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*Original Article*

# Screening of brucellosis in goats by RBPT and c-Elisa in organized farms in Chennai, India

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**Abstract**

The prevalence of brucellosis among goats was studied in organized farms in and around Chennai, Tamil Nadu, by conventional Rose Bengal Plate Test (RBPT) and also with c-ELISA using Bru Alert® *Brucella* antibody kit. In a total of 161 sera samples screened for the presence of *Brucella* antibody, the overall positivities of 4.97% and 5.59 % were respectively found by RBPT and c-ELSA. The study also recorded high seropositivity in animals past 5 years of age and in female animals. A comparative analysis showed excellent agreement of the two tests and the chi-square test indicated strong ($P<0.01$) association between the alternative tests. The study arrived at the conclusion that initial screening for brucellosis could be done with RBPT and confirmation should be made with c- ELISA test to eliminate false positives.

**Keywords**: abortion, organized farm, caprine brucellosis, c-ELISA, RBPT

## 1. Introduction

Goat rearing plays an important role in the socioeconomic conditions of traditional Indian society and is a source of family income. Many factors directly or indirectly affect the economic sustainability of farming, while those factors that directly influence reproductive performance can adversely affect the productivity of goats. Brucellosis is a most widespread and economically devastating contagious disease of sexually matured animals, caused by *Brucella spp*. (Saikia *et al.*, 2019). Caprine brucellosis is mainly caused by *Brucella melitensis*, is widespread in India, and is a major cause of abortion in goats as well as of brucellosis in humans (Shome *et al.*, 2015). Caprine brucellosis has also been reported to be caused by *B. abortus* (Wareth, Melzer, Tomaso, Roesler, & Neubauer, 2015), and it is endemic, highly transmissible to humans and reported to be dominantly responsible for human brucellosis (Mantur & Amarnath, 2008).

Brucellosis in humans and animals is known to be a worldwide problem and still it remains a major public health hazard of great economic importance (Charisis, 1998). The World Health Organization considers brucellosis a neglected zoonosis and classifies *Brucellae* as risk group III agents, because they can be easily transmitted via aerosols (World Health Organization [WHO], 2006).

Making a diagnosis of brucellosis is comparatively difficult because of the general symptoms that are largely

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shared with other febrile illnesses, the slow growth rate of the causative agent in blood culture, and the complexity of its sero-diagnosis (Memish, Mah, Al Mahmoud, Al Shaalan, & Khan, 2000). Accurate diagnosis of brucellosis could be made only after a battery of tests, and further the selection of the test is based on the purpose of study. Antigen detection is done with conventional culture isolation and it is correlated with PCR assay, either single or multiplex PCR. *Brucella* specific antibody detection is carried out using Rose Bengal plate agglutination test (RBPT), standard tube agglutination test (STAT), enzyme linked immunosorbent assay (ELISA), complement fixation test (CFT), or fluorescent polarization assay (FPA), which are routinely used in various combinations (World Organisation for Animal Health [OIE], 2009). In brucellosis, the diagnosis is quite cumbersome due to the various merits and demerits of each test. Rose Bengal plate agglutination test (RBPT) is simple, rapid, inexpensive, sensitive and specific, but has a low specificity in chronic cases and a relatively low specificity in endemic areas. Also, ELISA is simple and safe with high specificity, sensitivity and efficiency, but is expensive and laborious with high possibility of false positive and negative results. Competitive ELISA has a high sensitivity and flexibility with the best capability for detection of small antigens even when they are present in a low concentration, while indirect ELISA has a signal amplification and possibility of cross- reactivity from secondary antibodies. There is no single test to confirm brucellosis, except the incontrovertible diagnostic approach using culture isolation methods (Nielsen *et al.*, 1995). Even though culturing is a gold-standard diagnostic approach, it is not so easy to retrieve the isolate from infected animals due to poor sensitivity, facultative intracellular nature of the organism, and the risk of laboratory acquired zoonosis (OIE, 2009).

On the basis of extensive work done on serological tests, it has been reported that the error could be minimized using various tests according to the infective stage and type of sample (preferred samples include samples from blood, testicles, lymph nodes, mammary glands, secretions, aborted material, etc.) (Gall &Nielsen, 2004; Nielsen, 2002). It is generally agreed that a positive response in the agglutination test in an early stage of the infection, which detects mainly IgM, has to be further confirmed by a positive IgG response in a later stage of the infection (Bhanu Rekha, Gunaseelan, Subramanian, & Yale, 2013).

The present study aimed to screen brucellosis in goats by RBPT and c-ELISA in organized farms in and around Chennai, India.

## 2. Materials and Methods

### 2.1 Experimental design

This study was designed to screen for brucellosis in goats by RBPT AND c-ELISA in organized farms in and around Chennai, India. Farm locations and animals were sampled using simple random sampling to represent the target population. Most of the samples were collected randomly from apparently healthy animals of different ages and sexes. In a few of the animals, the sera samples were collected based on history or clinical evidence of brucellosis, like abortion.

### 2.2 Sample collection

Sera samples were collected from randomly selected goats from organized farms located in and around Chennai and from the clinical cases brought to Madras Veterinary College teaching hospital in Chennai, India. An about 3 ml blood sample was collected from each of the 161 goats by jugular vein puncture, into sterile test tubes of 5 ml capacity. The tubes were left undisturbed until the sera cleared, and centrifuged at 2000 rpm for 15 minutes. All the sera samples were numbered and stored at – 20$^{o}$C until further use.

### 2.3 Serological tests

#### 2.3.1 Rose bengal plate test (RBPT)

The colored antigen required for RBPT was obtained from the Division of Biological products, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, and the test was performed as per the standard protocol of agglutination test (OIE, 2009). Briefly, a drop of serum (30 µl) was placed on clean grease-free glass slide and an equal quantity of antigen was added and mixed thoroughly using an inoculation loop. The mixture was observed for clumping / agglutination for one minute and the results were recorded as agglutination (+) or no agglutination (-).

#### 2.3.2 Competitive ELISA test (c- ELISA)

Competitive ELISA test (c- ELISA) kit (Bru Alert®) for the diagnosis of brucellosis was obtained from TRPVB, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India, and was used for testing the sera samples. All the reagents were thawed before use and homogenized by inversion. The protocol given by the manufacturer was followed to perform the c-ELISA test.

Interpretation of c-ELISA: For each sample, the Percentage Inhibition (PI) was calculated as follows, using the sample and control values:

$$PI = 100 - (\text{Test sample OD} / \text{Negative control OD}) \times 100$$

#### 2.3.3 Statistical analysis

In order to compare the different diagnostic tests and calculate percentages, Chi-square test, kappa statistics, sensitivity, and specificity were calculated as per Thrus field (2005) using MS office 2007 Excel spreadsheet, and coded and analyzed by SPSS version 17. To determine whether the performance of the two tests (RBPT and c-ELISA) is statistically significant, we must conduct a test of significance called the Chi-Square Test. The kappa statistic is frequently used to test interrater reliability. The importance of rater reliability lies in the fact that it represents the extent to which the data collected in the study are correct representations of the variables measured. While there have been a variety of methods to measure interrater reliability, traditionally it was measured as percent agreement, calculated as the number of scores that agree divided by the total number of scores. Like

most correlation statistics, the kappa can range from -1 to +1 (1 indicates perfect agreement, whereas 0 indicates no agreement).

## 3. Results

In this study, overall seropositivities of 4.97% and 5.59 % were observed respectively by RBPT and c-ELISA. Among the 161 sera samples screened by RBPT and c-ELISA, the highest percentage of positivity was observed in the ≥5 years of age group, with 2.5% in each of RBPT and c-ELISA; followed by the 3-4 years of age group (Table 2). Sex distribution of the cases had high positivity in females, namely 4.34% and 3.73% by RBPT and c-ELISA, respectively (Table 3). In the aborted cases recorded in goat, only 2 out of 15 (13.34%) cases turned to be *Brucella* sero-positives.

Out of 8 samples detected as positive by RBPT, none was negative by c-ELISA and of the 9 samples positive by c-ELISA, 1 sample was negative by RBPT (Table 4). The concordance between these two tests was 99.38% with a kappa of 0.9441(Table 4).

## 4. Discussion

Brucellosis has recently been identified as one of the greatest problems in cattle and buffaloes in India and this infection is consistently found escalating. There are various reasons behind this problem, like the unavailability of testing facilities in the field, lack of awareness and ignorance of animal owners, and socio-economic and religious beliefs (Walunj, Mhase, Bhave, Muglikar, & Pawde, 2019).

In India, about 80% of people live in a close contact with domestic livestock or companion animals, a critical risk factor for zoonotic transmission of diseases such as brucellosis; yet, the true incidence rate of human brucellosis is unknown. Seroprevalence studies suggest infections may range between 0.9 % and 18.1 % in humans, with higher risk in veterinarians and farm workers (Agasthya, Isloor, & Prabhudas, 2007).

The RBPT and c-ELISA used in this study could detect the genus specific common epitope present in all the smooth strains of *Brucella* species, including *B. melitensis.* In this study, a seropositivity of 4.97 % and 5.59 % was observed respectively by RBPT and c-ELISA (Table 1). Rahman *et al.* (2011) found a similar level of seroprevalence of brucellosis in goat (5.83 %) by RBPT followed by a lower positivity (2.5 %) by I-ELISA. Also, Din *et al.* (2013) found 11.33 % positive rate by RBPT. A higher prevalence (25.8 %) of *Brucella* antibodies using RBPT was also recorded in goats by Kaltungo, Saidu, Sackey, and Kazeem (2013). In serosurveillance work conducted by Kanani *et al.* (2018), seropositivity rates of 7.79 % and 9.35 % were reported respectively for RBPT and i-ELISA tests in organized farms.

### 4.1 Demographic determinants for brucellosis in goats

#### 4.1.1 Age

Among the 161 sera samples screened by RBPT and c-ELISA, the highest positivity rate was observed in the ≥5 years of age group, with 2.5 % in each of RBPT and c-ELISA, followed by the 3-4 years of age group (Table 2). The finding is in agreement with the results of Saikia *et al.* (2019) in Assam, India, and by Rahman *et al.* (2011) for Bangladesh. In line with our finding, previous studies found that age can be regarded as an intrinsic factor influencing brucellosis seropositivity (Chimana *et al.*, 2010; Megersa *et al.*, 2011). This could be attributed to the biological fact that the clinical disease (brucellosis) mainly affects the actively producing animals, while the young animals have not yet reached reproductive age (Amin *et al.*, 2005). Also, younger animals tend to be more resistant to the infections, although latent infections have also been reported (Radostits, Gay, Hinchcliff, & Constable, 2007). Sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity.

Table 1. Serological results for brucellosis in goats

| Test | No. of samples | Positives | % positivity |
|---|---|---|---|
| RBPT | 161 | 8 | 4.79 |
| ELISA | 161 | 9 | 5.59 |

Table 2. Distribution of brucellosis in goats by age group

| % positivity | ELISA N=161 | % positivity | RBPT N=161 | Age (years) |
|---|---|---|---|---|
| 0.62 | 1 | 1.24 | 2 | 1-2 |
| 1.86 | 3 | 1.86 | 3 | 3-4 |
| 2.50 | 4 | 2.50 | 4 | ≥5 |

Table 3. Distribution of brucellosis in goats by sex

| Sex | RBPT N=161 | % positivity | ELISA N=161 | % positivity |
|---|---|---|---|---|
| 1.24 | 2 | 1.24 | 2 | M |
| 3.73 | 6 | 4.34 | 7 | F |

Table 4. Concordance of RBPT and ELISA in the detection of brucellosis in goats

| Test | | ELISA | | Total | Concordance | Kappa value |
|---|---|---|---|---|---|---|
| | | Positive | Negative | | 99.38 | 0.9441 |
| RBPT | Positive | 8 | 0 | 8 | | |
| | Negative | 1 | 152 | 153 | | |
| | | 9 | 152 | 161 | | |

### 4.1.2 Sex

Sex distribution of the cases showed high positivity rate in the females, with 4.34% and 3.73% by RBPT and c-ELISA, respectively (Table 3). Rahman *et al.* (2011) found a higher prevalence of brucellosis among female animals in Bangladesh. The higher rate of infection in females might be due to infection within the female reproductive tract providing a potential reservoir for the organism to propagate. Moreover, the erythritol content of the placenta facilitates the multiplication of *Brucella* organisms in gravid uterus and makes female animals more susceptible to brucellosis.

### 4.1.3 Aborted cases

In the aborted cases recorded in goat, only 2 out of 15 (13.34%) cases turned to be *Brucella* seropositives. The finding is in close agreement with the 12.99 % prevalence reported by Saikia *et al.* (2019) in goats with abortion in Assam region of India. Also, 27.1% prevalence of brucellosis among aborted goats in Jordan was reported by (Samadi, Ababneh, Giadinis, & Lafi, 2010). A higher prevalence of 66.67% of brucellosis in goats with abortion was also reported by Rahman *et al.* (2011) in Bangladesh.

### 4.2 Comparison of RBPT and c-ELISA techniques in goats

Out of 8 samples detected as positive by RBPT none was negative by c-ELISA, and of the 9 samples positive by c-ELISA only 1 sample was negative by RBPT (Table 4). The concordance between these two tests was 99.38% with a kappa of 0.9441 indicating RBPT to have almost perfect agreement with the c- ELISA. Statistical analysis using chi-square test indicated strong ($P<0.01$) association between the test performances (Table 4).

This result is consistent with finding from a study conducted in Kigali, Sudan, where the agreement between RBPT and c-ELISA was excellent with a kappa of 0.92 (Manishimwe, Ntaganda, Habimana, Nishimwe, & Byukusenge, 2015). Also from Sudan (Adil & Hind, 2012) reported an excellent agreement with a kappa of 0.86. In India, a close result has been reported where the kappa value of 0.72 indicated a very good agreement between the two tests (Islam, Pratap & Kaur, 2013).

## 5. Conclusions

The overall positive case rates detected by RBPT and c-ELISA were 4.97 and 5.59 % respectively, showing that there is a conspicuous presence of *Brucella* antibodies in the goat population in the study area, indicating the presence of *Brucella* infections in the population and justifying the need for continued sero-surveillance of the disease in the study area. We also recommend further *Brucella* screening and confirmatory programs in a wider region. Since a comparison of the two tests (RBPT and c-ELISA) showed a kappa of 0.9441 indicating RBPT to almost perfectly agree with the c-ELISA, it is recommended that RBPT could be a reliable test for the initial screening of brucellosis in goats and c-ELISA be used as a confirmatory test to eliminate false positives from it. A successful eradication drive always depends on precise diagnosis, for which it is necessary to have an easy, robust, sensitive, and specific test, which in turn might be useful for strategic planning to establish appropriate control measures and prevent further spread of an infection.

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