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Molecular Detection of *Brucella melitensis*: A Case of Sheep Abortion in Tirunelveli, Tamil Nadu

S. Rajagunalan[1*], C. Saranya Vellaiammal[2], A. Ganesan[3] and A. Sundar[4]

[1]Assistant Professor, Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, TANUVAS, Tirunelveli (Tamil Nadu), India.
[2]Project Technical Support, Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, TANUVAS, Tirunelveli (Tamil Nadu), India.
[3]Assistant Professor, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, TANUVAS, Tirunelveli (Tamil Nadu), India.
[4]Assistant Professor, Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, TANUVAS, Salem (Tamil Nadu), India.

(Corresponding author: S. Rajagunalan)*
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ABSTRACT: Reproductive loss in farm animals can be caused by various etiological agents investigation of the cause of abortion will help in reducing further loss in the farm. *Brucella melitensis* is an important zoonotic pathogen causing brucellosis. This disease poses serious economic and public health challenges worldwide. This study investigates a case of sheep abortion that was identified using conventional and molecular detection methods like serological, staining, culture, and PCR-based methods. The causative agent was identified using a *bcsp31* gene-based PCR assay and AMOS PCR and is supported by serological tests. The laboratory investigation revealed clear evidence of *B. melitensis* infection, providing critical insights into the epidemiology of brucellosis in sheep populations in this region. This case underscores the importance of routine molecular diagnostics for early detection of the causative agent and the need for public health interventions to mitigate the impact of this disease.

Keywords: *Brucella melitensis*, sheep, abortion, AMOS-PCR.

INTRODUCTION

India hosts the world's largest livestock population, with over 535.82 million animals. Despite this, livestock productivity remains significantly lower; this is primarily due to poor health caused by multiple endemic infectious diseases (Bardhan *et al.,* 2020; Khurana *et al.,* 2021). Sheep farming, a key livelihood for small and marginal farmers in India, plays a vital role in socioeconomic development. With 6.8% of the global sheep population (FAOSTAT, 2010), sheep are efficient utilizers of poor-quality grass and crop residues, converting them into valuable resources such as meat and skin (Singaravadivelan *et al.,* 2019). This is particularly relevant in the southern agro-climatic conditions of Tamil Nadu, where sheep farming sustains livelihoods under resource-constrained conditions (Ravimurugan *et al.,* 2012).

Reproductive losses remain one of the significant challenges in sheep farming, with brucellosis being a major concern. Brucellosis is a highly prevalent zoonotic disease caused by bacteria of the genus *Brucella*. The primary species, including *B. melitensis*, *B. abortus*, and *B. suis*, cause severe reproductive complications in animals and pose significant public health risks (Whatmore and Foster 2021; Shome *et al.,* 2021; Khurana *et al.,* 2021). Other common infectious causes of abortion are campylobacteriosis, salmonellosis, leptospirosis, listeriosis, chlamydiosis, coxiellosis, blue tongue virus, and others (Vidić *et al.,* 2007). In India, brucellosis leads to substantial economic losses due to abortion, retained placenta, endometritis, and infertility, with total losses estimated at Rs. 9,212 crores annually (Bardhan *et al.,* 2020).

In sheep, abortion caused by *B. melitensis* typically occurs in the later stages of pregnancy. The bacteria spread through contact with aborted materials, contaminated feed and water, and direct exposure to infected animals. Infected rams can also serve as sources of transmission. High-density flocking practices further accelerate the spread of infection (Buxton and Henderson 1999).

Although multiple species of *Brucella* can infect sheep, *B. melitensis* is the most common, and clinical signs are often absent in non-pregnant animals (Mantur and Amarnath 2008; Sonekar *et al.,* 2018). Transmission to humans occurs through direct contact with infected materials or consumption of unpasteurized milk and dairy products (Sonekar *et al.,* 2018; Islam *et al.,* 2023). Given the zoonotic potential and economic implications of brucellosis, early and accurate diagnosis is critical for effective disease control and management.

This study reports the detection of *B. melitensis* induced abortion in a sheep from Tirunelveli, Tamil Nadu, highlighting the importance of diagnosis in reducing

reproductive losses and preventing zoonotic transmission of the pathogen.

Case presentation: A non-descript sheep was presented to the Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli with the history of abortion and retained fetal membrane. The serum sample and a small piece of fetal membrane were collected from the ewe and submitted to the Department of Veterinary Public Health and Epidemiology, VCRI, Tirunelveli for laboratory investigation.

Laboratory investigation: The sample was processed under aseptic conditions.

Serological testing: The serum was subjected to the Rose Bengal Plate Agglutination test (RBPT) by testing 30 microliters of antigen and 30 microliters of serum. The antibody titer was determined by the *Brucella* micro-agglutination test in a 96-well V-bottom plate as described by Baum *et al.* (1995); Sundar *et al.* (2020) using *Brucella* plain antigen. The plate was incubated overnight at 37°C, and the wells were examined for agglutination reaction, and the titer was determined by examining for the highest dilution of serum showing button formation in the bottom of the well against an oblique light.

Pathogen detection: From the fetal membrane, an impression smear was prepared from the freshly cut surface and was allowed to air dry and heat-fixed. The smear was then stained using the modified Ziehl Neelsen method (Alton *et al.,* 1975) and examined under oil immersion. The freshly cut surface of the placenta was also used for inoculation of *Brucella* selective agar plates in duplicate, and one plate was incubated in a carbon dioxide incubator and the other under aerobic conditions at 37°C for 3 days. Bacterial colonies obtained were screened using PCR targeting the *bcsp31* gene of *Brucella*, using DNA extracted from the colonies.

PCR-based detection: A small piece of the fetal membrane was used for DNA extraction using the Nucelospin Tissue DNA Extraction kit (Mackeral Nagel) as per the manufacturer's protocol and stored at -20°C. A PCR reaction was set up targeting the *bcsp31* gene of *Brucella* in the presence of a positive control as described by Serpe *et al.* (1999). For identification of the species of *Brucella*, AMOS PCR was carried out as described by Bricker and Halling (1994). The details of the primers used are given in Table 1.

RESULTS AND DISCUSSION

Abortion in sheep can result from both infectious and non-infectious causes, with significant economic and public health implications. Investigating the cause of abortion becomes crucial, especially if abortion rates exceed 2% in a farm. However, identifying the etiological agent remains challenging, with detection rates ranging between 32% and 55% due to factors such as improper sampling, multiple etiologies, limited diagnostic facilities, and high costs (Vidić *et al.*, 2007).

In this case report, *B. melitensis* was identified as the causative agent of abortion in a sheep based on molecular diagnostic methods. Serological testing using the Rose Bengal Plate Agglutination Test (RBPT) revealed a positive result (Fig. 1). The *Brucella* Micro-Agglutination Test (B-MAT) indicated an antibody titer of 1:1280, supporting the diagnosis of brucellosis (Figure 2). Modified Ziehl-Neelsen (MZN) staining of the fetal membrane smear showed pink, cocco-bacillary organisms arranged individually or in small clusters, consistent with *Brucella* morphology (Figure 3). Although isolation of *Brucella* is considered the gold standard diagnostic method, it was unsuccessful in this case due to overgrowth of contaminating flora on selective media. This emphasizes the importance of collecting samples promptly and handling them under aseptic conditions to minimize contamination. Despite these challenges, PCR targeting the *bcsp31* gene successfully detected DNA specific to *Brucella* organisms in the fetal membrane (Figure 4). Furthermore, AMOS-PCR identified the species as *B. melitensis* based on the presence of specific amplicons, confirming the diagnosis (Fig. 5). The use of DNA-based methods can overcome the difficulties of culture-based methods, facilitating accurate and rapid identification of *Brucella* (Londhe *et al.,* 2010). Polymerase chain reaction-based methods are useful in the identification of *Brucella* from culture and clinical samples (Yu and Nielsen 2010). These methods are sensitive and rapid in providing results (Navarro *et al.,* 2004). Several PCR assays have been developed for the diagnosis of brucellosis. Some of the genes targeted include *bcsp31, 16S rRNA, IS711, BMEI1162, BMEII0466, alkB, eryC*, and *per* (Liu *et al.,* 2023). Amongst them, the *bcsp31* gene is the most commonly used target, as it is present as a single-copy gene in the genome of *Brucella* (Ghodasara *et al.,* 2010; Al-Dahouk *et al.,* 2013; Liu *et al.,* 2023). The AMOS PCR can give specific identification of *Brucella* species, and this assay has been used by several authors in the identification of the species of *Brucella* (Ewalt and Bricker 2000; Senthil *et al.,* 2019; Parthiban *et al.,* 2019; Saravanan *et al.,* 2021).

Although *B. ovis* is the species specific to sheep (Olsen and Palmer 2014; Sarvanan *et al.,* 2021), infection with *B. melitensis* is reported to be the most common species causing abortion in sheep in India (Upadhyay *et al.,* 2019; Sarvanan *et al.,* 2021). Sheep can acquire B. *melitensis* when these are raised along with goats, which is reported by other authors (Parthiban *et al.,* 2019; Parthiban *et al.,* 2021), and it is also a common practice in southern districts of Tamil Nadu that every sheep flock has a few goats raised along with sheep that could have played a role in the transmission of the disease.

The findings align with previous studies indicating a high prevalence of brucellosis in Indian sheep populations. Nationwide surveillance has reported a seroprevalence of 11.55% in sheep (Shome *et al.,* 2018), while a recent study in Tirunelveli district documented a prevalence of 19.25% using various serological tests (Sundar *et al.,* 2020). Senthil *et al.* (2019) also detected *B. melitensis* in samples collected from aborted ruminants. Sharma *et al.* (2018) recorded a total of 15 abortions in two sheep flocks in Udaipur,

Rajasthan, and isolated *B. melitensis* in these cases. Sarvanan *et al.* (2021) also detected *B. melitensis* among Mecheri sheep in Namakkal, Tamil Nadu. These findings highlight the endemic nature of brucellosis in the region and the urgent need for the implementation of control measures. This study underscores the utility of the molecular diagnostic method PCR in identifying *Brucella* species accurately. While conventional methods like culture remain valuable, they are time-consuming and resource-intensive. Combining molecular and serological diagnostics provides a more reliable approach to identifying brucellosis, especially in resource-limited settings. Early diagnosis and segregation of infected animals are crucial to prevent the spread of infection within flocks and to humans. This report of *B. melitensis*-induced abortion highlights the role of brucellosis in reproductive losses among sheep and its public health significance. The findings reinforce the importance of implementing robust diagnostic and control strategies to mitigate the impact of brucellosis on animal health, livelihoods, and public health.

Table 1: Details of the primes used in the present study.

Name	Sequences (5'-3')	Amplicon size	Reference(s)
BCSP31-F *BCSP31-R*	GGGCAAGGTGGAAGATTT CGGCAAGGGTCGGTGTTT	443 bp	Serpe *et al.* (1999)
AMOS-Ab AMOS-Me AMOS-Ov AMOS-Su AMOS-IS711	GACGAACGGAATTTTTCCAATCCC AAATCGCGTCCTTGCTGGTCTGA CGGGTTCTGGCACCATCGTCG GCGCGGTTTTCTGAAGGTTCAGG TGCCGATCACTTAAGGGCCTTCAT	498 bp 731 bp 976 bp 285 bp -	Bricker and Halling (1994)

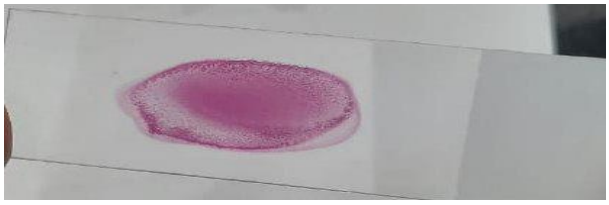

Fig. 1. RBPT test showing characteristic agglutination reaction.

Fig. 2. B-MAT showing positive reaction.

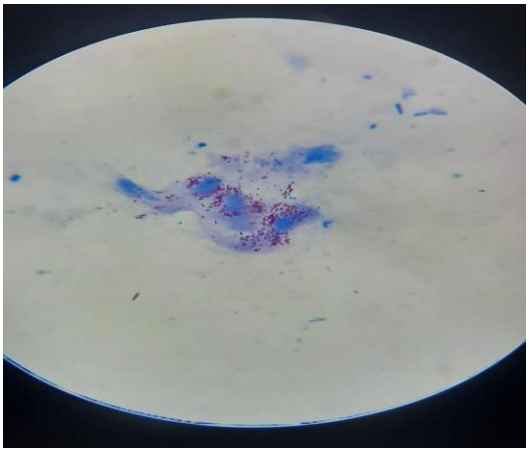

Fig. 3. Modified Ziehl Neelsen stained smear showing characteristic cocco-bacillary organisms against a blue background.

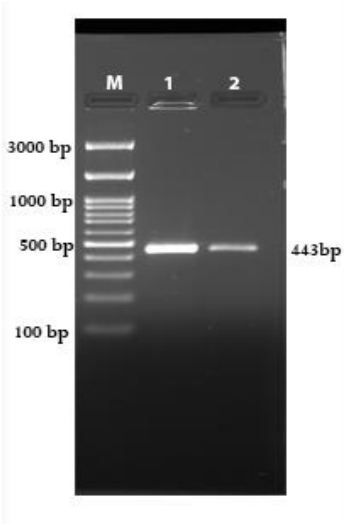

Fig. 4. Agarose gel showing *bcsp31* specific amplicons, Lane M: 100 bp ladder, Lane 1: Positive control, Lane 2: Sample.

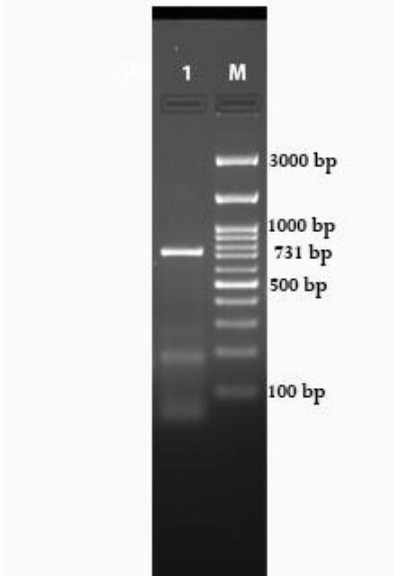

Fig. 5. Agarose gel showing AMOS PCR amplicons, Lane M: 100 bp ladder, Lane 1: Sample.

CONCLUSIONS

This report of *B. melitensis*-induced abortion highlights the role of brucellosis in reproductive losses among sheep and its public health significance. The findings reinforce the importance of implementing robust diagnostic and control strategies to mitigate the impact of brucellosis on animal health, livelihoods, and public health.

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Conflict of Interest. None.

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