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Frequency of Occurance of Brucellosis in Goats in Ludhiana District of Punjab State of India

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Authors' contributions

This work was carried out in collaboration between all authors. Author BBS designed the study. Author NS performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NS, BBS and JPSG managed the analyses of the study. Author RSA managed the literature searches. All authors read and approved the final manuscript.

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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.

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1. 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 and is prevalent in all parts of the country [1]. The causative agent of Brucellosis in goats is *Brucella melitensis* [2]. In a study conducted in various districts of Punjab in 2000, the overall prevalence of Brucellosis was found to be 3.60% in goats [3]. The incidence of Brucellosis was reported to be 5% in goats, in Sangrur district of Punjab, India [4]. However, no recent information is available on the prevalence of the disease in goats in other districts of Punjab, particularly Ludhiana district. Humans are exposed to Brucellosis by handling raw milk and meat from infected goats and animals get infected by direct contact with them or through common attendants. Mutton is consumed in large quanity in Ludhiana and goats are brought here for slaughter from nearby states also. Brucellosis in animals is endemic in India in general and Punjab state in particular.

The present study was undertaken to determine the frequency of Brucellosis in goats in Ludhiana district of Punjab state of India employing the common serodiagnostic methods – Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT), Microagglutination Test (MAT) and Competitive Enzyme Linked Immunosorbant Assay (cELISA). Four techniques were employed to compare their efficacy with each other in diagnosis. Samples positive by two different techniques (agglutination viz RBPT, and ELISA) were considered positive taking into account body's immune response to both particulate and soluble antigens simultaneously.

2. MATERIALS AND METHODS

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

2.1 Location

Ludhiana is the most centrally located district in the Malwa region of the State 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].

2.2 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℃ till use.

2.3 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.

2.4 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.

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

2.6 Estimation of Antibody Titers by Microtiter Agglutination Test (MAT)

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

2.7 Competitive Enzyme Linked Immunosorbant Assay (cELISA)

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

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

Calculations:

1. The mean OD values were calculated for each of the controls and samples.

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

PI = 100 – (OD of samples or control x 100) / OD of conjugate control.

2.8 Statistical Analysis

The results were analyzed by ANOVA and students' 't' test using online software.

ELISA was used as a gold standard. Samples positive by ELISA were considered as true positives while those negative by ELISA but positive by another test were considered as false positives [9]. MAT was not considered as the gold standard because agglutination tests detect antibodies to particulate antigens only and not soluble antigens. Sensitivity and specificity were calculated using the formulae given below:

Sensitivity: A/(A+C) × 100
Specificity: D/(D+B) × 100

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

3. RESULTS

3.1 Seroepidemiology of Brucellosis in Goats

This study aimed to find out the frequency of occurrence of Brucellosis in goats in Ludhiana district of Punjab state of India using RBPT, STAT, MAT and C-ELISA. A total of 191 samples from goats were subjected to analysis with four different serodiagnostic tests i.e. RBPT, STAT, MAT and ELISA to identify Brucellosis affected animals. Out of 191 samples, 31 were found to be positive by one or more tests (Table 1). A total of 160 serum samples were found to be negative by all the four serodiagnostic methods. Among the positive samples (Fig. 1), 14 were found to be positive by RBPT, 17 by STAT, 21 each by

MAT and ELISA. ELISA and MAT detected the highest number of samples followed by RBPT and STAT, respectively. Some samples were positive by two serologic methods with other two tests being negative. Ten samples were positive by both RBPT and ELISA. Ten samples were positive by all the four tests i.e. RBPT, STAT, MAT and ELISA. Frequency of occurrence of the disease in goats was estimated to be 5.23% by RBPT and ELISA.

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

4. DISCUSSION

The results of our study indicate that caprine Brucellosis is a major problem of concern in Ludhiana district of Punjab state of India. Based on various serologic analyses on 191 serum samples of goats collected from slaughter houses and local small meat shops in Ludhiana, the frequency of occurrence of the disease was estimated to be 5.23% by RBPT and ELISA, respectively. This finding is important because currently goats are not vaccinated for Brucellosis in this part of the country and treatment of infected goats is neither practiced not economic. The disease causes great economic losses and is a public health hazard.

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

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	Gl8	-ve	-ve	**+ve**	-ve
31	Ga 36sm	**+ve**	**+ve**	**+ve**	**+ve**

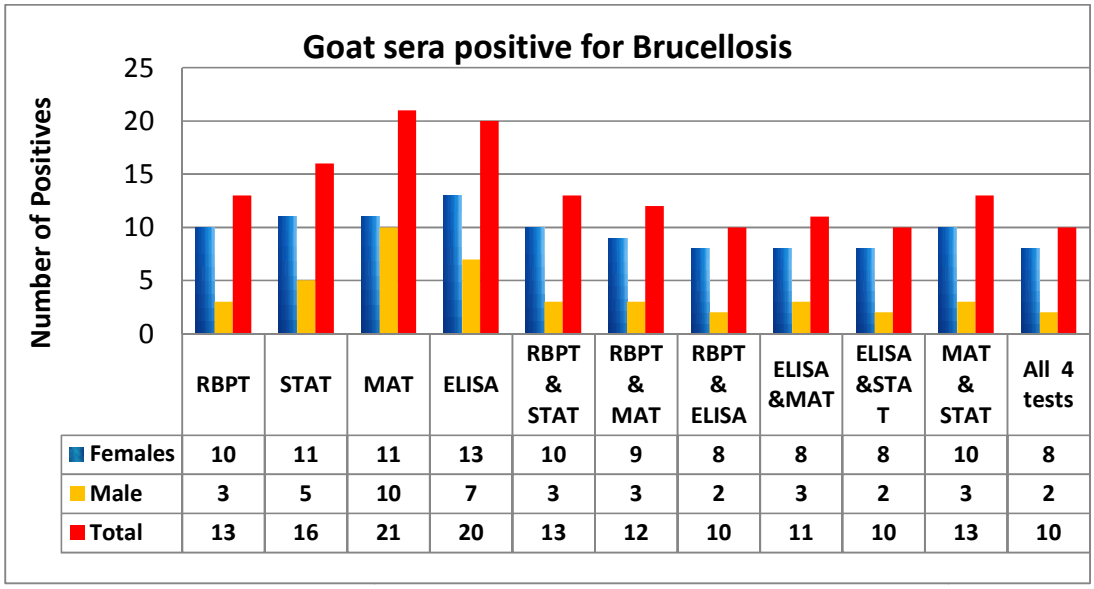

Fig. 1. Goat sera positive for Brucellosis by different serodiagnostic tests

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**

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

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

The frequency of occurrence of the disease was estimated to be 11.40% by MAT, 11.11% by STAT and ELISA, and 10.34% by RBPT, respectively. RBPT was found to have the highest positive predictive value of 76.92% followed by STAT (73.33%) and MAT (50%), respectively. STAT was found to have the highest negative predictive value of 94.86% followed by MAT (94.71%) and RBPT (94.38%), respectively. RBPT had the highest positive likelihood ratio of 28.50 while MAT had the lowest value of 9.00. RBPT had the highest negative likelihood ratio of 0.51 while STAT had the lowest value of 0.46 (Table 3). It has emerged that one single serodiagnostic method may not be reliable and to get a more accurate diagnosis, a combination of RBPT and ELISA may be recommended.

Brucellosis is endemic in India and is prevalent in all parts of the country. Several serological surveys of small ruminant Brucellosis have indicated varying levels of infection in different states. Incidence of 7.6% in goats in Karnataka [11], 18% in goats in Delhi, 16% in goats in Punjab and 30% in goats in Rajasthan [12], 55% in goats in Andhra Pradesh [13] and 24% in goats in Uttar Pradesh [14] have been reported earlier.

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

In a study [15] in Maharashtra an overall prevalence of 7.32% was reported in goats. In a study in Gujarat state the prevalence in goats was reported to be 8.80% by I-ELISA, 11.30% by RBPT and 11.10% by STAT [16].

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

The prevalence of Brucellosis in Himachal Pradesh has been reported to be 16% among goats which is much higher than our figures. In a study conducted in Maharashtra [18], the prevalence was recorded to be 7.32% in goats. Kerala had the least seroprevalence of 5.7% in goats [19]. In a national survey conducted on Brucellosis in goats from 10 states [1], the cumulative incidence in goats was 2.2% indicating widespread prevalence of Brucellosis in small domestic ruminants in the country.

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

5. CONCLUSIONS

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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