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## Sero-prevalence of brucellosis in small ruminants of Western Maharashtra

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**Abstract**

Brucellosis in small ruminants is caused due to *Brucella melitensis* but, the pathogens are not entirely species specific, e.g., Goat and sheep may be infected with *Brucella abortus* which generally caused disease in bovines. The present study taken up to ascertain the sero-prevalence of brucellosis in small ruminants of Western Maharashtra. Total 300 animals were included from the flocks of small ruminants having history of abortion, retention of placenta, metritis and swelling of the testicles from Shirwal, Dahiwadi and Baramati. Serum samples were collected. Sero-prevalence was calculated by using i-ELISA. Out of 300 animals 32 were positive by i-ELISA and the overall sero-prevalence was found to be 10.66% with highest sero-prevalence was observed from Dahiwadi region as 16%. Maximum number of sero-positive cases found in the age group above 6 years as 13.72% and high sero-prevalence was noticed in males as 17.77% when compared to females.

**Keywords:** Brucellosis, small ruminants, ELISA, sero-prevalence

**1. Introduction**

Brucellosis is a highly contagious bacterial disease in small ruminants and having zoonotic importance but remains a neglected disease in India. The risk of the disease is directly correlated with the changing farming practices in small ruminants. Migration of the flocks from one geographic area to other remains a major concern for the spreading of the disease in small ruminants. Brucellosis is usually transmitted through contact with infected birthing fluids and tissues (e.g., vaginal discharges, aborted fetuses, placenta, fetal fluids) and also be spread through blood, milk, semen and urine of infected animals. Brucellosis can also be transmitted by contaminated objects (fomites) such as, clothing, hay, feed, water, equipment and shoes. Some animals act as a carrier; they will have the bacteria but show no signs of illness. These animals can shed the bacteria into the environment for long periods of time. Abortion remains the common signs in sheep and goat during the late pregnancy period. A systemic signs like fever, depression, loss of weight may also occur in natural outbreaks in small ruminants and many times accompanied by mastitis, lameness and hygroma. As the organism is a facultative intracellular parasite and the pathogenesis depends upon localization in lymph node, udder and uterus and an initial bacteremia.

Generally, diagnosis of brucellosis is based on clinical examination of the animals, isolation of the organism from infected individual but this is a cumbersome and time consuming task, because organisms grow slowly on primary isolation <sup>[1]</sup> Moreover, it is not always practically possible to isolate *Brucella* every time even from infected individual, therefore, assessment of antibody response employing serological test play a key role in the routine diagnosis of brucellosis and supported where appropriate by bacteriological examination <sup>[2]</sup>.

Interests have been increasing to maintain brucellosis under control in endemic regions, because of the health and economic impact of brucellosis <sup>[3]</sup>. It is important to identify new endemic regions and to implement strict eradication programs beyond national boundaries. There is increasing reports on the incidence of brucellosis. Studies have revealed that in a few years to come, there is likely to be geometric increase in the incidence of brucellosis <sup>[4]</sup>. The small ruminants are considered as poor farmer's economic backbone. The study will focus on sero-prevalence of Brucellosis in western Maharashtra region, which in turn will help the farmers to abide preventive measures to lower the incidence of brucellosis in their flocks. Moreover, this study will help Veterinarians to learn about the distribution pattern of the disease.

## 2. Materials and methods

### 2.1 Study Area

For the present study, a total of 300 animals included from the flocks of small ruminants having history of abortion, retention of placenta, metritis and swelling of the testicles from Shirwal, Dahiwadi and Baramati. Present research work was carried out in the Department of Veterinary Epidemiology and Preventive Medicine, Krantisingh Nana Patil College of Veterinary Science, Shirwal, District-Satara.

### 2.2. Sample collection

Serum samples were collected in clot activator vials in morning hours and transported to the laboratory under chilling conditions. Data related to age, sex, location were collected on sampling day.

### 2.3. Elisa

Collected serum samples were subjected to Indirect Enzyme linked Immunosorbent Assay (i-ELISA) on the same day and results were calculated. The serum samples were stored at -20 °C till further use.

Indirect Enzyme Linked Immunosorbent Assay (i-ELISA) was performed by using ID Screen® Brucellosis Serum Indirect Multi-Species kit manufactured by ID. Vet Innovative Diagnostics. Microwells are coated with purified *Brucella* LPS. Specimen to be tested and controls were added to the microwells diluted at 1/20. Anti-*Brucella* antibodies, if present, form an antigen-antibody complex. After elimination of the sera by washing, a multi-species horseradish peroxidase (HRP) conjugate was added to the wells. It fixes to the anti-*Brucella* antibodies, forming an antigen-antibody-conjugate-HRP complex. Again after eliminating the excess conjugate by washing the substrate solution Tetramethylbenzidine (TMB) was added this gives a blue colour reaction product. The Intensity of this product is proportional to the amount of *Brucella*-specific IgG antibodies in the specimen. Sulphuric acid was added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm was read using an ELISA microwell plate reader. Interpretation was done calculating the S/P percentage (S/P%) using following formula:  $S/P = \frac{OD_{\text{sample}} - OD_{\text{NC}}}{OD_{\text{PC}} - OD_{\text{NC}}} \times 100$ , then it was interpreted as % SP  $\leq 120\%$  (positive), 110-120% (doubtful) and  $\geq 110\%$  (negative).

## 3. Results

### 3.1 Overall prevalence of Brucellosis

Total 300 serum samples (226 goats and 74 sheep) were collected from Shirwal, Dahiwadi and Baramati out of which 32 (18 goats and 14 sheep) serum samples were positive for brucellosis with overall sero-prevalence of 10.66%, Species wise as 7.96% in goats and 18.91% in sheep by i-ELISA (table 1 and fig. 1).

### 3.2 Area wise sero-prevalence of brucellosis in small ruminants

Total 300 serum samples were collected for the present study, out of which 100 samples (67 goats and 33 sheep) were collected from Dahiwadi, 100 samples (70 goats and 30 sheep) from Shirwal and 100 samples (89 goats and 11 sheep) from Baramati. Highest numbers of sero-positive samples were obtained from Dahiwadi 16 (7 goats and 9 sheep), followed by Baramati 9 (7 goats and 2 sheep) and Shirwal 7 (4 goats and 3 sheep) which gives overall 16%, 9% and 7% sero-prevalence in small ruminants, respectively. Species wise

the sero-prevalence in goats of these areas was 10.44%, 7.86% and 5.71%, respectively; whereas, the sero-prevalence in sheep was 27.27%, 18.18%, and 10%, respectively when subjected to i-ELISA (table 1).

### 3.3 Age wise sero-prevalence of brucellosis in small ruminants

The age wise sero-positivity sheep and goats were divided into 3 age groups i.e., 0.5-3 years, 3-6 years and above 6 years. A high percent of overall sero-prevalence in small ruminants was observed in the age group above 6 years as 13.72% followed by 3-6 years age group as 12.06% and 0.5-3 years age group as 5.33%. Species wise the sero-prevalence in goats of these age groups i.e. 0.5-3 years, 3-6 years and above 6 years was 3.57%, 9.16% and 10.25% respectively; whereas age wise sero-prevalence in sheep was 10.25%, 20.93% and 25% respectively when subjected to i-ELISA. The present study shows that adult age group of sheep and goats are more susceptible than young age group of sheep and goats for brucellosis (table 1).

### 3.4 Sex wise sero-prevalence of brucellosis in small ruminants by i-ELISA

In present study the sex wise overall sero-prevalence in small ruminants was found higher in males as 17.77% when compare to females as 10.66% respectively. Species wise sero-prevalence in goats was observed higher in males as 16.12% when compare to female 6.66% and in sheep it was also higher in males as 21.42% when compare to female as 18.33% respectively when subjected to i-ELISA (table 1).

## 4. Discussion

The overall sero-prevalence in the present study was 10.66%. The results of present study were in agreement with the Teshale *et al.* (2006) collected serum samples from 2000 sheep and goats and these samples were evaluated by i-ELISA, results showed 193 (9.7%) serum samples were found positive for brucella antibodies [5] and similar observations was noted by Sadhu *et al.* (2015) performed i-ELISA on total 1000 serum samples (485 sheep and 515 goats) and calculated sero-prevalence of brucellosis as 8.80% [6]. Brucellosis is endemic in western Maharashtra region and spread of infection generally takes place through tracking of animals from one place to another place.

The results of area wise sero-prevalence were in accordance with Hussain *et al.* (2014) who studied the sero-prevalence of brucellosis in 100 sheep in Kohat district, and found 12.12%, 09% and 08.82% sero-prevalence in Lachi, Seni Gumbat and Kohat tehsils respectively, with an overall sero-prevalence of 10% [7]. Similarly, Negash *et al.* (2012) investigated sero-prevalence of brucellosis in small ruminants at selected sites of Dire Dawa region, Eastern Ethiopia. Highest sero-prevalence was found in Aseliso 11.11% and lowest in that of Gedenser 6.84% [8]. Higher sero-prevalence was observed in Dahiwadi region because farmers use to graze their animals in free range, where transmission of infection takes place between different flocks. Whereas, in Shirwal and Baramati region farmers practiced stall feeding in which feeding in which movement of animals is restricted.

The results of age wise sero-prevalence were in accordance with Chahar *et al.* (2016) reported higher sero-prevalence of 33.33% in adult goats as compared to 12.72% in goats below one year of age group [9]. Similarly, Jarikre *et al.* (2015) observed higher sero-prevalence of 92% in adult goats above

two-years-old as compare to 8% in below two-years -old goats [10]. Sharma *et al.* (2017) found higher sero-prevalence in 6-9 years age group by i-ELISA [11] also, Mittal *et al.* (2005) reported animals of 4-6 years age (57.89%) showed maximum positive cases whereas, animals of 0-2 years showed 23.44% sero-positivity [12]. Higher sero-prevalence observed in adult animals because, sexually mature, pregnant animals were more susceptible to infection with the organism than sexually immature animals [13]. Erythritol is a four carbon sugar produced by the fetus preferentially consumed by *Brucella spp.* which leads to localization and subsequent accumulation of large amounts of bacteria to these sites, ultimately cause abortion [14] and also adult animals remains a disease carrier for their entire lifespan.

The result of sex wise sero-prevalence was in agreement with Suryawanshi *et al.* (2014) observed that sex wise sero-prevalence was higher in males of sheep and goats as 25.00% and 24.00% as compare to 17.16% and 4.32% in female sheep

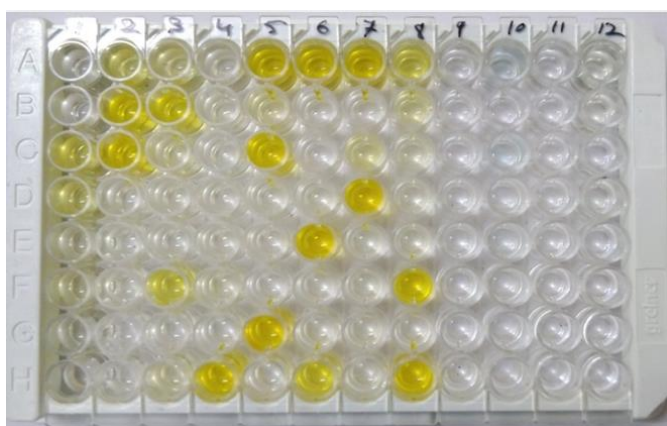
and goats [15]. Similarly, Nguna *et al.* (2019) noticed that out of 451 human samples tested, sex wise sero-prevalence in males was 6.4% (11/173) and in females 3.2% (9/278) [16]. Male animals was more susceptible to brucella infection because farmers generally exchange their breeding males between farms to avoid inbreeding therefore, males had more chances to get infection than females.

## 5. Conclusion

The overall sero-prevalence in these 3 areas observed was 10.66% with highest sero-prevalence was observed from Dahiwadi as 16%. The prevalence of brucellosis was recorded highest in the age group above 6 years as 13.72% and high sero-prevalence was noticed in males as 17.77% when compared to females. To prevent economic losses due brucellosis surveillance and monitoring of the diseases is important. Effective vaccination should be done in endemic areas to prevent and control incidence rate.

**Table 1:** Overall prevalence, area wise age wise and sex wise prevalence of brucellosis in sheep and goat

Animals	Overall	Area wise prevalence			Age wise prevalence			Sex wise prevalence	
		Shirwal	Dahiwadi	Baramati	0.5-3 years	3-6 years	Above 6 years	Male	Female
Sheep and Goat	10.66%	7	16	9	5.33	12.06	13.72	17.77	9.41
Goat	7.96%	5.71	10.44	7.86	3.57	9.16	10.25	16.12	6.66
Sheep	18.91%	10	27.27	18.18	10.52	20.93	25	21.42	18.33



**Fig 1:** ELISA module showing positive and negative reactions for IgG class antibody detection in small ruminants by Indirect ELISA, Wells A1 and B1: Negative Control Wells C1 and D1: Positive Control, Wells E1 to H12: Test serum samples. Yellow coloured wells denotes presence of Antibodies whereas; colourless wells denotes absence of antibodies.

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