

MOLECULAR AND SEROLOGICAL DETECTION OF *BRUCELLA MELITENSIS* IN CAPRINE POPULATIONS: BANGLADESH AGRICULTURAL UNIVERSITY GOAT FARM

A. A. Mamun,¹ I. A. Ema,¹ M. Afrose,⁶ R. R. Sarker,¹ A. H. Shuvo,¹ S.M. S. Ashraf,² F. I. Siddique,³ K. A. Konok,⁴ M. S. Ahmed,² A. Al-Maruf,¹ F. Yasmin,⁵ B. S. Ahmed,¹ and M. S. Rahman^{1*}

¹Department of Medicine, Faculty of Veterinary Science (FVS), ²Department of Pathology, ⁴FVS, and Interdisciplinary Institute for Food Security (IIFS), Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh; ³Armed Forces Medical College, Dhaka Cantonment, Dhaka, ⁵Bangladesh; Training, Planning & Technology Testing Division, Bangladesh Livestock Research Institute, Dhaka, Bangladesh; ⁶Department of Animal Science and Veterinary Medicine, Gopalganj Science and Technology University-8100, Gopalganj, Bangladesh

*Corresponding author: siddique.medicine@bau.edu.bd

ABSTRACT

Background: Brucellosis is one of the most important zoonotic diseases affecting a range of animals caused by *Brucella* species. Reported to have zoonotic importance include *B. abortus*, *B. melitensis*, *B. suis*, and *B. canis*. The causative agents of brucellosis in small ruminants are *B. abortus*, *B. melitensis*, and *B. ovis*, mainly associated with abortion, stillbirth, placentitis, and weak offspring, with the development of yellowish, sticky layers on the placenta in females, while in males, orchitis, epididymitis, as well as inflammation of the joints and bursae, are affected. Most serological studies have reported a high prevalence of caprine brucellosis in Bangladesh. However, molecular and serological detection of *B. melitensis* in caprine populations involves using a combination of antibody tests and DNA-based methods to identify infection.

Objective: The main aim of this study was to confirm RBPT- and ELISA-positive cases of *B. melitensis* infection in goats at BAUGF using a combination of serological (RBPT, i-ELISA) and molecular (PCR) tests.

Materials and Methods: A total of 18 blood samples were collected from goats of BAUGF and tested by Rose Bengal Plate Test (RBPT), the indirect enzyme-linked immunosorbent assay (i-ELISA), and polymerase Chain Reaction (PCR).

Results: Serological analysis of 18 goats revealed decreasing positivity rates across methods: 33.33% (Rapid Kit), 22.22% (Rose Bengal Plate Test), and 16.67% (both ELISA and PCR), with females (23.07%) showing higher infection rates favorable with all the used four tests, than males, which are negative with two tests (RKT & RBPT), and positive with two tests (i-ELISA & PCR) with only one case (20.0%) positive. Of the 18 goats screened, PCR detected *B. melitensis*-specific omp2 genes in 11.11% of samples.

Conclusions: Serological evidence of caprine brucellosis in goats and molecular detection of *B. melitensis* in Bangladesh warrant regular screening, surveillance, and reporting of disease in goats and human risk groups.

Keywords: RBPT, i-ELISA, PCR, *Brucella melitensis*, zoonosis, goats, Bangladesh

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INTRODUCTION

Brucellosis is a globally distributed zoonotic bacterial disease that is endemic in humans and animal populations in Bangladesh.¹ Brucellosis has been recognized as an occupational hazard for livestock farmers of brucellosis-positive herds, who had a significantly higher probability of being seropositive for brucellosis. A study emphasized that contact with livestock, especially goats, is a significant risk factor for brucellosis transmission among individuals in the high-risk occupational groups.² A review of the analysis of 16 reports on the sero-prevalence of caprine brucellosis revealed that 1.56% goats were found positive for *Brucella* infections by using different serological tests, including TAT, RBPT, PAT, iELISA, MET, and SAT.¹ However, molecular and serological detection of *B. melitensis* in caprine populations involves using techniques like the Rose Bengal Plate Test (RBPT), ELISA, and Polymerase Chain Reaction (PCR) to identify the presence of antibodies and bacterial DNA, respectively. These methods are used to screen for the zoonotic disease of goats, which can cause abortions and is a public health concern for those who handle them. Studies have shown that molecular techniques are highly sensitive and can detect infections in even serologically negative animals, highlighting the importance of using both methods for comprehensive surveillance. Combining the molecular and serological methods is crucial because molecular tests can detect infection even in serologically negative animals, such as those with recent infections, and serological tests provide a broader view of the herd's exposure to the disease. A recent study on the sero-prevalence of caprine brucellosis at Bangladesh Agricultural University goat farm (BAUGF) by using RBPT (2.67%) and i-ELISA (2.0%) has reported a significantly higher seropositivity of *Brucella* infection in goats with a previous history of abortion, with both the RBPT (30.0%) and i-ELISA (20.0%) in the BAUGF.³ Considering these facts, the main objective of this study was to confirm the RBPT and ELISA positive cases of *B. melitensis* infection in goats of BAUGF by using a combination of serological (RBPT, i-ELISA) and molecular (PCR) tests.

MATERIALS AND METHODS

A cross-sectional study was conducted at BAUGF in Mymensingh from April to September 2024. Blood samples (5-7 ml) were collected aseptically from the jugular vein of 18 goats (5 males, 13 females; age distribution: <1 year [n=3], 1-2 years [n=9], >2 years [n=6]). The samples were left to clot at room temperature for 30 minutes, incubated overnight at 4°C, and then centrifuged at 2500 rpm for 10 minutes. The obtained sera were stored at -20°C until further analysis.

Serological screening

All the samples were initially screened via the *Brucella* Antibody Rapid Kit Test (sensPERT) (Fig. 1) and Rose Bengal Plate Test (RBPT). For the rapid test, two drops of serum and two drops of test buffer were applied to the sample well, and the results were read after 15 minutes. RBPT was performed according to OIE (2004) procedures, involving mixing 30 µL of serum with 30 µL of Rose Bengal antigen on a glass plate, rocking for 4 minutes, and examining for agglutination (Fig. 2).

Indirect ELISA

Samples that were positive in the initial screening were subjected to an indirect ELISA (Svanova Biotech AB, art. No. 10--2700--10, Uppsala, Sweden) according to the manufacturer's instructions. PBS-Tween buffer was prepared by diluting the 20 × concentrate 1:20 in distilled water (Figure 4). Serum samples and controls (4 µl) were added to wells containing 100 µl of sample dilution buffer, incubated at 37°C for 1 hour, rinsed three times with PBST, and subsequently treated with 100 µl of HRP conjugate at 37°C for 1 hour. After washing, 100 µl of the substrate mixture was added, and the mixture was incubated at room temperature for 10 minutes; the reaction was then terminated by adding 50 µl of stop solution. The optical density was then recorded at 450 nm within 15 minutes.

Molecular confirmation

DNA was isolated from blood samples via a GeneJet Genomic DNA Purification Kit (catalog # K0722-250). The DNA concentration was determined spectrophotometrically (NanoDrop ND-2000). PCR was used to target the IS711 sequence region specific for *B. melitensis* (expected amplicon: 731 bp) via the following primers: 5'-AAATCGCGTCCTTGCTGGTCTGA-3' (forward) and 5'-TGCCGATCACTTAAGGGCCTTCAT-3' (reverse). The vaccine strain RB51 was used as a positive control. Amplification was performed using a commercial PCR master mix (Thermo Scientific #K0171) with an initial denaturation at 95°C for 10 minutes, followed by 35 cycles of 95°C for 45 seconds, 50°C for 45 seconds, and 72°C for 45 seconds, with a final extension at 72°C for 10 minutes.⁴ The PCR products were electrophoresed at 100 V for 20 minutes and compared against a 100 bp DNA ladder (Fig. 4).

RESULTS

Molecular & serological detection of *B. melitensis* in goats

In goats (n=18), the detection rates for *B. melitensis* were 33.33% with the rapid kit, 22.22% with RBPT, 16.67% with ELISA, and 16.67% with PCR. The seroprevalence was highest in animals >24 months (33.33%) and was higher in females (23.07%) than in males (Table 1).

A total of 18 goat serum samples from the BAUGF were screened for *B. melitensis* via multiple diagnostic methods. The overall seroprevalence varied by test methodology, with the highest detection rate observed using the Rapid Kit Test (6/18, 33.33%), followed by RBPT (4/18, 22.22%), while both i-ELISA and PCR detected identical prevalence rates (3/18, 16.67%). These findings suggest potential differences in test sensitivity and specificity, with implications for selecting diagnostic strategies in caprine brucellosis surveillance programs. (Table 1)

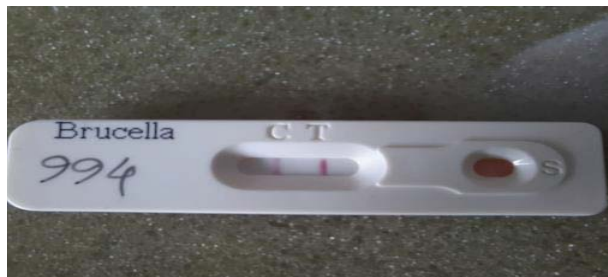


Fig. 1. *Brucella melitensis* Rapid Antibody Kit Test positive result on goat serum showing two bands (Control line C and positive line T).

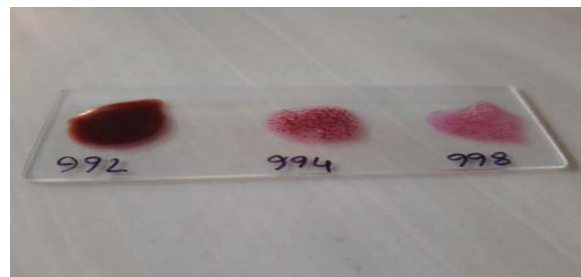


Fig. 2. Rose Bengal Plate Test on serum of goats showing positive for antibodies of *Brucella melitensis* infection in the goat.



Fig. 3. ELISA test on goat serum showing positive reactions to *Brucella melitensis* infection in goats

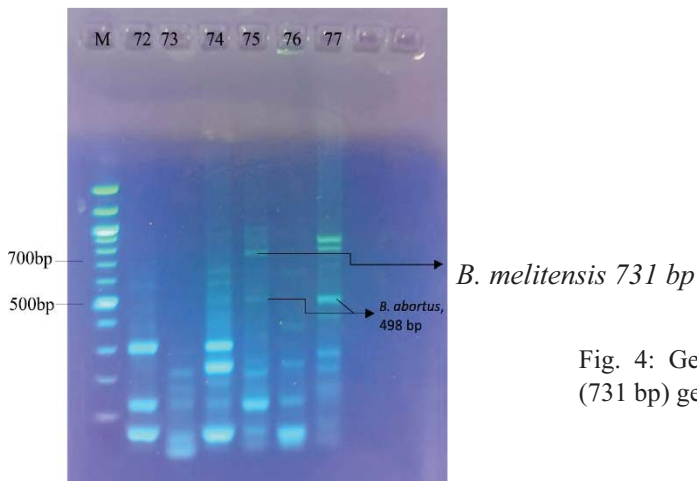


Fig. 4: Gel electrophoresis image showing amplicons (731 bp) generated by the IS711 PCR assay.

Table 1. Gender and age-wise prevalence of caprine brucellosis at the BAUGE, Mymensingh							
SN	Variables	Factors	No. of Samples tested	Tests used with No. (%)	No. of positive and percentage	ELISA positive No. (%)	PCR positive No. (%)
①	Gender	Male	05	1 (20.00)	1 (20.00)	0	0
		Female	13	5 (38.46)	3 (23.07)	3 (23.07)	3 (23.07)
②	Age (months)	06-12	03	0	0	0	0
		13-24	09	3 (33.33)	2 (22.22)	1 (11.11)	1 (11.11)
		>24	06	3 (50.00)	2 (33.33)	2 (33.33)	2 (33.33)
	Overall	-	18	6 (33.33)	4 (22.22)	3 (16.67)	3 (16.67)

The gender-wise analysis of brucellosis sero-prevalence revealed notable differences between male and female goats. Among the five male goats tested, positivity was detected only by the Rapid Kit Test and RBPT (20% each), while i-ELISA and PCR showed no positive results (Table 1). In contrast, female goats (n=13) demonstrated consistently higher sero-positivity across all the tested methods: Rapid Kit Test (38.46%), RBPT (23.07%), i-ELISA (23.07%), and PCR (23.07%). These findings suggest that female goats may be more susceptible to *B. melitensis* infection, potentially linked to reproductive factors, with important implications for herd management and disease control strategies (Table 1).

Age-wise analysis of brucellosis sero-prevalence demonstrated a clear age-dependent pattern of infection. Young goats (6-12 months, n=3) presented no evidence of *B. melitensis* infection across all the diagnostic platforms (Table 1). In contrast, middle-aged goats (13-24 months, n=9) exhibited moderate sero-positivity: rapid kit test (33.33%), RBPT (22.22%), and both i-ELISA and PCR (11.11%). The highest sero-prevalence was observed in older goats (>24 months, n=6), with positivity rates of 50% (Rapid Kit Test), 33.33% (RBPT), and 33.33% (both i-ELISA and PCR). This progressive increase in seropositivity with age suggests cumulative exposure over time, highlighting the importance of age-targeted surveillance and control measures for caprine brucellosis management (Table 1).

DISCUSSION

Caprine brucellosis caused by *B. melitensis* is a significant zoonotic disease, particularly prevalent in tropical and subtropical regions worldwide. It is recognized as a neglected tropical disease with a

substantial economic impact in developing countries, including Bangladesh. The disease manifests itself in the goat populations, primarily in the reproductive and placental tissues, leading to abortion in goats. At the same time, generalized bacteremia affecting a wide range of organ systems is also possible. The most virulent cause of brucellosis in human subjects is *B. melitensis*. Human risk groups for brucellosis include those with occupational contact with animals, such as veterinarians, farmers, butchers, and laboratory personnel, who may contract infections from goat populations. Individuals who consume unpasteurized milk or raw cheese exhibit a wide range of clinical symptoms affecting various organ systems, with undulating fever (pyrexia of unknown origin) being the most manifested. Although isolation by culture is the gold standard for diagnosing brucellosis, the zoonotic nature of the bacteria, long incubation periods, and the need for biosafety cabinets pose considerable drawbacks. Thus, various indirect (serological) and direct (molecular) tests are envisaged for screening and diagnosing brucellosis in both goats and human subjects.⁵

Combining a serological test for antibodies with a molecular test for the bacterium's DNA provides a more complete and accurate diagnosis. Serological tests can be negative in animals in the very early or late stages of infection, or even positive due to cross-reaction. In contrast, molecular tests may detect DNA present in tissues or body fluids, thus providing a more complete picture of infection. Molecular testing can confirm serological test results, especially in borderline cases. Molecular and serological methods are used together to detect *B. melitensis* in goats, as they complement each other by identifying antibodies (serology) and the pathogen's DNA (molecular). Serological tests such as RBPT and ELISA detect the immune response to bacteria. At the same time, molecular techniques such as real-time PCR or PCR-RFLP can identify the specific species and even biovar of *B. melitensis* in samples from serum, milk, or tissues. This combined approach is crucial for accurate diagnosis and effective disease management. RBPT is a rapid screening test often used to detect the seroprevalence of brucellosis in animals. In contrast, ELISA is a standard screening and confirmation method that detects antibodies in serum and milk.

The overall prevalence of brucellosis in goats in the present study was 33.33% by RKT, 22.22% by RBPT, 16.67% by i-ELISA, and 16.67% by PCR. These results on the sero-prevalence of brucellosis in goats support the earlier report of 30.0% prevalence by RBPT and 20% by i-ELISA in the same goat flock.³ A review of 16 reports on the sero-prevalence of caprine brucellosis in Bangladesh reported 7.27% sero-positivity by TAT, 4.41% by RBPT, 7.69% by PAT, 3.7% by i-ELISA, 2.53% by MET, and 4.17% by SAT with multiple sources of samples.¹ The higher prevalence of caprine brucellosis in a single goat flock might be due to the limited number of samples tested in a single caprine flock exposed earlier to a source of *Brucella* infection. Livestock farmers do not cull and dispose of brucellosis-positive animals; instead, they sell them to other farmers, allowing infected animals to enter a *Brucella*-free herd and transmit infection to other animals. However, these findings support the results of 13% prevalence of caprine brucellosis by RBPT, 9.75% by i-ELISA, and 6.5% by PCR in multiple flocks of Pakistan.⁶

While comparing different diagnostic tests, a higher percentage of positive cases was detected by RKT (33.33%) and RBPT (22.22%) compared to i-ELISA (16.67%) and PCR (16.67%). The higher sero-prevalence detected by RKT and RBPT might be due to RBPT detecting both IgM and IgG antibodies, which may lead to some cross-reaction with other bacteria. A review of research reports showed that i-ELISA is more sensitive than conventional tests such as RKT and RBPT.^{7,8} The higher sensitivity of i-ELISA due to its recognition of cytosolic antigen S-LPS fragments may decrease cross-reaction with other Gram-negative bacteria.⁹ PCR detects DNA, which may be present in small amounts in serum samples. Alternatively, the titer in serum may be undetectable, but the amount of DNA present in the sample may be sufficient for PCR detection. As little as 5fg of DNA can be detected using PCR. PCR is more reliable and sensitive because it can detect antigens rather than antibodies. Accordingly, PCR is

more sensitive than conventional tests for detecting *B. melitensis* in goats.¹⁰

The sero-prevalence of *B. melitensis* infection was recorded as higher in females (i-ELISA 23.67%) than in males (i-ELISA 0%) animals. A higher sero-prevalence of caprine brucellosis has been reported in female (RBPT 10.4%) than in male (RBPT 1.5%) goats in Pakistan.¹¹ In contrast, an insignificant difference in the seropositivity between male (RBPT 3.33%) and female (RBPT 3.93%) goats has been reported in Bangladesh.¹² In the present study, the number of male goats was lower than the number of female goats, because positive bucks of the same herd might have served female goats or were older in age, increasing the risk of getting infected.

CONCLUSION

This study detected *B. melitensis* in goats at the BAUGF, with an overall sero-prevalence of 16.67% by i-ELISA and PCR. These findings suggest that female goats and older animals are more susceptible to infection, highlighting the need for targeted surveillance and control strategies. This study provides critical baseline epidemiological data for implementing evidence-based strategies to reduce the economic losses and public health risks of brucellosis in goat populations in Bangladesh. The results emphasize the importance of continued monitoring and control efforts to reduce the prevalence of this zoonotic disease.

ETHIC STATEMENT

All animal-related procedures and methods were carried out following the guidelines of the Animal Welfare and Experimentation Ethical Committee of the Bangladesh Agricultural University, Mymensingh (Ethical approval number - AWEEC/BAU/2023(55))

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CONFLICT OF INTEREST

There is no conflict of interest to disclose.

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