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SEROLOGY AND ANTIBIOTIC SUSCEPTIBILITY IN BRUCELLOSIS

AMARESH NIGUDGI[1], PRAVEEN DODDAMANI[2], VINAY HAJARE[3], SREEKANTHA[4], SUNIL BIRADAR[5] AND KANTHA R C[6]

1,5,6-Department of Microbiology,M.R.Medical College,Gulberga,Karnataka.
2- Department of Microbiology,Vinayaka Mission Medical College,Karikal,Pondychery.
3- Department of Microbiology,Navodaya Medical College,Raichur,Karnataka.
4- Department of Biochemistry,Navodaya Medical College,Raichur,Karnataka.

ABSTRACT

Background and Objectives: Brucellosis is the most common disease noted among people who handle animals and human beings are indirectly infected. This study was aimed to know the serological profile and antibiotic susceptibility pattern in patients with brucellosis.Methodology and Results: 1120 patients with Pyrexia of unknown origin (PUO), among which 360 patients of high risk group having either history of contact with animals or their products were screened. 2% of cases revealed positively among the 1120 samples and 15.3% revealed positively among the 360 high risk group. Conclusion: Brucellosis is one of the important zoonotic disease usually diagnosed in patients with PUO. Serological screening will help in the accurate diagnosis and antibiotic susceptibility profile will help in the better treatment and limit the complications of brucellosis.

KEY WORDS: Brucellosis, Serology, Antibiotic susceptibility

SREEKANTHA
Department of Biochemistry,Navodaya Medical College,Raichur,Karnataka,India.

INTRODUCTION

Brucellosis is an important zoonotic disease which has worldwide distribution. It is caused by bacillus belonging to the genus Brucella. Brucella in human beings is caused by Brucella melitensis, Brucella abortus and Brucella suis and occasionally by Brucella canis. The animals that are known to serve as sources of human infection are goat, sheep, cattle, buffalo and swine. Human beings are secondarily infected. It is acquired by consuming pasteurized dairy products or by close contact with ruminants and canines.Majority of people in our country are engaged in agriculture related activities like seasonal agriculture labours, dairy product selling and animal meat selling.Brucellosis is a disease more often seen in specific occupational groups like dairy workers, shepherds, goatherds veterinarians, laboratory workers, butchers and abattoir workers.Brucellosis has been known by several names like undulant fever, Malta fever, Mediterranean fever, goat fever, Bang's disease and Gibraltar fever. The organism was first isolated in 1887 by Sir David Bruce, who recovered organism from spleens of British soldiers dying of Malta fever[1].The disease in human is characterized by an acute bacteremic phase and irregular fever, followed by chronic stage that may extend over many years and may involve many tissue[2]. Common symptoms are fever, muscular and articular pain, chill, sweat, weakness, loss of weight, abdominal pain, respiratory illness, central nervous system infections, heart disease, urogenital infection, etc. Brucellosis is amenable to treatment with the antibiotics now available. Brucella organism requires long time to grow in vitro, hence a presumptive diagnosis can be made by serological tests[2]. Routinely used serological tests are agglutination tests. The present study was undertaken to know the prevalence of brucellosis in this area. The findings of this work might be a great value for health authorities in circumventing the disease.

MATERIALS AND METHODS

The present study was conducted in the department of Microbiology M.R. Medical College, Gulbarga.Blood samples received in our laboratory for various serological test viz widal, RA, VDRL, Brucellosis etc., from patients attending Basaveshwar Teaching and General Hospital, Gulbarga were included for the study. Patient's sera which were positive for various tests like Widal, VDRL, RA factor, ASLO were excluded from the study.Blood samples of veterinary staff working at district and taluka level veterinary hospitals were collected during the monthly review meeting. 3ml blood of all veterinary persons was collected by venepuncture with sterile precautions for serological tests.Blood samples of butchers, farmers, shepherds and healthy individuals were also collected.

Methods

1) Rose Bengal Plate Test (RBPT)
2) Standard Tube Agglutination Test (STAT).
3) Blood Culture
4) Biochemical Reaction
5) Growth in Presence of Dyes.
6) Antibiotic Susceptibility

Around 3ml of blood was collected under strict aseptic conditions in a bottle was allowed to clot at room temperature for half an hour, after which serum was separated by dislodging the clot and centrifuged at 3000 rpm for 5 minutes.

Antibiotic susceptibility test[5,6,7,8]

Antibiotic susceptibility testing was carried out according to Kriby and Beuer disc diffusion method on trypticase soy agar. The following antibiotics from Himedia, Mumbai, were used – Tetracycline (30mcg), Erythromycin (15mcg), Doxycycline (30mcg), Ciprofloxacin (5mcg), Streptomicin (10mcg) and Co-trimoxazole (23.75mcg).

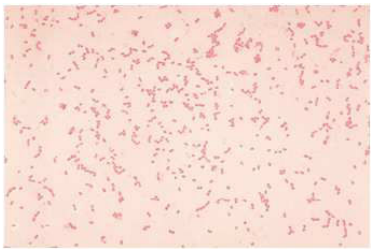


Figure 1
Gram's staining

Figure 2
Rose Bengal plate test RBPT

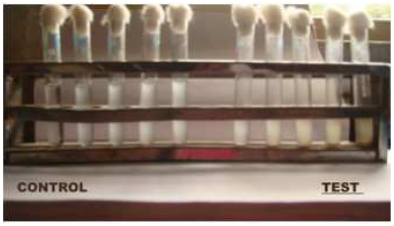


Figure 3
Standard tube agglutination test STAT

Figure 4
Brucella melitensis colonies on blood agar

Figure 5
Brucella melitensis colonies on MacConkey's agar

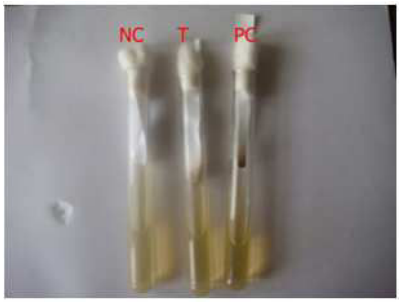


Figure 6
Production of H_2S

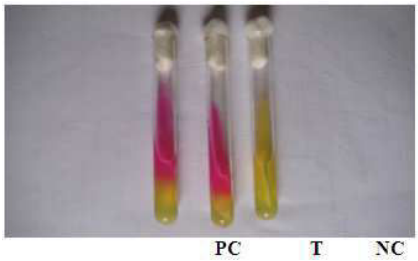
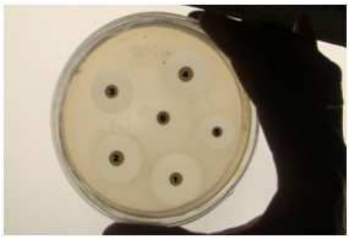


Figure 7 ***Urease test***

PC – Positive control; T – Test; NC – Negative Control

Figure 8
Antibiotic susceptibility test



1-Tetracycline (30 mcg); 2-Streptomicin (10mcg); 3-Cotrimoxazole (23.75 mcg); 4-Doxycycline (30 mcg); 5-Ciprofloxacin (5 mcg); 6-Erythromycin (15 mcg)

Growth in presence of dyes

Figure 9
Basic Fuchsin – 1:25000

Figure 10
Basic Fuchsin – 1:100000

Figure 11
Safranine – 1:5000

Figure 12
Methyl violet – 1: 50000

RESULTS

The study material comprised 1480 blood samples which were screened for brucellosis and 50 blood samples from healthy individuals as controls.The blood samples from 1480 individuals were divided into two groups. One group included 1120 samples which were received in laboratory for serological test suspected of pyrexia of unknown origin. Other group comprised high risk individuals and included veterinary personnel, butchers, shepherds and farmers (Table III).

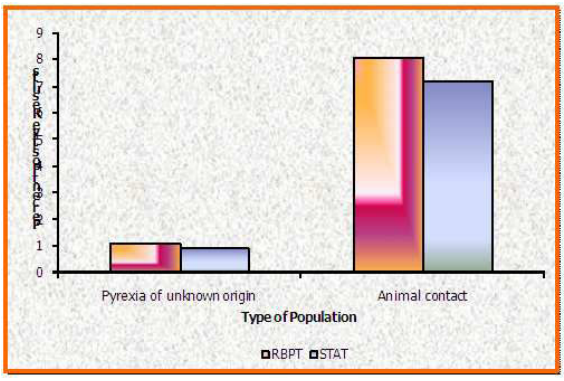
Table III
Seroprevalence of Brucellosis

Type of Population	No. of sample screened	Positive Result		Negative Result	
		RBPT (%)	STAT (%)	RBPT (%)	STAT (%)
Pyrexia of unknown origin	1120	12 (1.1%)	10 (0.9%)	1108 (98.9%)	1110 (99.1%)
Animal contact	360	29 (8.1%)	26 (7.2%)	331 (91.94%)	334 (92.8%)
Total	1480	41 (2.8%)	36 (2.43%)	1439 (97.23%)	1444 (97.57%)

For STAT x^2 = 45.99 P< 0.001 High Significant
For RBPT x^2 = 49.34 P< 0.001 High Significant

The difference in seroprevalence between the pyrexia of unknown origin and animal contact was statistically significant, as P value is <0.005.Out of 1120 serum samples of PUO, RBPT and STAT showed 12 (1.1%) and 10 (0.9%) positivity, respectively, where as 360 samples of animal contact individuals revealed 29 (8.1%) and 26 (7.2%) positivity by RBPT and STAT, respectively.

Figure 13
Seroprevalence of Brucellosis



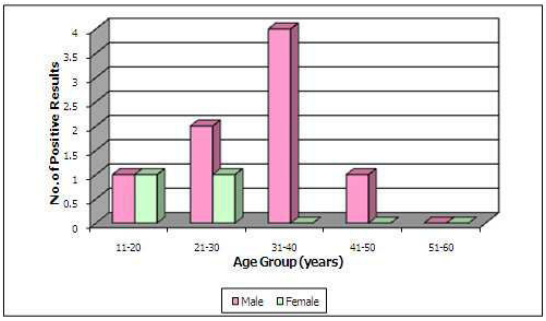
The prevalence of Brucellosis in different age group among PUO cases was studied. Individuals between 11-60 years of age comprised the study group. Both sexes were included. Age and sex wise distribution of PUO cases was also included in the study. The study included 719 (64.2%) males and 401 (35.8%) females (Table IV).

Table IV
Prevalence of Brucellosis in different age group in male and female individual with pyrexia of unknown origin

Age Group (years)	No. of Serum Samples Screened	Serum Samples Positive	Male		Female	
			No. of Serum Samples	Positive	No. of Serum Samples	Positive
11-20	262	2	160	1	102	1
21-30	306	3	192	2	114	1
31-40	327	4	217	4	110	0
41-50	145	1	88	1	57	0
51-60	80	0	62	0	18	0
Total	1120	10	719	8	401	2

The result indicated a total of 10 cases were positive for presence of Brucella antibodies of which 8 were males and 2 were females. The age group of 31-40 years revealed four out of ten cases (40%) which has highest. All the four individuals were males.

Figure 14
Prevalence of Brucellosis in different age group in male and female individual with pyrexia of unknown origin



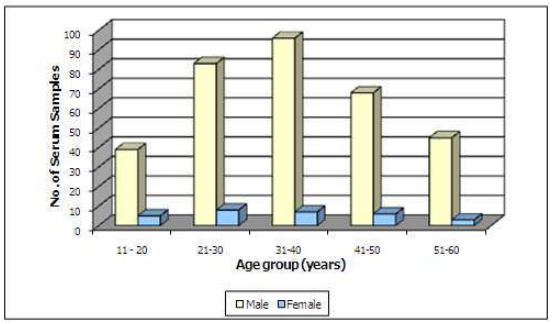
The prevalence of Brucellosis in different age group among animal contact was studied. Individuals between 11-60 years of age comprised the study group. Both sexes were included. Age and sex wise distribution of animal contact was also included in the study. The study included 331 (91.94%) males and 29 (8.06%) females (Table V).

Table V
Prevalence of Brucellosis in different age group in male and female in animal contact

Age Group (years)	No. of Serum Samples Screened	Serum Samples Positive	Male		Female	
			No. of Serum Samples	Positive	No. of Serum Samples	Positive
11-20	44	2	39	2	5	0
21-30	91	10	83	9	8	1
31-40	103	11	96	11	7	0
41-50	74	2	68	2	6	0
51-60	48	1	45	1	3	0
Total	360	26	331	25	29	1

The study included 331 (92%) were males and 29 (8%) were females. Majority of seropositive cases in animal contact were between 31-40 years age group and all of them were males.

Figure 15
Prevalence of Brucellosis in different age group in male and female in animal contact



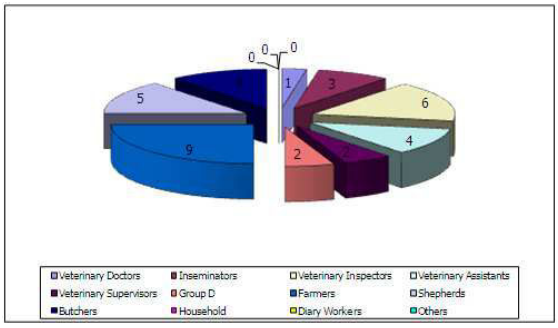
The study on prevalence of Brucellosis among various occupation groups were screened. This includes the cases of PUO and those also who come directly or indirectly in contact with animals. A total of 1480 cases were screened. (Table VI).

Table VI
Occupation wise seroprevalence of Brucellosis

Category	No. of Serum Samples Screened	STAT Positive Cases (%)
Veterinary Doctors	20	1 (5.0)
Inseminators	48	3 (6.6)
Veterinary Inspectors	57	6 (10.5)
Veterinary Assistants	56	4 (7.1)
Veterinary Supervisors	39	2 (5.0)
Group D	26	2 (7.7)
Farmers	880	9 (1.0)
Shepherds	40	5 (12.5)
Butchers	40	4 (10.0)
Household	118	0 (0.0)
Diary Workers	08	0 (0.0)
Others	148	0 (0.0)
Total	1480	36 (2.43)

Out of 36 (2.43%) positive samples the highest prevalence was seen among shepherds 5 (12.5%) followed by veterinary Inspectors 6 (10.5%). Seropositive cases were not found in household and diary workers.

Figure 16
Occupation wise seroprevalence of Brucellosis



Efforts were made to know seropositivity for Brucella agglutinins from sera received for various serological investigations which were clinically diagnosed as PUO (Table VII).

Table VII
Seropositivity of Brucella agglutination among serum received for diagnosis of PUO

Name of Test	No. of Serum Samples Screened	No. of Positive test	Percentage
Brucella agglutination test	42	3	7.14
Widal Test	973	6	0.62
VDRL Test	30	0	0.0
RA test	75	1	1.33
Total	1120	10	0.89

Out of 1120 cases, 10 cases (0.89%) were positive for Brucella antibodies, out of 42 cases sent for Brucella agglutination test, 973 for Widal test, 75 for RA test and 30 for VDRL test and they showed

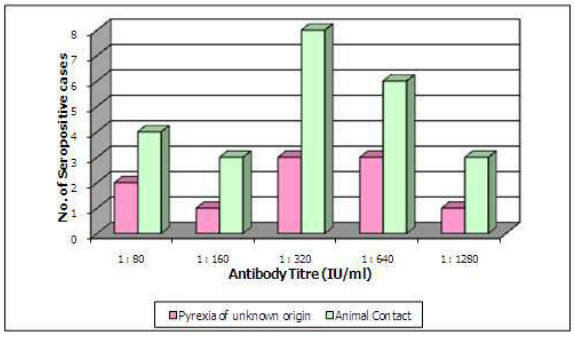
positivity rate of 3 (7.14%), 6 (0.62%), 1 (1.33%) and 0 (0%) respectively by STAT.Antibody titer level in seropositive cases in our study ranged from.1:80 IU/ml to 1: 2560 IU/ml. The diagnostic titer suggested by IVRI is 1: 80 IU/ml (Table VIII).

Table VIII
Antibody titre range distribution of Brucella seropositive cases

Antibody Titre (IU/ml)	No. of Seropositive cases (Percentage)		
	Pyrexia of unknown origin (%)	Animal Contact (%)	Total (%)
1 : 80	2 (20)	4 (15.38)	6 (16.66)
1 : 160	1 (10)	3 (11.53)	4 (11.11)
1 : 320	3 (30)	8 (30.77)	11 (30.55)
1 : 640	3 (30)	6 (23.07)	9 (25.00)
1 : 1280	1 (10)	3 (11.53)	4 (11.11)
1 : 2560	0 (00)	2 (07.69)	2 (05.56)
1 : 5120	0 (00)	0 (00.00)	0 (00.00)
Total	10	26	36

The study shows that 11 cases (30.55%) were at 1:320 IU/ml followed by 9 cases (25%) at 1: 640 IU/ml and 2 (5.56%) cases were at 1:2560 IU/ml.

Figure 17
Antibody titer range distribution of Brucella seropositive cases



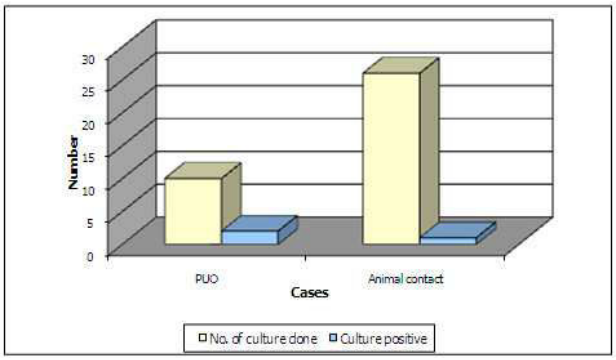
Blood culture was done for those cases only which were positive by STAT. Out of 1530 cases, 36 seropositive cases therefore, were subjected for blood culture (Table IX).

Table IX
Results on Blood Culture Study

Cases	No. of Serum Samples	Seropositive	No. of culture done	Culture positive	Percentage
PUO	1120	10	10	2	20.00
Animal contact	360	26	26	1	03.84
Healthy control	50	0	0	0	00.00
Total	1530	36	36	3	8.33

The culture positivity among seropositive cases of 10 seropositive cases of PUO was 2 (20%) and among the 26 seropositive cases of animal contact, 1 (3.84%) was culture positive. Out of 3 cases of culture positive 2 were shepherds and 1 was farmer. Br. melitensis was isolated from all the 3 (8.33%) cases.None of the healthy controls showed the presence of Brucella antibodies.

Figure 18
Results on Blood Culture Study



Antibiotic susceptibility testing was done for all the 3 culture positive samples using Kirby and Bauer disc diffusion method on trypticase soy agar. The following antibiotics from Himedia, Mumbai were used.

Table X
Antibiotic Susceptibility of Brucella Species Isolated by Blood Culture

Antibiotics Used	Isolate No. 1	Isolate No. 2	Isolate No. 3
Tetracycline (30mcg)	S	S	S
Doxycycline (30mcg)	S	S	S
Erythromycin (15mcg)	R	R	R
Ciprofloxacin (5mcg)	I	S	I
Streptomicin (10mcg)	S	S	S
Co-trimoxazole (23.75mcg)	S	I	I

It was found that all the 3 samples were sensitive to tetracycline, streptomycin, doxycycline. 2 samples showed intermediate to ciprofloxacin and co-trimoxazole and all 3 samples were resistant to erythromycin.

DISCUSSION

Brucellosis which is an important public health problem is a zoonotic disease seen all over the world as well as in our country. Patients who are generally diagnosed as PUO are not routinely screened for Brucella antibody. This is because of the perception that Brucella is not commonly seen in our region. Such cases of PUO are treated non specifically and symptomatically. An effort has been made to include veterinarians and various occupational segments of animal care and contact. The present study was done to establish Brucellosis in clinically undiagnosed or misdiagnosed cases. In the present study prevalence of Brucellosis was found to be 2.4% [Table – III].Studies done by various workers were Mathur T.N. (1969)[9] in Karnal (6.06%), Joshi D. V. and Omprakash (1971)[10] in Delhi (5.8%) Phadke S.A. and Phadke A.R (1974)[11] in Pune (6.5%) and Handa R. et al (1998)[12] in Delhi (6.6%) shows positive range of around 6%. This could be because agriculture activities, cattle rearing and dairy farming is the main occupation in those areas with higher prevalence. Our prevalence rate was found to be low, where as in comparison to study done by Koshi et al (1971)[13] in Vellore (0.5%), Bal A and Tyagi S.P.(1971)[14] in Aligarh (0.09%), Sharma K.D. et

al (1974)[15] in Aurangabad (1.08%) and Nagalotimath et al (1978)[16] in Belgaum (0.8%), our prevalence rate was higher. Our prevalence 36 (2.4%) correlates approximately with the result of Mantur B.G. et al (1994)[4] from Bijapur (2.2%) and Ajay Kumar V.J. (2005)[17] from Kerala (2.45%). The reason for this could be because the identical geographical and occupational features in neighbouring Bijapur district and higher health awareness in people of Kerala. Out of 1120 cases of PUO in our study 10 (0.9%) cases were seropositive for Brucella antibodies. This is found to be marginally lower than the prevalence reported by Anand (1968)[18] 1.4% and Sharma et al (1974)[15] 1.05%. The reason for this may be that patients are misdiagnosed as enteric fever and protean manifestations and atypical presentations. In our study 360 persons of high risk group were screened for antibrucella antibodies. Out of this 26 (7.2%) cases were sero positive. In a study done by Thakur S.D. (2002)[76] in Uttaranchal revealed a prevalence rate of 4.97% in persons occupationally exposed to animals. In another study the prevalence rate among high risk individuals was found to be 2.26%.In the present study the male and female ratio correlates well with Thakur S.D. et al (2002)[19] (2:1), Kadri S.M. et al (2000)[20] (3:1) and Randhawa et al (1974)[21] (3:2) 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 the majority of the males are exposed to animals compared to females. The prevalence of Brucellosis in different age groups in the present work among PUO (Table IV), was highest in 31-40 age groups 4 (1.2%) followed by 21-30 age groups 3 (1%) of life. Randhawa et al (1974)[21] have reported third decade (14.2%) and fourth decade (7%) as the commonest age group is affected. Spink W.W. (1956)[22] in his classical monograph, described that the persons in third decade were frequently affected by Brucellosis. Our study is in concurrence with observations of above workers. More prevalence in this age group could be due to increased activities with regards to this occupation, thereby enhancing the risk of acquiring the disease.

In the present study the seroprevalence in persons with animal contacts (Table V) majority of cases were in the age group of 21-30 years 10 (11%) cases followed by 31-40 years 11 (10.6%) cases. This is in line with the result of D.K. Kochar et al (2007)[23]. The seropositivity in our study in high risk group is found to be higher in males (7.55%) than females (3.44%). This is in concordance with the study done by A.S. Agasthy (2007)[24] in Bangalore, where prevalence in male is more than females. This is different from the Kapoor et al who reported higher sero prevalence in females compare to male. However in our study difference in seropositivity between male and females is statistically not significant which may be due to less number of female subjects in the occupationally exposed group. Brucellosis is a disease more often seen in specific occupational groups like veterinarians, shepherds, dairy workers, butchers, farmers etc. The present study reveals highest prevalence of Brucellosis among shepherds 5 (12.5%) followed by veterinary Inspectors 6 (10.5%) and butchers 4 (10%) (Table VI). Spink W.W. et al(1956)[22] and Mathur T.N. et al (1964)[23] has similarly noted these occupational groups to have a higher prevalence. Agasthy A.S. et al (2007)[24] reported that among veterinarians, veterinary inspectors have got the highest prevalence. Which is similar to our finding. In another study by Kumar P. et al (1997)[26] reported highest prevalence among blood collectors. Direct contact (occupational contact with animals in field, handling animals, engaged in parturition of animals) is found to be significantly most important predisposing risk factor than indirect contact (ingestion of raw milk, raw meat and laboratory personnel) which explains our results.In our study among the sera received for diagnosis of PUO by various serological tests in our laboratory (Table VII), seropositivity was highest by Brucella agglutination test 3 (7.14%) followed by sera sent for RA test 1 (1.33%) however the sample size for RA test was small (75 out of 1120).

Where as the seropositivity of sera sent for Widal test was 6 (0.62%). These results indicate that screening of Brucella antibody should not be neglected, in sera negative by other serological test (Widal, VDRA and RA test) in PUO cases.Antibody level among the seropositive persons in our study ranged from 1:80 IU/ml to 1:2560 IU/ml (Table VIII). Active Brucellosis is more commonly associated with a diagnostic titre as low Brucella agglutinins can be found in healthy individuals (Spink 1956)[22]. The diagnostic titre suggested by IVRI is 1: 80 IU/ml.

In the present study majority of positive cases showed titre of 1:320 IU/ml. (30.55%) followed by titre 1:640 IU /ml. (25%).In the present study, all 36 samples which showed seropositivity by STAT were subjected to blood culture. Out of these, 3 (8.33%) samples showed positive blood culture. This positive culture included 2 samples from patients of PUO and 1 sample from the cases of animal contact giving the prevalence of 20% and 3.84% respectively.Joshi et al (1971)[10] from Delhi and Koshi et al (1971)[13] from Vellore reported the culture positive results as low as 0.00% and 0.77% respectively.In a study done by Singh Balbir and Saxena (1964)[27] in Delhi showed blood culture positive as 25.92% and Mathur TN (1969)[9] reported culture positivity as high as 62.35%. In another study done by J. Staszkiewicz et al (1988)[28]. Blood culture positivity was 63%. Recently Mantur BG. et al (2006)[29] from Bijapur reported 55.3% positive blood culture of Brucella. He documented that the diagnosis of Brucella in 495 patients was based on serology and one case by blood culture isolation alone.In comparison to all the above studies our rate of isolation of Brucella by blood culture was low [Table – IX]. The reason for low percent isolation of organism from blood might be due to inadequate or inappropriate antibiotic therapy for PUO. Another reason probably could be the presence of few Brucella organisms per unit of the blood reducing chances of recovery of bacteria from blood.Antibiotic susceptibility testing was done for all the 3 culture positive samples using Kirby and Bauer disc diffusion method on trypticase soy agar. The following antibiotics from Himedia, Mumbai, were used [Table – X]– Tetracycline (30mcg), Erythromycin (15mcg), Doxycycline (30mcg), Ciprofloxacin (5mcg), Streptomicin (10mcg) and Co-trimoxazole (23.75mcg). It was found that all the 3 samples were sensitive to tetracycline, streptomycin and doxycycline. 2 samples showed intermediate to ciprofloxacin and co-trimoxazole and all 3 samples were resistant to erythromycin. Good intracellular penetration is essential for invivo activity against Brucella and thus there is limited correlation between invitro performance and therapeutic efficacy.

CONCLUSION

Prevalence of Brucellosis in this area is significant. Males were affected more commonly than females. Shepherds and Veterinary personnel were largely affected. Persons in the third and fourth decade were predominantly affected. Among the veterinary personnel, veterinary inspectors and veterinary assistants are more commonly affected group. A proportion of sera received for various serological investigations like Widal and RA were positive for Brucella agglutinins, suggesting a need to investigate all sera for Brucella agglutinins. Blood culture for isolation of Brucella organism was found to be less successful. Therefore serological methods proved to be more reliable for diagnosis of Brucellosis. Antibiotic susceptibility testing is mandatory for each isolate has a separate susceptibility pattern. Therefore, since the disease has been seen as a major occupational hazard, it is advisable to propagate the information to all those involved. Another major point of concern is that the disease is seen in uneducated / semi-educated / individual from rural / semi-urban population, who have the least knowledge of the disease. It is the need of the hour, on the part of the health authorities, to provide the relevant message to the needy.

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