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Seroprevalence of brucellosis in ovines of Ganderbal district of Kashmir Valley

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Abstract

The present study was carried out on ovines of four administrative blocks of Ganderbal i.e., Lar, Ganderbal, Wakura and Kangan from May 2016 to July 2017. A multi-stage simple random sampling technique was used to determine sample size. The total sample size as calculated turned out to be 223 sheep. Samples of blood in clot activator tubes were collected and serum was harvested. The Microagglutination test (MAT) was performed in 96-well U-shaped microplates. The overall prevalence of ovine brucellosis was 35.87%. Prevalence was non-significantly higher in females (37.82%) having 1.99 times greater odds than males (23.33%). Age-wise prevalence was significantly higher in sheep >2 years of age. The sheep aged >2 years had 2.35 times greater odds (80.6/34.21) of having a higher prevalence of brucellosis than sheep aged < 2 years. The results of this study suggest a high prevalence of ovine brucellosis in this region, whose economic effect has not been investigated till date.

Keywords: Brucellosis, microagglutination test, prevalence, serum tube agglutination test

1. Introduction

Small ruminants play a pivotal role in rural economy in developing countries like India. Their importance in income generation and households' social and financial security becomes evident by its substantial contribution of around ₹ 24,000 million annually to the rural economy and ₹ 80,000 million to national economy of India [1]. Their importance is even greater in hill and mountain areas like Jammu and Kashmir where it provides means of sustainable production and food security [2].

Brucellosis in sheep is a serious problem in many parts of the world particularly in developing countries where the disease is prevalent but vaccination is not practiced. Prevalence rate of 1.2-2.6% has been reported by various authors from different parts of the world [3], however in India 6.3-7.9% has been reported [4, 5]. Brucellosis may cause considerable economic losses in small and marginal farmers of Jammu and Kashmir.

Screening for Brucellosis in sheep like most species is done using serological tests viz., Rose Bengal plate agglutination test, microagglutination test and enzyme linked immunosorbent assays however the presence of pathogen in host is confirmed by polymerase chain reaction [6,7] as isolation of *Brucella* species require biosecurity level 2 laboratories.

In places where brucellosis is endemic, humans can get infected via contact with infected animals or consumption of their products, mostly milk and milk products especially cheese made from unpasteurized milk of sheep and goats [8]. Some specific occupational groups including farm workers, veterinarians, ranchers, and meat packing employees are considered at higher risk [9]. Jammu and Kashmir is one of the highest mutton consuming states of India and hence the chances of zoonosis are higher as compared to other parts of India. However no systemic study has been conducted regarding prevalence of ovine brucellosis in J & K. Brucellosis is emerging as a serious concern in last few years in J & K as has been reported by several unpublished outbreaks and a non-systemic study [4]. Keeping in view, the above facts, the present study was conducted to assess the current status of ovine brucellosis in one of the important sheep rearing areas of the valley i.e. Ganderbal district, using Microagglutination test.

2. Materials and Methods

2.1 Study design

The present study was carried out in Ganderbal district of Kashmir valley from May 2016 to July 2017. Animals were selected randomly from four administrative blocks of Ganderbal i.e., Lar, Ganderbal, Wakura and Kangan, the number of animals selected from each block being proportionate to percentage of ovine population they hold. For determining prevalence a multi-stage simple random sampling technique was used with single animal as the epidemiological unit of concern, to draw a simple random sample from ovines of Ganderbal district.

The total sample size was calculated as described by Thrushfield [10]. The sample size was based on the following parameters: 95.0% level of confidence, $\pm 5\%$ desired level of precision and the expected prevalence of ovine brucellosis of 6.5 % [4]. By using the following formula:

$$n = Z^2 \times p(1-p)/d^2$$

Where: n = required sample size p = expected prevalence (25 %) d = desired absolute precision (5%) and for 95% confidence interval Z will be taken as 1.96, the sample size required to determine the prevalence was 203 animals. A correction fraction of 10% was included to account for the effect of randomness and representativeness in multistage sampling strategy [10]. Thus, total sample size of 223 was selected for prevalence study.

2.2 Collection of samples

Samples of blood in clot activator tubes were collected from

selected animals. The samples were immediately transported on ice packs to the laboratory. The serum was harvested from clot activator tubes. A total of 223 serum samples were collected from 23 small ruminant farms of the Ganderbal district. (Table 1). The sheep breed reared in the region is indigenously developed Kashmir merino (cross of delaine merino and local Poonchi, Gaddi and Bhakerwal). The animals are reared intensively in winter months and transhumance takes places in months of June to October.

Table 1: Number of samples collected from various Blocks.

S. No.	Block	Serum samples	No. of farms
1.	Ganderbal	83	8
2.	Lar	66	6
3.	Zazna	28	4
4.	Kangan	46	5

2.3 Serological diagnosis

The Microagglutination test (MAT) was performed with a commercial Brucella plain antigen as described previously [11], in 96-well U-shaped microplates. Serial two-fold dilutions of the sera were made in saline from 1:10 and to 1:1280. The MAT was performed by incubating the sera at 37 °C for 24 hours. Appropriate positive and negative serum controls were used for all tests as well as for controls. Dilutions of sera from 1:5 to 1:640 were made directly in micro titer plates and antibody titers of $\geq 1:20$ in MAT were considered infective (Fig. 1).

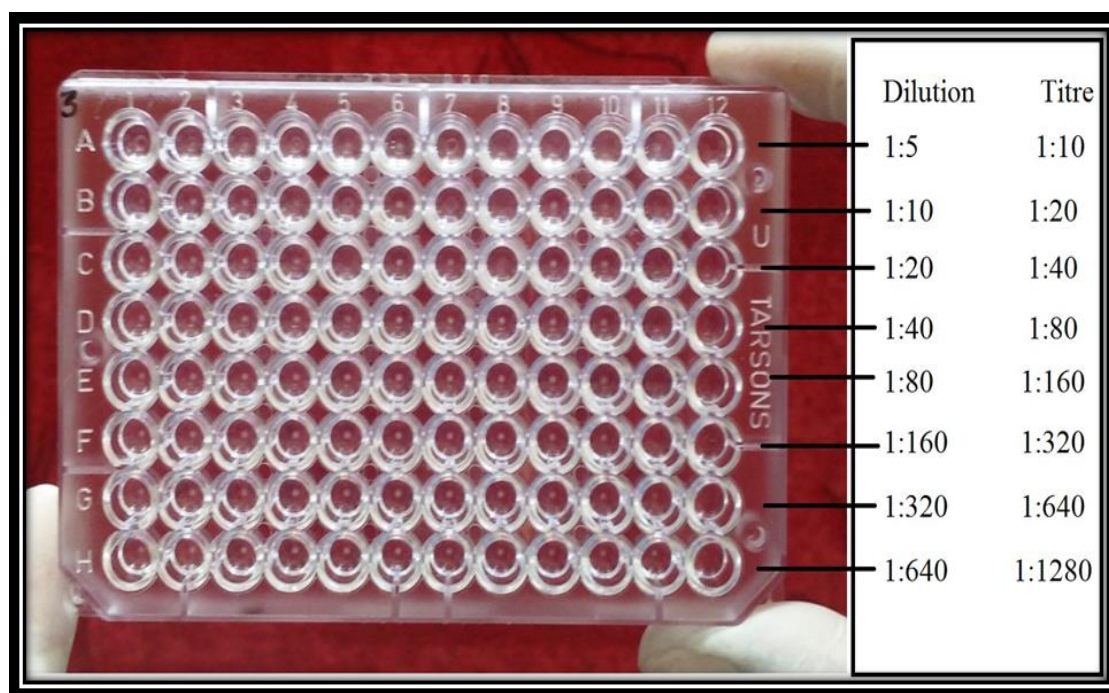


Fig 1: Serial dilution in micro plates (U-Bottom).

2.4 Statistical Analysis

The categorical data were presented as frequency and/or percentage. Chi square test was used for comparisons between binary outcome and binary explanatory variable. The prevalence was compared between two sexes and three age groups. For all statistical procedures a value of $P < 0.05$ was considered significant.

3. Results

A total of 223 sera samples were screened by MAT for prevalence of ovine brucellosis. The age of the screened sheep ranged from one to 8 years. Out of 223 sheep, 30 were male and 193 were female. The overall prevalence of ovine brucellosis 35.87% (80/223), being non-significantly higher in females (37.82%) than males (23.33%) (Table 2). Female sheep had 1.99 times greater odds (60.83/30.43) of having a higher prevalence of brucellosis than male sheep.

Table 2: Prevalence of brucellosis in male and female sheep.

Sex	Brucellosis positive	Brucellosis negative	Total	Odds	
Male sheep	7 (23.33)	23 (76.67)	30 (100)	7/23=30.43	p=0.49
Female sheep	73 (37.82)	120 (62.18)	193 (100)	73/120=60.83	
Total	80 (35.87)	143 (64.13)	223 (100)		

The numbers in parenthesis represent row percentages

The prevalence was significantly higher in sheep aged > 2 years of age (Table 3). The sheep aged > 2 years had 2.35 times greater odds

(80.6/34.21) of having a higher prevalence of brucellosis than sheep aged < 2 years.

Table 3: Prevalence of brucellosis in different age groups of sheep.

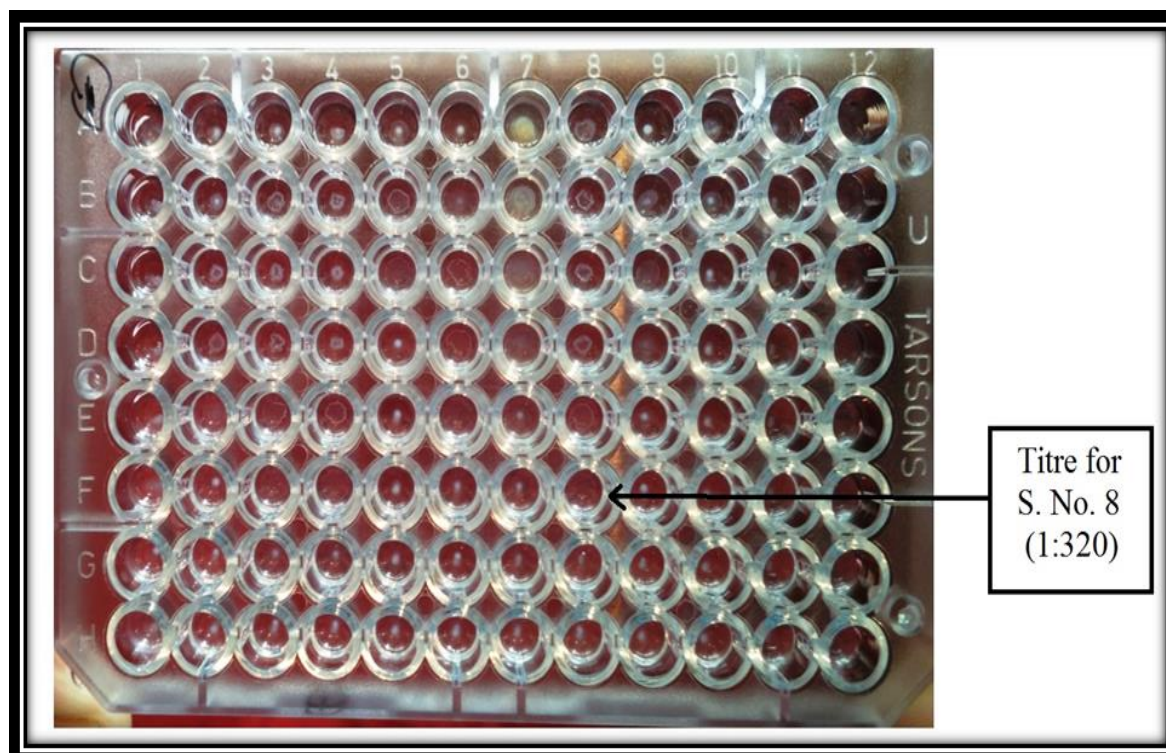
Age	Brucellosis positive	Brucellosis negative	Total	Odds	
≤ 2years	26 (25.49)	76 (74.51)	102(100)	26/76=34.21	p=0.031
> 2 years	54 (44.63)	67 (55.37)	121 (100)	54/67=80.60	
Total	80 (35.87)	143 (64.13)	223 (100)		

The numbers in parenthesis represent row percentages

4. Discussion

Rose Bengal plate agglutination test (RBPT) is the commonly employed method for diagnosis of brucellosis in animals. However RBPT cannot differentiate between infective and non-infective titers. So MAT was employed in the current

study for determining the prevalence of brucellosis in sheep. Antibody titers of $\geq 1:20$ in MAT are infective and it is less time consuming and requires less antigen and serum than the Standard Agglutination Test ^[11] (Fig. 2).

**Fig 2:** Agglutination in micro plate (U-Bottom).

To our best knowledge, this is the first systemic study to describe prevalence of ovine brucellosis in Kashmir valley, so the required sample size was calculated by using standard epidemiological method. The sample size of this study meant that we selected a random sample of 223 from an infinite population of sheep, and determined that 10% of subjects had the factor of interest; we were 95% confident that between 5% and 15% of subjects in the population had the factor of interest. The prevalence of ovine brucellosis in this study was much higher than the expected, and we suggest that brucellosis in sheep of this region occurs frequently, without noticeable clinical symptoms. The earlier literature indicates a low prevalence of 6.5% brucellosis in sheep of Kashmir valley ^[4]. The prevalence of ovine brucellosis varies in different studies and is hard to compare due to differences in the examined sample size and study design. We believe that

the findings of this study are true representative of sheep in this region because the sample size was calculated by standard method.

Agreeing to findings of present study, Kumar *et al.*, ^[12] also reported incidence of sheep brucellosis was 50% in Punjab and 32.73% in Rajasthan. Also Hawari ^[13] screened 620 sheep sera from 15 flocks and 145 goats sera from 5 flocks and reported true prevalence of *Brucella* seropositive in sheep was 21.1% and in goats was 24.6%. However, contrasting to findings of present study, Sharma *et al.*, ^[14] studied seroprevalence of brucellosis in sheep from the Mehsana and Patan districts of Gujarat, India and reported a lower prevalence rate of 20.45% in Mehsana district and 10.41% in Patan district. Suryawanshi *et al.*, ^[15] also reported a prevalence rate 7.32% which also was lower than present study. This could be attributed to practice of raising sheep in

highland communal pastures during summer months which provides a platform for spread of brucellosis in sheep of various flocks. Agreeing to this finding Mekonnen *et al.*,^[16] opined that as brucellosis is contagious in nature sharing grazing land and drinking water facilitates transmission of the disease. Another study by Mir *et al.*,^[2] also reported a contrastingly lower overall prevalence of 10.23% by RBPT and 8.83% by STAT of brucellosis in Changra goats.

Among various risk factors studied, communal pastures, large flock size, high stocking density, sharing water facilities, age > 2 yrs, addition of new animals, contact with other sheep flocks and lending lambs during breeding season have been found to be associated with spread of brucellosis in sheep^[17, 18]. However, in this preliminary study these risk factors were not taken into consideration except age and sex and it was found that age and not sex may be an important risk factor for ovine brucellosis in Kashmir valley.

Abdallah *et al.*,^[19] investigated potential individual risk factors for brucellosis in sheep and found that with exception of age none of the other individual or management risk factors had an effect on the occurrence of brucellosis in sheep which was in agreement with findings of present study. Also agreeing with results of present study, Sulima *et al.*,^[20] reported that seroprevalence of brucellosis was highest in 2-3 years (27.9%) age group followed by more than 3 years (19.2%) and less in 1-2 years (14.2%) age group. In contrast Akhter *et al.*^[21] opined that numerous risk factors, such as: age, sex, breed, lactation number, herd size and living conditions might determine the seroprevalence of brucellosis in animals, however none of these risk factors was significantly ($p < 0.05$) associated with brucellosis in sheep. Agab^[22] and Wadood *et al.*,^[23] also concluded that although age was potentially not a significant risk factor that could probably influence the epidemiology of brucellosis in animals, although they did observe significant statistical differences in seroprevalences among various age groups and the antibody titre against brucellosis appeared to be lower in younger animals than in adults.

5. Conclusion

The results of present study suggest a much higher prevalence of ovine brucellosis in this region of valley than what was expected as per current literature. Females and animals more than 2 years appeared more susceptible. Microagglutination test is an easy and reliable test for determining seroprevalence of brucellosis in sheep. Lastly, the economic effect of ovine brucellosis has not been investigated in this region till date and may be taken into consideration in future studies.

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