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Seroprevalence studies of Brucellosis among Goats using different serological tests

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Abstract
A study was conducted to determine the prevalence of Brucellosis in goats in and around border areas of Jammu, J&K, India using different serological tests *viz*., RBPT, STAT and I-ELISA. A total of 350 serum samples from goats were tested by RBPT, STAT and I-ELISA. Overall sero-prevalence of 1.14% was recorded in goats. Higher prevalence rates in 6-9 yr age group in goats (1.42%, 2.85% and 8.57%) were obtained by RBPT, STAT and I-ELISA, respectively. Goats of unorganized sector observed higher prevalence compared to organized sector, whereas sex-wise the does had higher prevalence than bucks. Upon odd ratio analysis in goats, 1-2 yr age group of animals was most susceptible.

Keywords: Brucellosis, sero-prevalence, goats, RBPT, STAT, I-ELISA

1. Introduction
Brucellosis is a major bacterial zoonosis of global, economic and public health significance. [1] The disease has also been reported in recent years from wild and marine mammals and birds. The presence of brucellosis in wild animals, with a potential for continuous transfer to domestic animals and from them to humans is another epidemiological issue. [2] In cattle, *B. abortus* causes abortion, stillbirth and weak calves with abortions usually occurring during the third trimester of gestation. In goats, *B. melitensis* can cause abortion, retained placenta, orchitis and epididymitis. Abortions usually occur during the fourth month of gestation in goats. Clinical manifestations of disease in man are weakness, fever, profuse sweating especially in night, loss of weight, generalized body ache etc. Swelling in testes and burning micturition due to orchitis and urethritis, respectively, are also peculiar symptoms of disease in man [3].

There are six recognized species for Genus *Brucella*: *B. melitensis, B. abortus, B. suis, B. canis, B. ovis* and *B. neotomae*; and two provisional species *B. pinnipediae* and *B. cetaceae* (Table 1). This taxonomic classification is mainly based on the difference in host preference and pathogenicity which can be attributed to various proteomes, as exemplified by specific outer-membrane protein markers [4].

Cross-infection between species further adds to the complexity of the disease. The disease has been managed successfully in the developed countries by efficient diagnosis, vaccination and test and slaughter/segregation policy but it still remains a problem in India including J&K State. The incidence of human brucellosis is significantly high where caprine brucellosis is endemic [5]. Since goat is very important source for transmission of *B. melitensis* to human being, it becomes very imperative to screen goats and in-contact human beings for brucellosis which can be helpful in generation of epidemiological data regarding this disease in an area, which can be further used for designing strategic control measures towards eradication of this disease from country. The soundness of such programmes depends upon quick and accurate diagnosis. Active and continuous surveillance and monitoring programmes are of utmost importance in formulating and implementing the disease intervention strategies for brucellosis. A number of serological tests have been employed to determine the status of the disease in an area. However, each test has its own advantages and limitations in terms of sensitivity and specificity. Therefore, a battery of serological tests is preferable in determining the status of the disease during mass surveys.

Thus, due to paucity of epidemiological data of humans and goat brucellosis in and around border areas of Jammu region and unavailability of simple and sensitive method for diagnosis of humans and goat brucellosis, this study has been proposed with the objective to study the prevalence of brucellosis in goats in and around border areas of Jammu.

2. Materials and Methods

A total of from 350 sera samples from goat were collected from in and around border areas of Jammu region. The distribution of collected samples as per the area and species has been shown in the Table 1. The sampling in animals was done in a randomized manner, from healthy animals to animals having history of abortion, retained placenta, fever, epididymitis and orchitis in males. Moreover, in goats, particulars in terms of age, sex were also collected as shown in Tables 2 and 3. All the samples were subjected to RBPT, STAT and I- ELISA tests.

In goats, for serum collection the blood was withdrawn aseptically by jugular vein puncture in disposable syringes (10ml) and kept in slanting position for 2 hr. at room temperature and about 30 minutes at 4 °C for clot retraction. The separated serum was collected in duly labeled sterilized screw capped vials (2ml).

Table 1: Details of serum samples collected from goats in and around border areas of Jammu

Place	No. of sample
Govt. Dairy Goat Research Farm-Rajbagh, Kathua	60
Vijaypur, Samba	140
R.S. Pura, Jammu	50
Kanachak, Akhnoor	100
Veterinary Professionals/Animal Handlers	-
Community Health Centre- R.S. Pura, Jammu	-
Total	350

Table 2: Age-wise distribution of goat serum samples

Species (No. of samples)	Age-group (yr)	No. of samples
Goats (350)	1-2	100
	3-5	180
	6-9	70

Table 3: Sex-wise distribution of goat serum samples

Species (No. of samples)	Sex	No. of samples
Goats (350)	Male	150
	Female	200

For RBPT and STAT, the antigens *viz.*, Rose Bengal antigen and *B. abortus* plain antigen, respectively, were procured from Biological Products Division (B.P. Division), Indian Veterinary Research Institute (IVRI), Izatnagar. Both antigens were stored at 4°C before and after use. For I-ELISA, the antigen (smooth- lipopolysaccharide) along with other technical requirements and expertises were kindly provided by Division of Veterinary Public Health, IVRI, Izatnagar.

The RBPT was performed according to the method described by Alton *et al.* (1975). The Rose Bengal Antigen was procured from Biological Products (BP) Division, IVRI.

Before the test, both, serum and antigen were allowed to come at room temperature. Then, the test was performed by mixing 30 µl each of serum and antigen on a glass plate. With continuous shaking, the plates were looked for any appearance of agglutination. Appearance of agglutination within 4 min of mixing of reagents was taken as positive while absence of agglutination was recorded as negative result.

The test was performed in clean glass tubes (14 mm x 100 mm) according to the method described by Alton *et al* [6, 7].

The ELISA was performed as per the method standardized by Singh [8], using S-LPS extracted from *B. abortus* S 99.

Smooth LPS was extracted from heat-killed cells of *B. abortus*, by the hot water/hot phenol method as described by OIE with minor modifications [9].

For the extraction, 5g of lyophilized cells of *B. abortus* strain 99 was suspended in 170ml of distilled water (DW) and heated to 66 °C. An equal volume of phenol (90%; v/v) in DW, also heated to 66 °C, was added and the solution was stirred continuously for 20 min. It was then cooled to 4 °C and centrifuged at 12,000g for 20 min at 4 °C. The phenol phase (bottom layer) was recovered and filtered through Whatman #1 to which three volumes of chilled methanol reagent was added. It was mixed thoroughly and left to precipitate at 4 °C for 2 h. The precipitate was recovered by centrifugation at 12,000x g at 4 ^{0}C and re-suspended in the 80 ml of DW and centrifuged at 6,000g for 20 min. The pellet was re-suspended in 80ml of DW and stirred at 4 °C overnight. The solution was then centrifuged at 10,000g for 15 min at 4 °C and the supernatant was decanted. Another 80 ml of DW was added to the pellet, which was then stirred for 1 h and centrifuged as before. The two supernatants were pooled, filtered through membrane filter (0.3µm), and 50-100µg each of ribonuclease, deoxyribonuclease and proteinase K were added. This mixture was incubated for 18 h at 20 °C. It was re-precipitated with methanol and re-suspended as above in 2 ml of DW. The solution was dialyzed extensively against DW until free of phenol. The resultant antigen was lyophilized, weighed and resuspended in DW to give 1mg LPS/ ml. This was finally freeze dried in 1 ml volume and stored at 4 ^{0}C for future use.

The relative sensitivity and relative specificity of the test was calculated using the method described by Mcdiarmid and Hellstrom [10].

$$\text{Relative Sensitivity (\%)} = \frac{\text{Serum samples positive to both test compared and standard test}}{\text{Serum samples positive to standard test}} \times 100$$

$$\text{Relative Specificity (\%)} = \frac{\text{Serum samples negative to both test compared and standard test}}{\text{Serum samples negative to standard test}} \times 100$$

2.1 Statistical Analysis: The kappa value, odd's ratio, accuracy, predictive value and likelihood ratio were calculated using JavaStat-2-way Contingency Table Analysis at 95% confidence interval.

3. Results

On analysis of 350 samples of goats by RBPT, STAT and I-ELISA, the overall prevalence obtained was 1.14% in goats (Tables 4 and 5). Individual tests wise, out of 350 serum samples of goats 6 (1.71%), 12 (3.42%) and 20 (5.71%) samples were positive to RBPT, STAT and I-ELISA, respectively (Table 6). The epidemiological pattern of brucellosis was estimated for goats for which the records on age, sex and animal rearing systems were available. In goats, the animals were divided in 3 age groups *i.e.,* 1-2 yr, 3-5 yr and 6-9 yr. In goats, the highest prevalence was observed in 6-9 yr age group as 1.42%, 2.85% and 8.57% by RBPT, STAT and I-ELISA, respectively (Table 7). In general, the prevalence of brucellosis was higher in females than in males in goats (Table 8). A higher prevalence was observed among goats from the unorganized sector as 1.72%, 3.44% and 6.20% by RBPT, STAT and I-ELISA, respectively, as compared to 1.66%, 3.33% and 3.33% of organized sector (Table 9). Odds ratio which depicts risk factor for acquiring the disease was calculated for goats in relation to age. More

susceptible animals fell in 1-2 years age group (Table 10). The results obtained in different serological tests *viz.,* RBPT, STAT and I-ELISA were analyzed statistically in Table 11 in terms of (a) relative sensitivity (b) relative specificity (c) accuracy, (d) positive predictive value, (e) negative predictive value, (f) positive likelihood ratio, (g) negative likelihood ratio, (h) Kappa values and by analyzing the presence of anti-*Brucella* antibodies in different tests combinations. The relative sensitivities and specificities of the tests was calculated using the method described by Mcdiarmid and Hellstrom [10].

As I-ELISA are the prescribed test for international trade [11, 12] in goats the statistical analysis *i.e.,* (a) to (h) were determined by taking I-ELISA as standard. The same pattern was followed in human as well. On interpretation of results of goats, interestingly, it was observed that there were 8 samples exclusively positive in I-ELISA, while negative in RBPT and STAT. Moreover, only two samples were found positive in RBPT and STAT but negative in I-ELISA. Further, not even a single sample positive exclusively in RBPT was reported and also none of the samples positive exclusively in STAT was found. Four samples were found positive in all the 3 tests. In goats, upon statistical analysis of RBPT and STAT using I-ELISA as standard, interestingly, RBPT and STAT observed just 20% and 60% relative sensitivity with high values of relative specificity i.e., 99.39% and 100% respectively. However, both tests observed appropriate positive and negative likelihood ratio. Further, in comparison, STAT recorded higher positive and negative predictive value than RBPT. The kappa was found higher in STAT and STAT observed 0.40 negative likelihood ratio and 100% positive predictive value in comparison to I-ELISA (Table 12). In goats, accuracy of STAT (97.71%) was higher than RBPT (94.85%) using I-ELISA as standard (Table 12).

Table 4: Sero-prevalence of brucellosis in goats

Species (No. of samples)	RBPT Positive (%)	STAT Positive (%)	I-ELISA Positive (%)
Goat (350)	6 (1.71)	12 (3.42)	20 (5.71)

Table 5: Overall sero-prevalence of brucellosis in goats

Species	Samples examined	Samples positive	% positive
Goat	350	4	1.14

Table 6: Sero-prevalence of brucellosis among goats (n=350) in different places of border areas of Jammu as detected by RBPT, STAT and I-ELISA

TESTS	RBPT		STAT		I-ELISA	
PLACE (Number of samples)	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Govt. Dairy Goat Research Farm Rajbagh, Kathua (60)	1 (1.66)	59 (98.33)	2 (3.33)	58 (96.66)	2 (3.33)	58 (96.66)
Vijaypur, Samba (140)	3 (2.14)	137 (97.85)	6 (4.28)	134 (95.71)	10 (7.14)	130 (92.85)
KanachakAkhnoor (100)	1 (1.0)	99 (99.0)	3 (3.0)	97 (97.0)	6 (6.0)	94 (94.0)
RS Pura, Jammu (50)	1 (2.0)	49 (98.00)	1 (2.0)	49 (98.00)	2 (4.0)	48 (96.00)
TOTAL (350)	6 (1.71)	344 (98.28)	12 (3.42)	338 (96.57)	20 (5.71)	330 (94.28)

Table 7: Age-wise sero-prevalence of brucellosis in goats

Age-group (yr)	No. of animals	RBPT +ve (%)	STAT +ve (%)	I-ELISA +ve (%)
1-2	100	2 (2.0)	3 (3.0)	6 (6.0)
3-5	180	3 (1.66)	7 (3.88)	8 (4.44)
6-9	70	1 (1.42)	2 (2.85)	6 (8.57)

Table 8: Sex-wise sero-prevalence of brucellosis in goats

Species (No. of samples)	Sex (No. of samples)	RBPT +ve (%)	STAT +ve (%)	I-ELISA +ve (%)
Goat (350)	Male (150)	1 (0.66)	4 (2.66)	8 (5.33)
	Female (200)	5 (2.50)	8 (4.0)	12 (6.0)

Table 9: Sero-prevalence of brucellosis in goats in different rearing system

Sector (No. of samples)	RBPT +ve (%)	STAT +ve (%)	I-ELISA +ve (%)
Organized (60)	1 (1.66)	2 (3.33)	2 (3.33)
Unorganized (290)	5 (1.72)	10 (3.44)	18 (6.20)

Table 10: Age-wise Odds ratio in goats Using I-ELISA as standard

Age(yr)	1-2	3-5	6-9
1-2	-	1.37 95%CI=0.41-4.52 P- value=0.58	0.68 95%CI=0.18-2.52 P- value=0.55
3-5	-	-	0.49 95%CI=0.15-1.69 P- value=0.23
6-9	-	-	-

Table 11: Statistical analysis of RBPT and STAT taking I-ELISA as standard in goats (*n*=350)

Test	Kappa Value	Relative sensitivity (%)	Relative specificity (%)	+ve predictive value (%)	-ve predictive value (%)	+ve likelihood ratio	-ve likelihood ratio
RBPT	0.29	20	99.39	66.66	95.34	33	0.81
STAT	0.74	60	100	100	97.63	Inf	0.40

Table 12: Accuracy of RBPT and STAT taking I-ELISA as standard in goats (*n*=350)

Test	Accuracy (%)
RBPT	94.85
STAT	97.71

Table 13: Presence of anti-*Brucella* antibodies in different serological test combinations in goats (*n*=350)

Test	1	2	3	4	5	6	7	8
RBPT	-	+	-	-	+	-	+	+
STAT	-	-	+	-	+	+	-	+
I-ELISA	-	-	-	+	-	+	+	+
Total (350)	330	0	0	8	2	6	0	4

4. Discussion

In the present study, on analysis of 350 serum samples of goats, an overall prevalence of 1.14 percent was observed. The results are comparable to Sharma *et al.* [13] in Jammu who found 2.85 percent of overall prevalence in goats. However, the prevalence rate was observed to be higher in his study. The results are also comparable to Sharma [14, 15] who found 4.14 percent of overall prevalence in goats higher than the present study. The reason could be lower sample size in our study compared to Sharma [16, 17].

Individual test wise, on analysis of goat serum samples (350), the sero- prevalence observed was 1.71%, 3.42% and 5.71% by RBPT, STAT and I-ELISA, respectively, less than that of Reddy *et al.* [18] in Karnataka who reported 5.15% prevalence by RBPT, 6.34% by STAT and 9.52% by I-ELISA. The results are also comparable to Sharma *et al.* [13-15] in Jammu who found 4.2%, 21.4% and 34.2% samples positive by RBPT, STAT and I-ELISA, respectively and also to Sharma [16, 17] who found 7.73%, 10.22% and 12.98% samples positive by RBPT, STAT and I- ELISA, respectively higher than the present study. The reason behind the lower test wise prevalence in present study compared to others studies of Jammu may be due to that in present study samples were taken from border areas of Jammu where grazing of animals in open area was prevalent while in others studies samples were taken from areas of Jammu where zero grazing (intensive farming) was prevalent. As we know that the chances of transmission of brucellosis are higher in intensive farming system than the free grazing system, the prevalence in former is expected to be greater than latter.

In goats the prevalence was higher in females than male animals; similar observations were recorded by Sharma *et al.* [13, 14], who ascribed higher resistance of the male animals as compared to female animals. Similar results were those of Sharma [15-17].The age wise prevalence in goats was higher in 6 yrs-9 yrs age group. The findings of present study are not in concordance with Sharma *et al.* [13-15] who reported that the prevalence of brucellosis in goats was more in the age group of 3yrs-5yrs.The results also differed to that observed by Singh *et al.* [19] who reported higher prevalence in 1-2 yr age group in goats. But the study was in concordance with Sharma [13] who found higher prevalence in mature goats.

A higher sero-prevalence was observed in unorganized sector in comparison to organized sector that may be attributed to better managemental practices like routine screening of goats for brucellosis in organized sector. The results are in contradiction to those observed by Singh *et al.* [19] and Sharma *et al.* [15-17] who reported higher sero-prevalence in organized sector as compared to unorganized sector of Jammu region, ascribing it to higher transmission rate of infectious agent between susceptible and infected animals in organized rearing system than the unorganized and similar observations were also made by Kellar *et al.* [20] and Nicoletti [21]. But the results are comparable to Sharma [13-17] who also observed higher prevalence in unorganized sector in comparison to organized sector.

Nevertheless, I-ELISA was observed to be a more sensitive test over RBPT and STAT and should be applied on a large scale to evaluate it for screening purposes for diagnosis of brucellosis in the country.

5. Conclusion

On analysis of 350 samples of goats by RBPT, STAT and I - ELISA, the overall sero-prevalence obtained was 1.14% in goats. Individual tests wise, 6 (1.71%), 12 (3.42%) and 20 (5.71%) samples were positive to RBPT, STAT and I-ELISA, respectively.

In goats, the highest prevalence was in 6-9 yr age group as 1.42%, 2.85% and 8.57% by RBPT, STAT and I-ELISA, respectively. Also, the prevalence was higher in females (2.50%, 4.0% and 6.0% by RBPT, STAT and I-ELISA, respectively) and in unorganized sector animals (1.72%, 3.44% and 6.20% by RBPT, STAT and I-ELISA, respectively). In humans, 36-50 yr persons (3.33%, 0.0% and 10.0% by RBPT, STAT and I-ELISA, respectively) and males (3.33%, 1.66% and 6.66% by RBPT, STAT and I-ELISA, respectively) recorded highest prevalence.

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