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Full Length Article

Seroprevalence of Brucellosis in Humans in Contact with Camels in Bikaner and Nearby Villages in Rajasthan State of India

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Abstract

Brucellosis is a dreadful zoonotic disease of livestock. In camels, the causative organisms are *Brucella melitensis* and *B. abortus*, both of which can cause the disease in humans. We investigated its prevalence in men and women associated with camels in the city of Bikaner and some surrounding villages in Rajasthan province in India. Blood from 188 human beings (109 men and 79 women) were tested by Rose Bengal Plate Agglutination Test (RBPT) and ELISA. 17 humans (4 women and 13 men) were found to be positive by RBPT. Prevalence by RBPT was 9.04% (11.92% in males and 5.06% in females). Prevalence by RBPT in Bikaner, Gadwala and Gadola was 11.90, 3.44 and 16.66%, respectively. Age-wise prevalence by RBPT was 8.0% in humans of age less than 20 years and 11.40% in those between 20–40 years, respectively. Out of the 188 human sera analyzed by ELISA, 11 (2 females and 9 males) were positive (three were veterinarians). Prevalence by ELISA was 2.25% (males 0.92% and females 4.34%). Location-wise prevalence by ELISA was 3.57% in Bikaner, 10.34% in Gadwala and 13.88% in Gadola, respectively. Age-wise prevalence by ELISA was 4.0% in humans less than 20 years of age and 7.89% in those between 20–40 years of age. Six human sera were positive by both ELISA and RBPT, 11 samples positive for RBPT were negative by ELISA and 5 samples negative by RBPT were positive by ELISA. Seroprevalence by ELISA and RBPT combined was 3.19%. The results indicate that Brucellosis is prevalent in those persons who routinely come in close proximity of domestic camels in Bikaner and surrounding villages of Rajasthan.

Keywords: Brucella; Brucellosis; Human Brucellosis; Prevalence; Seroprevalence; Camel

Introduction

Nomadic people in African and Asian regions rear camels for milk, meat, wool and hair and for transport purpose. Its dung is commonly used as fuel (FAO 2019). Camel is the common livestock reared by rural and nomadic people in several countries in the arid regions of Asia and Africa (Gwida *et al.* 2012).

Brucella organisms cause Brucellosis which is one of the most dreaded zoonotic diseases. In camels, *B. melitensis* and *B. abortus* cause Brucellosis, which also cause the disease in man (Omer *et al.* 2010). Brucellosis may be spread to human beings through milk of infected camel or products of such milk. Brucellosis in humans due to use of milk and meat of infected camel occurs in different regions of the world and hence is of public health concern (Dawood 2008). Brucellosis is prevalent in the Middle Eastern countries, and parts of Northern and Eastern Africa, the Mediterranean region of Europe, Central Asia, Southern Asia, Southern America and Central America (Corbel 2006).

Brucellosis is of importance from public health point of view around the world (Radostits *et al.* 2007) because of substantial reduction in man power, foods and livestock productivity caused by this disease. Brucellosis is an occupational disease affecting Veterinarians, animal handlers, workers from slaughter houses and meat-packaging units and laboratory staff (CDC 2015). Infection can be spread to humans from animals infected with the disease by close contact and intake of raw, unpasteurized milk and products made from such infected milk or consuming or handling contaminated meat.

There have been few studies on prevalence of Brucellosis in human beings who routinely come in close contact with camels in Thar desert of India, particularly in Bikaner city and villages in its close vicinity in Rajasthan state of India. Therefore, we carried out this study to understand the frequency of occurrence of Brucellosis in human beings who come in contact with camels viz. farmers, animal handlers and veterinarians in and around Bikaner district of Rajasthan. Serological tests, RBPT and ELISA, commonly used for the diagnosis of Brucellosis (Alton 1990), were employed in the present study to analyze human sera for diagnosis of Brucellosis.

Materials and Methods

Serum samples from human beings in contact with camels (especially persons reporting a history of fever, joint pain, arthritis, weakness and sweating) were collected from Bikaner, Gadwala, Gadola and Naurangdesar villages. The experimental work for the study was carried out at the Departments of Veterinary Public Health and Microbiology and Biotechnology, COVAS, RAJUVAS, Bikaner, India.

Human serum samples analyzed

Sera from 188 human beings (109 men and 79 women) were collected from Bikaner city and Gadola, Gadwala and Naurangdesar villages (Table 1). The subjects included animal owners, veterinarian and laboratory staff. Their ages ranged from 1 to 75 years.

Serum samples

Blood was collected aseptically from humans in contact with camels. After retraction of the clot, serum was obtained by spinning the clotted blood at 1200 rpm for 15 min. The serum samples were stored in vials in a deep freezer at -20ºC till use for serological studies.

Rose bengal plate test

The method of Morgan *et al.* (1978) was employed for carrying out RBPT. Colored antigen from Punjab Veterinary Vaccine Institute (PVVI), Ludhiana, India was used. Known brucellosis negative serum was kept as the Negative Control and known brucellosis positive serum was the positive control. Positive samples displayed clumping or agglutination whereas negative samples revealed no clumping.

ELISA on human sera

All the human sera were analyzed by indirect ELISA (I-ELISA) using a kit from ABCAM limited.

Procedure: A 96-well microtiter plate precoated with *Brucella* antigens was employed. Test sera and control sera were added to the respective wells and incubated. After incubation and washing, Horse Radish Peroxidase conjugated anti-Human IgG antibody was added to the wells of the plate. A dilution of 1:1 was performed to predilute the sample with PBS. It was then diluted with IgG Sample Diluent to 1:100 and multiplied by 2 in Standard Units. All samples were assayed in duplicate. 100 μL of samples were added into appropriate wells. One well carrying only the substrate served as the blank. The wells of the plate were covered with the foil and kept in the incubator for 1 h at 37°C. The reactant mixtures in the wells were aspired and the wells were washed 3 times with 300 μL of 1x Wash Solution.

Soak time was > 5 sec in each wash cycle. The remaining solution was aspirated by suction after the last wash. The plate was agitated to remove excess liquid and blotted against clean paper towels.100 µL of HRP conjugated anti-*Brucella* IgG was poured into each well sparing the well-kept as blank. It was then covered with the foil to avoid exposure to direct sunlight and incubated at room temperature for half an hour. This step was repeated and then 100 μL of TMB Solution (Substrate) was poured into all the wells. It was then kept for incubation at room temperature in the dark for exactly 15 min. 100 μL of a solution to stop the reaction was added in all the wells. The blue color turned to yellow. The absorbance at 450 nm was read within half an hour of adding the stop solution using an ELISA Microtiter plate reader.

Determination of results*:* Calculated the mean value of the background and subtracted absorbance for every sample and compared to mean value of cut-off control *i.e.*, the mean absorbance of the control wells.

Read-out of results*:* Samples were taken to be positive if the value of absorbance was higher than 10% above the cut-off value, negative if the absorbance value was lower than 10% under the cut-off and inconclusive (*i.e.* neither positive nor negative) if absorbance was smaller than 10% above or below the cut-off control value.

Statistical analysis of data

MedCalc Statistical analysis software was employed online for analyzing the data for calculation of specificity, sensitivity, false positive and false negative values.

Results

RBPT analysis of human sera

17 sera from humans were found positive and 171 were found negative by RBPT (Table 2 and Fig. 1). Out of the 17 positive samples, 10 were from Bikaner, 1 from Gadwala and 6 from Gadola, respectively (Table 3 and Fig. 2). The positive samples were from 4 females and 13 males (Table 4 and Fig. 3) and included animal owners and two veterinarians. The age of the positive humans ranged from one year to 35 years. The mean age of RBPT positive persons was 19.07 in men and 21.25 in women, respectively (Table 5 and Fig. 4).

Location-wise prevalence by RBPT

Prevalence in humans by RBPT was 11.90, 3.44 and 16.66% in Bikaner, Gadwala and Gadola, respectively (Table 3 and Fig. 2).

Sex-wise seroprevalence by RBPT

Overall prevalence in humans by RBPT was 11.92% in males and 5.06%.in females, respectively (Table 4 and Fig. 3). In Bikaner, it was 13.46% in males and 9.37% in females, In Gadwala, it was 5.0% in males and in Gadola it was 16.12% in males and 20.0% in females, respectively.

Table 1: Sex and age-wise distribution of humans included in the study

S. no.	Location	Numbers		Total	Age range (years)
		Males	Females		
1	Bikaner city	52	32	84	1 – 70
2	Gadwala	20	9	29	20 – 63
3	Gadola	31	5	36	2 - 75
4	Naurangdesar	6	33	39	2 – 57

Table 2: Human sera positive for Brucellosis by RBPT

S. n.	Case no.	Age (yrs)	Sex	RBPT	Location
1	HB1*	30	M	+	Bikaner
2	HB5	30	M	+	Bikaner
3	HB8	5	M	+	Bikaner
4	HB11	6	M	+	Bikaner
5	HB57	34	F	+	Bikaner
6	HB72	25	F	+	Bikaner
7	HB73	9.5	M	+	Bikaner
8	HB82	3	F	+	Bikaner
9	HB83	1	M	+	Bikaner
10	HB84	1.5	M	+	Bikaner
11	HW4*	27	M	+	Gadwala
12	HO11	23	F	+	Gadola
13	HO15	22	M	+	Gadola
14	HO21	29	M	+	Gadola
15	HO24	17	M	+	Gadola
16	HO27	35	M	+	Gadola
17	HO29	35	M	+	Gadola

* Veterinarian

Table 3: Location – wise prevalence of Brucellosis in humans by RBPT

Location	Count (percentage)		Total	Prevalence
	RBPT Negative	RBPT Positive		
Bikaner	74	10 (58.8%)	84	11.90%
Gadwala	28	1 (5.9%)	29	3.44%
Gadola	30	6 (35.3%)	36	16.66%
Naurangdesar	39	0 (0.0%)	39	0.0%
Total	171	17	188	9.04%

Table 4: Sex – wise prevalence of Brucellosis by RBPT in humans in different locations

Location	Males			Females		
	RBPT Positive	Total examined	Prevalence	RBPT Positive	Total examined	Prevalence
Bikaner	7	52	13.46%	3	32	9.37%
Gadwala	1	20	5.0%	0	9	0.0%
Gadola	5	31	16.12%	1	5	20.0%
Naurangdesar	0	6	0.0%	0	33	0.0%
Total	13	109	11.92%	4	79	5.06%

Age-wise seroprevalence in humans by RBPT

Age-wise prevalence in humans by RBPT was 8.0% in humans of age less than 20 years and 11.40% in those between 20–40 years, respectively (Table 5 and Fig. 4).

ELISA on human serum samples

All the human samples were analyzed by ELISA (Table 6 and Fig. 5). Out of the 188 samples, 11 (9 males and 2 females) were positive by ELISA. The overall prevalence by ELISA was 5.85%. Location-wise prevalence in humans by ELISA was 3.57% in Bikaner, 10.34% in Gadwala and 13.88% in Gadola, respectively (Table 7 and Fig. 6).

Table 5: Age – wise prevalence of Brucellosis in humans by RBPT

Age	Count (percentage)		Total	Prevalence
	RBPT Negative	RBPT Positive		
< 20 Years	46	4 (23.5%)	50	8.0%
20-40 Years	101	13 (76.5%)	114	11.40%
40-60 Years	20	0 (0.0%)	20	0.0%
> 60 Years	4	0 (0.0%)	4	0.0%
Total	171	17	188	9.04%

Fig. 1: Analysis of serum for Brucellosis by RBPT
Left: Brucellosis positive serum; Right: Brucellosis negative serum

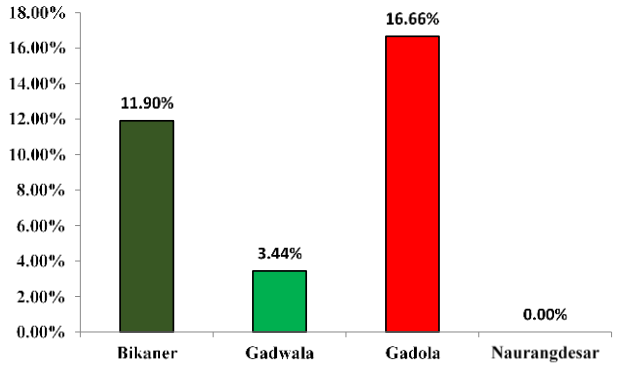

Fig. 2: Location – wise prevalence of Brucellosis in humans by RBPT

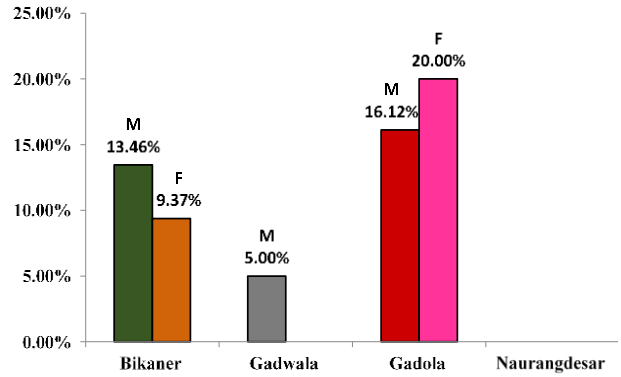

Fig. 3: Sex-wise prevalence of Brucellosis in humans by RBPT

Table 6: Human serum samples positive for Brucellosis by ELISA

S. n.	Case no.	Age (yrs)	Sex	ELISA	Location
1	HB13	3	M	+	Bikaner
2	HB57	34	F	+	Bikaner
3	HB73	9.5	M	+	Bikaner
4	HW4*	27	M	+	Gadwala
5	HW6*	28	F	+	Gadwala
6	HW29*	36	M	+	Gadwala
7	HO15	22	M	+	Gadola
8	HO24	17	M	+	Gadola
9	HO27	35	M	+	Gadola
10	HO34	23	M	+	Gadola
11	HO36	23	M	+	Gadola

*Veterinarian

Table 7: Location – wise prevalence of Brucellosis in humans by ELISA

Location	Count (percentage)		Prevalence
	Total examined	ELISA Positive	
Bikaner	84	3 (27.3%)	3.57%
Gadwala	29	3 (27.3%)	10.34%
Gadola	36	5 (45.5%)	13.88%
Naurangdesar	39	0 (0.0%)	0.0%
Total	188	11	5.85%

Table 8: Sex-wise prevalence of Brucellosis by ELISA in humans in different locations

Location	Males			Females		
	ELISA Positive	Total examined	Prevalence	ELISA Positive	Total examined	Prevalence
Bikaner	2	52	3.84%	1	32	3.12%
Gadwala	2	20	10%	1	9	11.11%
Gadola	5	31	16.12%	0	5	0.0%
Naurangdesar	0	6	0.0%	0	33	0.0%
Total	9	109	8.25%	2	79	2.53%

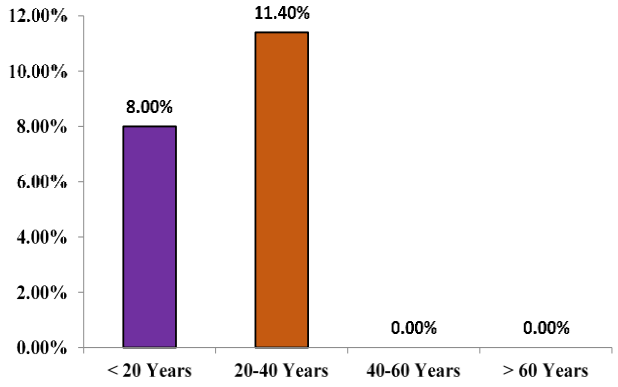

Fig. 4: Age-wise prevalence of Brucellosis in humans by RBPT

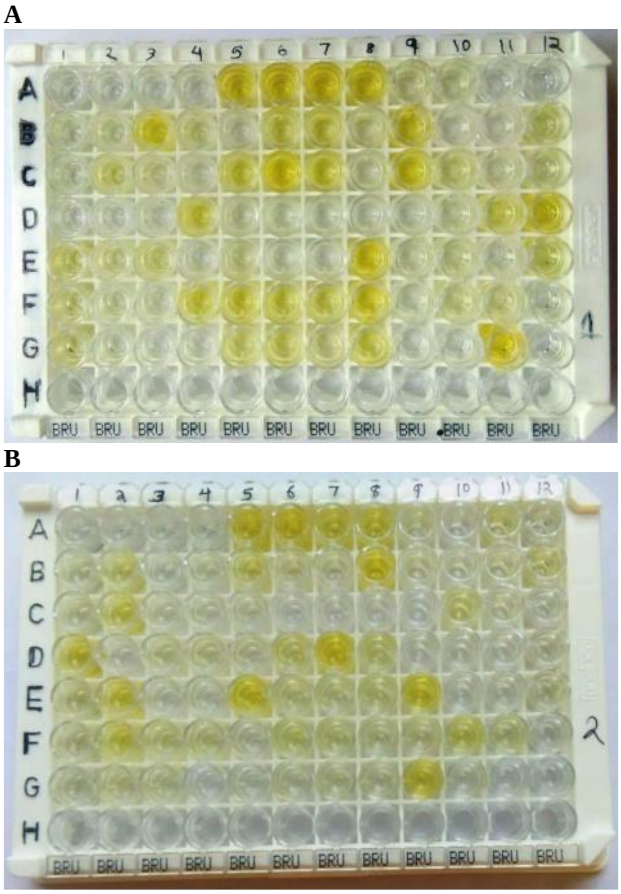

Fig. 5: ELISA on human sera: plates A & B show positive (yellow) samples

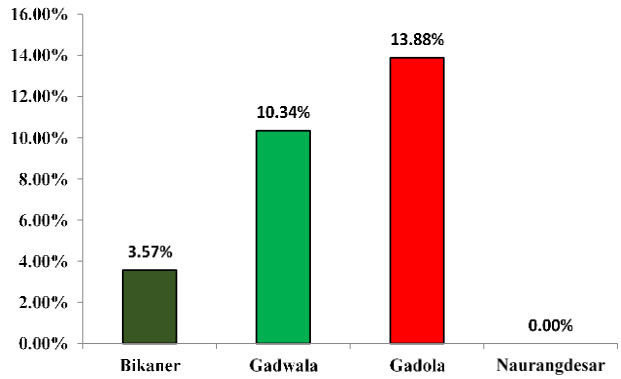

Fig. 6: Location – wise prevalence of Brucellosis in humans by ELISA

Sex-wise prevalence was 8.25% in males and 2.53% in females (Table 8 and Fig. 7). Sex-wise prevalence in Bikaner was 3.84% for males and 3.12% for females, in Gadwala, it was 10.0% for males and 11.11% for females and in Gadola, it was 16.12% for females, respectively. Age-wise prevalence in humans by ELISA was 4.0% in humans less than 20 years of age and 7.89% in those between 20–40 years of age (Table 9 and Fig. 8). The average age of ELISA positive humans varied from 15.5 in Bikaner to 24 in Gadola and 30.33 in Gadwala, respectively. Out of the total population, the younger people ranging from 3 to 35 were more likely to be infected with Brucellosis due to close contact with animals. The average age of infection in women was 20.66 and in males it was 20.58 years. In males, the adolescents showed more predilections to Brucellosis.

Table 9: Age –wise prevalence of Brucellosis in humans by ELISA

Age	Count (percentage)		Prevalence
	Total examined	ELISA Positive	
< 20 years	50	2 (18.2%)	4.0%
20-40 years	114	9 (81.8%)	7.89%
40-60 years	20	0 (0.0%)	0.0%
> 60 years	4	0 (0.0%)	0.0%
Total	188	11	5.85%

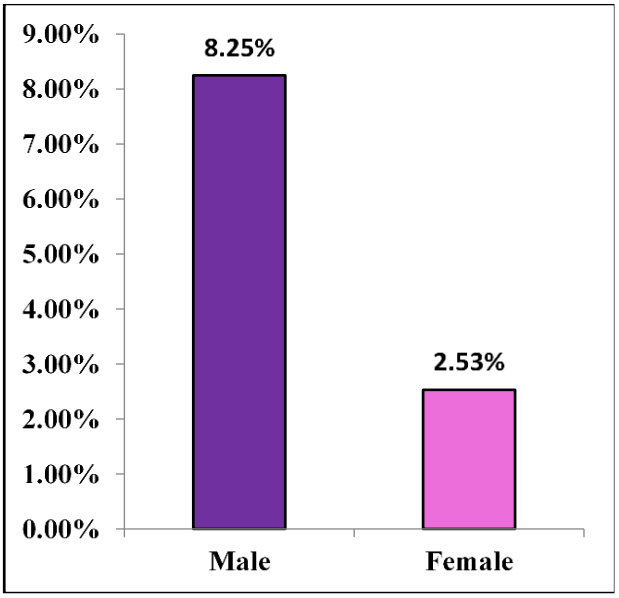

Fig. 7: Sex – wise prevalence of Brucellosis in humans by ELISA

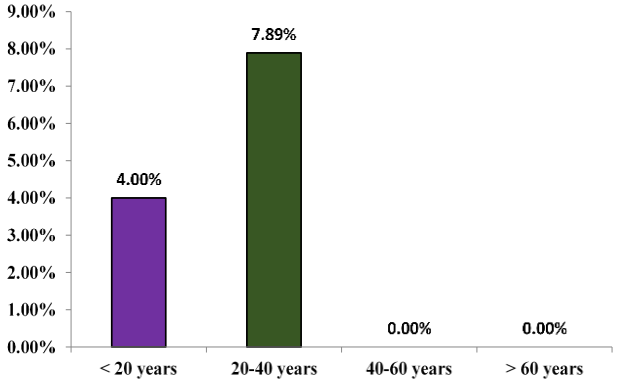

Fig. 8: Age – wise prevalence of Brucellosis in humans by ELISA

Out of the 11 ELISA positive samples, six samples were positive by both ELISA and RBPT. On the other hand, 11 samples positive for RBPT were negative by ELISA. Interestingly, 5 samples negative by RBPT were found to be positive by ELISA (Table 10).

Prevalence by RBPT and ELISA taken together

Since RBPT detects antibodies to particulate antigens whereas ELISA detects antibodies to soluble antigens, prevalence was calculated taking into account results of both RBPT and ELISA for confirmation. The overall prevalence by ELISA and RBPT taken together was 3.19%.

Table 10: Human serum samples positive for Brucellosis by RBPT and/or ELISA

S. n.	Case no.	Age (yrs)	Sex	RBPT	ELISA	Location
1	HB1	30	M	+	-	Bikaner
2	HB5	30	M	+	-	Bikaner
3	HB8	5	M	+	-	Bikaner
4	HB11	6	M	+	-	Bikaner
5	HB13	3	M	-	+	Bikaner
6	HB57	34	F	+	+	Bikaner
7	HB72	25	F	+	-	Bikaner
8	HB73	9.5	M	+	+	Bikaner
9	HB82	3	F	+	-	Bikaner
10	HB83	1	M	+	-	Bikaner
11	HB84	1.5	M	+	-	Bikaner
12	HW4	27	M	+	+	Gadwala
13	HW6	28	F	-	+	Gadwala
14	HW29	36	M	-	+	Gadwala
15	HO11	23	F	+	-	Gadola
16	HO15	22	M	+	+	Gadola
17	HO21	29	M	+	-	Gadola
18	HO24	17	M	+	+	Gadola
19	HO27	35	M	+	+	Gadola
20	HO29	35	M	+	-	Gadola
21	HO34	23	M	-	+	Gadola
22	HO36	23	M	-	+	Gadola

Table 11: Location – wise prevalence of Brucellosis in humans by both RBPT and ELISA

Location	Count (percentage)				Prevalence
	Total examined	RBPT +	ELISA+	Both +	
Bikaner	84	10 (58.8%)	3 (27.3%)	2 (33.33%)	2.38%
Gadwala	29	1 (5.9%)	3 (27.3%)	1 (16.66%)	3.44%
Gadola	36	6 (35.3%)	5 (45.5%)	3 (50.0%)	8.33%
Naurangdesar	39	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0%
Total	188	17	11	6	3.19%

Table 12: Sex – wise prevalence of Brucellosis in humans by RBPT and ELISA combined

Sex	Count (percentage)				Prevalence
	Total examined	RBPT +	ELISA+	Both +	
Male	109	13 (76.5%)	9 (81.8%)	5(83.33%)	4.58%
Female	79	4 (23.5%)	2 (18.2%)	1 (16.66%)	1.26%
Total	188	17	11	6	3.19%

Location-wise prevalence in humans by both RBPT and ELISA

Prevalence of Brucellosis in humans by positivity for both RBPT and ELISA was found as 2.38% in Bikaner, 3.44% in Gadwala and 8.33% in Gadola respectively (Table 11, Fig. 9).

Sex-wise prevalence in humans by RBPT and ELISA combined

Prevalence in humans by RBPT and ELISA combined was 4.58% in males and 1.26% in females, respectively (Table 12, Fig. 10).

Table 13: Age – wise prevalence of Brucellosis in humans by RBPT and ELISA combined

Age	Total examined	RBPT +	ELISA+	Both +	Prevalence
< 20 years	50	4 (23.5%)	2 (18.2%)	2 (33.33%)	4.0%
20-40 years	114	13 (76.5%)	9 (81.8%)	4 (66.66%)	3.50%
40-60 years	20	0 (0.0%)	0 (0.0%)	-	0.0%
> 60 years	4	0 (0.0%)	0 (0.0%)	-	0.0%
Total	188	17	11	6	3.19%

Table 14: Statistical evaluation of RBPT as compared to I-ELISA in humans

Statistic	Value	95% CI
Sensitivity	68.75%	41.34% to 88.98%
Specificity	96.51%	92.56% to 98.71%
Positive Likelihood Ratio	19.71	8.40 to 46.23
Negative Likelihood Ratio	0.32	0.16 to 0.67
Disease prevalence (*)	8.51%	4.94% to 13.45%
Positive Predictive Value (*)	64.71%	43.87% to 81.14%
Negative Predictive Value (*)	97.08%	94.13% to 98.57%
Accuracy (*)	94.15%	89.77% to 97.04%

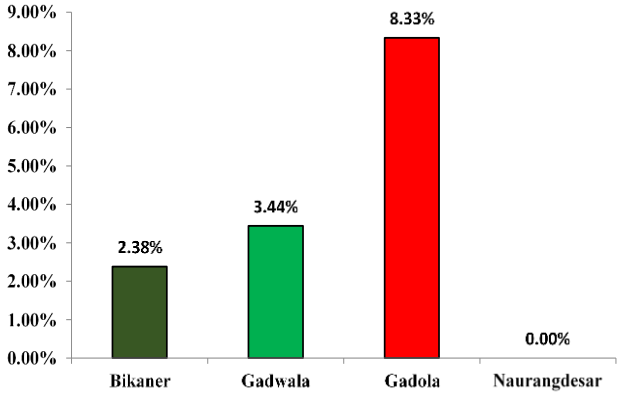

Fig. 9: Location–wise positivity for Brucellosis in humans by both RBPT & ELISA

Age-wise prevalence in humans by RBPT and ELISA combined

Prevalence in humans by RBPT and ELISA taken together was 4.0% in humans less than 20 years of age and 3.50% in humans aged between 20–40 years, respectively (Table 13 and Fig. 11).

Considering ELISA as gold standard, RBPT yielded a sensitivity of 68.75% and specificity of 96.51%. Its positive predictive value was 64.71% and negative predictive value was 97.08% in our present study on human sera (Table 14).

Discussion

In our study, occurrence of Brucellosis in human beings was 9.04% by RBPT. The humans positive by RBPT included 76.5% men and 23.5% women, respectively. Positive humans were from Bikaner (58.8%), Gadwala (5.9%) and Gadola (35.3%), respectively. Among the positives, 23.5% humans were aged less than 20 years and 76.5% were

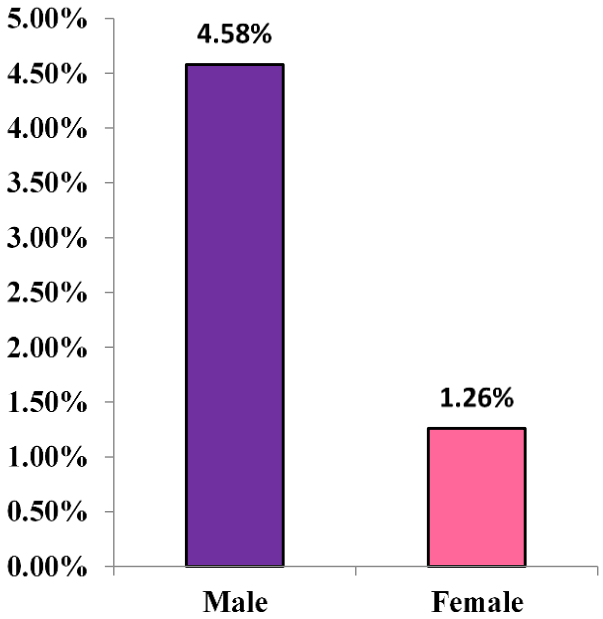

Fig. 10: Sex – wise prevalence of Brucellosis in humans by both RBPT and ELISA

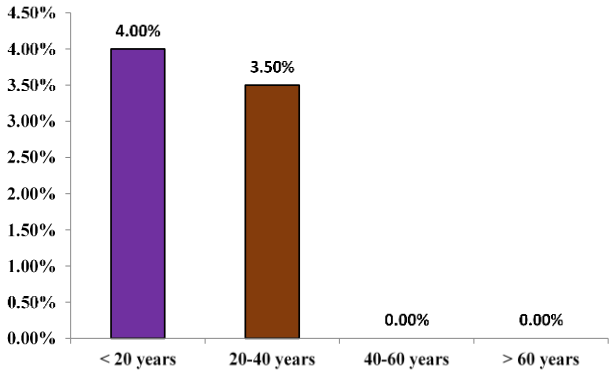

Fig. 11: Age – wise prevalence of Brucellosis in humans by both RBPT and ELISA

between 20–40 years, respectively.

Kataria *et al.* (2011) carried out a study in 10 districts in Rajasthan on the seroprevalence of brucellosis among 366 veterinarians and 719 para-veterinary staff. The serum samples were screened by RBPT and the RBPT positive samples were analyzed for antibody titre by tube agglutination test. The overall seroprevalence in veterinary professionals was 3.68% (3.00% in veterinarians and 4.03% in para-veterinary staff). However, our results show a nearly 3-fold higher rate of prevalence in humans in the same state after 10 years compared to the above-mentioned study.

In an outbreak of disease manifesting polyarthritis in 48 persons in village Kanvari in district Churu in Rajasthan, 91.6% of the people were found to be positive for Brucellosis (Kalla *et al.* 2001). Kochar *et al*. (2007) tested 175 people in Bikaner (155 were villagers) for Brucellosis. Among the

infected persons, two were veterinary officers. The risk factors identified included ingestion of unpasteurized or unheated milk (86.86%) and contact (occupational – 62.28% and household contact – 16%) with infected animal.

In a study conducted in western Rajasthan by Ali *et al.* (2014), 350 people (Veterinary Officers, milk vendors and slaughter house employees) were screened. It was revealed that meat handlers (42%), veterinarians and milkmen (28%) (13% of them suffering from pyrexia of unknown origin) and 4% of normal healthy people were positive for Brucellosis. Thus, people who are in contact with animals were much more susceptible to Brucellosis compared to those not in contacts.

In a study at an organized dairy farm at Karnal, Mathur (1964) found that 8.5% of the employees had antibodies against *Brucella* with titres of 80IU and above. In a study at Pune, 133 (21.8%) out of 611 serum samples received for VDRL and 19 (3.1%) out of 46 serum samples received for Widal were found to be positive for *Brucella* agglutinins (Phadke and Phadke 1974).

In a study conducted by Kadri *et al.* (2000), 28 (0.8%) out of 3,532 patients of PUO were found to be positive for brucellosis. Thakur and Thapliyal (2002) screened a total of 352 human sera in Uttaranchal and found 4.97% persons occupationally exposed to animals positivefor brucellosis. In a study by Kumar and Nanu (2005) in Kerala,1.6% were found to be seropositive for brucellosis. Frequency of occurrence was 17.39% in field veterinarians, 2.45% in common people and 1.14% in veterinary students. However, the prevalence rate in humans estimated in our study was almost twice as that reported from Uttaranchal and about four times as that reported from Kerala.

Agasthya *et al.* (2007) tested 618 persons for occupational Brucellosis. The disease was detected in Veterinary inspectors (41.23%), veterinary assistants (30.92%), veterinary officers (12.37%), veterinary supervisors and group D workers (6.18%), shepherds (2.06%) and butchers (1.03%), respectively.

Our study has yielded data that shows the rate of prevalence of Brucellosis in humans in this region is greater than the national level, comparable to Kerala, Uttaranchal and Haryana but lesser than some of the earlier reports from Rajasthan. However, it is much higher than those reported in some other studies from Rajasthan.

ELISAs have a sensitivity similar to or more than RBT and Complement Fixation Test, but cannot differentiate recently vaccinated animals from the infected ones (Jiménez de Bagüés *et al.* 1992; Blasco *et al.* 1994; Diaz-Aparicio *et al.* 1994; Delgado *et al.* 1995; Ficapal *et al.* 1995; Marín *et al.* 1999; Ferreira *et al.* 2003) or infections with bacteria known to cross-react. The ELISA has earlier been found to have a sensitivity of 99.4% and specificity of 98.9% in camels and humans (Biancifiori *et al.* 2000).

Xu *et al.* (2020) reported that out of 235 Brucellosis affected humans, 51 (21.7%) were culture positive, 150 (63.8%) positive by agglutination test, and 232 (98.7%) by ELISA. ELISA was the most sensitive method and yielded the maximum positives. Determination of level of IgG was more informative than that of IgM level. They opined that ELISA has higher sensitivity and specificity in diagnosing Brucellosis in humans. ELISA had a higher sensitivity and specificity compared to agglutination test. This was consistent with other studies (El-Rab and Kambal 1998; Osoba *et al.* 2001; Ulu-Kilic *et al.* 2013). With the progression of disease, culture positivity and positivity by agglutinin test decrease substantially while ELISA is unaffected. El-Rab and Kambal (1998) reported that IgM ELISA had a significant positive correlation with SAT, compared to IgG ELISA.

It has been recommended by Mayo Clinic that ELISA positive specimens should be confirmed by agglutination test. High levels of IgG antibodies may be found in circulation even in the absence of active disease. ELISA positive samples not confirmed by *Brucella*-specific agglutination may be false-positive. ELISA should be used for screening purpose only. Positive results by ELISA should be confirmed using an agglutination assay. CDC has recommended that samples positive by ELISA should be confirmed by a *Brucella*-specific agglutination test.

The results of our study on 188 human serum samples indicate that Brucellosis is a serious public health problem in people directly in contact with camels affected with Brucellosis in Bikaner and adjacent villages of Indian state of Rajasthan. The finding is important because this disease is zoonotic and currently there is no vaccine or cure for humans Brucellosis.

Conclusion

The present study has revealed that Brucellosis is prevalent at a significant rate (9.04% by RBPT and 2.25% by ELISA) in human beings associated with camels in Bikaner city and adjoining villages of Rajasthan. This is of public health significance.

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Author Contributions

NS did all the experimentation, analyzed the results and wrote the manuscript. RJ checked the manuscript and approved it.

Conflicts of Interest

All the authors declare no conflicts of interest.

Data Availability

Data presented in this study will be available on request to the corresponding author.

Ethics Approval

Ethics approval was taken from the ethics committee.

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