JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2021.h494
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

March 2021
VOL 8, NO. 1, PAGES 123–128

ORIGINAL ARTICLE

Seroprevalence of Brucellosis in goats in some selected areas of Bangladesh

Md. Nuruzzaman Munsi[1], Sonia Akther[1], Md. Habibur Rahman[1], Md. Zakir Hassan[2], Md. Zulfekar Ali[2], Md. Ershaduzzaman[3]

[1]Goat and Sheep Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka-1341, Bangladesh
[2]Animal Health Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka-1341, Bangladesh
[3]Socio-Economic Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka-1341, Bangladesh

ABSTRACT

Objective: This study aimed to explore the seroprevalence of *Brucella* spp. in goats in some selected areas of Bangladesh.
Materials and Methods: This study was conducted in different goat-populated regions of Bangladesh from July 2017 to June 2018. A total of 208 serum samples were randomly collected from goats in Jashore (n = 50), Jhenidah (n = 22), Tangail (n = 40), Savar (n = 46), Thakurgaon (n = 18), and Bandarban (n = 32) areas. The samples were subjected to determine the presence of antibodies against *Brucella* spp. by rose bengal plate test (RBPT) and competitive enzyme-linked immunosorbent assay (c-ELISA).
Results: Overall, the seroprevalence of Brucellosis in goats was 4.33% (n = 9/208) by RBPT and 2.40% (n = 5/208) by c-ELISA. The seroprevalence of brucellosis on the basis of RBPT was 6% (buck: 0%, doe: 6%) in Jashore, 4.5% (buck: 0%, doe: 4.5%) in Jhenidah, 2.5% (buck: 0%, doe: 2.5%) in Tangail, 4.35% (buck: 0%, doe: 4.35%) in Savar, 6.25% (buck: 0%, doe: 6.25%) in Bandarban, and 5.56% (buck: 0%, doe: 5.56%) in Thakurgaon. On the other hand, the seroprevalence of brucellosis by c-ELISA was 4% (buck: 0%, doe: 4%) in Jashore, 4.5% (buck: 0%, doe: 4.5%) in Jhenidah, 3.13% (buck: 0%, doe: 3.13%) in Bandarban, and 5.56% (buck: 0%, doe: 5.56%) in Thakurgaon. Brucellosis was more prevalent ($p > 0.001$) in does aging 3–4 years.
Conclusion: Goats from different areas of Bangladesh are caring antibodies against *Brucella* organisms. Further bacteriological investigations are necessary.

ARTICLE HISTORY

Received September 10, 2020
Revised November 09, 2020
Accepted December 20, 2020
Published March 06, 2021

KEYWORDS

Brucellosis; seroprevalence; goat; RBPT; c-ELISA

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Introduction

Brucellosis is a zoonotic bacterial disease caused by *Brucella* spp. The bacteria are usually transmitted through sexual contact from infected mature animals with aborted placenta, fetal fluids, and adult male testes [3]. Brucellosis has been a global threat to the animal kingdom, especially cattle, sheep, and goats [1]. In sheep and goats, Brucellosis is caused by *Brucella melitensis*. It is a Gram-negative facultative coccobacillus, which is morphologically short-rod in shape. Besides, it is an intracellular bacterium that includes three biovars [1,3]. *B. melitensis* and rarely *B. abortus* cause Brucellosis in sheep and goats, but other biovars may cause Brucellosis according to area and species [2,3]. In 1887, a famous bacteriologist, named David Bruce, first isolated *B. melitensis* [4] from the spleens of soldiers who died of Mediterranean fever in the island in Malta.

Brucellosis is an endemic disease reported in humans and livestock populations in Bangladesh [5]. In most parts of the Mediterranean Basin, the Middle East, Central Asia [6,7], Latin America, and parts of Africa [8], ovine and caprine Brucellosis is endemic. The cross-border trading center of Thailand near Cambodia is facing a problem with Brucellosis in cattle, goats, and sheep that may have a considerable impact on human and animal health [9], socio-economic factors, and may create a problem regarding the development of the livestock sector [10]. A small ruminant population of Gujarat in India is facing Brucellosis, which is causing a public health hazard. It spreads to humans

Correspondence Sonia Akther soniahossain77@gmail.com Goat and Sheep Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka-1341, Bangladesh.

How to cite: Munsi MN, Akther S, Rahman MH, Hassan MZ, Ali MZ, Ershaduzzaman M. Seroprevalence of Brucellosis in goats in some selected areas of Bangladesh. J Adv Vet Anim Res 2021; 8(1):123–128.

and other animals due to their close association with the human community [11]. Hence, considerable economic losses occur in the small ruminant industry [8,12].

On the other hand, the swine industry is also facing significant financial losses due to reproductive failure worldwide [13–15]. Brucellosis causes substantial economic losses through abortion, decreased calf production, low milk yield, and finally, livestock infertility [16]. Humans get an infection from the infected animal by handling, milking, and drinking unpasteurized or raw milk or dairy products [17]. The seropositivity against ovine Brucellosis was reported to be 2.59% in Nilphamari Sadar and 33.33% in Kishoreganj by I-enzyme-linked immunosorbent assay (ELISA) [10,18–20]. Various diagnostic techniques have been used for the detection of Brucellosis in goat samples. From the biosafety perspective, *Brucella* is under risk group III, which requires a biosafety level 3 (BSL-3) laboratory facility for the bacteriological culture [21]. These drawbacks make the serological test the most practical epidemiological technique used in the laboratory to detect *Brucella* organisms [22].

There are some well-known serological tests against Brucellosis, such as the rose bengal plate test (RBPT), ELISA, serum agglutination test, and complement fixation test [23]. BSL-3 facilities are required for culturing, routine identification, differential diagnosis, and molecular and phenotypic characterization of *Brucella* spp. from suspected samples for mitigating laboratory-acquired infections and environmental safety [24]. Polymerase chain reaction is the most sensitive assay for detecting *Brucella* than other standard microbiological tests [25]. According to OIE, ELISA and RBPT are reliable and safe methods for *Brucella* identification [12]. RBPT is used as a screening technique, while competitive enzyme-linked immunosorbent assay (c-ELISA) is used as a confirmatory method. The current investigation was attempted to evaluate seropositivity against Brucellosis in blood samples of goats collected from some areas of Bangladesh by using RBPT and c-ELISA.

Materials and Methods

Ethical approval

This research project was affirmed by the Animal Experimentation and Ethics Committee of Bangladesh Livestock Research Institute ([Reference no.: Bangladesh Livestock Research Institute (BLRI)0007)].

Study design

A cross-sectional examination was directed between July 2017 and June 2018 to evaluate the seropositivity against *Brucella* organisms in goats. Data were collected based on the previous history of abortion, retention of placenta, and multiple breeding. However, semi-intensive goat farming was usually practiced in the study area. According to the biosafety point of view, *Brucella* organisms in the risk group 3 require a BSL-3 laboratory for culture. However, the mentioned type of competence is limited in the experimental design. After that, the culture-free serological diagnosis was made through RBPT and competitive enzyme-linked immunosorbent assay (c-ELISA). The tests were carried out in the serology laboratory in the Animal Health Research Division, BLRI.

Sample collection and preparation

The study was designed to collect samples and information from goat populations in six regions of Bangladesh, namely Jashore, Jhenidah, Tangail, Savar, Thakurgaon, and Bandarban. A total of 208 blood samples were collected, comprising Jashore (*n* = 50; buck: 3, doe: 47), Jhenidah (n = 22; buck: 1, doe: 21), Tangail (*n* = 40; buck: 2, doe: 38), Savar (*n* = 46; buck: 10, doe: 36), Thakurgaon (*n* = 18; buck: 1, doe: 17), and Bandarban (*n* = 32; buck: 2, doe: 30) by puncturing the jugular vein of goats using disposable 5 ml plastic syringes gently after wiping with 70% ethyl alcohol or tincture of iodine without any anticoagulant. After that, the collected blood samples were transferred to the respective laboratory within a cool box containing an ice pack. The sera were separated through centrifugation at 3,000 rpm for 3 min, then decanted the clear serum into 1.5 ml Eppendorf tubes with labeling. For mentioning the history of abortion and retained placenta, the blood samples were collected via face-to-face conversation.

Serological test

Rose bengal plate test (RBPT)

RBPT is a quick serological test carried out according to the manufacturer's guidelines of Lilli Test RBT Antigen kit of *Brucella* organisms (Lilidale Diagnostics, product code: V2001, Pig Oak Farm, Wimborne BH21 7DG, UK). About 30 μl of serum and 30 μl of the rose bengal-colored antigen was taken on a glass plate by a micropipette and mixed with the serum. Afterward, the plate was shaken for 4 min and then read. Definite clumping/agglutination to slight agglutination was considered a positive reaction, whereas no clumping/agglutination was regarded as a negative result.

Competitive enzyme-linked immunosorbent assay (c-ELISA)

c-ELISA was used to detect *Brucella* antibodies (IgG) in the serum sample of goats. Hence, c-ELISA was carried out using available commercial kits by following the manufacturer's guidelines from SVANOVIR *Brucella*-Ab cELISA kit (SVANOVA®, where the article no: 10-2701-02 and 10-2701-10 in Boehringer Ingelheim Svanova Box 1545 SE-751 45 Uppsala, Sweden). According to the

manufacturer's protocol, each collected serum sample was subjected to testing for the *Brucella* antibodies. A 96-well ELISA microtitre plate was used. At first, 45 µl of dilution buffer was added to each well of the ELISA microtitration plate. Then, 5 µl of positive, weak positive, and negative control was added in A1A2, B1B2, and C1C2, respectively. Another two wells were filled with 5 µl sample dilution buffer, where the total volume was 50 µl. Meanwhile, the rest of the wells were filled with collected sera samples at 5 µl. After that, all wells contained 50 µl reaction fluid. In the 96 wells, each 50 µl mAb solution was added individually; tapping, shaking, and incubation was carried out at 18°C–25°C for 30 min. After that, the ELISA plate was washed four times with the supplied PBS Tween buffer.

Consequently, 100 µl of the conjugate solution was added to each well and incubated for 30 min at 18°C–25°C. However, each well was filled with 100 µl substrate solutions, and incubation was carried out at room temperature. Then, the addition of 50 µl stop solution was carried out in each ELISA plate well and mixed properly. After that, optical density (OD) was measured at 450 nm in a multichannel spectrophotometric ELISA plate reader. Microsoft® Excel program was used to read the datasheet and was saved in the computer's hard disk with respective identification marks.

Data analysis

Data were processed by calculating OD values and percent of inhibition (PI) according to c-ELISA kit protocol in a microplate photometer (AccuReader, Taiwan), and the results were put into Microsoft Excel (Microsoft Corporation, Redmond, WA) spreadsheet.

$$PI = 100 - \frac{(\text{OD sample or control} \times 100)}{\text{OD conjugate control cc}}$$

where PI is the percent inhibition as per protocol. OD conjugate control was (−-0.75)–2.0. PI in the positive control, weak positive control, and negative control was 80–100, 30–70, (−10)–15, respectively. However, the PI result of supplied serum samples for the detection of *Brucella* antibody titer was interpreted as follows: <30% was negative and ≥ 30% was positive. The probability of Brucellosis prevalence among parameters like sampling location and age and RBPT vs. c-ELISA was statistically analyzed by the chi-square test using Statistical Package for the Social Sciences software, version 22.0.

Results

Many factors like breed, geographic location, diagnostic method, management, and environmental would vary the disease prevalence or seropositivity and infection rate.

However, the seroprevalence of Brucellosis in Jashore, Jhenidah, Tangail, Savar, Thakurgaon, and Bandarban was calculated by RBPT and c-ELISA. The diagnostic procedure also lead to variations in the interpretation of the results. However, RBPT is a rapid screening test used to identify animals infected with *Brucella* organisms and where c-ELISA confirms the presence of *Brucella* antibodies. Usually, RBPT is a higher sensitivity with lower specificity serological test compared to c-ELISA. Moreover, as a screening test, RBPT shows a higher prevalence rate.

For this reason, the results varied from RBPT to c-ELISA. In this study, a test for *Brucella*'s antigen in goats gave a representative view of seropositivity against *Brucella* organisms carried out by RBPT and c-ELISA. In the case of RBPT, the overall seroprevalence was 4.33%, and in the case of c-ELISA, it was 2.40%, respectively (Table 1).

Among the different samples of different regions of Bangladesh, the seroprevalence of Brucellosis was higher in Bandarban district (6.25%), which was followed by 6% in Jashore, 5.56% in Thakurgaon, 4.5% in Jhenidah, 2.5% in Tangail, and 2.17% in Savar, respectively (Table 1). For more confirmation, c-ELISA was carried out. In the Jashore district cases, 4% (2/50) serum samples were found positive; in the case of Jhenidah, it was 4.5% (1/22); in the case of Tangail and Savar, no positive cases were detected (Table 1). In Bandarban and Thakurgaon, 3.13% (1/32) and 5.56% (1/18) were found, respectively (Table 1). However, the overall seroprevalence of Brucellosis by c-ELISA was 2.40%. On the other hand, Brucellosis was 4.33% by RBPT.

Table 1. Seroprevalence of caprine brucellosis according to RBPT and c-ELISA.

Location	Total no. of sera tested	Total no. of RBPT-positive reactors	Percentage (%)	Total no. of c-ELISA-positive reactors	Percentage (%)
Jashore	50 (buck 3; doe 47)	3 (doe)	6 (doe)	2 (doe)	4 (doe)
Jhenidah	22 (buck 1; doe 21)	1 (doe)	4.5 (doe)	1 (doe)	4.5 (doe)
Tangail	40 (buck 2; doe 38)	1 (doe)	2.5 (doe)	0	0
Savar	46 (buck 10; doe 36)	1 (doe)	2.17 (doe)	0	0
Bandarban	32 (buck 2; doe 30)	2 (doe)	6.25 (doe)	1 (doe)	3.13 (doe)
Thakurgaon	18 (buck 1; doe 17)	1 (doe)	5.56 (doe)	1 (doe)	5.56 (doe)
Total	208 (buck 19; doe 189)	9 (doe)	4.33 (doe)	5 (doe)	2.40 (doe)

According to age, Brucellosis in various districts showed different results by RBPT and c-ELISA. In Jashore, according to RBPT and c-ELISA, goats aged between 1.5 and 2 years had 6.6% and 0% prevalence, while between 2 and 3 years had 6.6% and 6.6% and between 3 and 4 years had 15% and 10% *Brucella* seropositivity, respectively.

In Jhenidah, goats aged between 1.5 and 2 years had 0% prevalence, between 2 and 3 years revealed 12.5% and 12.5% prevalence, and between 3 and 4 years showed 28.5% and 42.2% *Brucella* prevalence. In Tangail district, goats aged between 1.5 and 2 years revealed 0% prevalence, between 2 and 3 years also showed no prevalence, and between 3 and 4 years had 20% and 0% *Brucella* prevalence by RBPT and c-ELISA. In Savar, 1.5–2-year-old goats had no prevalence, whereas 2–3-year-old individuals revealed 5%, and 3–4-year-olds had 10% seropositivity in RBPT. In Bandarban, goats aged between 1.5 and 2 years showed 5% and 0% prevalence, between 2 and 3 years experienced 10%, and between 3 and 4 years had 50% and 50% *Brucella* seropositivity by RBPT and c-ELISA, respectively. In Thakurgaon, goats aged between 1.5 and 2 years had 0% prevalence, between 2 and 3 years revealed 16%, and between 3 and 4 years showed 50% and 33.3% *Brucella* seropositivity by RBPT and c-ELISA, respectively (Table 2).

Table 2. Seroprevalence of Brucellosis according to age in different districts.

District	Age group (year)	Number of serum tested (*n*)	Numer RBPT-positive sample (%)	Number c-ELISA-positive sample (%)
Jashore (*n* = 50)	1.5–2	15	1 (6.6)	0 (0.0)
	2–3	15	1 (6.6)	1 (6.6)
	3–4	20	3 (15.0)	2 (10)
Jhenidah (*n* = 22)	1.5–2	7	0 (0.0)	0 (0.0)
	2–3	8	1 (12.5)	1 (12.5)
	3–4	7	2 (28.5)	3 (42.21)
Tangail (*n* = 40)	1.5–2	20	0 (0.0)	0 (0.0)
	2–3	15	0 (0.0)	0 (0.0)
	3–4	5	1 (20)	0 (0.0)
Savar (*n* = 46)	1.5–2	16	0 (0.0)	0 (0.0)
	2–3	20	1 (5.0)	0 (0.0)
	3–4	10	1 (10)	1 (10)
Bandarban (*n* = 32)	1.5–2	20	1 (5)	0 (0.0)
	2–3	10	1 (10)	1 (10)
	3–4	2	1 (50%)	1 (50)
Thakurgaon (*n* = 18)	1.5–2	6	0 (0)	0 (0.0)
	2–3	6	1 (16)	0 (16)
	3–4	6	3 (50)	2 (33.3)

These findings indicate that Brucellosis was more prevalent between 3 and 4-year-old does than other age groups among the six regions – Jashore, Jhenidah, Tangail, Savar, Bandarban, and Thakurgaon. Goats in Jhenaidah district showed more prevalence of Brucellosis, which was about 28.5% and 42.21% RBPT- and c-ELISA-positive, whereas RBPT of Bandarban was 50% positive. Thakurgaon had 33.3% and 50% positive goats aged between 3 and 4 years, respectively (Table 2). Therefore, our present findings revealed that goats aged 3–4 years were more susceptible to Brucellosis. Furthermore, Brucellosis was significantly ($p > 0.001$) higher in does than bucks tested by RBPT and c-ELISA (Tables 1 and 2).

Discussion

From the public health perspective, Brucellosis is a significant, economically essential, and zoonotic bacterial disease of many countries in the world [26]. Crawford et al. [27] showed that Brucellosis causes substantial losses to many developed and less developed countries and poses a severe public health threat to human beings. Brucellosis in goats mainly causes abortion in the late stage of pregnancy, retention of placenta, and orchitis in male animals. The present findings revealed that the studied area indicated that the overall seroprevalence of *Brucella* antibodies in goats was 4.33% and 2.40% in RBPT and c-ELISA, respectively.

Al-Griw et al. [28] revealed that *Brucella* seropositivity in goats was 33.4%, and in sheep it was 9.2% in North West Libya. However, 30%–40% seroprevalence results varied in RBPT and c-ELISA because c-ELISA was more specified and sensitive than RBPT. Usually, RBPT was a screening test and c-ELISA was a confirmatory test [28]. Furthermore, EL-Sayed et al. [29] reported that the prevalence of Brucellosis in goats was 8.91% in Egypt according to RBPT, which was higher than the present study. Additionally, Ahasan et al. [30] and Rahman et al. [18] specified that the prevalence rate against Brucellosis was 1.98% and 3.15%, respectively. However, Rahman et al. [10] found a 4.72% RBPT-positive sample and 3.15% i-ELISA-positive sample, which was relatively higher than our present study, where the c-ELISA result was 2.40%.

A study on Brucellosis by Islam et al. [19] found a 13.64% seroprevalence of Brucellosis in the milk of Black Bengal goats by the milk ring test. With regard to RBPT, they found a 3.85% prevalence in serum which was slightly lower than our present findings. According to age, 3–4-year-old animals in the Jhenidah district showed more prevalence of Brucellosis, which were 28.5% and 42.21% in RBPT and c-ELISA, where RBPT of Bandarban revealed a 50% prevalence, and Thakurgaon indicated 33.3% and 50% prevalence with animals aging between 3 and –4 years,

respectively. Meanwhile, among the six selected areas of this study, the Thakurgaon district's goats were most susceptible to Brucellosis than other regions. This might be due to the close contact with the India–Bangladesh border and the high possibility of animal movement.

On the other hand, the seroprevalence of Brucellosis in Savar was low compared to other respective study regions, which was 2.17% and 0% in RBPT and c-ELISA, respectively. It might be due to a low abortion history, and this area is a central point of Bangladesh. Hence, the low cross-contamination rate in border-crossing areas like Thakurgaon. The present findings can be compared with the results of Islam et al. [19], where the prevalence of Brucellosis found in goats was 12.50% and 3.70% in the age group of above 4 years and below 4 years, respectively. Older goats showed higher seropositivity against Brucellosis than younger ones [19]. This result was almost similar to the findings of Amin et al. [31], where it is stated that the seroprevalence of *Brucella* antibody was higher in backyard farms (5.0%) and intensive or semi-intensive goat farms (2.5%), and hence, higher in pregnant animals (5.9%) than non-pregnant (4.7%). Moreover, *Brucella* antibody was higher (4%) in goats above the age of 4 years [31]. However, this result was similar to the reports of Nahar and Ahmed [32] and Solorio-Rivera et al. [33].

Here, Brucellosis was higher ($p > 0.001$) in does than bucks tested by RBPT and c-ELISA. It is an essential zoonotic bacterial disease and significantly affects economic aspects, requiring close observation to reduce the loss and eliminate the occurrence. According to the current study, since caprine Brucellosis was present in some areas of Bangladesh with an overall seroprevalence of 4.33% and 2.40% by RBPT and c-ELISA, respectively, further investigation should be carried out to identify the circulating species of *Brucella* in Bangladesh by adopting molecular techniques. However, early diagnosis, medication, and farm biosecurity are required to prevent animal and zoonotic diseases [34].

Conclusion

It can be concluded that Brucellosis is a silent zoonotic disease that affects the livestock industry. However, a lower prevalence of Brucellosis occurs in goats of the study areas of Bangladesh. Therefore, more scientific work would be needed for specifying the disease area through surveillance for combating the zoonoses.

List of abbreviations

c-ELISA - Competitive enzyme-linked immunosorbent assay; RBPT - Rose bengal plate test; PI - Percent inhibition; OD - Optical density; BLRI - Bangladesh Livestock Research Institute; OIE-World Organisation for Animal Health; PBS- Phosphate Buffered Saline,.

Acknowledgments

The authors express their special thanks to the scientists and lab personnel of the Goat and Sheep Production Research Division for funding and Animal Health Research Division, BLRI, for laboratory support for conducting this research work.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contribution

MNM designed and lead the study; SA and MHR were involved in the sample collection, laboratory testing, data analysis, and manuscript writing. MZH, MZA, and ME were involved in laboratory works, data analysis, and finalization of the manuscript writing.

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