

FREQUENCY OF OCCURANCE OF BRUCELLOSIS IN GOATS IN LUDHIANA DISTRICT OF PUNJAB STATE OF INDIA

ABSTRACT

We investigated the frequency of Brucellosis in goats in Ludhiana district of Punjab state of India because it is of great public health concern yet there is hardly any report in this regard from this region recently. 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. Frequency of Brucellosis in goats in Ludhiana was found to be 5.23% by RBPT and ELISA. This is alarming considering the zoonotic potential of the disease, handling of raw milk and goat mutton and consumption of goat cheese in this region. 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 by 't' test. Thus, Brucellosis is prevalent in a small proportion of goat population in Ludhiana and needs regular monitoring to enable effective measures for its control.

Key words: Brucellosis, goats, frequency, epidemiology, MAT, competitive ELISA.

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. Brucellosis is endemic in India

24 and is prevalent in all parts of the country (1). The causative agent of Brucellosis in goats
25 is *Brucella melitensis* (2). In a study conducted in various districts of Punjab in 2000, the
26 overall prevalence of Brucellosis was found to be 3.60% in goats (3). The incidence of
27 Brucellosis was reported to be 5% in goats, in Sangrur district of Punjab, India (4).
28 However, no recent information is available on the prevalence of the disease in goats in
29 other districts of Punjab, particularly Ludhiana district. Humans are exposed to
30 Brucellosis by handling raw milk and meat from infected goats and animals get infected
31 by direct contact with them or through common attendants. Mutton is consumed in large
32 quantity in Ludhiana and goats are brought here for slaughter from nearby states also.
33 Brucellosis in animals is endemic in India in general and Punjab state in particular.
34 The present study was undertaken to determine the frequency of Brucellosis in goats in
35 Ludhiana district of Punjab state of India employing the common serodiagnostic methods
36 – Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT),
37 Microagglutination Test (MAT) and Competitive Enzyme Linked Immunosorbant Assay
38 (cELISA). Four techniques were employed to compare their efficacy with each other in
39 diagnosis. Samples positive by two different techniques (agglutination viz RBPT, and
40 ELISA) were considered positive taking into account body's immune response to both
41 particulate and soluble antigens simultaneously.

42 **Materials and methods**

43 The guidelines of Institutional Animal Ethics Committee of GADVASU, Ludhiana were
44 followed throughout the study.

45 **Location:** Ludhiana is the most centrally located district in the Malwa region of the State
46 of Punjab between North Latitude 30°-34' and 31°-01' and East longitude 75°-18' and 76°-

20. It is bounded on the north by River Sutlej which separates it from Jalandhar district and Hoshiarpur district. It is flanked by Rupnagar district in the East, Moga district in the West, and Sangrur and Patiala districts in the South and South East, respectively. The District is an Alluvial plain and can be divided into the flood plain of the Sutlej, and the up land plain. The climate of the district is characterized by dryness except a brief spell of monsoon season between a very hot summer and a bracing winter. Total geographical area of the district is 3767 Sq. Kms. The total human population of the district as per 2011 census is 34,98,739. According to 19th Indian livestock census – 2012, the Punjab state has 327272 goats out of which 23924 goats are in Ludhiana district (5).

Serum samples: Blood samples (5 ml each) from jugular vein were collected over a year in 2013 from adult goats from five slaughter houses and meat shops located at different representative regions in or around Ludhiana. These were big shops / slaughter houses and animals from nearby districts were also brought. Serum was separated from clotted blood and stored at -20°C till use.

Seroepidemiological studies on caprine Brucellosis:

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

Rose Bengal Plate Agglutination Test (RBPT)

RBPT was carried out by colored antigen with the method of Morgan *et al.* (6). 10 µl of antigen was mixed with 10 µl of serum and observed for clumps as positive.

Estimation of antibody titers by Standard Tube Agglutination Test (STAT): The standard method recommended by OIE (7) was followed. *Brucella abortus* plain antigen (Punjab Veterinary Vaccine Institute, Ludhiana) was employed for the test. The highest

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

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

73 MAT was performed as per the standard method (8) with suitable minor adaptation. A
74 microtiter plate was appropriately labeled and 80 μ L of 0.85% normal saline was added
75 to the first row and 50 μ L to the rest of the rows. To each well of the first row was added
76 20 μ L of a serum sample. The contents in the first row, i.e. the serum and saline, were
77 thoroughly mixed and 50 μ L of this mixture was transferred to the corresponding well in
78 the second row. The process was repeated until the last row. From the last row 50 μ L of
79 the mixed contents was discarded. This was followed by addition of 50 μ L of plain
80 antigen to each well. The microtiter plate was incubated at 37 °C for 24 h before the
81 results were read. Controls were run using known positive and known negative sera. The
82 formation of matt signified agglutination while button formation was indicative of a
83 negative reaction. Titers were recorded as the reciprocal of the highest dilution of the
84 serum giving at least 50 percent agglutination. The titer was expressed in the unit system
85 by doubling of the serum titer as International Units (I.U.) per milliliter of serum.

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

87 All the serum samples were subjected to analysis by competitive ELISA employing a
88 commercial kit Svanovir Brucella Antibody cELISA Kit (Svanova). The kit is based on a
89 solid phase competitive ELISA. It is subjected to treatment by with a monoclonal
90 antibody (mAb) for the o-polysaccharide portion of the S-LPS antigen, are exposed to
91 *Brucella abortus* smooth lipopolysaccharide (S-LPS) coated wells on microtiter plates. If
92 *Brucella* antibodies are present in the test sample they will bind to the antigens in the well

93 and block these antigenic sites. If *Brucella* antibodies are absent in the sample, these sites
94 will remain free and the monoclonal antibody which was added together with the sample
95 will bind to these free antigenic sites. After an incubation period the unbound materials
96 are removed by rinsing and a goat anti- mouse IgG conjugated with horseradish
97 peroxidase (HRP) is added to the plate. The HRP conjugate will bind to the specific mAb
98 in absence of *Brucella* antibodies in the sample. Unbound materials are removed by
99 rinsing prior to the addition of the substrate. Development of a blue color shows negative
100 result. The reaction is stopped by addition of stop solution; the color changes to yellow.
101 The result is read at 450 nm.
102 The samples were run in duplicates. The optical density (OD) of the controls and samples
103 was measured at 450 nm with photometer. After 15 minutes we measured OD after the
104 addition of Stop Solution to prevent fluctuation in OD values.
105 Calculations: 1. The mean OD values were calculated for each of the controls and
106 samples.
107 2. The percent inhibition (PI) values were calculated using the following formula:
108
$$PI = 100 - (OD \text{ of samples or control} \times 100) / OD \text{ of conjugate control}$$

109 **Statistical analysis**

110 The results were analyzed by ANOVA and students' 't' test using online software.
111 ELISA was used as a gold standard. Samples positive by ELISA were considered as true
112 positives while those negative by ELISA but positive by another test were considered as
113 false positives (9). MAT was not considered as the gold standard because agglutination
114 tests detect antibodies to particulate antigens only and not soluble antigens. Sensitivity
115 and specificity were calculated using the formulae given below:

116 Sensitivity: $A/(A+C) \times 100$

117 Specificity: $D/(D+B) \times 100$

118 Where A = True positive, B = False positive, C = False negative and D = True negative.

119 Results

120 Seroepidemiology of Brucellosis in goats

121 This study aimed to find out the frequency of occurrence of Brucellosis in goats in
122 Ludhiana district of Punjab state of India using RBPT, STAT, MAT and C-ELISA. A
123 total of 191 samples from goats were subjected to analysis with four different
124 serodiagnostic tests i.e. RBPT, STAT, MAT and ELISA to identify Brucellosis affected
125 animals. Out of 191 samples, 31 were found to be positive by one or more tests (Table 1).
126 A total of 160 serum samples were found to be negative by all the four serodiagnostic
127 methods. Among the positive samples (Figure 1), 14 were found to be positive by RBPT,
128 17 by STAT, 21 each by MAT and ELISA. ELISA and MAT detected the highest
129 number of samples followed by RBPT and STAT, respectively. Some samples were
130 positive by two serologic methods with other two tests being negative. Ten samples were
131 positive by both RBPT and ELISA. Ten samples were positive by all the four tests i.e.
132 RBPT, STAT, MAT and ELISA. Frequency of occurrence of the disease in goats was
133 estimated to be 5.23% by RBPT and ELISA.

134 The mean titers of anti –Brucella antibodies in goat sera were 36.66 ± 16.32 by STAT
135 and 49.16 ± 29.47 by MAT (Table 2), the difference was non – significant by 't' test.

136 Discussion

137 The results of our study indicate that caprine Brucellosis is a major problem of concern in
138 Ludhiana district of Punjab state of India. Based on various serologic analyses on 191

139 serum samples of goats collected from slaughter houses and local small meat shops in
140 Ludhiana, the frequency of occurrence of the disease was estimated to be 5.23% by
141 RBPT and ELISA, respectively. This finding is important because currently goats are not
142 vaccinated for Brucellosis in this part of the country and treatment of infected goats is
143 neither practiced nor economic. The disease causes great economic losses and is a public
144 health hazard.

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

149 Maximum false positive results were obtained by MAT (7.53%), followed by RBPT
150 (6.03%), ELISA (5.02%) and STAT (2.01%), respectively. Highest number of false
151 negatives were obtained with MAT (6.03%) followed by ELISA (5.52%), RBPT and
152 STAT (5.02% each), respectively. It is evident from the above results that STAT yielded
153 highest numbers of true positives and true negative results while MAT gave highest
154 numbers of false positives and false negative results. Reports on such comparative studies
155 are lacking in the available literature. STAT was found to have the highest sensitivity
156 (59.09%) followed by MAT (54.55%), RBPT (52.38%) and ELISA (50%), respectively.
157 STAT had the highest specificity (97.73%) followed by ELISA (94.32%), RBPT
158 (93.41%) and MAT (92.98%), respectively. The cELISA has been reported earlier to
159 have a sensitivity of 97.75% and specificity of 90.5%, respectively (10).

160 The frequency of occurrence of the disease was estimated to be 11.40% by MAT, 11.11%
161 by STAT and ELISA, and 10.34% by RBPT, respectively. RBPT was found to have the

162 highest positive predictive value of 76.92% followed by STAT (73.33%) and MAT
163 (50%), respectively. STAT was found to have the highest negative predictive value of
164 94.86% followed by MAT (94.71%) and RBPT (94.38%), respectively. RBPT had the
165 highest positive likelihood ratio of 28.50 while MAT had the lowest value of 9.00. RBPT
166 had the highest negative likelihood ratio of 0.51 while STAT had the lowest value of 0.46
167 (Table 3). It has emerged that one single serodiagnostic method may not be reliable and
168 to get a more accurate diagnosis, a combination of RBPT and ELISA may be
169 recommended.

170 Brucellosis is endemic in India and is prevalent in all parts of the country. Several
171 serological surveys of small ruminant Brucellosis have indicated varying levels of
172 infection in different states. Incidence of 7.6% in goats in Karnataka (11), 18% in goats in
173 Delhi, 16% in goats in Punjab and 30% in goats in Rajasthan (12), 55% in goats in
174 Andhra Pradesh (13) and 24% in goats in Uttar Pradesh (14) have been reported earlier.
175 In a study (15) in Maharashtra an overall prevalence of 7.32% was reported in goats. In a
176 study in Gujarat state the prevalence in goats was reported to be 8.80% by I-ELISA,
177 11.30% by RBPT and 11.10% by STAT (16).

178 In a study on caprine Brucellosis in Wayanad district of Kerala, a total of 24 out of 420
179 sera were positive by the RBPT with a seroprevalence of 5.7%. Upon STAT testing, 18
180 out of 24 RBPT positive samples (4.3%) showed the presence of *Brucella* antibodies
181 (17). Of the 420 sera examined, 24 (5.7%) were seropositive by RBPT, out of which only
182 18 (4.3%) reacted positively to STAT.

183 The prevalence of Brucellosis in Himachal Pradesh has been reported to be 16% among
184 goats which is much higher than our figures. In a study conducted in Maharashtra (18),

185 the prevalence was recorded to be 7.32% in goats. Kerala had the least seroprevalence of
186 5.7% in goats (19). In a national survey conducted on Brucellosis in goats from 10 states
187 (1), the cumulative incidence in goats was 2.2% indicating widespread prevalence of
188 Brucellosis in small domestic ruminants in the country.

189 Our study has yielded data that shows the frequency is much higher than the national
190 level figures, comparable to some states like Kerala, Karnataka and Maharashtra but far
191 less than other states like Gujarat, Delhi, Rajasthan, Andhra Pradesh and Uttar Pradesh.

192 **Conclusions**

193 The present study revealed the frequency of occurrence of Brucellosis in goats in
194 Ludhiana district of Punjab to be 5.23% by RBPT and ELISA. This is alarming
195 considering the zoonotic potential of the disease and high consumption of mutton and
196 milk in this region. Our result is different from the figures reported from several other
197 states which could perhaps be due to the combination of tests relied upon instead of a
198 using a single serodiagnostic test or due to different animal husbandry practices adopted
199 in different places.

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254 **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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256

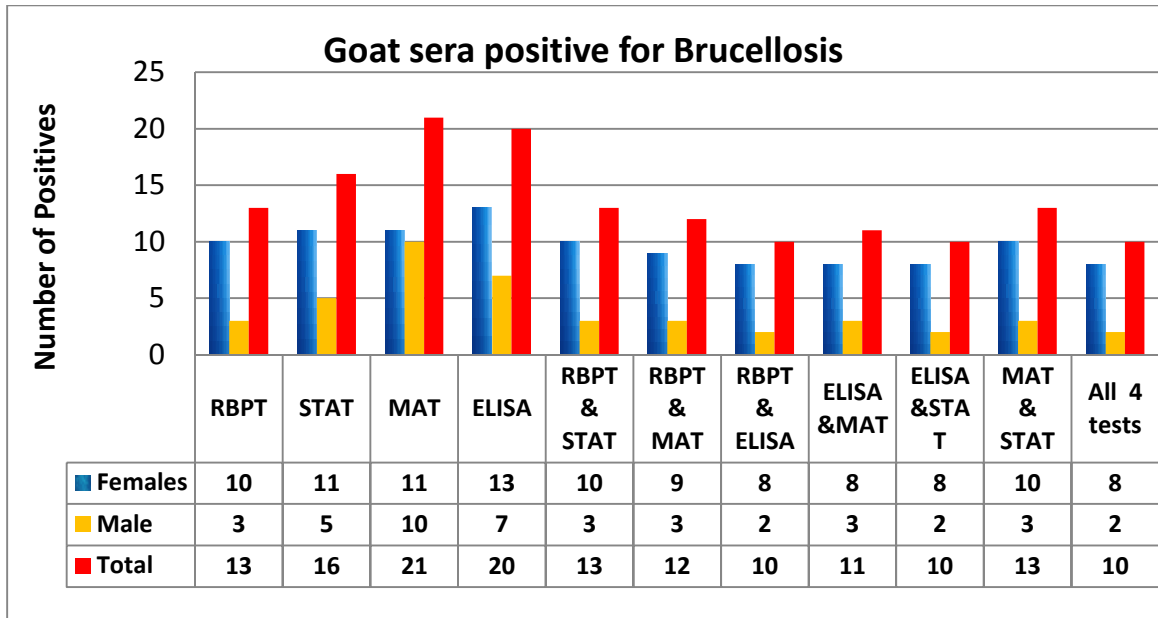


Figure 1: Goat sera positive for Brucellosis by different serodiagnostic tests

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

Frequency	STAT titer	MAT titer
7	20	40
1	20	20
2	40	20
3	40	80
9	40	40
1	80	80
1	80	160
Mean±SD	36.66 ± 16.32	49± 29.47

274 Difference between the two means is not significant by 't' test.

275 **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

276