

Original Research Article

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A Comparative Study of Microplate Agglutination Test (MAT) with Enzyme Linked Immunosorbant Assay (ELISA) for Diagnosis of Brucellosis

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ABSTRACT

Keywords

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Brucellosis is one of the important Zoonotic diseases in India and continues to be a major public health concern globally. Human brucellosis is difficult to diagnose and requires laboratory testing for confirmation. Previously Standard Agglutination Test (SAT) and Rose Bengal Plate Test (RBPT) were used to detect Brucellosis. Objective of the study is the determination of antibody titres in the positive sera by ELISA and MAT. The study was conducted in Department of Microbiology RIMS, Raichur. Blood samples were collected from the patients attending orthopedic OPD of RIMS teaching hospital with complaints of fever, joint pain and backache. RBPT, MAT and ELISA IgM and IgG were carried out on serum samples. It was done using Chi square test. Out of 116 blood samples tested 23 cases were positive for Brucella antibodies by RBPT and MAT. Twenty two cases were positive for ELISA IgM and twenty cases were positive for ELISA IgG. All the 40 healthy controls were negative by RBPT, MAT and ELISA. Considering ELISA as gold standard, sensitivity and specificity of MAT when compared to IgM ELISA was 95% and 98.92% respectively. Sensitivity and specificity of MAT when compared to IgG ELISA was 86.95% and 96.77% respectively. To conclude along with MAT, ELISA should also be done to rule out false positive cases.

Introduction

In India 80% of population resides in rural areas in close contact with livestock like cattle, sheep, goat etc.,¹ and agriculture is the main occupation in the rural area. The dairy farming and animal husbandry are one of the major small-scale industries employing a large portion of the country's population.

History of animal contact is expected in these profession *i.e.*, agriculture, veterinary, dairy farming and animal husbandry. Brucellosis is a Zoonotic disease and is not uncommon in the rural population of our country.

Brucellosis is one of the most common zoonosis and continues to be a major public health concern globally especially in the developing countries. The disease can be missed in the early stage for the other diseases due to great variety of clinical manifestation. It is difficult and often impossible for a physician to give clinical diagnosis with certainty.² important reasons could be due to multiplicity of illness it mimics, lack of adequate facilities in most hospitals for establishing the diagnosis and lack of awareness of the disease. Though it is primarily a disease seen in animals and can be

transmitted to humans, it can lead to a substantial public health problem. Brucellosis is usually transmitted from animals to man by direct contact with infected animals or their products and by consumption of raw unboiled milk infected with *Brucella* organisms. Other rare modes of transmission includes through the placenta, breast-feeding and sexual intercourse. Infection occurs when *Brucella* organisms present in vaginal discharges, fetuses, placenta, manure or carcasses enter through skin, mucosa and conjunctiva.

Human brucellosis has a wide spectrum of clinical manifestations, earning it a place alongside syphilis and tuberculosis as one of the great imitators. It is often misdiagnosed as typhoid or Pyrexia of Unknown Origin (P.U.O). Common symptoms are fever, chills, sweats, weakness, loss of weight and abdominal pain but it is not rare for brucellosis to present musculoskeletal system involvement, respiratory disease, central nervous system infection, cardiovascular disease, urogenital infection, or as chronic localized lesions. Backache and joint pain are important presenting complaints of the brucellosis.²

The complications which may develop include spondylitis, arthritis, neurobrucellosis, and endocarditis etc. The most frequent suppurative complications of human brucellosis are of osteoarticular. These include spondylitis, arthritis, osteomyelitis, tendinitis and bursitis.³ Low backache of *Brucella* spondylitis closely simulates pain of prolapsed intervertebral disc and Pott's spine. So the diagnosis of Brucellosis is often not considered in such cases.⁴

A high index of suspicion by the clinicians and optimal use of a sophisticated laboratory is essential for accurate diagnosis. Isolation of the organism causing the disease by doing the culture gives a definitive diagnosis.⁵ *Brucella*

organism grow very slowly in vitro, so serological tests are used as screening tests for preliminary diagnosis of brucellosis.⁶ The most widely used serological tests are agglutination tests which are very good screening procedures as they give specific and reliable preliminary diagnosis and are also cost effective. PCR could be a significant breakthrough in the near future but the cost effectiveness may still be a hurdle in India.

Brucellosis is susceptible to treatment with the antibiotics presently available so it is of great importance a proper diagnosis be made during the early stage of disease. The present study has been carried out to screen the patients attending orthopedic out-patient department, for *Brucella* antibodies.

Serological tests like Enzyme-linked Immunosorbant assay (ELISA) is capable of readily identifying individual IgM and IgG antibody allowing for a better correlation with the clinical situation. A micro agglutination test (MAT) variant of the SAT or Enzyme-linked Immunosorbant assay (ELISA) is recommended for serological diagnosis since it is more specific and sensitive and simpler to perform and the results are easier to read.⁷ this study is undertaken to compare the most sensitive tests like MAT and ELISA for demonstration of *Brucella* antibodies.

Materials and Methods

The present study was carried out at the Department of Microbiology, R.I.M.S, Raichur during the year January 2015 to December 2015. Serum samples from the patients with complaints of fever, joint pain and backache attending orthopedic outpatient department as well as from individuals who are at occupational risk like Veterinarians, Para veterinarians, Farm workers, Slaughter house workers etc were subjected to Rose Bengal Plate test (RBPT) for detection of

antibrucella antibodies. The samples positive by RBPT were further tested by MAT and ELISA. In all a total of 116 patients belonging to various age groups and either sex were included in this study. 40 healthy individuals of different age and sex were also studied as controls.

Rose Bengal Plate Test (RBPT)

Reagents and materials

Rose Bengal Plate Test (RBPT) antigen. (Vircell microbiologists, Granada, Spain).

Serum.

Test was performed as per manufactures guidelines.

Observation

The observation made by examining the agglutination in a good light. Reading was often facilitated when the mixture was observed as it flowed away on the slide.

Interpretation

The result was read as positive if there is agglutination or negative when no agglutination was seen. Positive and negative control was run with each test.

Storage

All the components were at a temperature of 2-8^o C.

ELISA

ELISA IgG and IgM were performed and interpreted using a commercial kit (Vircell microbiologists, Granada, Spain) according to the manufacturer's instruction. All serum samples, a total of 116 suspected cases and 40

controls were subjected for indirect ELISA, which used S-LPS of *Brucella abortus* 99 strain.

Reagents

Serum diluents, IgM Positive control, IgG Positive control, IgM and IgG cut off control, IgM and IgG Negative control, IgM conjugate, IgG conjugate, TMB Substrate solution, Stop reagent and Wash buffer, ready to use reagents were supplied in the kit.

Validation

Positive, negative and cut off controls was run with each test. Optical densities (O.D) of positive, negative and cut off controls as shown in the table 1 was taken for interpretation of results of the test.

Interpretation

Mean O.D for cut off serum was calculated.

Antibody index was calculated by using the formula

Antibody index= (sample O.D/ cut off serum mean O.D) x 10 (Table 2).

Storage

Reagents and Wells in the kits are stored at 2 – 8^o C.

Microplate agglutination test

The test consists of U-bottom well strips coated with anti-human immunoglobulin's. After addition and dilution of serum, the antigen is added and strips are incubated for 24 hours until agglutination takes place. This assay allows the detection of both agglutinating and incomplete antibodies.

Material

All reagents are ready to use. Break-apart 2 plates of 12 U-bottom 8 well-strips are in the kit, so that the same number of wells is consumed than the number of samples to be performed. The result of the test is obtained in 24 hours in a single step.

14ml of stained serum diluents

12 ml of stained *Brucella abortus* bacterial suspension formaldehyde treated.

250 µl of positive control containing Proclin.

250µl of negative control containing Proclin.

Observation

The observation made by examining the agglutination in a good light.

Interpretation

The result was read as positive if a net covering the whole well surface appears.

A button of bacteria in the center of the well indicates a negative result.

Titers higher than 1:320 suggests brucellosis.

Storage

The antigen and plates were stored at 2-8 °C

Results and Discussion

Out of 116 cases of backache and joint pain there were 70 males and 46 females cases (Table 3).

A total of 23(19.8%) cases were positive for the presence of *Brucella* antibodies, in which 20 cases were males and 3 cases were

females. Age group of 31-40 years included maximum number of cases (34/116 i.e 29.31%), age group of 31-40 years also included highest number of seropositive cases (8/23 i.e., 34%) of which 5 were male and 3 were female, followed by 6 cases each in 21-30 and 41-50 years age group (Table 3). The control group included students and voluntary blood donors, which were apparently healthy and completely asymptomatic. None of the controls gave history of any significant illness in the recent past, which required antibiotic therapy. The control group included 73 % males as compared to 27% females (Table 4).

In all 116 cases of backache & Joint pain, patients were serologically screened for Brucellosis by RBPT of which 23 were positive for brucellosis, out of which 10 cases had the history of fever, none of the healthy controls showed presence of *Brucella* antibodies (Table 5).

MAT and ELISA was also performed on the total samples and the results of both the test were compared. Out of 23 MAT positive cases IgM ELISA was positive in 22. One ELISA negative was falsely reported as positive by MAT. Sensitivity and specificity of MAT when compared to IgG ELISA shows 95% and 98.92% respectively (Table 6).

Out of 23 MAT positive cases IgG ELISA was positive in 20. Three cases of ELISA negative were falsely reported as positive by MAT. Taking ELISA as gold standard, sensitivity and specificity of MAT when compared to IgG ELISA shows 86.95% and 96.77% respectively (Table 7).

In the present study 116 patients attending Orthopedic out-patient department with complaints of multiple joint pain and backache were screened for anti-brucella antibodies. Out of 116 patients 70 were males and 46 were females (Table 3).

Out of 116 patients studied, 23(19.82%) showed the presence of *Brucella* antibodies by both Rose Bengal Plate Test (RBPT) and MAT. Out of 23 Seropositive cases 20 (87 %) were males and 3(13%) were females. This male preponderance can be explained by higher exposure of males to infected animals and their occupation. In a study carried out by Koshy and Myers (1967) 65% patients were males and 35% were females. In Mathur's (1968) study 70% were males and 30% were females.^{2,5} However Cooper (1991) has reported a higher incidence amongst women than men and a remarkable increase in brucellosis with increasing age. This may be due to either an increased exposure to infected livestock, or to an increased susceptibility to the disease in women and with increasing age.¹⁶

In our study it was observed that the disease is more common in 31-40 years (34.7%) age group, followed by third and fifth decade. Youngest patient was 22 years male and the eldest patient was 55 years male. Adults in general are more likely than children to be involved in the day-to-day care of livestock and particularly to be involved in certain high-risk practices such as assisting in animal parturition.¹⁶ Koshi and Myers (1967) have

also reported that maximum number of cases belonged to the third decade of life.²

The control group

Forty healthy individuals were also screened for *Brucella* antibodies. Out of them 29 (72.5%) were males and 11(27.5%) were females. Majority were between 21-30 years of age (Table 4).

The controls included students and voluntary blood donors, who were apparently healthy and asymptomatic and who had no history of animal contact. None of them showed the presence of *Brucella* antibodies. This indicates that the healthy individuals, who were not exposed to animals and who had a healthy life style, are unlikely to suffer from *Brucella* infections.

Sharma *et al.*, (1974) have studied 488 healthy blood donors as controls and showed no incidence of *Brucella* antibodies in healthy individuals.⁹ Roy *et al.*, (1965) had screened 25 medical students as healthy controls for anti-brucella antibodies. They have also showed no incidence of *Brucella* antibodies in healthy individuals.¹⁰ similar findings has been recorded in the present study.

Table.1 Cut off values

Control	Optical density (O.D)
Positive control	>0.9
Negative control	<0.5
Cut off control	>0.55
	<1.5

Table.2 Interpretation

Antibody index	Interpretation
<9	Negative
9-11	Equivocal
>11	Positive

Table.3 Age / sex wise distribution of Brucellosis in backache and joint pain patients

Age group	No. of patients (Positive Cases)	Males (Positive Cases)	Females (Positive Cases)
21-30	23 (6)	17(6)	06(0)
31-40	34(8)	19(5)	15(3)
41-50	34(6)	19(6)	15(0)
51-60	25(3)	15(3)	10(0)
Total	116(23)	70(20)	46(3)

Table.4 Age/sex wise distribution of healthy controls.

Age	Total Persons Screened	Males (%)	Females (%)
21-30	20	15 (75%)	05(25)
31-40	12	08(66.7%)	04(33.3)
41-50	05	04 (80%)	01(20)
51-60	03	02(66.7%)	01(33.3)
Total	40	29(72.5%)	11(27.5)

Table.5 Cases studied and their results (shows the total cases screened by RBPT and their results)

Source of samples	Males		Females	
	Seropositive	Seronegative	Seropositive	Seronegative
Backache, Joint pain patients (n=116)	20	50	3	43
Healthy controls (n=40)	00	29	00	11

Table.6 MAT vs ELISA (IgM)

	ELISA +ve	ELISA -ve	TOTAL
MAT (+ve)	22	01	23
MAT (-ve)	01	92	93
TOTAL	23	93	116

$\chi^2 = 103.75$ P= <0.001 df= 1

Sensitivity = (22 x 100) / 23 = 95 %

Specificity = (92 x 100) / 93 = 98.92 %

Positive Predictive Value = (22 x 100) / 23 = 95 %

Table.7 MAT vs IgG

	ELISA (positive)	ELISA (negative)	TOTAL
MAT (positive) n=23	20	03	23
MAT (negative) n=93	03	90	93
TOTAL	23	93	116

$\chi^2 = 81.325$ P = < 0.001 df = 1
 Sensitivity = (20 x 100) / 23 = 86.95 %
 Specificity = (90 x 100) / 93 = 96.77 %
 Positive Predictive Value = (20 x 100) / 23 = 86.95 %
 Negative Predictive Value = (90 x 100) / 93 = 96.77 %

Evaluation of seropositive cases

Out of 23 seropositive cases of brucellosis, all the 23(19.82%) patients showed the presence of *Brucella* antibodies in diagnostic titres. The serological methods used in present study were RBPT, MAT and ELISA from Vircell microbiologists, Granada, Spain. The serological methods are the most generally used tests for diagnosis. A rise in titre in the presence of clinical illness is strongly suggestive of active Brucellosis. The diagnostic titre in MAT as per Vircell microbiologists suggestive of *Brucellosis* is 1:320.

In a study by Roy *et al.*, (1965) out of 351 samples screened, 21 were positive in diagnostic titres (6%).¹⁰ Joshi and Omprakash screened 800 samples and have found diagnostic titres in 39 cases (4.9%)¹¹. In study by Gokhale *et al.*, (1999) out of 72 patients 6 were Seropositive for brucellosis (8.3%).⁴ In our study all the 23 Seropositive cases showed titres more than 1:320

Relation of backache & joint pain in patients with Brucellosis

In our study 23 out of 116 (19%) patients suspected of brucellosis presented with features of multiple joint pain and backache. Khateeb *et al.*, (1990) found that out of 400

patients with brucellosis, 104 (26%) had arthritis, of which 96 could be followed up. The spine (8%), sacroiliac joint (26%) involvement was the major joint involvements.¹² Malik *et al.*, (1997) studied 104 patients of brucellosis. He found that most common presenting symptoms were fever (100%), sweating, headache (76.9%), joint pains (76.9%) and backache (73.1%).

All the patients had *Brucella melitensis* infection.¹³ Nagalotimath has studied 213 cases of brucellosis with symptomatology referring to orthopedics. He found 57cases with spine involvement and 14 cases with sacroiliac joint involvement.¹⁴

Though there are reports of brucellosis with backache & joint pain in patients from various parts of the world, large numbers of cases remain undiagnosed. They can be diagnosed by serological test and blood culture. Hence we recommend that serum of all cases of backache and joint pain should be routinely screened for brucellosis¹⁵ (Table 5).

Comparison between MAT and ELISA (IgM) (Table 6)

Out of 23 MAT positive cases IgM ELISA was positive in 22. One ELISA negative was falsely reported as positive by MAT. Sensitivity and specificity of MAT when

compared to IgG ELISA shows 95% and 98.92% respectively.

Comparison between MAT and IgG (Table 7)

Out of 23 MAT positive cases IgG ELISA was positive in 20. Three cases of ELISA negative were falsely reported as positive by MAT. Taking ELISA as gold standard, Sensitivity and specificity of MAT when compared to IgG ELISA shows 86.95% and 96.77% respectively.

It is suggested that the individuals engaged in an occupation, which brings them in close, constant contact with *Brucella* infected animals should be screened periodically for *Brucella* antibodies.¹⁶

Due to the ignorance of the people, it is very difficult to get enough co-operation which is most necessary in the study of brucellosis. The disease can be eradicated from India with a comprehensive study.¹⁵

The clinicians need to work with coordination to eradicate human brucellosis in India.

A prudent approach to the disease will reduce the impact of brucellosis as a worldwide problem. Perhaps a close liaison between veterinarians and clinicians may help to control the problem of brucellosis.

To conclude brucellosis should not be overlooked in the differential diagnosis of Joint pain and backache, as the disease is curable.

The disease requires a high index of suspicion, which can result in early diagnosis and treatment.

Rose Bengal Plate test was used for screening and MAT and ELISA was used to detect antibodies.

Sensitivity and specificity of MAT when compared to IgM ELISA shows 95% and 98.92% respectively.

Sensitivity and specificity of MAT when compared to IgG ELISA shows 86.95% and 96.77% respectively.

In addition to MAT, ELISA should be done to rule out false positive cases.

Accurate and rapid diagnosis of Brucellosis in the diagnostic laboratory still holds challenge and requires additional testing and standardization especially with the development of more recent diagnostic assays.

A precise diagnosis of *Brucella* species infection is, important for the control of the disease in animals and consequently in man. Clinical diagnosis is based usually on the history of reproductive failure in livestock, but it is a presumptive diagnosis that must be confirmed by laboratory methods.

Considering the economic losses and human suffering produced by Brucellosis it may be worthwhile to say that rigorous steps are necessary to

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