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Comparative Evaluation of Rose Bengal Plate test and ELISA in lab Diagnosis of Human Brucellosis

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ABSTRACT

Introduction: Human brucellosis is a major bacterial zoonosis reported worldwide. It is mainly an occupational disease reported in farmers, veterinarians, slaughterhouse workers, animal handlers and meat inspectors. Unavailability of automated blood culture systems makes isolation difficult and diagnosis mainly depends on serological and molecular methods. **Materials and Methods:** In a prospective study, a total of 127 serum samples, 28 from pyrexia of unknown origin (PUO) cases and 99 from high risk cases like veterinary personnel, butchers, nomads, farmers and gawalas, were serologically tested by Rose Bengal Plate Test (RBPT) and enzyme-linked immumosorbent assay (ELISA) and results were analyzed. **Results:** Out of 127 samples, 20 were tested positive by RBPT while 24 tested positive by ELISA. None of the PUO cases and veterinary personnel tested positive by RBPT or ELISA. Positivity was more among males, urban area, history of animal contact and consumption of raw milk. ELISA was found to be more sensitive and specific than RBPT. **Conclusion:** In our study significant prevalence of brucellosis was found among occupationally exposed persons. Most of the positive cases were found to be asymptomatic and those who were symptomatic had non-specific complaints. The findings signified silent presence of disease in our society. RBPT can be used as a rapid screening test in the peripheral resource poor labs and in absence of culture ELISA can be used for further confirmation.

Keywords: Human Brucellosis, Rose Bengal Plate Test, enzyme-linked immunosorbent assay.

INTRODUCTION

Brucellosis, also known as "undulant fever", "Mediterranean fever" or "Malta fever" is a zoonosis and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products.[1] Human brucellosis is a major bacterial zoonosis reported worldwide. B. melitensis, B. abortus, B. suis and B. canis are the four pathogenic species affecting humans.[2] It is mainly an occupational disease reported in farmers, veterinarians, slaughterhouse workers, animal handlers and meat inspectors.[3,4] The infection occurs through the ingestion of unboiled milk of infected animals, contact with vaginal discharge, urine, faeces and blood of infected animals, through unbreached skin and mucous membrane of conjunctiva and also by inhalation.[4] Human-to-human transmission is rarely encountered.[2] It affects people of all age groups and of both sexes but rare in childhood.[1] Males are affected more commonly than females which may be due to risk of occupational exposure.[5]

Brucellosis is a systemic infection characterized by protean clinical manifestations. There are no specific manifestations pointing to the diagnosis, leading to missed diagnosis. Brucellosis 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.[6] A definitive diagnosis of Brucellosis is established by recovering the bacteria in blood or demonstration of elevated levels of humoral antibody. Although automated blood culture systems have shortened the isolation time from weeks to days but due to its unavailability in most laboratories in our country makes isolation often difficult and a positive diagnosis usually depends on clinical,

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serological and epidemiological data.[7] In this study results of Rose Bengal Plate Test and ELISA in the serodiagnosis of human brucellosis were compared. In this study seroprevalence of brucellosis among pyrexia of unknown origin cases and occupationally exposed persons in Amritsar was surveyed and results of serological tests were compared.

MATERIALS AND METHODS

A prospective study was carried out in the Department of Microbiology, Govt Medical College, Amritsar for a period of 1½ years after obtaining the Institutional Ethics Committee's approval and informed consents from the patients. The study comprised of cases of Pyrexia of Unknown Origin (PUO) coming to Out Patient Department (OPD)/Indoor Patient Department (IPD) of various departments and health centres associated with Govt Medical College, Hospital, Amritsar. The study also included the high risk cases like veterinary personnel, butchers, farmers/dairy farmers, gawalas and animal handlers. The demographic data like age, sex, duration of fever, any other symptoms, and the risk factors were noted.

With proper aseptic precautions, patient's blood was collected in numbered gel vacutainers (VacutechTM gel clot activator- Vacuum Blood Collection Tubes, manufactured by Labtech Disposables, Gujarat, India) by venipuncture and then transported to the laboratory. The patient's serum was tested for Rose Bengal plate test (RBPT) and Brucella IgG and IgM ELISA.

RBPT was done by using Brucel-RB, Brucellosis Positive Control (Tulip Diagnostics (P) Ltd). Serum was also tested for Brucella IgM and IgG ELISA (Demeditec Diagnostics GmbH). All the tests were carried out as per manufacturer's guidelines.

RESULTS

The study comprised of 127 serum samples received in the Department of Microbiology, Govt Medical College, Amritsar. The samples were taken from cases of Pyrexia of Unknown Origin (PUO) coming to Out Patient Department (OPD)/Indoor Patient Department (IPD) of various departments and health centres associated with Govt Medical College, Hospital, Amritsar and also from the high risk cases like veterinary personnel, butchers, nomads, farmers and milkmen/gawalas.

All samples were collected and stored as per standard procedures. Brucella antibodies were detected by Bruel-RB (for Rose Bengal Plate Test i.e. RBPT). Seropositivity was ultimately confirmed by IgM & IgG ELISA. These tests were then compared. By RBPT, maximum seropositivity was seen in nomads 66.67% followed by gawalas 50% and butchers 43.75%. Lowest seropositivity of 5.36% was seen in farmers. None of the samples from veterinary practitioners and PUO patients tested positive. [Table 1]

By ELISA, maximum seropositivity was seen among butchers (68.75%) followed by nomads (66.67%). None of the samples tested positive for only IgM while 16.54% seropositivity was seen for only IgG. None of the PUO samples tested positive with ELISA. [Table 2]

In our study maximum seropositivity was seen in 11-20 and 61-70 age groups at 33.33% by RBPT wheras by ELISA it was 33.33% for 11-20 age group. None of the cases below 10 and above 70 years tested positive. [Table 3]

Table 1: Seroprevalence Among Risk Groups According To Brucel-Rb/ Rbpt (Rose Bengal Plate Test - Slide Agglutination Test)

Risk Group	RBPT (Slide Agglutination Test)		Total
	Negative	Positive	
Butcher	9 (56.25%)	7 (43.75%)	16 (100.00%)
Farmer	53 (94.64%)	3 (5.36%)	56 (100.00%)
Gawala	6 (50.00%)	6 (50.00%)	12 (100.00%)
Nomad	2 (33.33%)	4 (66.67%)	6 (100.00%)
PUO	28 (100.00%)	0 (0.00%)	28 (100.00%)
Veterinary practitioner	9 (100.00%)	0 (0.00%)	9 (100.00%)
Total	107 (84.25%)	20 (15.75%)	127 (100.00%)

Table 2: Seroprevalence among Risk Groups According To Elisa

Risk group	ELISA				Total
	Both IgM and IgG negative	Both IgM and IgG positive	Only IgG positive	Only IgM positive	
Butcher	5 (31.25%)	0 (0.00%)	11 (68.75%)	0 (0.00%)	16 (100.00%)
Farmer	54 (96.43%)	1 (1.79%)	1 (1.79%)	0 (0.00%)	56 (100.00%)
Gawala	6 (50.00%)	2 (16.67%)	4 (33.33%)	0 (0.00%)	12 (100.00%)
Nomad	2 (33.33%)	0 (0.00%)	4 (66.67%)	0 (0.00%)	6 (100.00%)
PUO	28 (100.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	28 (100.00%)
Veterinary practice	8 (88.89%)	0 (0.00%)	1 (11.11%)	0 (0.00%)	9 (100.00%)
Total	103 (81.10%)	3 (2.36%)	21 (16.54%)	0 (0.00%)	127 (100.00%)

Seroprevalence of 17.59% and 5.26% was seen with RBPT among males and females respectively. With ELISA, it was 21.30% and 5.26% among males and females respectively. [Table 4].

Table 4: Seroprevalence By Rbpt And Elisa According To Sex

SEX	Number of cases	Test	
		RBPT (Slide Agglutination Test)	IgG ELISA
Female	19 (100.00%)	1 (5.26%)	1 (5.26%)
Male	108 (100.00%)	19 (17.59%)	23 (21.30%)
Total	127 (100.00%)	20 (15.75%)	24 (18.90%)

Table 3: Seroprevalence By Rbpt And Elisa According To Age Groups

Age distribution (in years)	Number of cases	Test	
		RBPT (Slide Agglutination Test)	IgG ELISA
1) <=10	5 (100.00%)	0 (0.00%)	0 (0.00%)
2) 11-20	9 (100.00%)	3 (33.33%)	3 (33.33%)
3) 21-30	24 (100.00%)	1 (4.17%)	1 (4.17%)
4) 31-40	31 (100.00%)	3 (9.68%)	6 (19.35%)
5) 41-50	32 (100.00%)	7 (21.88%)	8 (25.00%)
6) 51-60	12 (100.00%)	2 (16.67%)	3 (25.00%)
7) 61-70	12 (100.00%)	4 (33.33%)	3 (25.00%)
8) >70	2 (100.00%)	0 (0.00%)	0 (0.00%)
Total	127 (100.00%)	20 (15.75%)	24 (18.90%)

DISCUSSION

Brucellosis is an important re-emerging infectious disease. The disease is closely associated with the evolution of mankind as an agrarian society linked to the practice of shepherding and popularization of animal husbandry.[8] It remains endemic in many developing regions of the world.[7] In India, brucellosis was first recognized in 1942 and is now endemic throughout the country. Brucellosis is an important zoonotic problem in India accounting for a loss of about 30 million man days per year.[9] In India, the serological evidence of Brucellosis in humans has been reported from various parts of the country, where the incidence varies from0.9% to 18%.[10,11]

The clinical features and presentation of human brucellosis overlap with many other infectious and noninfectious diseases such as typhoid fever, rheumatic fever, spinal tuberculosis, pyelitis, cholecystitis, thrombophlebitis, autoimmune disease, and tumours.[12,13] Brucellosis can lead to severe debilitation and disability. Because of the deceptive nature, the disease may be easily misdiagnosed or diagnosis may be delayed thereby making clinical diagnosis a challenge. In the absence of specific clinical features, serological testing forms the mainstay of diagnosis.[7]

In our study out of total 127 samples 20 (15.75%) were positive by RBPT [Table 1] which is in concordance with study done by Dias and Dias and in contrast to studies by Pathak et al, Agasthya et al, Aniyappanavar et al, Metri et al, Mangalgi et al which reported less than 7% seropositivity.[3,4,6,7,14]

In our study all 20 (20.20%) RBPT positive samples were from occupationally exposed risk group which is in concordance with the study by Pathak et al. Maximum seropsitivity was observed among Nomads (66.67%) followed by gawalas of gaushala (50%), butchers (43.75%) and famers (5.36%). None of the PUO case and village veterinary practitioners tested positive through RBPT/Brucel-RB. Seropositivity among farmers is in concordance with the study done by Metri et al.[6] Combined seropositivity in village veterinary practitioners and gawalas (milkman) is 28.75% which is in contrast to study by Prakash et al.[9]

In this study maximum seropositivity by ELISA was seen among butchers 11 (68.75%) followed by nomads (66.67%). None of the 127 samples tested positive for only IgM whereas both IgM and IgG were detected in 3 (2.36%). Only IgG was detected in 21 samples (16.54%) [Table 2]. So, total seroprevalence (IgG + Both IgM and IgG) through ELISA was observed as 18.89%. This is in concordance with the study done by Agasthya et al and in contrast to studies by Pathak et al and Aniyappanavar et al.[3,4,15]

Maximum seropsitivity among butchers, nomads and gawalas can be due to higher rate of exposure to animals and animal products and small sample size compared to sample size of farmers.

In our study maximum seropositivity was seen in 11-20 and 61-70 age groups at 33.33% by RBPT wheras by ELISA it was 33.33% for 11-20 age group. Maximum seropositivity in 11-20 years age group can be due to small sample size compared to other age groups and consumption of raw milk by the positive cases which belonged to nomads and gawalas. Maximum cases were from 41-50 years age groups i.e. 8 out of total 24 IgG ELISA positive cases because this group had persons who were occupationally exposed and coming in greater contact with animals. None of the cases below 10 and above 70 years tested positive. This is in contrast to studies by Prakash et al, Agasthya et al, Dias and and Metri et al.[4,6,7,9]

In our study, seroprevalence of 19 (17.59%) and 1 (5.26%) was seen with RBPT among males and females respectively. With ELISA, it was 23 (21.30%) and 1(5.26%) among males and females respectively, though not statistically significant

because of more males involved in occupations involving contact with animals. This in concordance with the studies by Mangalgi et al, Metri et al, Dias & Dias, Thakur SD et al and Kadri et al which reported male preponderance.[6,7,14,16,17]

In our study, 65% of the positive cases were asymptomatic which is in corcordance with the study done by Prakash et al in which high positivity was observed even in aymptomatic healthy controls and study by Agasthya et al which reported 50% asymptomatic among seropsitive patients.[4,9] Lowbackache was observed in 20% positive cases which is in concordance with study done by Dias and Dias.[7] Fatigue was reported by 15 % positive cases which is in concordance with the study done by Mangalgi et al.[14]

In our study seroprevalence of 8.64% with RBPT and ELISA was seen among persons belonging to rural area. It was 28.26% with RBPT and 36.96% with ELISA for persons belonging to urban area. More seropositivity for urban area is because of the fact that all the gawalas were from urban area and 15 out of 16 butchers were from urban area. Both the risk groups i.e. gawalas and butchers were found to have seropsitivity of 50% and 68.75% respectively.

Seroprevalence of 18.69% with RBPT and 22.43% with ELISA was seen among persons exposed to animals while none of the samples of the cases, without history of contact with animals, tested positive. The findings indicate that exposure to animals is a major risk factor for Brucella infection.

In our study Seroprevalence of 11.88% with RBPT and 12.87% with ELISA was seen among persons not consuming raw milk or raw animal products whereas for persons consuming raw milk, it was 30.77% with RBPT and 42.31% with ELISA. The findings of our study indicate that raw milk consumption is a major risk factor for Brucella infection. In our study, findings for risk factor i.e. stay in rural area is in contrast and findings for risk factors like history of contact with animals or consumption of raw milk are in concordance with studies done by Mangalgi et al, Tumwine et al, Nikokar et al, Praksh et al, Kochar et al and Awad.[9,14,18-21]

In our study, when compared with IgG ELISA, RBPT showed sensitivity of 75% and specificity of 98%. This is in contrast with the study by Dias and Dias which reported sensitivity of 55.55% and specificity of 98.6% for RBPT.[7]

Brucellae have slow growth rate, and the culture result are not available for several days or weeks. The number of bacteria in clinical samples may vary widely, with the isolation of Brucella being highly dependent on the stage of disease (acute vs. chronic), antibiotic pretreatment, the existence of an appropriate clinical specimen and the culturing methods used.[22] Blood culture provides definite proof of brucellosis but it may not be positive for all patients. Lysis centrifugation and blood clot culture techniques have yielded encouraging results in some studies in terms of sensitivity and rapidity. Bone marrow cultures are considered the gold standard in some studies.[8,23-27] Antigen detection by enzyme linked immunosorbent assay (ELISA) is an acceptable alternative to blood culture. Although antigen detection methods are potentially useful but have not been validated.[8] Polymerase chain reaction (PCR) has been explored for the rapid detection and confirmation of Brucella, but the clinical role of these tests remains to be defined.[28-34]

The limitations of aforementioned tools make serology directed against antibody detection the most useful tool.

CONCLUSION

In our study significant prevalence of brucellosis was found among occupationally exposed persons. Most of the positive cases were found to be asymptomatic and those who were symptomatic had non-specific complaints. The findings signified silent presence of disease in our society. In our study, all PUO cases and most of veterinary cases have tested negative while in other studies significant prevalence among these groups was observed. This cannot be said to be statistically significant because of small sample size. Culture was not done as it's a hazardous procedure and requires biosafety level 3 lab. As the disease has significant prevalence in our society, study of larger sample size especially of PUO cases and veterinary personnel is required to ascertain prevalence among these groups. RBPT can be used as a rapid screening test in the peripheral resource poor labs and in absence of culture ELISA can be used for further confirmation.

For brucellosis control, mass vaccination and regular screening of animals should be carried out. Protective clothing should be used while handling animals. Regular screening of high risk groups should be done regularly. Physicians should keep a high index of suspicion in case of PUOs for timely detection and treatment. Education and awareness regarding brucellosis should be given to the general population.

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