Seroprevalence Of Brucellosis In Davangere, Karnataka

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ABSTRACT

Context: Brucellosis is 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 Davangere (Karnataka) and to compare Rose Bengal plate test with Standard tube agglutination test. **Materials and Methods:** Three hundred eighty Blood samples received at Microbiology Department for various serological tests and 45 Blood samples collected from veterinary staff were used for 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 3.3%, it was 2.4% in general population and 11.1% in veterinary staff. Prevalence was more among males than in females . 20-40 years age group was more affected (3.6%). Shepherds had the highest prevalence rate (5.9%), followed by farmers (5.2%). Sera received for Widal test and Brucella agglutination test, showed a prevalence of 1.6% and 6.0% respectively. When compared with STAT, RBPT showed sensitivity of 100% and specificity of 99%. **Conclusion:** Prevalence of brucellosis in Davangere was found to be significant. It was fairly common among the veterinary personnel in this area. 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.

Key Words: Brucellosis, Seroprevalence, Rose Bengal plate test, Standard tube agglutination test

Key Messages:

- Prevalence of brucellosis in Davangere is high.
- Veterinary staff, shepherds, farmers are more commonly affected.
- RBPT is highly reliable and has a close relation with STAT in the diagnosis of human brucellosis.

INTRODUCTION

Brucellosis is worldwide in distribution [1]. It is chronic, contagious and zoonotic disease [2]. An outstanding characteristic of the disease is its protean manifestations. Common symptoms are fever, chills, sweats, weakness, loss of weight and abdominal pains, but it is not rare for the disease to present as respiratory illness, central nervous system infections, heart disease, urogenital infection or as chronic localized lesions. It is usually difficult to diagnose clinically. Brucellosis is amenable to treatment with the antibiotics now available, and so it is highly important that the proper diagnosis be made early [3].

Brucellosis is usually diagnosed in the laboratory by means of blood culture or demonstration of elevated level of antibodies. Since blood cultures can take weeks and is often unsuccessful, a positive diagnosis usually depends on clinical, serological and epidemiological data [4],[5].

Tests used in serological diagnosis are Enzyme linked immunosorbent assay (ELISA) , Standard tube agglutination test (STAT), Polymerase chain reaction (PCR), The Complement fixation test (CFT), Antihuman globulin test, Radioimmunoassay, Rose Bengal plate test (RBPT), Indirect hemolysis test and Western blot [6],[7],[8],[9],[10],[11].

STAT is the most commonly used and is the standard test [12]. RBPT is most rapid and convenient test. Hence the present study was done to know seroprevalence of brucellosis in Davangere, to study epidemiology of human brucellosis in relation to occupation, age, religion and to compare Rose Bengal plate test (RBPT) and Standard tube agglutination tests (STAT).

MATERIALS AND METHODS

The study was done on 380 blood samples received at Microbiology Department for a period of one year for various serological tests and 45 blood samples from veterinary staff. Details about age, sex, occupation, religion of subjects were also collected.

Inclusion Criteria:

Patient's sera received in our department, which were negative for various serological tests.

Exclusion Criteria :

Patient's sera which were positive for various tests like Widal, VDRL, RA Factor, ASLO and HbsAg were excluded from the study.

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) [13]:

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.
6. 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) [14]:

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 ranged 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.

Interpretation:

The tubes were taken out of the water bath and kept on the bench at room temperature for an hour, for observation. All the tubes were examined against light and tubes of test series were compared with antigen control tubes for degree of opacity of the supernatant fluid. The result of test was recorded as shown in [Table/Fig 1].

Antigen control Tubes	0.5% phenol Saline	Antigen	Degree of agglutination
Tube-I	Nil	2.0 ml	No agglutination
Tube-II	1.25 ml	0.75 ml	25% agglutination
Tube-III	1.50 ml	0.50 ml	50% agglutination
Tube-IV	1.75 ml	0.25 ml	75% agglutination
Tube-V	2.0 ml	Nil	100% agglutination

[Table/Fig 1]: Dilution of antigen control tubes

The highest dilution of test serum tube comparable with tube-III of antigen control tube was taken as the end titre of the serum. The result was expressed in international units per ml of serum by doubling the serum titre showing 50% agglutination. 80 IU/ml or above was considered significant, 40 IU/ml as doubtful and less than 40 IU/ml as negative.

RESULTS

[Table/Fig 2] shows seroprevalence among General population and veterinarians. Rose Bengal plate test and Standard tube agglutination test showed 11(2.9%) and 9(2.4%) of serum sample as positive in general population and 6(13.3%) and 5(11.1%) of serum samples as positive in veterinarians respectively. The difference in seroprevalence between the general population and veterinarians was statistically significant, as P value is < 0.005 for both RBPT and STAT.

Type of Population	No of Positives (%)		No of negatives(%)		Total screened
	RBPT	STAT	RBPT	STAT	
Gen population	11(2.9)	9(2.4)	369(97.1)	371(97.6)	380
Veterinary	6(13.3)	5(11.1)	39(86.7)	40(88.9)	45
Total	17(4.0)	14(3.3)	408(96)	411(96.7)	425

[Table/Fig 2]: Seroprevalence of brucellosis

RBPT: Rose Bengal plate test, STAT: Standard tube agglutination test
For STAT X 2 = 9.65 df= 1 p value = 0.002 Significant
For RBPT X 2 = 11.41 df= 1 p value = 0.001 Highly Significant

Males were more commonly affected (6.5%) than females (1.5%).

[Table/Fig 3] shows seroprevalence of brucellosis in different age groups among General population. Both RBPT and STAT showed that that brucellosis is more common among persons in the age group of 21-40 years followed by persons in the age group of 41-60.

Age in years	Total screened	No of Positives		Prevalence in Percentage	
		RBPT	STAT	RBPT	STAT
Below 20	120	1	1	0.8	0.8
21 – 40	197	7	7	3.6	3.6
41 – 60	47	2	1	4.3	2.1
61 & Above	16	1	0	6.3	0
Total	380	11	9	2.9	2.4

[Table/Fig 3]: Seroprevalence of brucellosis in relation to age among general population.

[Table/Fig 4] shows, In veterinarians brucellosis is more common among persons in the age group of 31-40 years followed by persons more than 41 years.

Age in years	Total screened	No of Positives		Prevalence in Percentage	
		RBPT	STAT	RBPT	STAT
21-30	9	0	0	0	0
31-40	29	5	4	17.2	13.8
41 And above	7	1	1	14.3	14.3
Total	45	6	5	13.3	11.1

[Table/Fig 4]: Seroprevalence of brucellosis in relation to age among veterinarians

[Table/Fig 5] shows seroprevalence of brucellosis in sera sent for various tests.

[Table/Fig 6] shows, seroprevalence of brucellosis with respect to occupation. The highest prevalence was seen in shepherds

Sample sent for	Total Screened	No of Positives		Prevalence in Percentage	
		RBPT	STAT	RBPT	STAT
Brucella agglutination test	116	9	7	7.8	6.0
Widal	129	2	2	1.6	1.6
VDRL	113	0	0	0	0
Others	22	0	0	0	0
Total	380	11	9	2.9	2.4

[Table/Fig 5]: Seroprevalence of brucellosis in sera sent for various tests

followed by farmers by both Rose Bengal plate test and Standard tube agglutination test.

Occupation	Total screened	No of Positives		Prevalence in Percentage	
		RBPT	STAT	RBPT	STAT
Farmer	115	6	6	5.2	5.2
House hold	187	3	2	1.6	1.1
Shepherd	17	2	1	11.8	5.9
Others	61	0	0	0	0
Total	380	11	9	2.9	2.4

[Table/Fig 6]: Seroprevalence of brucellosis with respect to occupation

Hindu and Muslims were equally affected by brucellosis in the current study by STAT but more of Hindus were affected by brucellosis by RBPT, as shown by [Table/Fig 7].

Religion	Total screened	No of Positives		Prevalence in Percentage	
		RBPT	STAT	RBPT	STAT
Hindu	362	15	12	4.1	3.3
Muslim	63	2	2	3.3	3.3
Total	425	17	14	4	3.3

[Table/Fig 7]: Seroprevalence of brucellosis with respect to religion

Comparison of RBPT with STAT is shown in [Table/Fig 8]. RBPT was compared with STAT and sensitivity of RBPT was 100%, specificity was 99%, Positive predictive value was 82%, Negative predictive value was 100%, Overall accuracy was 99% when compared with STAT.

		STAT		
		+	-	Total
RBPT	+	14 (T.P)	3 (F.P)	17
	-	0 (F.N)	408 (T.N)	408
	Total	14	411	425

[Table/Fig 8]: Comparison of Rose Bengal plate test with Standard tube agglutination test.

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

DISCUSSION

Brucellosis is an important zoonotic disease of world wide distribution. In India the disease has been found wherever it is looked for [15].

In the present study prevalence of brucellosis among general population in Davangere by serology is 2.4%. The results are comparable with the studies conducted by Balbir Singh [16]. Low percentage positivity has been reported by Koshi G et al [3], Shukla R N et al [17], Mathur T N et al [18]. Roy P B et al [19] have reported high percentage positivity. Shukla R N et al [17], Mathur T N et al [18] have used the antigen supplied by Hafkine Institute Bombay, where 1/10 is taken as significant titre. Hence this could be the reason for the high percentage positivity and another reason may be that these parts of the country are agriculturally progressive and dairy farming is extensive, whereas Davangere District is part of the country where dry agriculture is the chief occupation. The prevalence of brucellosis in this area is similar to those parts of our country with identical geographical and occupational features like the Bijapur district in Karnataka and in Southern Orissa.

The seroprevalence in high risk groups, i.e. veterinarians was 11.1%. This is in conformity with those of Handa R et al [20] who reported a seropositivity of 14% in occupationally exposed individuals.

The present study correlates well with studies done by Thakur SD et al [9], Kadri SM et al [21] and Randhawa et al [22] which show 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 the animals compared to females. This fact also explains the occupational hazard of the disease.

The prevalence of brucellosis in different age groups in the present work was highest in 20-40 yr age groups (3.6%) followed by 41-60 yr age group (2.1%) of life. Randhawa et al [22] have reported third decade (14.2%) and fourth decade (7.0%) as the commonest age group affected. The high prevalence in their study was due to a higher overall prevalence of brucellosis (7.8%). Spink W W [23] 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 above workers. More prevalence in this age group could be due to increased activities with regards to their occupation, thereby enhancing the risk of acquiring the disease. Similar results of relation between age and prevalence of brucellosis was observed among veterinary personnel.

Overall prevalence of *Brucella* agglutinins among sera received for different serological investigations was 2.4%. 2 cases were detected among sera received for Widal test (Brucella tests were done on Widal negative samples), with a clinical diagnosis of PUO and prevalence rate was 1.6%. Our finding of prevalence of brucellosis among PUO cases is similar to the finding of Kadri SM et al [21] (0.8%), and B.K. Nanda et al [24] (1.4%). In the present study, a small but significant number of PUO patients (1.6%) were diagnosed as brucellosis after their sera were tested for Brucella antibodies. Many a times, differential diagnosis of brucellosis in PUO cases is not considered by clinicians due to several reasons such as physicians and surgeon's failure to suspect the disease, reluctance of the physician to send the patient's serum for investigation, misdiagnosis as enteric fever, misconception about rising titre, failure to isolate organism on culture, absence of epidemiological approach, protean manifestations and atypical presentations [18]. Patients are treated presumptively as enteric fever, without recovery and prolonging the suffering. Hence, this indicates the need for screening of *Brucella* agglutinins in sera of patients with pyrexia of unknown origin even if not suspected of brucellosis.

Brucella agglutinins were not observed among the sera received for VDRL test and other tests like RA, ASLO and HBsAg in our study. Prevalence was highest in sera of patients, in whom clinical suspicion of brucellosis was made (6.0%).

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 (5.9%). Farmers and persons employed as seasonal farm workers constituted the bulk of brucellosis cases among the general population (6 out of 9), prevalence was (5.2%). Spink WW et al [23] and Mathur T N et al [25] have similarly noted these occupational groups to have a higher prevalence. People with household work were found to be suffering from brucellosis with a prevalence rate of 1.1%.

Hindus (4.1%) were more affected than Muslims (3.3%). Significance of this finding is not well understood, but could be due to the fact that in our study most of farmers and shepherds were Hindus.

RBPT showed sensitivity of 100% and specificity of 99% in our study which correlates with study conducted by Waghele S. et al [26], who observed sensitivity of 99.78% and specificity of 97% for RBPT when compared with STAT. Similar observation was made by Barbuddhe S B et al [27] who reported 96.91% specificity of RBPT in 1994. 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.

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