Full Length Article

PREVALENCE OF BRUCELLOSIS IN SHEEP IN CAUVERY DELTA REGION OF TAMIL NADU

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ABSTRACT

The present study was aimed to assess the seroprevalence of brucellosis in sheep of Cauvery Delta region by collecting serum samples from apparently healthy sheep and sheep with the history of abortion. Serum samples from a total of about 123 animals including 64 from apparently healthy animals, 54 from animals with previous history of abortion and 5 from aborted animals were subjected to serological tests like Rose Bengal Plate Test (RBPT), Standard Plate Agglutination Test (SPAT) and indirect Enzyme Linked Immuno-Sorbent Assay (i-ELISA). Molecular identification was carried out with the aborted materials from 5 animals with Bcsp31 gene. The prevalence rate of Brucellosis was 26.01 %, 22.76 % and 41.46 % by RBPT, SPAT and i-ELISA respectively. Among the 5 aborted animals, two were found to be positive for brucellosis with all the four diagnostic tests. Among the three serological tests, the highest seroprevalence of 41.46 % was observed when i-ELISA was used. However, RBPT was considered to be cost effective and easy to test. Hence, from this study it can be concluded that periodical screening of animals with cost effective serological tests for diseases like brucellosis, which is of economic and public health significance is mandatory to prevent the disease outbreak and economic loss to the farmers.

Key words: Brucellosis, RBPT, SPAT, i-ELISA, *Bcsp31* gene.

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INTRODUCTION

Brucellosis is a chronic, bacterial zoonotic disease caused by different species of *Brucella sp.* affecting a wide range of hosts. Brucellosis in small ruminants is mainly caused by *Brucella melitensis* and is characterized by various clinical signs like abortion, infertility, hygroma, retained foetal membrane, orchitis, epididymitis and rarely arthritis (Constable *et al*., 2017). For the diagnosis of brucellosis, various diagnostic tests available include Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT), 2- Mercapto ethanol test, Enzyme Linked Immuno-Sorbent Assay (ELISA), Milk Ring Test (MRT), Fluorescent Polarization Assay (FPA), Complement fixation Test, culture, isolation and molecular identification. Till now, no data is available on the seroprevalence of brucellosis in sheep in Cauvery Delta region. Hence, the objective of the present study was to determine the seroprevalence of ovine brucellosis using serological tests, *viz*., RBPT, SPAT and i- ELISA followed by confirmation with molecular technique. So, in this study, samples from Cauvery Delta region were subjected to different serological tests and molecular identification, to know the prevalence rate of brucellosis among sheep of Cauvery delta region.

MATERIALS AND METHODS

Sample collection and processing

Sample collection from sheep was done in and around Thanjavur, Thiruvarur and Nagapattinam districts. Serum samples were randomly collected from about 64 apparently healthy animals and 54 animals having previous history of abortion from different areas of Thanjavur, Thiruvarur and Nagapattinam districts and 5 aborted animals presented to Veterinary Clinical Complex (VCC), Veterinary College and Research Institute (VC&RI), Orathanadu.

A volume of four ml of blood was aseptically collected by jugular vein puncture and transferred to a sterile clot activator vial from apparently healthy animals. From aborted animals, both foetal membrane and blood for serum were collected for molecular identification and serological tests, respectively.

Reagents

Rose Bengal Plate Test antigen (Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh), *Brucella abortus* coloured (plate) reagent (Institute of Veterinary Preventive Medicine, Ranipet, Tamil Nadu) and Asur Dx ELISA Kit (Biostone Animal Health) were used for RBPT, SPAT and i-ELISA respectively. Molecular identification was done with *Bcsp31* gene as per the protocol followed by Kumar *et al*. (2016). Serum samples were subjected to RBPT, SPAT as per the standard protocol (Alton *et al*., 1988), i-ELISA as per the protocol given by kit manufacturer. The stored serum samples and the reagents were brought to room temperature 30 minutes prior to start of the procedure.

Rose Bengal Plate Test (RBPT)

In a clean white tile, about 30 µl of RBPT antigen and equal volume of serum were added and mixed together using a glass rod and by rotating the tile. After four minutes, the result was read by the presence (positive) or absence (negative) of agglutination as shown in figure 1A.

Standard Plate Agglutination Test (SPAT)

Equal volume of serum and *Brucella abortus* coloured (plate) antigen was mixed and result read by the appearance of agglutination after four minutes and is shown in figure 1B.

Indirect Enzyme Linked Immuno-Sorbent Assay (i-ELISA)

i- ELISA was done as per the kit manufacturer's protocol in the antigen coated plate to detect the presence of antibodies and the OD value was measured by using an ELISA reader. Percent Positivity value was calculated with the formula as given below:

Percent positivity = [(OD450 of test sample) / (Mean OD450 of positive control)] × 100 Samples with a PP value greater than 40 and less than 40 were considered as positive and negative, respectively.

Molecular identification

Deoxyribo nucleic acid was extracted from tissue samples using the QIAmp® Fast DNA Tissue kit procured from QIAGEN, as per the kit manufacturer's protocol. Nano drop was used to assess the purity and concentration of DNA extracts. DNA extracts were stored at -80ºC, until further processing.

The obtained DNA extracts were subjected to polymerase chain reaction, with the primer sequence of *Bcsp31* gene as follows:

Gene	Sequence	Product size	Reference
*Bcsp*31 gene	5'-TGG CTC GGT TGC CAA TAT CAA-3'- F	**224 bp**	(Kumar *et al*., 2016)
	5'-CGC GCT TGC CTT TCA GGT CTG-3'- R		

Polymerase chain reaction was performed in a 25 µl volume mixture, with 12.5 µl of PCR master mix, 10 pM of forward and reverse primer each, 2 µl of template DNA and 8.5 µl of nuclease free water. The PCR cycling condition followed was initial denaturation for 5 minutes at 94ºC, 35 cycles of denaturation for 30 seconds at 94ºC, primary annealing for 45 seconds at 55ºC, extension for 1 minute at 72ºC followed by final extension for 10 minutes at 72ºC.

The obtained PCR product was run in 1.5% agar gel electrophoresis at 70V for 40 minutes along with 100 bp DNA marker. To visualize the DNA, the gel was placed and visualized in a Gel Doc imaging system (Fig. 2).

RESULTS AND DISCUSSION

Out of the 123 animals, the seroprevalence was 26.01, 22.76 and 41.46 per cent by RBPT, SPAT and i-ELISA respectively and is shown in table 1. In female animals, the seroprevalence was 25.21, 21.84 and 40.35 per cent by RBPT, SPAT and i-ELISA respectively. The prevalence of brucellosis in aborted animals was 40 per cent by all the four diagnostic tests used.

The higher sero-prevalence observed in the present study might be due to the system of rearing i.e. nomadic system of rearing with increased movement being practised by most of the shepherds of Cauvery Delta region, where they get mixed with other herds resulting in increased probability of acquiring infection (Motta *et al*., 2018: Olufemi *et al*., 2018). The higher sero-prevalence might also be due to close interaction between animal herds, sharing of grazing sites (Tulu, 2022) and watering points.

Out of 54 animals with the history of abortion in the last gestation, the prevalence of brucellosis in sheep with the history of abortion in the previous gestation was 51.85, 44.44 and 72.22 per cent by RBPT, SPAT and i-ELISA respectively. In addition to this, none of the animals without history of abortion were positive for brucellosis. Animals showing positive in all the serological tests were considered as positive (Chand and Chhabra, 2013). So, the prevalence of brucellosis in sheep of Cauvery Delta region was 44.44 per cent in the present study. Rahman *et al*. (2013) reported that brucellosis was mostly prevalent in the area where the small ruminants are commonly reared.

Shome *et al*. (2020) reported a seropevalence of 11.55 % in sheep with higher seropositivity in females (14.85 %) compared to males (3.90 %). In contrary to the present study, Kanani *et al*. (2018) and Selim *et al*. (2019) reported the seroprevalence of 24.44 and 16.3 per cent, respectively in sheep by ELISA, which was lesser than our results. Higher prevalence in our study, might be due to the sampling of more number of animals with the previous history of abortion.

Few researchers recommended the combined use of RBPT and ELISA to obtain high sensitivity (Rekha *et al*., 2013; Mangtani *et al*., 2020). Serological tests can be used efficiently to study the serological prevalence and each serological test have its own merits and demerits. So a combination of two or more serological tests is needed for confirmation (Viswanathan *et al*., 2019).

The higher prevalence of brucellosis by ELISA than RBPT and SPAT might be due to variation in prescribed cut-off point per cent positivity to differentiate positive and negative samples (Wainaina *et al*., 2020) and according to clinical and epidemiological considerations such as the history, duration of illness and other risk factors (Yagupsky *et al*., 2020).

As brucellosis affect a wide range of hosts from animals to humans and is having public health significance with zoonotic potential, there is a need to go for periodical screening of animals and their in-contact persons to minimize its further prevention to nearby flocks and other humans, by proper culling and disposal of animals (Saravanan *et al*., 2021). Brucellosis control measures like sanitation, test and slaughter along with vaccination can be followed (Dadar *et al*., 2021).

Table 1. Details of sample collection and seroprevalence by different serological tests

	Total no. of serum samples	RBPT		SPAT		ELISA	
		Positive	Negative	Positive	Negative	Positive	Negative
Male	4	2	2	2	2	3	1
Female	114	28	86	24	90	46	68
Aborted animals	5	2	3	2	3	2	3
Total	123	32	91	28	95	51	72
Per cent		**26.01**	**73.9**	**22.76**	**77.23**	**41.46**	**58.5**

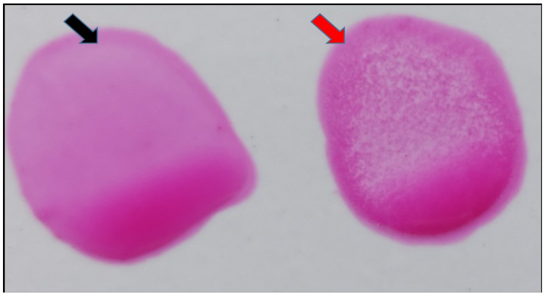

Fig.1A. Serum samples showing negative (black arrow – lack of agglutination) and positive (Red arrow – presence of agglutination) by RBPT

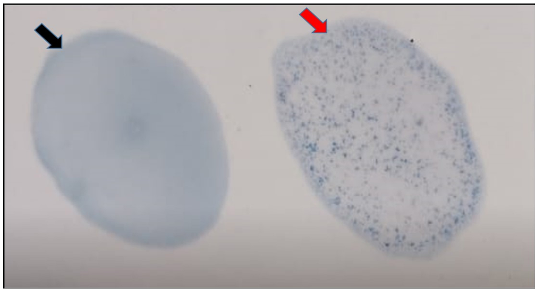

Fig. 1B. Serum samples showing negative (black arrow – no agglutination) and positive (Red arrow – presence of agglutination) by SPAT

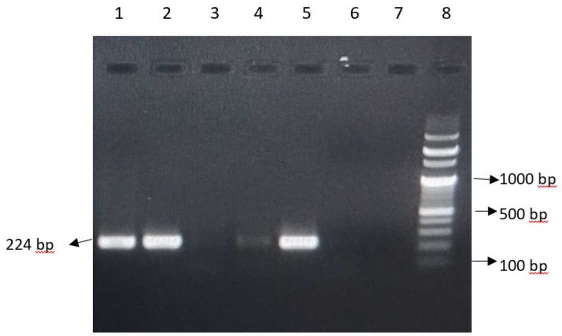

Fig 2. PCR product of 224 bp DNA of *Bcsp* 31 gene of *Brucella* sp. Lane 1 to 7 are samples and Lane 8 is 100 bp ladder. Lane 1, 2 & 5 are positive samples and Lane 7 is negative control

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