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Research Article

SEROPREVALENCE OF BRUCELLOSIS IN THE ORGANIZED SHEEP FARMS OF JAMMU, INDIA

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ABSTRACT

A study was carried out to determine the prevalence of Brucellosis in organized sheep farms of Jammu, India using different serological tests *viz.*, Rose Bengal Plate Test (RBPT) and Standard Tube Agglutination Test (STAT) for brucellosis. Total of 186 serum samples from sheep were tested. Upon analysis, an overall seroprevalence of 1.61% was obtained whereas test wise 3 (1.61%) and 6 (3.22%) were positive to RBPT and STAT, respectively. Higher prevalence was observed among sheep flocks from Government Sheep Breeding and Research Farm-Billawar (2.85% by RBPT and 4.28% by STAT) as compared to Government Sheep Breeding and Research Farm-Reasi (1.53% by RBPT and 3.07% by STAT) and Government Sheep Breeding Farm-Panthal (0 % by RBPT and 1.96% by STAT).

Keywords: Brucellosis, RBPT, Seroprevalence, Sheep, STAT

Brucellosis is a contagious disease that infects animals can be transmitted to humans and caused by different species belonging to the genus Brucella. Brucellosis is a bacterial zoonotic infection and is amongst the most important diseases, in terms of loss to economy that affects sheep and goat population in the developing countries. Brucellosis in sheep is primarily caused by *Brucella melitensis* though *Brucella abortus* and *Brucella suis* cause sporadic infections. *B. melitensis* is considered to have the highest zoonotic potential, followed by *B. abortus* and *B. suis*. The organisms are gram negative facultative intracellular parasites. Serological tests based upon the detection of anti-Brucella antibodies are commonly used to diagnose brucellosis (Ferreira *et al.*, 2003). Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) represent the most frequently applied tests for the serodiagnosis of brucellosis. The SAT provides quantitative data on immune responses (Hamidullah *et al.*, 2009). The RBPT is a quick, effective and less expensive test applicable to detect and diagnose brucellosis in large animal herds. As Brucellosis has public health and economic significance, the present study on seroprevalence of brucellosis was conducted to determine the impact of disease in sheep population of Jammu.

MATERIALS AND METHODS

A total of 186 samples from sheep were collected from organized sheep farms of Jammu. The distribution of collected samples as per the farm, age groups and gender has been detailed in Table 1. The blood samples were collected from the jugular vein of randomly selected apparently healthy animals as well as those having history of abortion. Samples were transported immediately on ice to the laboratory and sera were separated immediately by centrifugation of blood at 1,500×g for 10 min at room temperature and were kept at -20 °C until the day of analysis.

Rose Bengal Plate Test: 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 brought to 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.

Standard Tube Agglutination Test (STAT): The test was performed in clean glass tubes (14 mm x 100mm) according to the method described by Alton *et al.* (1975). For test, five sterilized tubes were used for each serum sample. The phenol saline was dispensed as 0.8 ml in the first tube and 0.5 ml in the rest of the tubes, followed by addition of 0.2 ml of test serum in the first tube. After mixing, 0.5 ml from the first tube was transferred to the next tube followed by mixing and transfer of 0.5 ml to the next tube. This step was repeated till the last tube from which 0.5 ml of mixture was discarded. Thus dilutions of 1:5, 1:10, 1:20, 1:40 and 1:80 of serum were obtained in five tubes. Thereafter each tube received 0.5 ml of *B. abortus* plain antigen giving final serum dilutions as 1:10, 1:20, 1:40, 1:80 and 1:160. The contents of tube were thoroughly mixed followed by incubation at 37 ºC for 18 hours. After incubation, the tubes were kept at room temperature for ½ hour followed by interpretation of results. A titre of ≥ 40IU was taken as positive in sheep.

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Table 1

Details of serum samples collected from sheep

Place	No. of Samples
Government Sheep Breeding and Research Farm-Reasi	65
Government Sheep Breeding Farm-Panthal	51
Government Sheep Breeding and Research Farm-Billawar	70
Total	186

Age-wise distribution of sheep serum samples

Age Group of Sheep	No. of samples
1-2 years	52
3-5 years	89
6-9 years	45

Sex wise distribution of sheep serum samples

Sex of Sheep	No. of Samples
Male	76
Female	110

RESULTS AND DISCUSSION

Upon analysis of 186 sheep serum samples, an overall seroprevalence of 1.61% was obtained whereas test wise 3 (1.61%) and 6 (3.22%) were positive to RBPT and STAT, respectively. Higher prevalence of brucellosis was observed among sheep flocks from Government Sheep Breeding and Research Farm-Billawar as 2.85% and 4.28% by RBPT and STAT as compared to Government Sheep Breeding and Research Farm-Reasi which was 1.53% and 3.07% by RBPT and STAT, respectively, followed by Government Sheep Breeding Farm-Panthal which was 0.00% by RBPT and 1.96% by STAT. The observed seroprevalence of brucellosis was 1.61% and 3.22% by RBPT and STAT, respectively, which is less than that reported by Reddy *et al.* (2014) from Karnataka who reported 5.15% prevalence by RBPT and 6.34% by STAT. However, much higher incidence was reported earlier from Jammu. Sharma *et al.* (2015) in Jammu found 4.2% and 21.4% samples positive by RBPT and STAT, respectively, and also by Sharma (2012) who found 7.73% and 10.22% samples positive by RBPT and STAT, respectively. The lower prevalence in the present study as compared to other studies may be attributed to collection of samples from organized sheep farms of Jammu where better managemental practices are followed.

Nevertheless, the reason may be due to the fact that the test fails to exclude cross-reacting non-Brucella IgM antibodies produced in response to immune stimulation by respiratory and gastro-intestinal microflora, resulting in false-positive results in STAT (MacMillan and Cockrem, 1985) as well in our study. The sero-positivity obtained in various tests in brucellosis was more in STAT as compared to RBPT in sheep.

Epidemiological pattern of brucellosis in sheep: The epidemiological pattern of brucellosis was estimated for sheep for which the records on age and sex were available.

Age : Out of three age groups of 1-2yr, 3-5yr and 6-9yr sheep, seroprevalence of brucellosis was higher in 6-9 yrs group (2.22% and 4.44% by RBPT, STAT, respectively) (Table 3 & 4). The results are comparable with those of Rahman *et al.* (2011) in Bangladesh who observed higher seroprevalence in sheep more than 24 months of age. Similar results have been observed by Shafy *et al.* (2016) in Bangladesh who observed higher seroprevalence in adult sheep than in young ones. Low seroprevalence in young animals may be explained on the basis that animal may harbor the organism without expressing any detectable antibodies until their first parturition or abortion.

Sex: Sex wise prevalence was higher in females by both

Table 2

Seroprevalence of Brucellosis in sheep by RBPT and STAT

No. of samples	Overall (%)	RBPT (%)	STAT (%)
186	3 (1.61)	6 (3.22)	3 (1.61)

Table 3

Age-wise Odds ratio for RBPT in sheep in Brucellosis

Age (yr)	RBPT (+)	RBPT (-)	O.R (95%CI)	p-value
1-2	1	51	0.863(0.023-32.702)	0.918
3-5	1	88	0.500(0.013-18.810)	0.620
6-9(Reference)	1	44	-	-

Table 4

Age wise Odds ratio for STAT in sheep in Brucellosis

Age (yr)	STAT(+)	STAT(-)	O.R (95%CI)	p-value
1-2	1	51	0.422(0.015-6.240)	0.474
3-5	3	86	0.750(0.097-6.706)	0.757
6-9 (Reference)	2	43	-	-

Table 5

Sex wise Odds ratio for RPBT in sheep in Brucellosis

Sex	RBPT (+)	RBPT (-ve)	O.R (95%CI)	p-value
Male	1	75	0.720 (0.025-10.367)	0.789
Female	12	108		

Table 6

Sex wise Odds ratio for STAT in sheep in Brucellosis

Sex	STAT (+)	STAT (-ve)	O.R (95%CI)	p-value
Male	1	75	0.280 (0.012-2.543)	0.220
Female	5	105		

RBPT and STAT with a prevalence of 1.81 % and 4.54% than in males with 1.31% and 1.31% by RBPT and STAT, respectively. Similar observations were recorded by Sharma *et al.* (2015), who ascribed higher resistance of the male animals as compared to female animals. Similar results were those of Sharma (2012) in Jammu.

Odds ratio: For seroprevalence of Brucellosis, odds ratio which depicts risk factor for acquiring the disease was calculated for sheep in relation to age using Java Stat-2 way contingency table analysis by taking 6-9yr as reference for RBPT and STAT (Table 3 and 4). Odds ratios were found to be non-significant similar results were obtained in relation to sex (Table 5 and 6).

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