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SEROLOGICAL INVESTIGATION OF CAPRINE BRUCELLOSIS ASSOCIATED WITH ABORTION HISTORY IN BANGLADESH AGRICULTURAL UNIVERSITY GOAT FARM WITH A BRIEF REVIEW

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ABSTRACT

Background: Brucella is an important and widespread intracellular zoonotic bacterium distributed globally that causes abortion and reproductive failure in livestock and zoonotic illness with serious chronic complications in affected humans. Brucellosis is recognized as re-emerging globally and neglected in low to medium-income countries in the world, including Bangladesh. Caprine brucellosis is caused by *Brucella melitensis* and is an important public health problem. Its diagnosis is mainly based on a primary screening test followed by confirmatory serologic tests. Several articles on the serological prevalence of brucellosis in goats associated with reproductive disorders have been published in Bangladesh and need to be reviewed for future research programs.

Objectives: This paper describes the seroprevalence of Brucella infection in farm goats with a previous history of abortion, including a brief review of caprine brucellosis in Bangladesh.

Materials and Methods: This study was conducted to detect the relationship between Brucella seropositivity and a history of previous abortion in goats, supported by a brief review of articles published on Brucella seropositivity in goats, especially associated with reproductive disorders in Bangladesh. A total of 26 articles published from Bangladesh and elsewhere have been reviewed to present this manuscript. Eight directly related inland reports have been examined to compare the results of the present findings. A total of 150 goat serum samples were obtained for the current investigation from BAU Goat Farm. A standardized questionnaire was used to collect information on host factors, management factors, and the occurrence of previous reproductive disorders. The serological diagnosis of brucellosis is performed using a screening test (e.g., RBPT) followed by a confirmatory test (e.g., ELISA) per kit manufacturer instructions.

Results: Overall seroprevalence of Brucella infection in goats was recorded as 2.67% (4/150) by RBPT and 2.0% (3/150) by I-ELISA on the serum samples collected from BAUGF, Mymensingh. The seroprevalence of Brucella infection was found higher in female goats (3.00%) than in male goats (2.00%), higher in adults (5.00%) than in young (1.25%) goats, pregnant (RBPT 3.33% & I-ELISA 1.67%) than non-pregnant (RBPT 2.2% & I-ELISA 1.67%) goats. A significantly higher seropositivity of Brucella infection was recorded in goats with a previous history of abortion with both the RBPT (30.0%) and I-ELISA (20.0%). These findings are compared with the results of the earlier inland reports and concluded.

Conclusions: This study has used RBPT as a screening test and i-ELISA as a confirmatory test, which has detected an overall 2.67% and 2.0% seropositivity to Brucella infection in goats, respectively, with significantly higher prevalence in goats with a previous history of abortion with both the RBPT (30.0%) and I-ELISA (20.0%) tests. Thus, the zoonotic prevalence and effect of Brucella infection in humans and animals have been documented in Bangladesh. The prevalence of Brucella infection at a low level (2.0%) in goats in Bangladesh is appropriate for launching and building a program for prevention and control through testing and slaughter, removing diseased animals from flocks. However, the 'One Health' approach with a targeted intervention policy should be implemented to break Bangladesh's Brucella transmission chain between animals and humans.

Keywords: Brucellosis, RBT, i-ELISA, goat, prevalence, BAU Goat farm and abortion history

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INTRODUCTION

Brucellosis is a worldwide distributed chronic infectious disease caused by bacteria of the genus *Brucella*, and 12 established species in the *Brucella* genus are recognized based on preferential host specificity.[1] The genus *Brucella* is named for Major-General Sir David Bruce, who 1886 led the Malta Fever Commission, which identified *Brucella melitensis* as the organism responsible for the disease.[2] Small ruminants are the preferred hosts for *Brucella melitensis*, although other Brucella species may infect small ruminants.[3] The *B. melitensis* was first isolated in 1887 by David Bruce in Malta Island from the spleens of four soldiers and is currently distributed globally; it causes abortion, stillbirths, and the birth of weak offsprings and is the most virulent zoonotic Brucella species for humans. The *B. melitensis* has been controlled in most industrialized countries. However, it remains endemic in low and medium-income nations, including Bangladesh, associated with an extensive negative impact on the productivity of flocks and public health threats due to zoonotic infection. The serological prevalence of Brucella infection was initially reported in Bangladesh in 1967 in cattle, in 1997 buffalo, 1983 in humans, and in 1988 in goats.[4] Although brucellosis is the most prevalent zoonotic disease worldwide, it severely hinders livestock productivity and human health, specifically in low-income countries, leading the WHO to classify it as one of the world's leading 'neglected zoonotic diseases.'[5] The seroprevalence of caprine brucellosis has been estimated to be 1.56% in goats by using RBPT, Rapid Brucella AB Test kit, and Mab-ELISA in the Black Bengal goats of Mymensingh,[6] 3.24% with RBPT and 2.59% with i-ELISA in Nilphamari[7] and with RBPT and c-ELISA in six more districts[8] in Bangladesh. The total economic losses attributable to caprine brucellosis have been estimated at BDT 46462900 (US$ 580786.25) annually in the district of Mymensingh.[6] It has been concluded that caprine brucellosis silently constitutes economic loss in goat farmers vis-a-vis the country due to insufficient knowledge and inadequate diagnostic facilities, lack of awareness, and an effective preventive and control strategy.[6] In addition, Brucella seropositivity has been reported significantly more frequently among goats with a history of abortion than among animals that have no history of abortion in multiple reports from Bangladesh[8-12] and elsewhere.[13] A similar incidence of Brucella infection in goats associated with a history of abortion in BAU goat farm has been observed. Considering these facts, a serological investigation of caprine brucellosis and its association with abortion history in the BAU goat farm was carried out with a brief review of similar inland research reports on goats.

MATERIALS AND METHODS

This study was conducted at the Bangladesh Agricultural University Goat Farm (BAUGF), Mymensingh. Of 150 goats, 50 were male, while 100 were female. Thirty goats were under one year of age, 80 were between one and two years of age, and 40 were above two years of age.

Collection of data and blood samples

Data from each of the randomly selected goats were collected using a questionnaire. The animal-level variables were age, sex, breed, and reproductive problem, and the farm-level variable was flooring systems such as Macha (made of bamboo) and brick flooring. After the

collection of data, about 5-7 ml of blood was collected aseptically from the jugular vein of each goat without any anticoagulant with the help of a sterile disposable syringe and needle. The syringe with a needle was kept undisturbed on a tray for at least 30 minutes at room temperature in a slightly inclined position to facilitate clotting and serum separation. After this period, the clotted blood samples with sera were transferred to the refrigerator at 4 °C and kept overnight. Later, the sera were poured into the separate labeled test tube. Then, the sera were centrifuged at 2500 rpm for 10 minutes. After centrifugation, clear sera were found, and then the sera were transferred to the vial. The vial was stored at -20 °C until further use.

Serological tests

All the collected serum samples were subjected to the Rose Bengal Plate test (RBPT).[14] The samples that were positive in RBPT were further confirmed by i-ELISA.[15]

Rose Bengal Plate test (RBPT)

The RBPT was performed according to the procedure described by EURLB[16] and WOAH.[17] The test sera samples and Rose Bengal antigen were kept for one hour at room temperature before the beginning of the test. Serum (30 µl) was taken on a Rose Bengal plate by micropipette. Then, 30 µl of Rose Bengal antigen was added to the serum. The antigen and serum were mixed thoroughly using a toothpick, and the plate was hand-rocked for 4 minutes. However, the plates were kept on the vortex (shaker) for smooth mixing. The sign of agglutination was considered positive, while no sign of agglutination was considered negative (Fig. 1).

Indirect Enzyme-Linked Immunosorbent Assay (i-ELISA)

The assay was performed according to the protocol and supplied by the manufacturer (Svanova Biotech AB, Art. No.10-2700-10, SE-751 83 Uppsala, Sweden).

Preparation of PBS-Tween Buffer

According to the procedure, 20X concentrate PBS-Tween solution (PBST) was diluted into 1/20 in distilled water (DW). A volume of 500 ml per plate was prepared by adding 25 ml PBST solution to 475 ml DW and was mixed thoroughly.

Preparation of conjugate

According to the procedure, lyophilized horse radish peroxidase (IIR.PO) conjugate was reconstituted with 11.5 ml PBS buffer. Buffer was added carefully to the bottle. Then the solution was left for one minute and mixed thoroughly. The solution was prepared immediately before use.

Test procedure

All reagents supplied by the manufacturer were equilibrated to room temperature at 18 to 25 °C before use. A volume of 100 µl of sample dilution buffer was added to each well that would be used for serum samples and serum controls. After that 4 µl of positive control serum (Reagent A) and 4 µl of negative control serum (Reagent B) were added, respectively to selected wells coated with *Brucella melitensis* antigen. For confirmation purposes, it was run with the control sera in duplicates. An amount of 4 µl of sera sample was also added to a selected well coated with

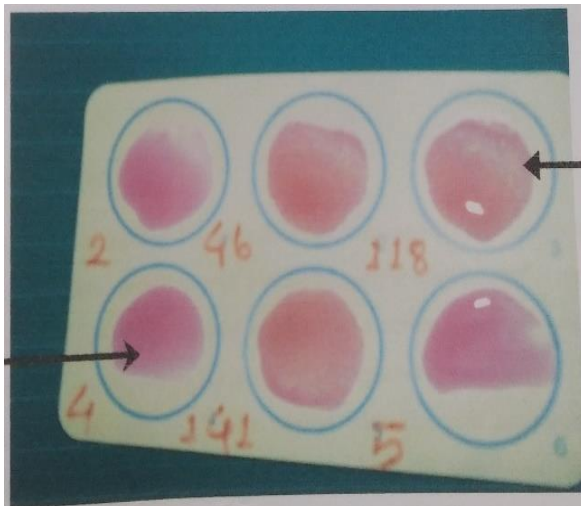

Fig.1 Results of positive and negative reaction in Rose Bengal Plate Test of brucellosis

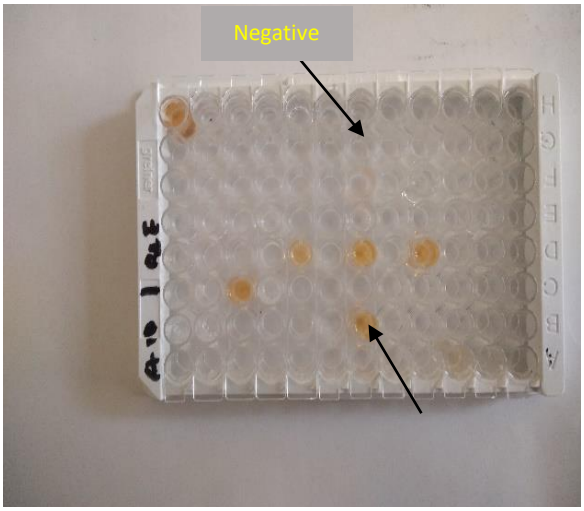

Fig.2 Brucellosis is positive and negative as shown in i-ELISA test plate

Brucella melitensis antigen. For confirmation purposes, it was also run with the samples in duplicate. The plate was shaken thoroughly sealed the plate strip and incubated at 37°C for 1 hour. The plates were rinsed 3 times with PBST buffer and filled in the wells at each rinse, emptied the plate, and tapped hard to remove all remaining fluid. Then 100 µl of HRP conjugate was added to each well and incubated at 37°C for 1 hour. Again, rinse the plate according to the previous way. Then 100 µl substrate solution was added to each well and incubated for 10 minutes at room temperature at 18 to 25 °C. The reaction was stopped by adding 50 µl of stop solution to each well and mixing thoroughly. The stop solution was added in the same order as the substrate solution was added. The optical density (OD) of the controls and samples was measured at 450 nm in a microplate photometer. The OD was measured within 15 minutes after the addition of stop solution to prevent fluctuation in OD values.

The percent positivity values (PP) were calculated using the following formula.

$$PP = \frac{\text{Test sample or Neg C (OD)} \times 100}{\text{Positive control (OD)}}$$

The assay was calibrated against the OIE ELISA Standard sera and Standardized against the EU derivatives 64/432/EEC, Annex C. The PP value of > = 40 was considered as positive.

Statistical analysis

Using SPSS version 17.0, the chi-square test was employed to find a significant relationship between the prevalence of brucellosis and demographic variables such as age, sex, breed, pregnancy status, and flooring system.

RESULTS

The collected serum samples were tested for Brucella infection with RBPT followed by i-ELISA which showed an overall 2.67% and 2.00% seropositivity in goats, respectively (Table 1). A review of the inland literature on the subject revealed seven types of serological tests have been applied for serological prevalence studies on caprine brucellosis, of which all the available eight articles used RBPT, followed by three articles used i-ELISA and other tests (c-ELISA, MAT, SAT, PAT & TAT) used one time by different authors (Table 1abc). Therefore, it is

Table 1a. Comparison of seroprevalence of brucellosis in goats between the present study and published reports based on some demographic factors in Bangladesh

S/N	Demographic factors	Sub-criteria	Findings of present study BAU DF, Mymensingh			Results of some published reports Nilphamari [7]			Dhaka, Rajshahi & M[10]		
			No. of goat tested	RBPT Positive No. (%)	i-ELISA Positive No. (%)	No. of goat tested	RBPT positive No. (%)	i-ELISA Positive No. (%)	No. of goat tested	RBPT Positive No. (%)	MAT Positive No. (%)
1.	Age (months)	6-12	30	0	0	045	0	0	-	-	-
		13-24	80	1 (1.25)	1 (1.25)	080	3 (3.75)	2 (2.50)	183!	6 (3.28)	5 (2.73)
		>24	40	3 (3.75)	2 (5.00)	029	2 (6.89)	2 (6.89)	025!!	2 (8.00)	7 (3.37)
2.	Gender	Male	50	1 (2.00)	0	050	1 (2.00)	0	030B	1 (3.33)	1 (3.33)
		Female	100	3 (3.00)	3 (3.00)	104	4 (3.84)	4 (3.84)	178D	7 (3.93)	6 (3.37)
3.	Breed	BBG	150	4 (2.67)	3 (2.00)	080	2 (2.50)	1 (1.25)	208	8 (3.85)	7 (3.37)
		Cross	-	-	-	074	3 (4.05)	3 (4.05)	-	-	-
4.	Pregnant status	Yes	90	3 (3.33)	2 (2.22)	090	4 (4.44)	3 (3.33)	048	5 (10.41)	4 (8.33)*
		No	60	1 (1.67)	1 (1.67)	064	1 (1.56)	1 (1.56)	130	2 (1.53)	2 (1.53)
5.	Reproductive disorders	PA	10	3 (30.0)	2 (20.0)	009	4 (4.44)	3 (33.33)	022	6 (27.27)	5 (22.72)*
		RP	05	0	0	008	0	0	020	6 (30.0)	5 (25.0)
		FC	15	0	0	018	0	0	-	-	-
		Others	-	-	-	119	1 (0.84)	1 (0.84)	-	-	-
		ND	120	1 (0.84)	1 (0.84)	-	-	-	-	-	-
6.	Housing flooring system	Kacha	-	-	-	137	4 (2.91)	3 (2.18)	-	-	-
		Brick	100	3 (3.00)	-	007	0	0	-	-	-
		Macha	50	1 (2.00)	1 (2.00)	010	1 (10.0)	1 (10.0)	-	-	-
7.	Rearing system	Farming	-	-	-	-	-	-	184	7 (3.93)	6 (3.37)*
		Rural	-	-	-	-	-	-	024	1 (4.11)	1 (4.11)
	Overall,		150	4 (2.67)	3 (2.00)	154	5 (3.24)	4 (2.59)	208	8 (3.85)	7 (3.37)

BAUGF = Bangladesh Agricultural University Goat Farm, M = Mymensingh
J, J, T, S, T & B = Jashore, Jhenidah, Tangail, Savar, Thakurgaon & Bandarban
MAT = Micro-agglutination Test
! = 0-4 years and !! = >4 years
B = Buck D = Doe PA = Previous abortion RP = Retained placenta FC = Failure to conceive
Others = Metritis, delayed heat, dystocia etc. ND = No disorders
RBPT = Rose Bengal Plate Test
ELISA = Enzyme-linked Immunosorbent Test
*Significantly high

Table 1b. **Comparison** of seroprevalence of brucellosis in goats between the present study and published reports based on some demographic factors in Bangladesh

S/N	Demo-graphic factors	Sub-criteria	Results of some published reports									
			J, J, T, S, T & B [8]			B, B, G, M & S [11]			Mymensingh & Bogra [12]			
			No. of goat tested	RBPT Positive No. (%)	c-ELISA Positive No. (%)	No. of goat tested	RBPT Positive No. (%)	i-ELISA Positive No. (%)	No. of goat tested	RBPT positive No. (%)	SAT Positive No. (%)	i-ELISA Positive No. (%)
1.	Age (years)	1.5-2	84	1 (1.19)	0	115	2 (3.22)	2 (3.22)	-	-	-	-
		> 2-3	74	4 (5.41)	3 (4.05)	011	2 (18.18)	2 (18.18)	-	-	-	-
		>3-4	50	10 (20)*	9 (18.0)*	-	-	-	-			
2.	Gender:	Male	19B	-	-	028	0	0	020	2 (10.00)	1 (6.25)	0
		Female	189D	9 (4.33)	5 (2.40)	099	4 (4.04) *	4 (4.04)*	100	5 (05.00)	4 (4.00)	3 (3.00)
3.	Breed:	BBG	-	-	-	-	-	-	-	-	-	-
		Cross	-	-	-	-	-	-	-	-	-	-
4.	Pregnant status	Yes	-	-	-	-	-	-	-	-	-	-
		No	-	-	-	-	-	-	-	-	-	-
5.	Repro-ductive disorders	PA: Yes	-	-	-	03	2 (66.67)	2 (66.67)	003	3 (100)	3 (100)	3 (100)*
		: No	-	-	-	96	2 (02.08)	2 (02.08)	117	4 (3.41)	2 (1.70)	0
		RP : Yes	-	-	-	-	-	-	-	-	-	-
		: No	-	-	-	-	-	-	-	-	-	-
		FC	-	-	-	-	-	-	-	-	-	-
		AUD : Yes	-	-	-	-	-	-	-	-	-	-
		No	-	-	-	-	-	-	-	-	-	
		ND	-	-	-	-	-	-	-	-	-	-
6.	Housing flooring system:	Kacha	-	-	-	-	-	-	-	-	-	-
		Brick	-	-	-	-	-	-	-	-	-	-
		Macha	-	-	-	-	-	-	-	-	-	-
7.	Rearing system	Farming-	-	-	-	-	-	-	-	-	-	-
		Rural	-	-	-	-	-	-	-	-	-	-
	Overall,		208	9 (4.33)	5 (2.40)	127	6 (4.72)	4 (3.15)	120	7 (5.83)	5 (4.17)	3 (2.50)

PAT = Plate agglutination test TAT = Tube agglutination test MAT = Microagglutination test
AUD = Abnormal uterine discharge B, B, G, M & S = Bagherhat, Bogra, Gaibandha, Mymensingh & Sirajgonj
*Significant at ($p < 0.01$)

justified to preliminary screening of Brucella infection by using RBPT followed by confirmation with i-ELISA (Table 1a, 1b & 1c).

The seroprevalence of brucellosis was relatively higher (5.00%) in > 24 months of age group compared to the other two age groups (Table 1a). Statistically, there was no significant association between age groups of goats and the seroprevalence of brucellosis. Groupings of goats based on age varied widely in the previous inland reports compared to the present study. Still, the findings of all the reports showed a higher prevalence of seropositivity of Brucella infection in higher age groups than in lower age groups (Table 1a,1b & 1c).

Gender-wise, the prevalence of Brucella infection revealed a comparatively higher prevalence in female (3.0%) than male (2.0%) goats, but the difference was found to be statistically insignificant. However, the prevalence of Brucella infection between buck and does vary widely in different published inland reports, in which the prevalence of this infection bucks a mostly negative lower rate of infection than female goats (Table 1a,1b & 1c).

Table 1c. Comparison of seroprevalence of brucellosis in goats between the present study and published reports based on some demographic factors in Bangladesh

S/N	Demo-graphic factors	Sub-criteria	Results of some published reports								
			Bangladesh[9]					Mymensingh[18]		Mymensingh[15]	
			No. of goat tested	RBPT Positive No. (%)	PAT Positive No. (%)	TAT Positive No. (%)	MAT Positive No. (%)	No. of goat tested	RBPT Positive No. (%)	No. of goat tested	RBPT positive No. (%)
1.	Age (years)	½ - 1	-	-	-	-	-	16	1 (6.25)	-	14 (08.6)x
		>1 - 2	-	-	-	-	-	35	2 (5.71)	-	21 (13.0)y
		> 2	-	-	-	-	-	62	4 (6.45)	-	38 (23.3)z
		> 2-5	-	-	-	-	-	-	-	-	90 (55.2)
2.	Gender	Male	-	-	-	-	-	36	2 (5.55)	-	40 (24.5)
		Female	-	-	-	-	-	77	5 (6.49)	-	123 (75.5)
3.	Breed	BBG	-	-	-	-	-	-	-	-	-
		Cross	-	-	-	-	-	-	-	-	-
4.	Pregnant status	Yes	-	-	-	-	-	-	-	-	-
		No	-	-	-	-	-	-	-	-	-
5.	Repro-ductive disorders	PA:	15	3 (20.0)	2 (13.3)	2 (13.3)	3 (20.0	-	-	-	-
		NO:	285	2 (00.7)	3 (01.1)	4 (01.4)	4 (01.4)	-	-	-	-
		RP: Yes	15	2 (13.3)	2 (13.3)	1 (6.7)	2 (13.3)	-	-	-	-
		No	285	3 (01.1)	3 (01.1)	5 (1.8)	5 (01.8)	-	-	-	-
		FC	-	-	-	-	-	-	-	-	-
		AUD: Yes	010	1 (10.0)	1 (10.0)	1 (10.0)	1 (10.0)	-	-	-	-
		No	290	4 (01.4)	4 (01.4)	5 (01.7)	6 (02.1)	-	-	-	-
		ND	-	-	-	-	-	-	-	-	-
6.	Housing flooring system	Kacha	-	-	-	-	-	-	-	-	-
		Brick	-	-	-	-	-	-	-	-	-
		Macha	-	-	-	-	-	-	-	-	-
7.	Rearing system	Farming	-	-	-	-	-	-	-	-	-
		Rural	-	-							
Overall,			300	5 (1.67)	5 (1.67)	6 (2.0)	7 (2.3)	113	7 (6.19)	1710	163 (9.53)

x = < 6 months y = > ½ - 1 year z = >1 -2 years

This study did not include the breed-wise prevalence of Brucella infection in goats. However, out of eight reports published from Bangladesh, only one report reported a higher prevalence in crossbred goats than in indigenous goats (Table 1a, 1b & 1c).

A relatively higher prevalence was found in pregnant goats (2.22%) than in non-pregnant goats (1.67%), which was not statistically insignificant. Out of eight articles published on the prevalence of Brucella infection based on goats' pregnancy, only two articles included the pregnancy factor to detect its influence on its prevalence, of which significantly higher prevalence rates were reported in both reports (Table 1a, 1b & 1c).

The prevalence of brucellosis was much higher (RBPT 30.00% & i-ELISA 20.0%) in goats with a history of previous abortion (Table 1a). Out of eight inland reports, six reports included the prevalence of Brucella infections in goats with a history of previous abortion, and in all these

reports showed a significantly higher prevalence of Brucella infection in goats with a previous history of abortion in comparison to goats without any history of previous abortion (Table 1a, 1b & 1c). The placental retention cases showed negative to Brucella infection in this study, but earlier some reports showed higher prevalence of Brucella infection in does with placental retention (Table 1a, 1b & 1c). However, statistically, there existed a significant ($p < 0.01$) effect of reproductive disorders on the seroprevalence of brucellosis in goats.

There was no positive reactor among the seven goats kept on the brick floor. More positive cases were found in goats kept on the Macha system floor (2.0%) than in goats kept on the Kacha floor (Table 1a).

DISCUSSION

Brucella melitensis is an infectious bacterial agent that can affect most domestic animals, but small ruminants are considered naturally susceptible hosts. Abortions in late pregnancy, birth of weak offspring, and reduced milk production are the common signs in newly infected flocks. Infected does and ewes usually only abort once but continue to shed bacteria in their birth products and only a few animals abort repeatedly. Goats become persistently infected and can shed bacteria in their milk throughout their lifetime. Healthy asymptomatic carriers are a source of infection for other animals and humans. Small ruminants infect themselves by licking aborted fetuses, placentas, newborn offspring, vaginal discharges, in utero, or by consuming milk or feed contaminated with these materials. Milkers can spread the infection via unsanitary milking practices. *B. melitensis* spreads to people from infected animals through raw milk, unpasteurized dairy products, processing meat from infected goats, and contact with aborted kids or infective reproductive tissues and /or secretions. Inhaling contaminated dust and aerosols, contact with carcasses, or handling wool from infected animals can also infect people.[19]

B. melitensis infection causes disease only in adult (sexually mature) females and males. Young animals may be infected but do not show any clinical signs and generally show only a weak and transient serological response. However, susceptibility increases after sexual maturity, especially with pregnancy.[20]

Tests currently used for the serological diagnosis of *B. melitensis* infections in small ruminants were initially developed to diagnose *B. abortus* infections in cattle. Although not formally validated for use in small ruminants, these tests, particularly the Rose Bengal Plate agglutination test, the complement fixation test, and the ELISA, have been used for the serological diagnosis of brucellosis in small ruminants. A combination of tests shows a sensitivity and specificity that appears sufficient to detect infected animals.[20] Considering these facts, this study describes the prevalence of Brucella infection in goats detected using RBPT followed by i-ELISA in the BAU GF, Mymensingh. An overall 2.67% and 2.0% seropositivity of Brucella infection was recorded with RBPT and i-ELISA), respectively. The seropositivity of 2.67% by using RBPT recorded in this study was found to be comparatively higher than 1.67% reported from Bangladesh[9] but lower than 3.24% reported from Nilphamari,[7] 3.85% reported from Dhaka, Rajshahi, and Mymensingh districts, 4.33% reported from six districts,[8] 4.72% reported from five districts,[11] 5.83% reported from Mymensingh and Bogra districts

,[12] 6.19% reported from Mymensingh[18] and 9.53% reported from Mymensingh[15] district. Similarly, with i-ELISA this study recorded an overall 2.0% prevalence of Brucella infection in goats which is found lower than the earlier reported findings detected by i-ELISA which includes 2.59% in Nilphamari district,[7] 3.15% in five districts,[11] and 2.50% in Mymensingh and Bogra districts.[12]

Although a higher prevalence of Brucella infection recorded in female goats (2.22-3.3%) than in male (0-2.0%) goats, no significant difference ($p > 0.05$) between males and females. This finding supports the observation of 3.84% Brucella infection in female and 0-2.0% in male[7] goats. However, some reports showed positivity of Brucella infection in female goats e.g. 2.40%,[8] 4.04% and 3.0%,[11,12] whereas bucks remain seronegative to Brucella infection in all these reports.

This study has recorded an insignificantly higher prevalence of Brucella infection in pregnant (2.22%) than in non-pregnant goats (1.67%) which is conformity with the earlier report of 3.33% prevalence of Brucella infection in pregnant and 1.56% in non-pregnant goats.[7] However, a significantly higher prevalence of Brucella infection in pregnant (8.33%) than in non-pregnant goats (1.53%) has also been reported in Bangladesh.[10]

Brucella seropositivity has been reported more frequently among small ruminants with a history of reproductive disorders than animals that have no history of reproductive disorders. This study has recorded higher seropositivity in does with a history of previous abortion (RBPT 30.0% & i-ELISA 20.0%) in comparison to normal does with 0.84% seropositivity with both the tests. These findings support the 33.33%[7] and 22.72%[10] seropositivity to Brucella infection in does with history of previous abortion in Bangladesh. However, Brucella infection could be a causative agent of 32.6% of abortion cases among small ruminants elsewhere[21]

Brucellosis is a highly infectious widespread zoonotic disease of global significance, and WHO considers it a neglected disease due to its lack of attention by the global health system.[22] Out of 12 identified species of Brucella, of which four species have moderate-to-significant human pathogenicity, which includes (a) *B. melitensis* (from small ruminants; highest pathogenicity), (b) *B. suis* (from pigs; high pathogenicity), (c) *B. abortus* (from cattle; moderate pathogenicity) and (d) *B. canis* (from dogs; moderate pathogenicity).

B. melitensis is the most virulent species, and ingesting unpasteurized goat milk and related dairy products is the main route by which this bacterium is transmitted to humans. As few as 10 to 100 organisms can cause the disease in humans.[2] David Bruce isolated the causative organism of Malta fever on the Island of Malta from four fatal human cases in 1887 and named it *Micrococcus melitensis*. A Greek physician working with Bruce, T. Zammit, demonstrated in 1905 that the Maltese goat- often with no clinical signs of illness- carried the organism and served as the source of infection through consuming unpasteurized milk by military personnel. Goat milk was banned for human consumption, and the troubling episode ended in 1906. Currently, the zoonotic Brucella infection has been controlled in most industrialized countries using this consumption principle of pasteurized milk. In contrast, it is still a great public health problem in medium and low-income countries, including Bangladesh, where people consume unpasteurized milk. Recently, an outbreak of zoonotic brucellosis in eight humans consuming raw milk of animals has been reported in Teknaf, Bangladesh, with symptoms of fever, joint

pain, fatigue, and headache with some chronic cases having chronic fatigue and arthritis.[23]

Consuming unpasteurized milk and milk products has been identified as the leading cause of human brucellosis, with prevalence ranged from 33.9 to 100%, with highest occurrence of Brucella organism in milk of cows (1.86-81.7%), buffaloes (10.4-61.67%), camel (0-24%), goat (0-88.8%), and cheese (0-39.1%) (Islam et al. 2023).[24] Although there are no published reports on the detection of *B. melitensis* in the milk of goats from Bangladesh, molecular detection of *Brucella* spp. from the milk of seronegative cows has been reported in Bangladesh.[25]

Review of literature reveals that both *B. abortus* and *B. melitensis* affect all livestock species, but B. abortus is most commonly in cattle and camels, while infection in sheep and goats is mainly associated with *B. melitensis*. Human infection with *Brucella* spp. (*B. abortus* & *B. melitensis*) may be transmitted from multiple livestock species (cattle, sheep, goats, and camels) reared in the same areas.[26] Among the *Brucella* spp. positive milk samples, 26.5% and 73.4% of the samples were infected with *B. abortus* and *B. melitensis*, respectively in small ruminants. *B. abortus* infection has been reported to be 6.8% and 12.5% respectively in sheep and goats. About 2.0% of samples (2 goats + 1sheep) were simultaneously infected with both *B. melitensis* and *B. abortus* elsewhere[21]

CONCLUSIONS

Brucellosis is a worldwide zoonotic disease, and the discovery of its causal agent of Malta fever by David Bruce in1887, which was controlled well in the late twentieth century in mostly industrialized countries but re-emerged in the past two decades even though it was controlled in the developed world. After over 137 years of its discovery, it remains one of the important zoonotic infections causing a serious public health threat, due to food-chain, and/or occupational exposure, and causing huge reproductive failure in animals and economic losses in low-and middle-income countries in the world. It is estimated that over one-fifth of the 1.4 billion worldwide cattle population is currently infected by Brucella, and this results in over ½ million new human cases annually in the world. The world population of 1.1 billion sheep and 0.5 billion goats, and the prevalence of Brucella infection varied widely from <1.0 to 15% in goats and <1 to 9.0% in sheep. Cross-species of Brucella infection is common especially where mixed farming of small ruminants and cattle is in practice in the developing world and serology has limitations in detecting whether the tested hosts are infected with *B. melitensis* or with *B. abortus* or with both Brucella species. Therefore, isolation, identification, and molecular characterization of Brucella species in humans and animals need to be detected to define a specific framework, identify the source of infection, and plan for appropriate control measures. A significantly ($p < 0.05$) higher prevalence of brucellosis was found in goats with a history of previous abortion in Bangladesh. The prevalence of brucellosis in the study flock was found to be low (2.0%) which will allow test and culling policy for eradication. A ‘One Health’ collaborative approach of multiple disciplines to attain optimal health for people, animals, and the environment is required to manage and prevent Brucella infections in humans and animals. This effort will involve continued collaboration among public health and veterinary services, microbiologists, medical and veterinary practitioners, animal breeders, and stakeholders to maintain brucellosis surveillance and prevention programs, recognize exposure risks and symptoms of clinical

disease, utilize appropriate diagnostic strategy, and determine optimal management of confirmed cases or infected populations. Challenges for 'One Health' implementation to control brucellosis in small ruminants include the plan and target-wise research, the need for a safer vaccine, filling in the infrastructure gap, realistic capacity building, the establishment of reference laboratories in critical areas, and the stepwise implementation of measures to prevent, control and eradicate brucellosis in developing countries including Bangladesh.

ETHICAL APPROVAL

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

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REFERENCES

01. Rajendhran J (2021). Genomic insights into Brucella. *Infection, Genetics and Evolution.* 87, 104635 [doi: 10.1016/j.meegid.2020.104635]

02. Hayoun MA, Muco E and Shorman M (2023). Brucellosis. Book- StatPearls Publishing, Treasure Island (FL). PMID: 28722861. https://pubmed.ncbi.nim.nih.gov/28722861/

03. Rossetti CA, Maurizio E and Rossi UA (2022). Comparative review of brucellosis in small domestic ruminants. Frontiers Veterinary Science 9, [doi: 10.3389/fvets.2022.887671]

04. Rahman MS, Sarker RR, Melzer F and Sprague LD (2014). Brucellosis in human and domestic animals in Bangladesh: A review. African Journal of Microbiology Research 8 (41): 3580-3594 [doi: 10.5897/AJMR2014.7074]

05. Franc KA, Krecek RC, Hasler BN and Arenas-Gamboa AM (2018). Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. BMC Public Health 18, 125 [doi: 10.1186/s12889-017-5016-y]

6. Ahmed BS, Osmani MG, Rahman AKMA, Hasan MM, Maruf AA, Karim MF, Karim SMA, Asaduduzzaman M, Hasan MR, Rahman MM and Rahman MS (2018). Economic impact of caprine and ovine brucellosis in Mymensingh district, Bangladesh. *Bangladesh Journal of Veterinary Medicine* 16 (2): 193-203 [doi: 10.33109/bjvm1805]

07. Rahman MS, Mithu S. Islam MT, Uddin MJ, Sarker RR, Sarker MAS and Akhter L (2012). Prevalence of brucellosis in Black Bengal goats in Bangladesh. *Bangladesh Journal of Veterinary Medicine* 10 (1-2): 51-56 [doi: 10.17221/1555-VETMED]

8. Munsi MN, Akther S, Rahman MH, Hassan MZ, Ali MZ and Ershaduzzaman M (2021). Seroprevalence of brucellosis in goats in some selected areas of Bangladesh. *Journal of Advanced Veterinary and Animal Research* 8(1): 123-128 [doi: 10.5455/javar.2021.h494]

09. Uddin MJ, Rahman MS and Akter SH (2007). Brucellosis of goats (*Capra hircus*) in Bangladesh. *Journal of Bangladesh Agricultural University* 5 (2): 287-282

10. Islam MA, Samad MA and Rahman AKMA (2010). Risk factors associated with the prevalence of brucellosis in Black Bengal goats in Bangladesh. *Bangladesh Journal of Veterinary Medicine* 8(2): 141-147

11. Rahman MS, Faruk MO, Her M, Kim JY, Kang SI and Jung SC (2011). Prevalence of brucellosis in ruminants in Bangladesh. *Veterinarni Medicina* 56 (8): 379-385

12. Rahman MS, Hahsin MFA, Ahasan MS, Her M, Kim JY, Kang S II and Jung SC (2011). Brucellosis in sheep and goat of Bogra and Mymensingh districts of Bangladesh. *Korean Journal of Veterinary Research* 51 (4): 277-280 [doi: 10.14405/kjvr.2011.51.4.267]

13. Megarsa B, Biffa D, Abunna F, Regassa A, Godfroid J and Skjerve E (2010). Seroprevalence of brucellosis and its contribution to abortion in cattle, camel and goats kept under pastoral management in Borana, Ethiopia. *Tropical Animal Health and Production* 43: 651-656 [doi: 10.1007/s11250-010-9748-2]

14. Mirza MA, Jalvi MA and Razzak A (1998). Screening of goat flocks for brucellosis using the Rose Bengal Plate test. *Pakistan Veterinary Journal*, 18 (3): 146-149.

15. Shafy NM, Ahmed BS, Sarker RR, Millat KSA, Hasan MT, Bhattacharjee PK, Chakrabartty A, Paul A, Sarker MAS, Truong T and Rahman MS (2016). Serological prevalence of ovine and caprine brucellosis in Bangladesh. *Bangladesh Journal of Veterinary Medicine* 14(2): 209-213

16. EURLB (2021). Brucellosis Rose Bengal Test Standard Operation Procedure. EU Reference Laboratory for Brucellosis (EURLB). Pp. 1-7. Sitesv2.anses.fr/sites/default/files/SOP%20Brucellosis% 20EU-RL%20RBT_Rev1_vf.pdf

17. WOAH (2023). Brucellosis (Infection with B. abortus, B. melitensis and B. suis). Chapter 3.1.4. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Twelfth edition. woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.04_BRUCELLOSIS.pdf

18. Akhter L, Islam MA, Das S, Khatun MM and Islam MA (2014). Seroprevalence of brucellosis and its associated risk factors in sheep and goats in the farms and slaughterhouse in Mymensingh, Bangladesh. *Microbes and Health* 3(1): 25-28 [doi: 10.3329/mh.v3i1.19778]

19. CDFA (2022). *Brucella melitensis* of goats and sheep public health issue. California Department of Food and Agriculture. www.cdfa.ca.gov

20. Garrido-Abellan F, Ahl R, Duran-Ferrer M, Macmillan A, Minas A, Nicoletti P and Vecchi G (2021). Brucellosis in sheep and goats (*Brucella melitensis*). Report of the Scientific Committee on Animal Health and Animal Welfare. SANCO.C.2/AH/R23/2001

21. Rostami S, Rashidan E, Jaydari A and Rahimi H (2023). Investigation of the proportion of Brucella abortus and Brucella melitensis in sheep and goat milk. *Veterinary Medicine International* 1/6751152 [doi: 10.1155/2023/6751152]

22. Woodcock TP, Frye E, Guarino C, Franklin-Guild R, Newman AP, Bennett J and Goodrich EL (2023). A one health review on brucellosis in the United States. *Journal of the American Veterinary Medical Association* 261 (4): [doi: 10.2460/javma.23.01.0033]

23. ICDDR'B (2023). Brucellosis outbreak sparks concern among health officials. https://www.icddrb.org/quick-lijks/press-releases?id=163&task=view

24. Islam MS, Islam MA, Rahman MM, Islam K, Islam MM, Kamal MM and Islam MN (2023). Presence of Brucella spp. in milk and dairy products: a comprehensive review and its perspective. *Journal of Food Quality* 1: [doi: 10.1155/2023/2932883]

25. Islam MS, Islam MA, Khatun MM, Saha S, Basir MS and Hasan MM (2018). Molecular detection of Brucella spp. from milk of seronegative cows from some selected areas in Bangladesh. *Journal of Pathogens* 7, 9378976 [doi: 10.1155/2018/9378976]

26. Akoko JM, Pelle R, Lukambagire AHS, Machuka EM, Nthiwa D, Mathew C, Fevre EM, Bett B, Cook EAJ, Othero D, Bonfoh B, Kazwala RR, Shirima G, Schelling E, Halliday JEB and Ouma C (2021). Molecular epidemiology of Brucella species in mixed livestock-human ecosystems in Kenya. *Scientific Reports* 11, 8881 [doi: 10.1038/s41598-021-88327-z]