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RESEARCH ARTICLE

Sero-prevalence and Risk Factors of Brucellosis in Goats in Bangladesh: Rose Bengal Plate Test and Enzyme-Linked Immunosorbent Assay Based Approaches

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ABSTRACT

Brucellosis is a zoonotic bacterial disease in goats. The causal agents are *Brucella* spp. and showing symptoms of abortions, stillbirths, and weak offspring in females and orchitis in males. The economic impact includes declined milk production and abortion, alongside the potential zoonotic transmission through milk and uterine discharges. The objective of this study was to determine the seroprevalence of caprine brucellosis in three districts of Bangladesh- Jhenaidah, Meherpur, and Mymensingh. Rose Bengal Plate Test (RBPT) and indirect Enzyme-Linked Immunosorbent Assay (i-ELISA) were used to detect *Brucella*-specific antibodies in infected animals. A total of 210 serum samples were collected. Goats were categorized based on geographic location, sex, breed, housing system, and rearing practices to evaluate potential risk factors. The overall prevalence of brucellosis was 3.81% by RBPT and 2.38% by ELISA. District-wise prevalence was highest in Mymensingh (RBPT: 4.123%, ELISA: 2.061%), followed by Jhenaidah (RBPT: 3.947%, ELISA: 2.631%), and Meherpur (RBPT and ELISA: 2.702%). Household-based farms exhibited the highest prevalence (RBPT: 6.976%, ELISA: 4.651%). Female goats showed a higher seroprevalence (RBPT: 4.081%, ELISA: 2.721%) compared to males (RBPT: 3.174%, ELISA: 1.587%). Among breeds, Black Bengal goats had the highest prevalence (RBPT: 4.032%, ELISA: 2.419%), and goats under free-ranging management showed the highest infection rates (RBPT: 4.545%, ELISA: 3.409%). This study emphasizes the prevalence and diversity of caprine brucellosis in Bangladesh across various regions, animal populations, and management approaches. The results highlight the necessity of regular surveillance and efficient control measures to mitigate the economic and public health impacts of brucellosis in goat farming systems.

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INTRODUCTION

Brucellosis is an important zoonotic disease with global implications for animal welfare and economics, and has been recognized since ancient times (Khurana *et al.,* 2021). This disease manifests significantly in animals, with males experiencing orchitis and epididymitis, and females suffering from abortion, reproductive disorders, and placenta retention (Ukwueze *et al.,* 2022). Outbreaks typically coincide with late pregnancy abortions, leading to weak calves and infertility in cattle (Yanti *et al.,* 2021).

The causative agents, *Brucella* spp., are facultative intracellular, non-motile bacteria devoid of flagella, capsules, and endospores (Coloma-Rivero *et al.,* 2021). In humans, *Brucella* infections cause febrile septicemia and localized infections in various organs, with diverse incubation periods (Ihsan Rashan *et al.,* 2022). Transmission among animals occurs through direct contact, sexual contact with infected animals, and exposure to contaminated materials such as aborted placentas and fetal fluids (González-Espinoza *et al.,* 2021).

Brucellosis is recognized as a significant zoonotic disease worldwide by the WHO, FAO, and OIE (Bansal *et*

al., 2023; Qureshi *et al.,* 2023). The disease causes economic losses through abortion, infertility, and stillbirth. It poses major public health risks through direct contact with infected animals or by consumption of dairy products from infected animals (Khan and Zahoor, 2018; Bansal *et al.,* 2023). Globally, over 500,000 people are infected by brucellosis each year (Laine *et al.,* 2023). In Bangladesh economic losses attributed to brucellosis are estimated to be over US$ 605, 455 annually, with losses due to mortality, decreased productivity, and cost of treatments (Ahmed *et al.,* 2018). In India alone, direct economic losses from brucellosis in livestock are estimated at approximately US$ 3.4 billion per year (Singh *et al.,* 2015).

Goats are vital to food production and rural livelihoods in South and Southeast Asia, with Bangladesh housing approximately 26 million goats that contribute 20–25% of total meat and 2–3% of milk output (DLS, 2023; Rakib *et al.,* 2022). Despite their importance, goat productivity is hindered by diseases such as brucellosis, PPR, and goat pox, resulting in substantial morbidity, mortality, and economic losses (Roy *et al.,* 2015, Munsi *et al.,* 2021). Brucellosis, which is endemic in both animals and humans in Bangladesh, leads to continuous economic losses through abortion, infertility, reduced milk production, stillbirths, prolonged inter-calving intervals, and international trade restrictions (Rahman *et al.,* 2012; Islam *et al.,* 2021; Munsi *et al.,* 2021; Bilal *et al.,* 2024). Although some districts report prevalence rates of 4.33% (RBPT) and 2.40% (c-ELISA), comprehensive data for goats remain limited.

Goats are primarily infected by *B. melitensis*, which is highly virulent in humans (Tekle *et al.,* 2019; Munsi *et al.,* 2021). *B. abortus* can also cause infection in goats. Diagnosis of brucellosis is based on clinical signs as well as biochemical and serological tests (Kurmanov *et al.,* 2022). Commonly used serological tests include Rose Bengal Plate Test (RBPT), ELISA, serum agglutination test, and complement fixation test (Gwida *et al.,* 2010). According to the OIE, ELISA and RBPT are reliable tools to detect *Brucella*-specific antibodies (Munsi *et al.,* 2021). PCR is also employed for the detection of *Brucella* spp., although it requires biosafety level-3 (BSL-3) laboratory facilities for culturing and molecular characterization (Munsi *et al.,* 2021). Loop-mediated isothermal amplification (LAMP) assay has also been evaluated as a potential field diagnostic method, showing promise due to its simplicity and sensitivity (Bilal *et al.,* 2024).

Brucella species exhibit marked host specificity. For example, *B. abortus* primarily affects cattle and buffaloes, occasionally infecting horses and humans; *B. melitensis* is the main pathogen in small ruminants and a major cause of human brucellosis; and *B. ovis* affects sheep. Other species such as *B. ceti*, *B. pinnipedialis*, and *B. microti* are associated with marine mammals and wildlife (Tulu, 2022; Bilal *et al.,* 2024). This host adaptation contributes to the complex epidemiology of brucellosis.

The disease causes severe reproductive issues in goats and poses serious zoonotic risks. Brucellosis remains a major threat to animal and public health even in countries with structured control programs. For example, in Kazakhstan, the highest brucellosis incidence has been attributed to insufficient epizootiological monitoring, environmental persistence of Brucella, and the widespread use of pasture-based livestock systems, which complicate eradication efforts (Ospanov *et al.,* 2024). Similar complexities are observed in Iran, where Bayesian approaches have been employed to estimate the true prevalence of brucellosis across various species, highlighting regional differences and surveillance gaps (Meletis *et al.,* 2024). In India, a recent sero-prevalence study in small ruminants also confirmed ongoing transmission in high-density livestock regions (SreeLakshmi *et al.,* 2024).

These challenges underscore the importance of localized surveillance and context-specific control strategies. In Bangladesh, where goat farming is crucial for rural livelihoods and food security, understanding these regional dynamics is essential for designing effective interventions. This study aims to detect the prevalence of brucellosis in goats across different districts of Bangladesh and to identify associated risk factors such as geographical location, housing practices, and animal demographics to enhance the management of this significant zoonotic disease.

MATERIALS AND METHODS

Ethics statement: The study adhered to ethical guidelines, with a strong emphasis on minimizing harm and safeguarding animal welfare throughout the process. Blood sample collection was performed by registered veterinarians.

Study area and period: The cross-sectional study was conducted across Jhenaidah, Meherpur, and Mymensingh districts of Bangladesh (Fig. 1) from April 2022 to April 2023. All experiments were conducted at the Bacteriology Laboratory of the Department of Microbiology & Hygiene, Bangladesh Agricultural University, Mymensingh-2200.

Collection of Epidemiological Information: Animal owners were informed about the epidemiological study, and data collection was conducted sequentially using a structured questionnaire. Information collected included animal sex, breed, housing type, farm classification, pregnancy status, and management practices (Table 1).

Table 1: Variables in epidemiological study and number of goats

Variables	Category	Number of collected samples
Sex	Male	63
	Female	147
Breed	Black Bengal	124
	Jamunapari	35
	Deshi/cross	51
Housing type	Intensive	44
	Semi-intensive	52
	Free ranging	66
Farm type	Household (5-10)	43
	Medium farm (6-10)	20
	Commercial (above 11)	62
Pregnancy	Pregnant	35
	Non pregnant	26
Raring with cattle	Yes	55
	No	65

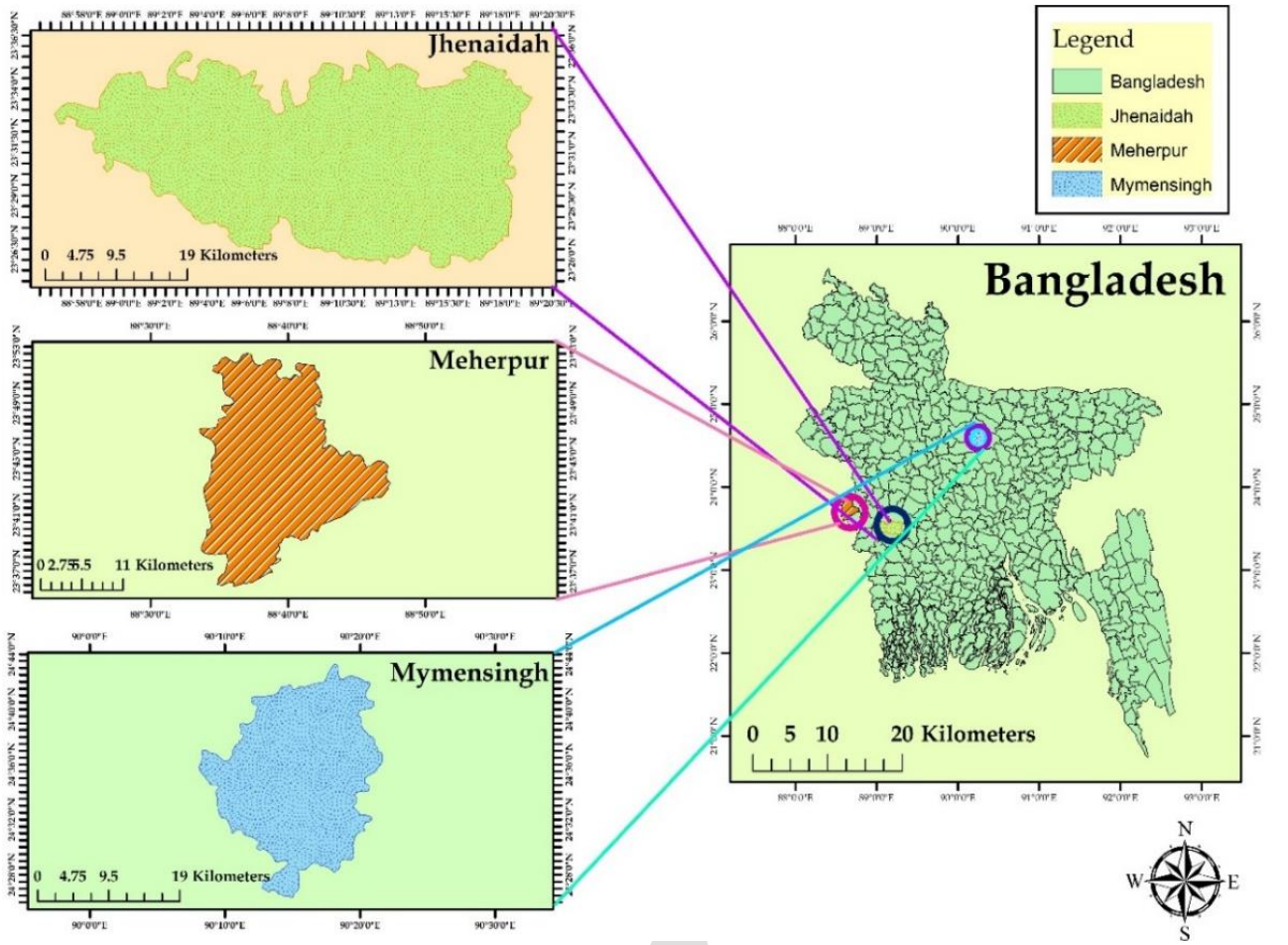

Fig. 1: Map of the study area.

Sample Size Determination: The number of animals selected for blood sampling was determined based on a standard formula for estimating sample size in prevalence studies, as described by Cochran (1963):

$$n = \frac{Z^2 \times P \times (1 - P)}{E^2}$$

Where:

- n = required sample size
- Z = 1.96 (standard normal deviate for 95% confidence interval)
- P = expected prevalence (4.33%, based on Munsi *et al.,* 2021)
- E = margin of error (5%)

Substituting the values:

$$n = \frac{(1.96)^2 \times 0.0433 \times (1 - 0.0433)}{0.05^2} = \frac{3.8416 \times 0.0433 \times 0.9567}{0.0025} = 191.17 \approx 192$$

To accommodate potential non-response or unusable samples, an additional 10% (≈19) was added to the minimum required sample size, resulting in a final target of 210 samples.

Collection and Preparation of Serum Samples: A total of 210 blood samples were collected from goats. Collected blood samples were allowed to clot in upright, undisturbed positions for 30min at optimum temperature and pressure. After clotting, samples were transferred to new sterile containers labeled with details. Serum samples were packed in secondary bags transported in ice-cooled containers to the testing laboratory. Clotted blood was centrifuged at 3000-4000rpm for 10min. The serum was collected from the top of the centrifuged blood. Then stored separately at -20°C for short term and -80°C for long-term (Guzmán-Bracho *et al.,* 2020).

Rose Bengal Plate Test (RBPT): The RBPT was conducted using a commercial antigen (IDvet Rose Bengal Antigen, France) following the manufacturer's protocol. In brief, 15µL of the antigen was added with 15µL of the serum sample and thoroughly mixed and incubated at room temperature (25°C). The reaction was observed for agglutination after 4min (Sharma *et al.,* 2017)

Indirect ELISA: A commercial kit (ID Vet Brucella Specific ELISA Kit, France; BRUS-MS) was used following manufacturer instructions. Briefly, 100µL of serum samples were diluted in sample diluent and added to microtiter plate wells. After incubation at room temperature (25°C), the plate was washed thrice with wash buffer, and 100µL of conjugate was added to each well, followed by another incubation and wash step. Substrate was subsequently added, incubated, and the reaction stopped using 100µL of stop solution. Plates were read at 450nm using an ELISA reader. Results were interpreted according to the manufacturer's instructions using the S/P% calculation:

S/P% = (OD sample − OD negative control) / (OD positive control − OD negative control) × 100
The interpretation criteria were: S/P% ≥ 120%: Positive; 110% ≤ S/P% < 120%: Doubtful; S/P% < 110%: Negative

Statistical Analysis: All data were recorded and initially processed in Microsoft Excel 365 (Microsoft/Office 365, Redmond, WA, USA). Statistical analysis of prevalence data was conducted using Statistical Package for Social Science (SPSS.v.25, IBM, Chicago, IL, USA) to calculate the overall and category-wise prevalence (%) of brucellosis as detected by RBPT and ELISA. The chi-square test (χ^2) was used to evaluate associations between seropositivity and various categorical risk factors including region, sex, breed, housing system, age group, pregnancy status, farm type, and cattle association. A P-value<0.05 was considered statistically significant.

RESULTS

Prevalence and Risk Factor Analysis: The overall prevalence of brucellosis in the sampled goat population was 3.81% based on the RBPT and 2.38% based on the i-ELISA), indicating the presence of *Brucella*-specific antibodies. Fig. 2 illustrates the RBPT results, where a positive reaction is characterized by visible agglutination. Fig. 3 presents the i-ELISA results, with positive samples exhibiting a yellow color change in the micro-wells.

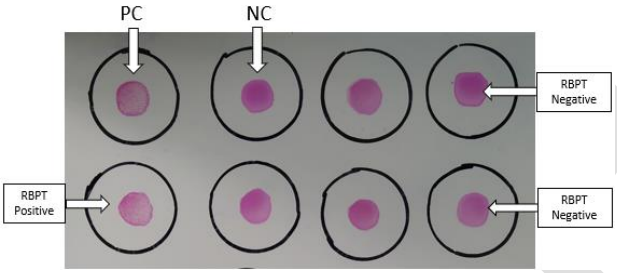

Fig. 2: Results of RBPT, positive reaction shows agglutination, PC, denotes positive control, and NC, denotes negative control.

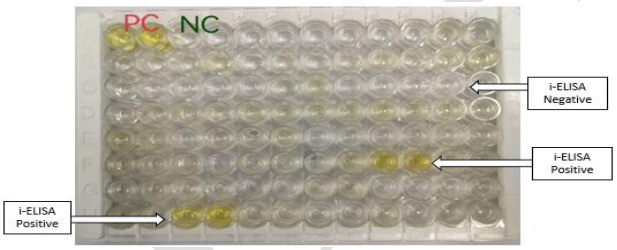

Fig. 3: Results of i-ELISA, positive reaction shows yellow color change in the microwells. PC, denotes positive control, and NC, denotes negative control.

There was a statistically significant association between region and brucellosis prevalence (P< 0.05). Varying rates were observed in Jhenaidah (RBPT: 3.947%, i-ELISA: 2.631%), Mymensingh (RBPT: 4.123%, i-ELISA: 2.061%), and Meherpur (RBPT: 2.702%, i-ELISA: 2.702%) (Fig. 4). Gender also showed a significant association with seroprevalence (P<0.05). In male goats, the prevalence was 3.174% by RBPT and 1.587% by ELISA, while in female goats it was 4.081% by RBPT and 2.721% by ELISA (Fig. 5).

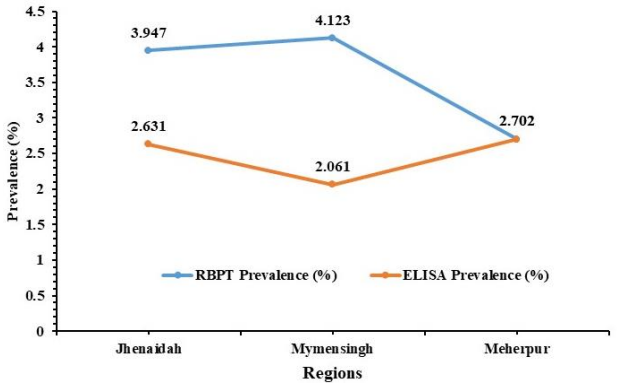

Fig. 4: The regional distribution of brucellosis prevalence in goats as detected by RBPT and i-ELISA across three study areas: Jhenaidah, Mymensingh, and Meherpur.

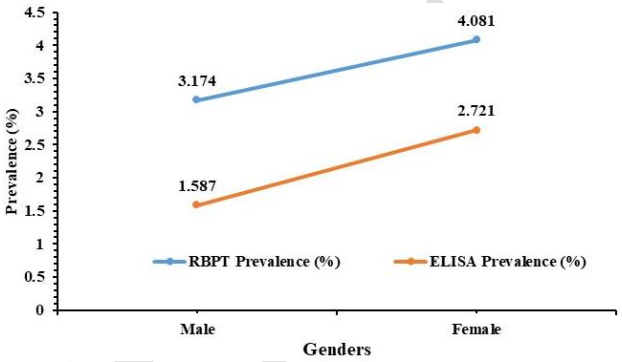

Fig. 5: The scatter graph represents the prevalence of brucellosis in goats based on genders determined by RBPT and i-ELISA.

Regarding breed, the differences were statistically significant (P<0.05). Black Bengal goats exhibited a higher prevalence (RBPT: 4.032%, i-ELISA: 2.419%) than Jamunapari (RBPT: 2.857%, i-ELISA: 2.857%) and Deshi/Crossbreeds (RBPT: 3.921%, i-ELISA: 1.960%) (Fig. 6).

Housing systems exhibited significant differences in brucellosis prevalence (P<0.05). Free-ranging goats had the highest prevalence (RBPT: 4.545%, i-ELISA: 3.030%), followed by semi-intensive systems (RBPT: 3.846%, i-ELISA: 1.923%) and intensive systems (RBPT and i-ELISA: 2.272%) (Fig. 7).

For age groups, no statistically significant association was observed (P>0.05). Goats aged 0–6 months had a prevalence of 3.125% by RBPT. The 6–12 month group showed 4.761% by RBPT and 2.380% by ELISA; 12–24 months group had 3.030% by both tests; and goats above 24 months_showed 4.285% by RBPT and 2.857% by ELISA.

Analysis of reproductive and management factors such as abortion history, retained placenta, infertility, pregnancy status, and co-rearing with cattle showed higher prevalence values in certain subgroups but did not show statistically significant associations (P>0.05). For instance, goats with a history of abortion showed a prevalence of 15.79% by RBPT and 10.53% by ELISA; goats with retained placenta had 11.11% by RBPT; those with infertility showed 6.25% by RBPT and 1.25% by ELISA. Pregnant goats showed 5.71% by RBPT and 2.86% by ELISA, while non-pregnant goats had 3.85% by both tests. Goats reared with cattle showed 3.64% (RBPT) and 1.82% (ELISA) prevalence, compared to 3.08 and 1.54% respectively in those not reared with cattle.

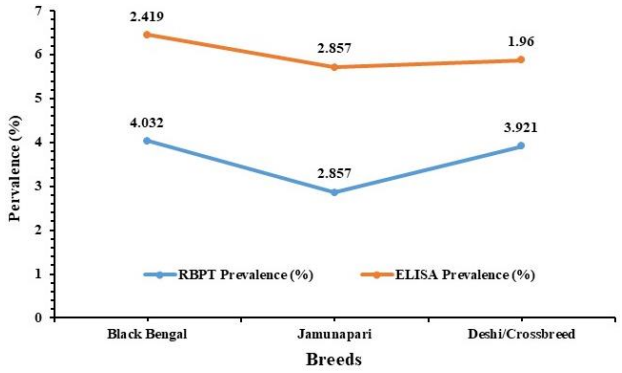

Fig. 6: The scatter graph depicts the breed wise prevalence of brucellosis in goats detected by RBPT and i-ELISA.

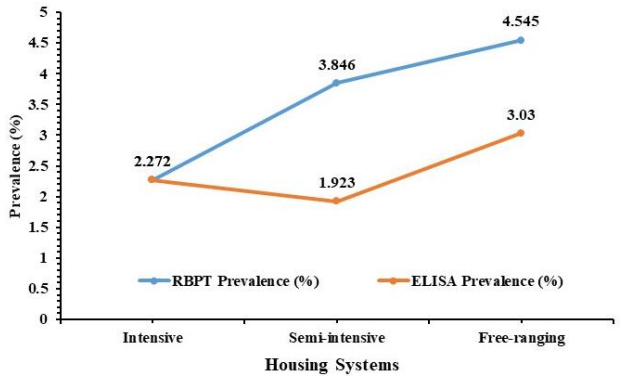

Fig. 7: The scatter graph shows the prevalence of brucellosis in goats reared at different housing systems as determined by RBPT and i-ELISA.

DISCUSSION

Brucellosis remains a significant zoonotic disease in Bangladesh, affecting both human and animal health, particularly in rural and peri-urban communities where small ruminants are integral to livelihoods. Multiple studies have confirmed its endemic status in Bangladesh, with serological evidence of Brucella exposure in livestock populations (Amin *et al.,* 2005; Rahman *et al.,* 2006; Uddin *et al.,* 2007; Nahar and Ahmed, 2009; Ahasan *et al.,* 2010; Rahman *et al.,* 2012; Islam *et al.,* 2019). Although *Brucella abortus* biovar 3 has been isolated from cattle (Islam *et al.,* 2019), no confirmed isolation of *Brucella* spp. from goats or sheep has yet been reported in the country, emphasizing the need for systematic surveillance, including molecular diagnostics.

In our study, the overall seroprevalence of brucellosis in goats was found to be 3.809% using RBPT and 2.380% using i-ELISA. These findings are in line with earlier reports from Bangladesh, including Munsi et al. (2021), who observed 4.33% (RBPT) and 2.40% (c-ELISA), and Ahasan et al. (2017), who reported a higher prevalence of 6.30%. Notably, the lower prevalence obtained via i-ELISA reflects its higher specificity and reduced cross-reactivity compared to RBPT, which may yield more false positives (Al-Griw *et al.,* 2017).

When compared to neighboring countries, the prevalence rates observed in our study are slightly lower but consistent with regional trends. In India, Lalrinzuala *et al.* (2023) reported brucellosis seroprevalence as 8% in goats. In Pakistan, lower prevalence has been reported, such as 0.55% by RBPT in in the district Quetta (Jamil *et al.,* 2020), suggesting a regional burden with varying epidemiological profiles possibly influenced by animal movement, vaccination status, and biosecurity practices.

Our analysis revealed a statistically significant association between geographical region and brucellosis prevalence ($P<0.05$). Jhenaidah, Mymensingh, and Meherpur districts showed variability in seropositivity, potentially attributable to differences in goat population density, inter-district animal trade, access to veterinary services, and biosecurity awareness. Similar findings were observed by Asmare *et al.* (2013), who emphasized the role of ecological and management factors in regional differences in Ethiopia. In Bangladesh, informal trade and lack of brucellosis control programs may exacerbate localized outbreaks.

Breed-wise variation in prevalence was statistically significant, with Black Bengal goats exhibiting the highest seroprevalence (4.032% by RBPT, 2.419% by i-ELISA), followed by Deshi/Crossbreed and Jamunapari. This pattern suggests possible genetic predisposition or differing susceptibility due to immune response, management intensity, or environmental exposure. Uddin *et al.* (2007) also reported breed-related differences in susceptibility, advocating for tailored management strategies.

The housing system was significantly associated with seroprevalence. Free-ranging goats had the highest brucellosis rates (4.545% RBPT, 3.030% i-ELISA), followed by semi-intensive and intensive systems. The increased exposure to contaminated environments, mixed-species herding, and poor sanitation in free-ranging systems could explain this trend. Similar patterns were reported in African and Asian studies, including Asmare *et al.* (2013), who highlighted the role of extensive management and wildlife contact in transmission risk.

Although age-related differences were not statistically significant, a higher prevalence in goats aged above 24 months (4.285%) aligns with previous findings suggesting cumulative exposure risk increases with age (Shafy *et al.,* 2016). Reproductive parameters such as abortion and retained placenta were associated with elevated brucellosis seropositivity (abortion: 15.79% RBPT, 10.53% i-ELISA), reflecting the reproductive tropism of *Brucella* spp. (Maurice *et al.,* 2013). Pregnant goats also showed higher prevalence than non-pregnant ones, indicating gestational vulnerability possibly due to immune modulation during pregnancy (Akhter *et al.,* 2014).

Goats reared on household farms showed higher seroprevalence (6.976% RBPT, 4.651% i-ELISA) than those in commercial or medium-sized farms, suggesting that smaller, less regulated units may have more frequent biosecurity lapses. Although goats reared alongside cattle had slightly higher prevalence, this interspecies interaction was not statistically significant, though it remains epidemiologically relevant due to shared environments and disease reservoirs (Islam *et al.,* 2010).

This study highlights the endemic presence of caprine brucellosis in Bangladesh and its association with key risk factors such as region, breed, housing system, and reproductive history. The findings support the implementation of region-specific surveillance, targeted biosecurity training, and improved diagnostic approaches. Future research should explore molecular detection and strain differentiation, along with longitudinal monitoring to

guide effective national brucellosis control strategies in both goats and cattle.

Conclusions: The current study confirms the endemic presence of caprine brucellosis in Bangladesh, with a seroprevalence of 3.809% by RBPT and 2.380% by i-ELISA, indicating active disease transmission within goat populations. Regional variation was statistically significant, with higher prevalence noted in Jhenaidah and Mymensingh, suggesting that geographic, ecological, and management factors may influence disease burden. Breed-specific susceptibility was observed, with Black Bengal goats showing higher infection rates, potentially due to genetic or husbandry-related vulnerabilities. Housing systems also played a critical role, with free-ranging goats experiencing the highest prevalence, likely due to increased exposure to contaminated environments and interspecies contact. Reproductive disorders—such as abortion, infertility, and retained placenta—were significantly associated with higher brucellosis seropositivity, directly implicating the disease in productivity losses among infected herds.

In Bangladesh, the scarcity of comprehensive prevalence data and active surveillance undermines control efforts. Vaccination programs targeting small ruminants, although not practiced, are critical for long-term disease mitigation. Coupled with systematic testing and culling of infected animals, awareness campaigns for farmers and veterinarians are vital to reduce transmission risks and ensure both animal and public health. This study underscores the urgent need for a coordinated, evidence-based brucellosis control strategy in endemic areas like Bangladesh.

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Author's contribution: KAS contributed in serum collection, formal analysis, writing original draft, and conceptualization; PB conducted data analysis, interpretation of result, and revised the final manuscript; MZR and MM contributed in serum and data collection, software, and validation; PPG conducted in review original draft; PS and TA helped in data curation, formal analysis, visualization; MMK contributed to validation, review, and editing. MAI, conceptualization, funding acquisition, research supervision, manuscript writing, and critical review. The final version of the manuscript was approved by all authors after they had critically reviewed it for significant intellectual content and analyzed the data.

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