

SEROPREVALENCE OF BRUCELLOSIS IN GOATS IN LUDHIANA DISTRICT OF PUNJAB STATE OF INDIA

ABSTRACT

We investigated the prevalence of Brucellosis in goats in Ludhiana district of Punjab state of India. A total of 191 serum samples of goats from slaughter houses were analyzed with RBPT, STAT, MAT and ELISA. Out of these, 31 goats were positive for Brucellosis by one or more of these tests. Among positives, 14 were detected by RBPT, 17 by STAT, 21 by MAT, and 21 by ELISA; 10 samples were positive and 160 negative by all methods. ELISA and MAT detected highest number of samples followed by STAT and RBPT. Prevalence of the disease in goats was 5.23% by RBPT and ELISA. The mean titers of anti Brucella antibodies in goats were 36.66 ± 16.32 by STAT and 49.16 ± 29.47 by MAT, with non-significant difference.

Key words: Brucellosis, prevalence, goats, seroprevalence, caprine, epidemiology.

Introduction

Brucellosis is an important zoonotic disease that causes huge economic losses to the livestock farmers and is of public health significance. It causes abortions, placentitis, epididymitis, orchitis and reproductive losses in animals. The causative agent of Brucellosis in goats is *Brucella melitensis*. Brucellosis is endemic in India and is prevalent in all parts of the country. In a study conducted in various districts of Punjab in 2000, the overall prevalence of Brucellosis was found to be 3.60% in goats (1). The incidence of Brucellosis was reported to be 5% in goats, in Sangrur district of Punjab,

23 India (2). However, no information is available on the prevalence of the disease in goats
24 in other districts of Punjab.

25 The present study was undertaken to determine the seroprevalence of Brucellosis in goats
26 in Ludhiana district of Punjab state of India employing the common serodiagnostic
27 methods – Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT),
28 Microagglutination Test (MAT) and Competitive Enzyme Linked Immunosorbant Assay
29 (cELISA).

30 **Materials and methods**

31 The guidelines of Institutional Animal Ethics Committee were followed throughout the
32 study.

33 **Serum samples:** Blood samples were collected from adult goats from slaughter houses
34 located in or around Ludhiana. Serum was separated from the clotted blood and stored at
35 -20°C till use.

36 **Seroepidemiological studies on caprine Brucellosis:**

37 All the serum samples from goats were subjected to analysis with four common
38 serodiagnostic techniques –RBPT, STAT, MAT and cELISA.

39 **Rose Bengal Plate Agglutination Test (RBPT)**

40 RBPT was carried out by colored antigen with the method of Morgan *et al.* (3). 10 µl of
41 antigen was mixed with 10 µl of serum and observed for clumps as positive.

42 **Estimation of antibody titers by Standard Tube Agglutination Test (STAT):** The
43 standard method recommended by OIE (4) was followed. *Brucella abortus* plain antigen
44 (Punjab Veterinary Vaccine Institute, Ludhiana) was employed for the test. The highest

45 serum dilution showing 50% agglutination was taken as the end point for the titer of
46 serum. A titer of 1:40 or above was considered positive.

47 **Estimation of antibody titers by Microtiter Agglutination Test (MAT)**

48 MAT was performed as per the standard method (5). The formation of matt signified
49 agglutination while button formation was indicative of a negative reaction. Titers were
50 recorded as the reciprocal of the highest dilution of the serum giving at least 50 percent
51 agglutination.

52 **Competitive Enzyme Linked Immunosorbant Assay (cELISA):**

53 All the serum samples were subjected to analysis by competitive ELISA employing a
54 commercial kit named Svanovir Brucella Antibody cELISA Kit (Svanova). The kit is
55 based on a solid phase competitive ELISA. It is subjected to treatment by with a
56 monoclonal antibody (mAb) for the o-polysaccharide portion of the S-LPS antigen, are
57 exposed to *Brucella abortus* smooth lipopolysaccharide (S-LPS) coated wells on microtiter
58 plates. If Brucella antibodies are present in the test sample they will bind to the antigens
59 in the well and block these antigenic sites. If Brucella antibodies are absent in the sample,
60 these sites will remain free and the monoclonal antibody which was added together with
61 the sample will bind to these free antigenic sites. After an incubation period the unbound
62 materials are removed by rinsing and a goat anti- mouse IgG conjugated with horseradish
63 peroxidase (HRP) is added to the plate. The HRP conjugate will bind to the specific mAb
64 in absence of Brucella antibodies in the sample. Unbound materials are removed by
65 rinsing prior to the addition of the substrate. Development of a blue color shows negative
66 result. The reaction is stopped by addition of stop solution; the color changes to yellow.
67 The result is read at 450 nm.

68 The samples were run in duplicates. The optical density (OD) of the controls and samples
69 was measured at 450 nm with photometer. After 15 minutes we measured OD after the
70 addition of Stop Solution to prevent fluctuation in OD values.

71 Calculations: 1. The mean OD values were calculated for each of the controls and
72 samples.

73 2. The percent inhibition (PI) values were calculated using the following formula:

74
$$PI = 100 - (OD \text{ of samples or control} \times 100) / OD \text{ of conjugate control}$$

75 **Results**

76 **Seroepidemiology of Brucellosis in goats**

77 This study aimed to find out the prevalence of Brucellosis in goats in Ludhiana district of
78 Punjab state of India using RBPT, STAT, MAT and C-ELISA. A total of 191 samples
79 from goats were subjected to analysis with four different serodiagnostic tests i.e. RBPT,
80 STAT, MAT and ELISA to identify Brucellosis affected animals. Out of 191 samples, 31
81 were found to be positive by one or more tests (Table 1). A total of 160 serum samples
82 were found to be negative by all the four serodiagnostic methods. Among the positive
83 samples (Figure 1), 14 were found to be positive by RBPT, 17 by STAT, 21 each by
84 MAT and ELISA. ELISA and MAT detected the highest number of samples followed by
85 RBPT and STAT, respectively. Some samples were positive by two serologic methods
86 with other two tests being negative. Ten samples were positive by both RBPT and
87 ELISA. Ten samples were positive by all the four tests i.e. RBPT, STAT, MAT and
88 ELISA. Prevalence of the disease in goats was estimated to be 5.23% by RBPT and
89 ELISA.

90 The mean titers of anti –Brucella antibodies in goat sera were 36.66 ± 16.32 by STAT
91 and 49.16 ± 29.47 by MAT (Table 2), the difference was non – significant.

92 **Discussion**

93 The results of our study indicate that caprine Brucellosis is a major problem of concern in
94 Ludhiana district of Punjab state of India. Based on various serologic analyses on 191
95 serum samples of goats collected from slaughter houses and local small meat shops in
96 Ludhiana, the prevalence of the disease was estimated to be 5.23% by RBPT and ELISA,
97 respectively.

98 The highest number of true positive samples were detected by STAT (6.53%) followed
99 by MAT (6.03%) and RBPT & ELISA (5.52%). The highest number of true negatives
100 were obtained with STAT (86.43%) followed by RBPT (85.42%), ELISA (83.41%) and
101 MAT (80.90%) respectively.

102 Maximum false positive results were obtained by MAT (7.53%), followed by RBPT
103 (6.03%), ELISA (5.02%) and STAT (2.01%), respectively. Highest number of false
104 negatives were obtained with MAT (6.03%) followed by ELISA (5.52%), RBPT and
105 STAT (5.02% each), respectively. It is evident from the above results that STAT is the
106 most reliable of the four methods with highest numbers of true positives and true negative
107 results while MAT emerged as the least reliable of the four methods with highest
108 numbers of false positives and false negative results.

109 STAT was found to have the highest sensitivity (59.09%) followed by MAT (54.55%),
110 RBPT (52.38%) and ELISA (50%), respectively. STAT had the highest specificity
111 (97.73%) followed by ELISA (94.32%), RBPT (93.41%) and MAT (92.98%),
112 respectively.

113 The prevalence of the disease was estimated to be 11.40% by MAT, 11.11% by STAT
114 and ELISA, and 10.34% by RBPT, respectively. STAT was found to have the highest
115 positive predictive value of 76.47% followed by ELISA (52.38%), MAT (50%) and
116 RBPT (47.83%), respectively. RBPT was found to have the highest negative predictive
117 value of 96.5% followed by STAT (95.03%), MAT (94.08%) and ELISA (93.79%)
118 respectively. STAT had the highest positive likelihood ratio of 26 while MAT had the
119 lowest value of 7.77. ELISA had the highest negative likelihood ratio of 0.53 while
120 STAT had the lowest value of 0.42 (Table 3).

121 Brucellosis is endemic in India and is prevalent in all parts of the country. Several
122 serological surveys of small ruminant Brucellosis have indicated varying levels of
123 infection in different states. Incidence of 7.6% in goats in Karnataka (6), 18% in goats in
124 Delhi, 16% in goats in Punjab and 30% in goats in Rajasthan (7), 55% in goats in Andhra
125 Pradesh (8) and 24% in goats in Uttar Pradesh (9) have been reported earlier.
126 In a study (10) in Maharashtra an overall prevalence of 7.32% was reported in goats. In a
127 study in Gujarat state the prevalence in goats was reported to be 8.80% by I-ELISA,
128 11.30% by RBPT and 11.10% by STAT (11).

129 In a study on caprine Brucellosis in Wayanad district of Kerala, a total of 24 out of 420
130 sera were positive by the RBPT with a seroprevalence of 5.7%. Upon STAT testing, 18
131 out of 24 RBPT positive samples (4.3%) showed the presence of *Brucella* antibodies
132 (12). Of the 420 sera examined, 24 (5.7%) were seropositive by RBPT, out of which only
133 18 (4.3%) reacted positively to STAT.

134 The prevalence of Brucellosis in Himachal Pradesh has been reported to be 16% among
135 goats which is higher than our figures. In a study conducted in Maharashtra (13), the

prevalence was recorded to be 7.32% in goats. Kerala had the least seroprevalence of 5.7% in goats (14). In a national survey conducted on Brucellosis in goats from 10 states (15), the cumulative incidence in goats was 2.2%. This survey indicated widespread prevalence of Brucellosis in small domestic ruminants in the country.

Conclusions

The present study revealed the prevalence of Brucellosis in goats in Ludhiana district of Punjab to be 5.23% by RBPT and ELISA.

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190 **Table 1: Goat sera positive for Brucellosis by one or more serological tests**

S. no.	Animal no.	RBPT	STAT	MAT	ELISA
1	G A56sm	-ve	-ve	+ve	-ve
2	G12	-ve	-ve	+ve	-ve
3	GM019	-ve	-ve	+ve	-ve
4	GM66	-ve	+ve	-ve	-ve
5	G A48sm	-ve	+ve	Strong +ve	-ve
6	Ga 41	-ve	-ve	+ve	-ve
7	G b36	weak +ve	+ve	+ve	-ve
8	GB19	+ve	+ve	+ve	+ve
9	GA 22sm	+ve	+ve	+ve	+ve
10	GM64	strong +ve	+ve	+ve	+ve
11	Gk 27	+ve	+ve	+ve	+ve

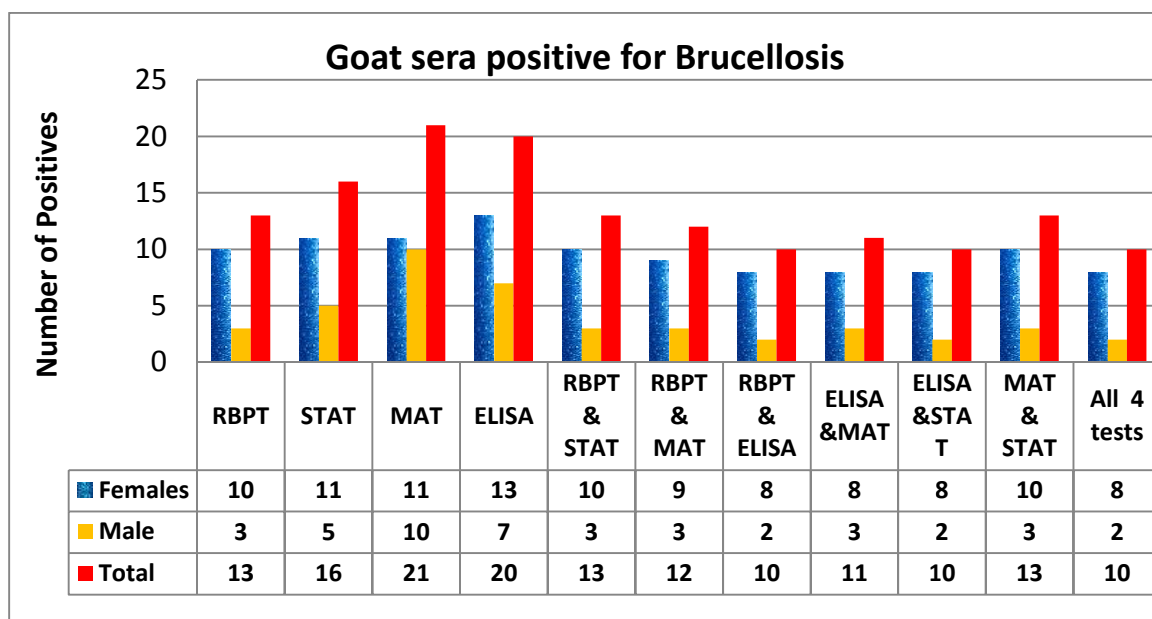
12	GM027	+ve	+ve	-ve	+ve
13	GA 18	+ve	+ve	+ve	+ve
14	BSD60	+ve	+ve	+ve	-ve
15	KNG R 1	-ve	-ve	-ve	+ve
16	KNG 12	-ve	-ve	-ve	+ve
17	KNG 10	-ve	-ve	-ve	+ve
18	KNG R 6	-ve	-ve	+ve	+ve
19	KNG 11	-ve	-ve	-ve	+ve
20	KNGRK4	-ve	-ve	-ve	+ve
21	KNG 18	-ve	-ve	-ve	+ve
22	GRK 6	-ve	-ve	-ve	+ve
23	GRK9	-ve	+ve	+ve	+ve
24	GRK 1	-ve	-ve	-ve	+ve
25	G A65sm	strong +ve	+ve	+ve	+ve
26	G A28sm	-ve	-ve	+ve	-ve
27	GA19	weak +ve	+ve	+ve	-ve
28	G A27sm	strong+ve	+ve	Strong +ve	+ve
29	G 9ABsm	strong +ve	+ve	+ve	+ve
30	GI8	-ve	-ve	+ve	-ve
31	Ga 36sm	+ve	+ve	+ve	+ve

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196 **Figure 1: Goat sera positive for Brucellosis by different serodiagnostic tests**

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199 **Table 2: Titers of anti-Brucella antibodies in goat sera by STAT and MAT**

Titer (I. U.)			
S. no.	Animal no.	STAT titer	MAT titer
1	GA56 sm	20	40
2	G12	20	40
3	GA 10 sm	20	20
4	GM019	20	40
5	GM 66	40	20
6	GA 48 sm	40	80
7	Ga 41	20	40

8	Gb 36	40	40
9	GB19	80	80
10	GA22 sm	40	40
11	GM64	40	40
12	Gk 27	40	40
13	GM027	40	20
14	GA18	40	40
15	BSD 60	80	160
16	KNG R 6	20	40
17	GRK9	40	40
18	GA65 sm	40	40
19	GA 28 sm	20	40
20	GA19	40	40
21	GA27 sm	40	80
22	G9ABsm	40	40
23	Gl8	20	40
24	GA36 sm	40	80
Mean $\pm$ SD		36.66 $\pm$ 16.32	49$\pm$ 29.47

200 Difference between the two means is not significant.

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204 **Table 3: Sensitivity and specificity of agglutination tests compared to C-ELISA**

Computed Value	RBPT	STAT	MAT
True Positives	10	11	10
False Positives	3	4	10
False Negatives	10	9	9
True Negatives	168	166	161
Sensitivity	50.00%	55.00%	52.63%
Specificity	98.25 %	97.65 %	94.15 %
Positive Likelihood Ratio	28.50	23.37	9.00
Negative Likelihood Ratio	0.51	0.46	0.50
Disease prevalence	10.47%	10.53%	10.00%
Positive Predictive Value	76.92%	73.33%	50.00%
Negative Predictive Value	94.38 %	94.86 %	94.71 %

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