Research Paper

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Seroprevalence of Brucellosis in a Tertiary Care Hospital in Shimoga, Karnataka

Microbiology

KEYWORDS : Brucellosis, Rose Bengal Plate Test, Standard Tube Agglutination Test

Dr Premalatha D.E Assistant Professor, Shimoga Institute of Medical Sciences, Shimoga

Dr Prakash N Assistant Professor, Shimoga Institute of Medical Sciences, Shimoga

ABSTRACT

Background : Brucellosis is a chronic, contagious and zoonotic disease. It is usually difficult to diagnose clinically in the absence of specific clinical features. Hence serological testing forms the mainstay of diagnosing the disease. Aim: The present study was done to know the seroprevalence of Brucellosis in Shimoga (Karnataka) and to compare Rose Bengal plate test with Standard tube agglutination test. Materials and Methods: Eight hundred and forty blood samples received at Microbiology Department for various serological tests were used for the study. Serum was separated from samples and Rose Bengal plate test (RBPT) and Standard tube agglutination test (STAT) were done. Results: The overall seroprevalence of brucellosis in the study was 1.4%. In positive cases majority were males (66.6%) compared to females (33.3%). 20-40 years age group were more affected (1.7%). Shepherds had the highest prevalence rate (5.5%), followed by farmers (3.3%). When compared with STAT, RBPT showed sensitivity specificity of 100%. Conclusion: Prevalence of brucellosis in Shimoga was not found to be significant. Shepherds and farmers were found to be largely affected. RBPT is highly reliable and has a close relation with STAT in the diagnosis of human brucellosis.

INTRODUCTION

Brucellosis is a zoonotic disease of wild and domestic animals in which man is an accidental host. It has a worldwide distribution, affecting humans and animals both in the developed and developing countries. The disease burden is more profound in the developing countries due to lack of effective public health measures, domestic animal health programs and appropriate diagnostic facilities[1].

Brucellosis is caused by small, fastidious gram-negative coccobacilli of the genus Brucella. There are four important species pathogenic to humans ; B. melitensis, ; B. abortus, B. suis and B. canis[2].

Humans are commonly infected through ingestion of raw milk, cheese or meat, or through direct contact with infected animals, products of conception or animal discharges (e.g., among shepherds, farmers and veterinarians), and through inhalation of infectious aerosols (e.g., by workers in abattoirs and microbiology laboratories)[3].

Human brucellosis can be an acute or a chronic febrile illness and presents with a variety of manifestations after an incubation period, which can vary from 1 to 6 weeks or several months. Diagnosis can be established by blood culture and/or serology.

The present study was taken up to find the prevalence of human brucellosis in Shimoga and to study the influence of age, sex and occupation in predisposing brucellosis and to compare Rose Bengal plate test (RBPT) and Standard tube agglutination tests (STAT). This kind of study was not done before in this part of Karnataka.

MATERIALS AND METHODS

The Study was conducted in the Department of Microbiology, SIMS Medical College, from May 2014 to October 2014 for a period of six months

Blood samples that were received at Microbiology Department for various serological tests (Widal, VDRL, HBs Ag, ASLO and RA tests) obtained from patients attending McGann hospital, formed the material for study.

All serum samples were first screened by Rose Bengal Plate Test (RBPT) and further analyzed by Standard Tube Agglutination Test (STAT). Details about age, sex, occupation of subjects were also collected.____

Serum was separated from blood sample and subjected to

1. Rose Bengal plate test (RBPT)
2. Standard tube agglutination tests (STAT)

1) Rose Bengal plate test (RBPT)[4]:

The antigen for the test was obtained from Indian Veterinary Research Institute (IVRI), Izatnagar. The RBPT antigen is an 8% suspension of pure, smooth killed cells of Brucella abortus strain 99 phenolized and stained with Rose Bengal dye. It is buffered at pH 3.65 using lactic acid buffer.

Procedure:

The test was performed according to information provided with the antigen kit.

1. Antigen bottle refrigerated at 4-8°C was removed and left at room temperature for half an hour.
2. Antigen was shaken to ensure homogeneous suspension
3. One drop of antigen (0.03 ml) was placed on each square of the plate.
4. With a 0.1ml pipette, one drop (0.03 ml) of serum was placed by the side of the antigen drop.
5. With a spreader the antigen and serum drops were thoroughly mixed and spread to an area of about 2.5 cm diameter. The plate was manually rotated for 4 minutes.
6. With each set of test sera, a known positive and negative control sera were also included.

Interpretation:

The test was examined for agglutination in bright light. Any degree of agglutination was taken as positive and no agglutination was taken as negative.

2) Standard Tube Agglutination Test (STAT)[5]:

The test was carried out with Brucella abortus plain antigen, procured from IVRI, Izatnagar. It is a suspension of pure, smooth Brucella abortus strain 99 in phenol saline, standardized against anti- Brucella abortus serum (1000 IU/ml) to give 50% agglutination with 1:500 dilution of the serum.

Procedure:

1. Doubling dilution of the test serum, starting from 1:5, was done with 0.5% phenol saline in 10 sugar tubes.
2. Antigen bottle was shaken well and brought to room temperature.
3. Equal amount of (0.5 ml) Brucella abortus antigen was

added to each tube. The contents of each tube were mixed by rolling in between the palms. The dilutions ranging from 1:10 in the first tube, to 1:5120 in the tenth tube.

4. The tubes were incubated in a water bath at 37°C for 24 hours.
5. Parallel to set of test sera, a set of 5 tubes was used with antigen in 0.5% phenol saline for comparing the result of the test sample. Antigen control tubes were also incubated in the water bath at 37°C for 24 hours. With each set of test sera, a known positive and negative were also included.

OBSERVATION AND RESULTS

The present study was conducted from May to October 2014 for the period of six months in the Department of Microbiology, SIMS Medical College, Shimoga. The sera received from 840 persons were examined by Rose Bengal plate test and Standard tube agglutination test.

Table – 1
SEROPREVALENCE OF BRUCELLOSIS

Total screened	No. of Positives		Prevalence in Percentage	
	RBPT	STAT	RBPT	STAT
840	12	12	1.42	1.42

RBPT: Rose Bengal plate test, STAT : Standard tube agglutination test

Table 2:
SEX DISTRIBUTION AMONG SEROPOSITIVE CASES

Sex	No. of cases (N= 06)	Percentage
MALE	08	66.6
FEMALE	04	33.3

Table2 shows that brucellosis was more common among males 08 (66.6%) compared to females 04 (33.3%)

Table 3:
SEROPREVALENCE OF BRUCELLOSIS IN RELATION TO AGE

Age in years	Total screened	No of Positives		Prevalence in Percentage	
		RBPT	STAT	RBPT	STAT
Below 20	220	02	02	0.9	0.9
21 – 40	454	08	08	1.7	1.7
41 – 60	128	02	02	1.5	1.5
61 & Above	38	0	0	0	0
Total	840	12	12	1.42	1.42

Table-3 shows seroprevalence of brucellosis in different age groups . Rose Bengal plate test and Standard tube agglutination test revealed that brucellosis is more common among persons in the age group of 21-40years followed by persons in the age group of 41-60 years.

Table 4
SEROPREVALENCE OF BRUCELLOSIS WITH RESPECT TO OCCUPATION

Occupation	Total screened	No of Positives		Prevalence in Percentage	
		RBPT	STAT	RBPT	STAT
Farmer	240	08	08	3.33	3.33
House hold	416	02	02	0.48	0.48
Shepherd	36	02	02	5.55	5.55
Others	148	0	0	0	0
Total	840	12	12	1.42	1.42

Table –4 shows seroprevalence of brucellosis in relation to occupation. The highest seroprevalence of brucellosis was found in shepherds (5.55%) followed by farmers (3.33%)

Table 5
COMPARISON OF ROSE BENGAL PLATE TEST WITH STANDARD TUBE AGGLUTINATION TEST

		STAT		
		+	-	Total
RBPT	+	12(T.P)	0(F.P)	12
	-	0(F.N)	828(T.N)	828
	Total	12	828	840

TP : True positive, FP : False positive, FN : False negative, TN: True negative

Sensitivity (true positive rate) - 100%

Specificity (true negative rate) - 100%

Positive predictive value - 100%

Negative predictive value - 100%

Overall accuracy - 100%

DISCUSSION

Brucellosis is an important zoonotic disease of worldwide distribution. Patients are often labelled as PUO and subjected to various laboratory investigations which generally do not include Brucella serology particularly in the fields.

In the present study prevalence of brucellosis among general population in Shimoga by serology was 1.4%.. This low percentage positivity is comparable to Shukla R N et al[6]. Mathur T N et al[7] have reported high percentage positivity ranging from 2.5 to 4%.

The present study correlates well with Thakur SD et al[8] and Randhawa et al[9] which shows that prevalence is more among males than in females. The increased incidence in males during the present study may be attributed to the fact that majority of the males are exposed to animals compared to females.

The prevalence of brucellosis in different age groups in the present work, was highest in 20-40 age groups (1.7%) followed by 41-60 age group (1.5%) of life. Randhawa et al[9] have reported third decade (14.2%) and fourth decade (7.0%) as the common-

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est age group affected. Spink W W[10]in his classical monograph, described that the persons in third decade and fourth decade were frequently affected by brucellosis .Our study is in concurrence with the observations of the above studies..

The significance of occupation as a risk for acquiring Brucella infection is an important aspect of epidemiology of brucellosis. Majority of the persons diagnosed in the present study, gave the history of contact with animals. The present study revealed highest prevalence of brucellosis among shepherds (5.5%). Farmers and persons employed as seasonal farm workers constituted the bulk of brucellosis cases (8 out of 12), prevalence was (3.3%). Randhawa et al[9] and Spink WW et al[10] have similarly noted these occupational groups to have a higher prevalence.

RBPT showed sensitivity and specificity of 100% in our study . Where as in other studies conducted by Waghele S. et al[11], observed a sensitivity of 99.78% and specificity of 97% for RBPT when compared with STAT. Similar observation was made by Barbuddhe S B et al[12] who reported 96.91% specificity of RBPT.

Though Standard tube agglutination test remains the standard test for serodiagnosis, Rose Bengal plate test may be considered highly reliable and has a close relation with Standard tube agglutination test in the diagnosis of human brucellosis.

CONCLUSION

Prevalence of brucellosis in this area was not found to be very significant. Though Standard tube agglutination test remains the standard test for serodiagnosis, Rose Bengal plate test may be considered as highly reliable and has a close relation with Standard tube agglutination test in the diagnosis of human brucellosis.

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