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Research Article

# SEROPREVALENCE OF BRUCELLOSIS IN SMALL RUMINANTS OF ANAND DISTRICT OF GUJARAT BY VARIOUS SEROLOGICAL METHODS

**PADHER R.R.*[1], NAYAK J.B.[1], ROY ASHISH[2], BHANDERI B.B.[2], PARMAR B.C.[1] AND MISTRY U.P.[1]**

[1]Department of Veterinary Public Health, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, 388001, Gujarat, India
[2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, 388001, Gujarat, India
*Corresponding Author: Email-radhapadher@gmail.com

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**Abstract-** The present study was taken up to ascertain the seroprevalence of brucellosis in small ruminants of Anand, districts of the Central Gujarat region. A total of 200 serum samples were collected from the goat and sheep of Anand district and subjected to different serological test i.e., Rose Bengal Plate test (RBPT), Standard Tube Agglutination Test (STAT) and Indirect ELISA to detect the brucella antibody. A total 200 sera sample including 100 from goats and 100 from sheep collected from the Anand district. Among 200 sera samples of sheep and goats, 94 (47.00%) samples were found to be positive. 93 sera samples were positive by I-ELISA, 27 by RBPT while 16 by STAT. One RBPT positive goat serum sample was found to be negative by I-ELISA. While species wise incidence was found to be 55 (55.00%) and 38 (38.00%) among goats and sheep, respectively. Fifty six goat sera samples were found to be sero-positive, out of which 55 samples were found to be positive by I-ELISA while 16 by RBPT and 10 by STAT. Among 38 sera samples of sheep found to be positive by I-ELISA while 11 by RBPT and 6 by STAT. Seroprevalence of brucellosis among goat was 16.00%, 10.00% and 55.00% higher than the sheep was 11.00%, 6.00% and 38.00% by RBPT, STAT and I- ELISA respectively. In small ruminantes sex wise seroprevalence was higher in female to be 18%, 13% and 65% then male 9%, 3% and 28% by RBPT, STAT and I-ELISA (*Brucella* spp.), respectively.

**Keywords**- Brucellosis, Zoonosis, Small ruminants, Seroprevalence.

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## Introduction

Brucellosis is one of the major zoonosis and cause economic losses in sheep and goat farming [1]. It is caused by bacteria of the genus *Brucella* and is reported throughout world. In female the symptoms like abortion, infertility, retained placenta, endometritis and in male produced orchitis and infection of the accessory sex glands [2]. Brucellosis is a zoonosis disease with a worldwide distribution that is important in public health [3]. It has been reported in different countries of the Asia including the India [4]. The contact with infected animals and the consumption of unpasteurized infected dairy products transmit the infection to humans [5, 6]. Abortion and retention of placenta are the most important sign of brucellosis and economic losses in ovine and caprine. Free grazing and movement with frequent mixing of flocks of sheep and goats are the main mode of disease transmission and resulting in high prevalence and wide distribution of brucellosis in India [7]. The objective of this study was to determine the seroprevalence of brucellosis in small ruminants, and also to create public awareness towards the zoonotic importance of the disease.

## Materials and Methods

The study was conducted to detect brucella antibodies for 200 sera samples comprising goat sera (100) and sheep sera (100) collected from various areas of Anand district, under aseptic precautions. These sera samples were tested for brucella antibodies using RBPT, STAT, and I-ELISA for detecting brucella antibodies from serum. Rose Bengal Plate Test was carried out by using rose Bengal plate test antigen. Using *B. abortus* agglutinating antigen carried out standard tube agglutination test. Both antigens were procured from Indian Veterinary Research Institute, Izatnagar. Indirect enzyme linked immunosorbent assay was carried out by using the smooth lipopolysaccharide (S-LPS) I-ELISA kits (for sheep and goat sera) procured from ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI) Bengaluru. The samples were collected in vacutainer with serum clot activator and transported to the departmental P. G. research laboratory on icebox for further processing and serological analysis. The vacutainer was kept in upright position at room temperature for about 2 hr. Then the tubes were centrifuged at 3000 rpm for 10 minutes to facilitate separation of serum, which was collected in a screw capped plastic vials. The sera were stored at -20ºC till subjected to I-ELISA [8-15].

## Results and Discussion

### Seroprevalence of Brucellosis in Goats

In present study, seroprevalence of brucellosis among goat was 16.00%, 10% and 55% by RBPT, STAT and I- ELISA, respectively [Table-1], [Fig-1, 2, 3, and 4]. The finding of the current study was in agreement with the finding of Din *et al.,* (2013) [16] who found 13.33% and 9.33% with RBPT and STAT in goat. Similarly Valarmathy *et al.,* (2007) who found 14.55%, 9.85% and 30.04% in goats by RBPT, STAT and I-ELISA respectively [17]. In addition, Sulima *et al.,* (2010) who reported 17.68% and 16.02% in goat by RBT and STAT respectively [18]. In addition, Bertu *et al*. (2010) who reported 17.68% in goat by RBT. Similarly Ebrahim *et al.* (2014) who reported 13.9% in goat by RBT. Sharma *et al*. (2015) who found 34.2% in goat by I-ELISA from Jammu which was somewhat similar to the present study [19]. In comparison to the present study higher seroprevalence of brucellosis with finding of Salama *et al.,* (2011) who recorded 29.30% and 27%

in ewes by RBT and SAT, respectively of Egypt [20]. In addition, Bertu *et al.* (2010) who reported 16.02% in goat by STAT [14]. Similarly, Kaltungo (2013) who got 25.80% in goat by RBPT [21]. Sharma *et al.* (2015) who find 21.40% in goat by STAT from Jammu which were higher than the present finding. In comparison to the present study lower seroprevalence of brucellosis with finding of Tayshete (2001) who recorded 4.00%, 2.85% and 2.85% with I-ELISA, RBPT and STAT, respectively, in the goats of North Gujarat [22]. In addition, Rahman *et al.,* (2011) who found 3.15% in goats by I-ELISA in five different districts *viz.* Bagerhat, Bogra, Gaibangha, Mymensingh and Sirajgonj of Bangladesh [23]. Similarly, Raju *et al.,* (2005) who found 18.79% in goat by dot-ELISA and Bertu *et al.* (2010) who reported 24.86% by I-ELISA which was lower than present study [24]. In addition Reddy *et al.,* (2014) who found 5.15% by RBPT, 6.34% by STAT, 9.52% by I-ELISA and 7.14% by Dot-ELISA in goats of the Karnataka, which was in contrast to present finding [25]. Moreover, the different species of animals can respond differently towards brucella infection. Alternatively, the immunoglobulins of the different species might have different reactivities in the test used for diagnosing brucellosis. These aspects are required to be thoroughly investigated under both controlled as well as natural conditions using a large number of serum samples.

**Table-1** *Seroprevalence of brucellosis in goats by various serological methods*

| Species | Sex | No. of sera samples tested | RBPT | | STAT | | I-ELISA | |
|---|---|---|---|---|---|---|---|---|
| | | | No. of samples positive | (%) | No. of samples positive | (%) | No. of samples positive | (%) |
| Goat | Male | 50 | 5 | 10.00% | 2 | 4.00% | 17 | 34.00% |
| | Female | 50 | 11 | 22.00% | 8 | 16.00% | 38 | 76.00% |
| Total | | 100 | 16 | 16.00% | 10 | 10.00% | 55 | 55.00% |

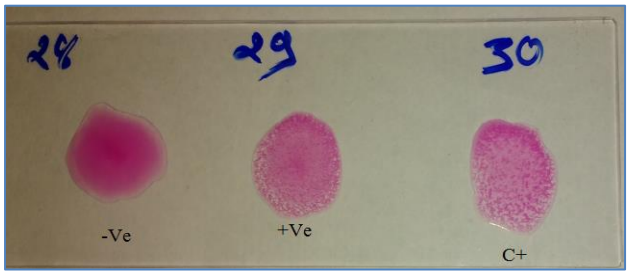


**Fig-1 Rose Bengal Plate Test (RBPT) for detection of *Brucella antibodies* Negative reaction=Homogenous mixture without any clumps. Positive Reaction = Definite clumping**

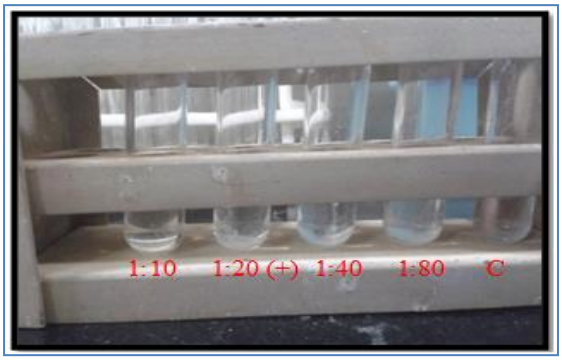


**Fig-2 Serum Tube Agglutination Test (STAT) for the detection of *Brucella* antibodies. Tube No. 1, 2 = Positive reaction, Tube No. 3, 4 = Negative reaction and Tube C = Control**

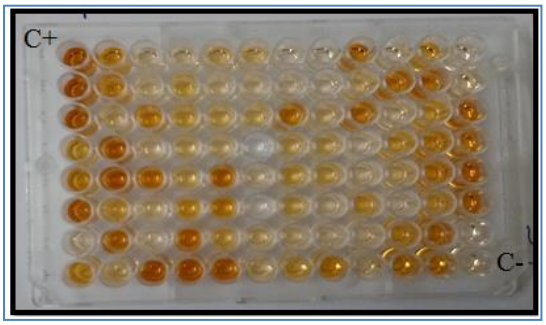


**Fig-3 Microtitre plate showing the results of I-ELISA for detection of *Brucella* antibodies. C+ (Positive control), C- (Negative control) and Rest of the well: Field serum samples.**
**Percent positive = (OD value of test serum/ OD value of positive control) x 100 More than 54%- Positive, Below 54% - Negative and 54% - To be re-samples.**

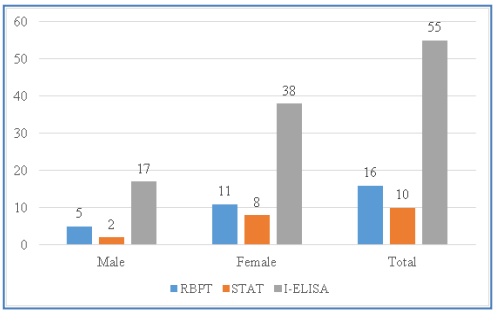


**Fig-4 Seroprevalence of Brucellosis in Goats by various serological methods**

**Seroprevalence of Brucellosis in Sheep**

Seroprevalence of brucellosis among sheep was 11.00%, 6.00%, 38.00% by RBPT, STAT and I- ELISA, respectively [Table-2] and [Fig-5].

The finding of the current study was in agreement with the finding of Raju *et al.,* (2005) who found 12.00%, 11.20% and 20.00% with RBPT, EDTA-STAT and dot-ELISA respectively. In addition Sharma *et al.,* (2006) who found 15.22% in sheep of Mehsana and Patan district of Gujarat by RBPT [26]. Similarly Al-Talafhah *et al.,* (2003) who reported 14.30% by RBT in Awassi sheep flocks of Northern Jordan [9]. In addition, Al-Mariri *et al.,* (2011) who got 60.00% in unvaccinated Syrian female sheep [8]. In comparison to the present study higher seroprevalence was obtained by Awandkar *et al.*, (2012) who observed 28.1% and 23.8% with RBPT and STAT, respectively in Decani sheep and Azmi, (2012) who also reported 21.1% in sheep of west bank by serially testing with RBPT and SAT [12]. In addition Onoja *et al.,* (2008) who found 76% in a sheep flock of Zaria by serially testing with RBPT and SAT and Al-Mariri *et al.,* (2011) who got 66% and 64% in unvaccinated Syrian female sheep by RBT and SAT, respectively [27]. Hawari (2012) who found 18.5% in sheep by RBT which was higher seroprevalence to present finding [28].

In comparison to the present study lower result was obtained by Sharma *et al.* (2015) who recorded 2.50%, and 15.50% with RBPT and I-ELISA, respectively from Jammu. In addition Al-Talafhah *et al.,* (2003) who reported 7.2% by ELISA in Awassi sheep flocks of Northern Jordan and Tayshete, (2001) who got 8.00%, 4.00%, 3.00% and 3.50% in the sheep of North Gujarat by dot-ELISA, I-ELISA, RBPT and STAT, respectively [29]. In addition Rahman *et al.,* (2011) who found 3.75%, 2.50%, and 1.25% by RBT, SAT and I-ELISA, respectively in sheep.

**Epidemiology of Brucellosis**

**Species Wise Seroprevalence of Brucellosis**

Seroprevalence of brucellosis among goat was 16.00%, 10.00% and 55.00% higher than the sheep was 11.00%, 6.00% and 38.00% by RBPT, STAT and I-ELISA respectively, in present study which were similar to the findings of Jarikre *et al.* (2015) 10.3% for goats were positive using RBPT but 3.00% in sheep by RBPT which was contrast to present finding[30]. In addition Rahman *et al.* (2011) who

found 5.83%, 4.17% and 2.50% seroprevalence of brucellosis in goats which was higher than sheep 3.75%, 2.50% and 1.25% by RBT, SAT and I-ELISA, respectively which was lower prevalence percentage but similar with present finding. Similarly, Dubad *et al.* (2015) who reported lower seroprevalence in sheep 8.85% (27/305) than goats 10.52% (10/95), respectively by RBPT [31].

The findings of the present study seems to be in contrast with findings of Andreani *et al.* (1982) who recorded 7.2% in sheep and 5.3% in goats by using SAT [10]. Shome *et al.,* (2006) who found higher seroprevalence of brucellosis in sheep (13.41% and 8.23%) than in goats (8.27% and 4.43%) by RBPT and STAT, respectively [32]. This difference could be due to differences in the sample size, species of animal and the tests used. It is possible that this is due to variations in animal management and production systems.

**Table-2** *Seroprevalence of brucellosis in sheep by various serological methods*

| Species | Sex | No. of sera samples tested | RBPT | | STAT | | I-ELISA | |
|---|---|---|---|---|---|---|---|---|
| | | | No. of samples positive | (%) | No. of samples positive | (%) | No. of samples positive | (%) |
| Sheep | Male | 50 | 4 | 8.00% | 1 | 2.00% | 11 | 22.00% |
| | Female | 50 | 7 | 14.00% | 5 | 10.00% | 27 | 54.00% |
| Total | | 100 | 11 | 11.00% | 6 | 6.00% | 38 | 38.00% |

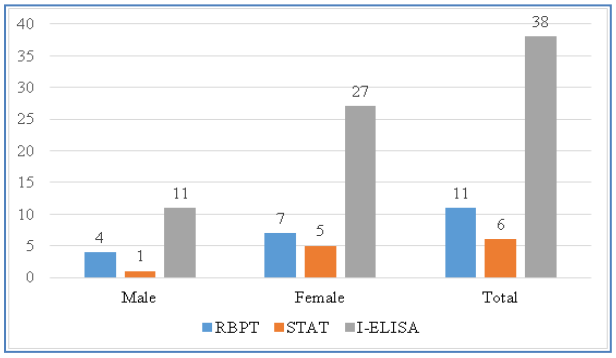


**Fig-5 Seroprevalence of Brucellosis in sheep by various serological methods**

**Sex Wise Seroprevalence of Brucellosis**

In small ruminantes sex wise seroprevalence was higher in female to be 18%, 13% and 65% then male 9%, 3% and 28% by RBPT, STAT and I-ELISA (*Brucella* spp.), respectively.

The results of present study seems to be similar with the finding of Din *et al.* (2013) who recorded sex-wise prevalence of brucellosis in male goats was 10.66%, 8.0% and 6.66% by RBPT, SPAT and STAT, respectively; while in females it was 16.0%, 14.66% and 12.0% by RBPT, SPAT and STAT respectively. In addition Kotadiya (2012) who recorded lower seroprevalence of brucellosis of 17.07%, 8.54% and 9.65% in male than 18.46%, 10.98% and 12.21% in female by I-ELISA, RBPT and STAT, respectively [33]. Similarly Valarmathy *et al.* (2007) reported overall prevalence of brucellosis was higher in females (36.92%) than in males (12.21%). In addition, Awandkar *et al.* (2012) who found higher seroprevalence in female (27.69%) than in Male (26.66%) by RBPT and STAT. Erythritol, a sugar alcohol synthesized in the ungulate placenta and stimulates the growth of virulent strains of brucella organisms, has been credited with the preferential localization of the bacteria within the placenta of ruminants.

In comparison to the present study slight lower seroprevalence was in female (3.84%) than the male (2.00%) of Black Bengal goat with I-ELISA by Rahman *et al.,* (2012). In addition Bekele *et al.,* (2011) [13] who observed higher prevalence of brucellosis in female of small ruminants (1.8%) than male of small ruminants (1.2%) by RBPT and Dabassa *et al.,* (2013) who found non-significant small ruminant brucellosis higher in female (2.10%) than male (0.68%) [15]. In addition Onoja *et al.,* (2008) who reported higher prevalence rate of brucellosis in ewe (69.2%) than ram (0.8%). In addition Bekele *et al.* (2011) observed 1.8% prevalence in female a bit higher than 1.2% in males by RBPT. Similarly Teshale *et al.* (2006) observed similar prevalence of brucellosis in female and male of both ovine and caprine species. 9.7 % females and 8.9 % males were positive by I-ELISA. In comparison to the present study slight higher seroprevalence was in female 12 (69.2 %) higher then male 1 (0.8%) with RBPT and STAT by Onoja *et al.* (2008). Similarly, Awandkar *et al.* (2012) found seroprevalence higher in female (27.69%) than in Male (26.66%) by RBPT and STAT [11].

In goats, seroprevalence of brucellosis was slight higher in female was 22.00%, 16.00% and 76.00% as compared to male of about 10%, 4.00% and 34% by RBPT, STAT, and I-ELISA (*Brucella* spp.) respectively.

Similar result obtained by Hawari (2012) who reported seroprevalence was 19.7% in female and 17.4% in male of goats by RBPT. In addition, Kaltungo (2013) reported prevalence in bucks was 32.3%, and 17.5% in does using RBPT, respectively. Kotadiya (2012) who reported 8.54% and 9.65% in male than 10.98% and 12.21% in female by RBPT and STAT, respectively in sheep. Din *et al.* (2013) who recorded sex-wise prevalence of brucellosis in male goats was recorded as 10.66% and 6.66% by RBPT and STAT, respectively; while in females it was 16.0% and 12.0% by RBPT and STAT respectively.

But contrast result was obtained by Kotadiya (2012) who recorded lower seroprevalence of brucellosis of 17.07%, in male than 18.46% in female by I-ELISA. In sheep, seroprevalence of brucellosis was slight higher in female was 14.00%, 10.00% and 54.00% as compared to male of about 8.00%, 2.00% and 22.00% by RBPT, STAT and I-ELISA, respectively.

Similar result obtained by Hawari (2012) who found seroprevalence was 17.2% in female and 12% in male of sheep by RBPT. In adition Kotadiya (2012) who reported seroprevalence of brucellosis was 8.54% and 9.65% in male than 10.98% and 12.21% in female by RBPT and STAT, respectively.

But contrast result was obtained by Teshale *et al.* (2006) who observed lower seroprevalence of brucellosis was 9.7 % females and 8.9 % males positive by I-ELISA in female and male of both ovine and caprine species. In addition, Priya *et al.* (2010) who found seroprevalence in female (6.1% and 4.7%) than males (3.9% and 2.6%) by RBPT and STAT, respectively in caprine [34]. Similarly, Ferede *et al.* (2011) who reported brucellosis higher in females (0.4%) than males (0%) by RBPT [35]. In addition, Rahman *et al.* (2012) who reported brucellosis in female sheep (3.41%) was higher than male (3.33%) by I-ELISA [36]. Kotadiya (2012) who reported seroprevalence of brucellosis of 17.07% in male and 18.46% in female by I-ELISA.

**Conclusions**

On the basis of the present study, we conclude that seroprevalence of brucellosis was prevalent in small ruminants of the study area. Seroprevalence of brucellosis was significantly more frequent in goats as compared to sheep. I-ELISA was a better serological test as compared to RBPT and STAT in the sense of sensitivity, specificity, and rapidity and it could be advocated for screening of brucellosis in sheep and goats.

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**Abbreviations**

**RBPR-** Rose Bengal Plate Test
**I-ELISA**- Indirect enzyme linked immunosorbent assay
**STAT-** Standard Tube Agglutination Test
**S-LPS** - Smooth lipopolysaccharide

**Author Contributions:** all author equally contributed

**Conflicts of Interest:** None declared

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