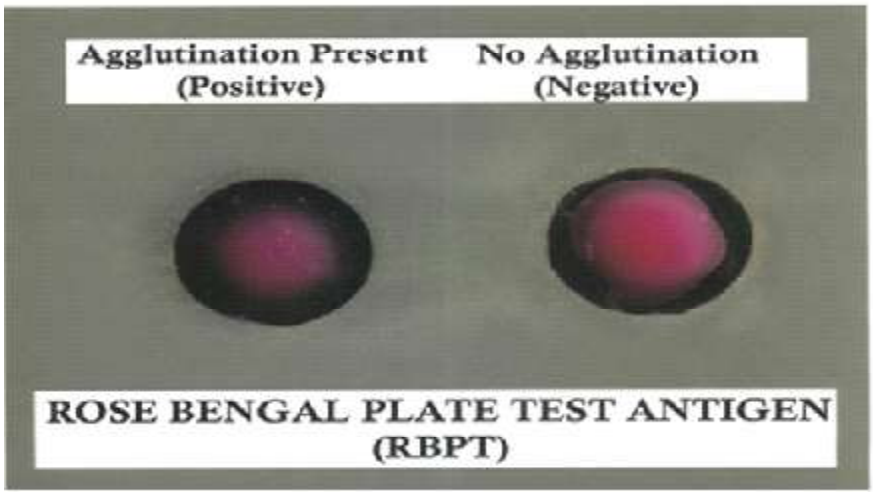

Fig. 2. Showing Rose Bengal Plate Test

access to specific therapy. In our study prozone phenomenon was found to be quite common with SAT.

In our study SAT had sensitivity and specificity of 50% and 89% respectively whereas RBPT was more sensitive (58.3%) and more specific (93.8%). RBPT is of value as a screening test especially in high risk rural areas where it is not possible to perform ELISA/SAT. RBPT also plays a great role in the rapid confirmation of neurobrucellosis, arthritis, epididymo orchitis, hydrocele. Many other workers like us have used *B. abortus* antigen for detecting antibodies to brucella. The advantage of using *B abortus* antigen is that it helps in detecting not only *B. abortus* but also *B. melitensis* and *B. suis*, however it can't detect *B canis*[5,8,12].

Other tests like Coombs test, *Brucella* IgM and IgG lateral flow and latex agglutination have been also recommended as field tests[7]. Some workers have recommended ELISA over other conventional methods since it is less labour intensive, require little standardization of reagents, can be automated and thus better suited for mass screening [5,6].

Overall prevalence of brucellosis was 20% in pyrexic patients by ELISA(15 % were IgM positive) which was quite high as compared to that reported by other Indian workers**.** Handa *et al* (1998)[5] from Delhi identified 3.3% cases with acute brucellosis and 6.6% cases of chronic brucellosis in a group of 121 patients with pyrexia of unknown origin (PUO). Sen *et al* (2002)[13] from Varanasi identified 26.8% seropositive cases in a group of 414 patients with PUO and Kadri *et al* (2000)[14] from Srinagar identified 20.8% seropositive cases in a group of 3,532 patients with PUO. However, the epidemiological data on this disease is frequently incomplete. The reasons for these variations may be the use of different selection/diagnostic criteria of pyrexic patients, different serological tests used for diagnosis and the regional endemicity of the disease.

An earlier study done from Kumaoun region of Uttarakhand has revealed a prevalence of 5% in human serum samples screened for brucellosis and a markedly higher prevalence of 17.4% was recorded among field veterinarians using a combination of RBPT, SAT and dot ELISA [15]. Our study also showed a high prevalence of Brucellosis in pyrexic patients. The prevalence of brucellosis in different geographical areas varies with standards of personal and environmental hygiene, animal husbandry practices, species of the causative agent and local methods of food processing.

It is possible that these cases of pyrexia report late after having received empirical antibiotics/anti tubercular therapy elsewhere and in absence of clinical improvement are referred to HIHT which is a major tertiary care referral center in this hilly state of Uttarakhand. This may have also led to higher detection rate of human brucellosis at HIHT.

Most of ELISA positive patients belonged to age group between 21-40 yrs (66.7%) with mean age of patients being 36.3 years , males were twice as effected as females probably because males are occupationally more exposed to farming activities, slaughter houses, abattoirs etc. Sixty two percent were from rural background. Out of 12 patients of brucellosis 83.3% gave history of exposure to animals or ingestion of raw milk or raw cheese and liver meat. Patel from Gujarat [12] Kochar *et al* from Bikaner[16] and Kadri et al from Kashmir[14] Frak *et al* from Saudi Arabia[17], Diaz *et al* from Spain[18] and Magee from Britain[19] showed that Brucella seropositive patients gave similar history of exposure.

In Haryana, 34% prevalence of human brucellosis was recorded among veterinarians, attendants and compounders in contact with animals[20] .In Gujarat, 8.5% prevalence of Brucella agglutinins was recorded in human cases[21]. The study conducted by Thakur and Thapliyal (2002) [15] in Uttarakhand, revealed a prevalence rate of 4.97% in samples obtained from persons exposed to animals and a markedly higher prevalence of 17.4% was recorded among field veterinarians. These observations support that occupation was an important risk factor for people with brucellosis.

All the 60 pyrexic patients were found negative for - HIV, HBs Ag, Widal test and malaria parasite . Human brucellosis is known for protean manifestations however, the most common presenting symptom is fever. The symptoms and signs most commonly reported are fever, fatigue, malaise, chills, sweats, headaches, myalgia, arthralgia, and weight loss[16,22]. Brucellosis is invariably under-diagnosed, likely because of

misleading clinical picture[4]. These pyrexic patients may be referred to as patients with PUO or the symptoms and signs be confused with those of other diseases. Thus to an unaware physician, the clinical diagnosis becomes a challenging one. Most of our subjects diagnosed in time showed good response to treatment with Doxycycline and Streptomycin (all of them responded).

CONCLUSION

Timely diagnosis of brucellosis prevents the initiation of empirical anti tubercular treatment in patients in whom brucellosis is diagnosed. Good clinical response is seen with appropriate antibiotic therapy

The detection of high sero prevalence of brucellosis in our study indicates that a larger study group including healthy population at risk and pyrexic patients be studied for brucella antibodies to assess the true endemicity and to investigate brucellosis as a cause of pyrexia in this predominantly rural/agricultural state. Alertness of medical staff and awareness of risk groups is required so as to recognize and adopt appropriate preventive measures and to control the disease. RBPT can be used for rapid mass screening in endemic areas.

REFERENCES

1. Gogia A, Duggal L, Dutta S .An Unusual Etiology of PUO. *JAPI.* 2011; **59**: 47-9.
2. Nagarathna S, Sharmada S, Veena Kumari HB, Arvind N, Sundar P, Sangeetha S. Seroprevalence of Brucella agglutinins: A pilot study. *Indian J Pathol Microbiol.,* 2009; **52**: 457-8.
3. Christopher S, Umapathy BL, Ravikumar KL. Brucellosis: Review on the recent trends in pathogenicity and laboratory diagnosis. *J Lab Physicians.* 2010; **2**: 55-60.
4. Corbel M.J. Brucella In : Albert Balows, Bian/ Duerden, editors. Topley and Wilson's Microbiology and Microbial infections, 9th ed. New York: Oxford University Press, 1998; 829-40.
5. Handa R, Singh S, Singh N, Wali JP. Brucellosis in North India: Results of a prospective study. *J Comm Dis* 1998; **30**: 85-7.
6. Klein JC, Behan KA. Determination of brucella immunoglobulin NG Agglutinating antibody titer with dithiothereitol. *Journal of clinical Microbiology* 1981; **17**: 24-5.
7. Mantur B G and Amarnath S K. Brucellosis in India – a review. *J. Biosci* 2008; **33**: 539-47.
8. Koshi G, Sulochana J, Pulimood BM, Chenan AM, John L, Swami Sk et al. Protean manifestation of brucellosis encountered at Vellore. *Indian J Med Res* 1988; **88**: 322 –9.
9. Gad El-Rab M O and Kambal A M. Evaluation of a Brucella enzyme immunoassay test (ELISA) in comparison with bacteriological culture and agglutination. *J. Infect* 1998. **36**: 197–201.
10. Araj GF, Lulu AR, Mustafa MY, Khateeb MI. Evaluation of ELISA in the diagnosis of acute and chronic brucellosis in human beings. *J Hyg Camb* 1986; **97**: 57-69.
11. Almuneef M and Memish Z A. Persistence of Brucella antibodies after successful treatment of acute brucellosis in an area of endemicity. *J. Clin. Microbio* 2002; **40:** 2313-8.
12. Patel P.R, Anjarial JM, Dave MR, Desai H. Serological evidence of Brucellosis in human being of Kaira District of Gujarat. *Ind J Public Health* 1986; **30**: 197-200.
13. Sen MR, Shukla BN, Goyal RK. Seroprevalence of brucellosis in and around Varanasi. *J Commun Dis* 2002; 34 :226-7.
14. Kadri MS, Rukhsana A, Laharwal MA, Tanvir M. Seroprevalence of Brucellosis in Kashmir among patients with pyrexia of unknown organization. Indian *Med. Assoc* 2000; **98**: 170-1 .
15. Thakur SD, Kumar R, Thapliyal DC. Human brucellosis: review of an under-diagnosed animal transmitted disease. *J Commun Dis* 2002; **34**: 287-301.
16. Kochar DK, Agarwal N, Neeti Jain, Sharma BV, Rastogi A, Chandra BM. Clinical profile of Neurobrucellosis. A report on 12 patients from Bikaner (North West India) JAPI 2000; **48**:376-8.
17. Frak KW, Yusuf Khan M. Analysis of 506 consecutive positive serologic test for Brucellosis in Saudi Arabia. J of Clinical Microbiology 1987; **25**: 1384-7.
18. Diaz, Pom EM, Riviro. A comparison of CIEP and other serological test in the diagnosis of human brucellosis. Bull world Health organization 1976; 417-22.
19. Magee JT. An enzyme labeled immunosorbent assay for Brucella abortus antibodies. *J. Medical Microbiology* 1980; **13**: 167-72.
20. Chauhan RS. Brucellosis in India and its impact on export of buffalo meat. *Indian J Ani Prod* 1999; **31**:316-7.
21. Panjarathinam R and Jhala CI. Brucellosis in

Gujarat state. *Indian J. Pathol. Microbiol* 1986; **29:** 53–60.

22. Mantur B G, Biradar M S, Bidri R C, Mulimani M S, Veerappa, Kariholu P, Patil S B and Mangalgi S S. 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