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Brucellosis in sheep and goat of Bogra and Mymensingh districts of Bangladesh

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Abstract : Brucellosis is the most important bacterial disease of livestock in Bangladesh. The present study was conducted to determine the seroprevalence of brucellosis in goat and sheep in Mymensingh and Bogra districts of Bangladesh using slow agglutination test and Rose Bengal test as screening test and indirect enzyme linked immunosorbent assay as confirmatory test. Questionnaire based data on age, gender, area, client's complaint, number of animals in herds, disease history, reproductive problems such as abnormal uterine discharge, abortion or previous abortion in sheep and goat and their reproductive diseases were recorded. A total of 200 sera samples were collected from 80 sheep and 120 goats. The prevalence of brucellosis in goat was 2.50% and 1.25% in sheep. Positive reactors were only detected in female of both goat and sheep. In this study, there existed a significant association among abortion and the prevalence of brucellosis ($p < 0.01$). The prevalence of brucellosis in sheep and goat in Bangladesh is not negligible, and it is therefore worth considering the adoption of preventive measures.

Keywords : Bangladesh, brucellosis, goat, seroprevalence, sheep

Introduction

Brucellosis is a zoonotic disease [13, 25] caused by *Brucella* that is pathogenic for a wide variety of animals and human beings [12]. Small ruminant brucellosis is mostly caused by *Brucella melitensis* [24]. *Brucella ovis* is also an important cause of orchitis and epididymitis in sheep but it is not recognized as a cause of natural infection in goats. Brucellosis in sheep and goats caused by *Brucella melitensis*, one of the most virulent species of *Brucella*, is a widespread zoonosis, especially in Mediterranean and the middle-east regions where it also constitutes a hazard for human [9]. This micro-organism is the main etiological agent of sheep brucellosis in Turkey [4, 11].

Brucellosis in human beings is caused by exposure to livestock and livestock products. Infection can result from direct contact with infected animals and can also be transmitted to consumers through raw milk and milk products. Brucellosis spreads between animals in a herd and the disease is a systemic infection that can involve many organs and tissues. Once the acute period of the disease is over, symptoms of brucellosis are mostly not pathognomonic, and the organism can be chronically located in the supramammary lymphatic nodes and mammary glands of 80% of infected animals. Thus they continue to secrete the *Brucella* organism in their body fluids [6, 24].

In Bangladesh, approximately 80% of people live in villages, and rural income is largely dependent on livestock; the people are in close contact with livestock on a daily basis. 6.5% of national income and 3.5% gross domestic product come from livestock. There are about 35 million of goats and 13 million of sheep in Bangladesh. A lot of undiagnosed cases of abortion, stillbirth and retained placenta were reported in sheep and goats which must be resulted from brucellosis. Brucellosis is an important constraint for the develop-

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ment of livestock in Bangladesh. The importance of brucellosis in Bangladesh is not known precisely, but it may have a considerable impact on both human and animal health.

In Bangladesh, brucellosis was first detected in cattle in 1967 [14], in buffalo in 1997 [16]. In human and goats brucellosis was first reported in 1983 [17]. However scant information is available about the sero-prevalence of brucellosis by agglutination tests in human beings, cattle, sheep, and goats [1-3, 13, 17, 19, 21-23]. Therefore, the present study was carried out for diagnosis of brucellosis in sheep and goats by the indirect enzyme linked immunosorbent assay (I-ELISA) in Mymensingh and Bogra districts of Bangladesh.

Materials and Methods

A total of 200 blood samples were collected from sheep and goats of 1~3 years old in Mymensingh and Bogra districts of Bangladesh. Among them goat (50) and sheep (35) sera were collected from Mymensingh and goat (70) and sheep (45) samples were collected from Bogra. The study recorded some clinical, epidemiological and reproductive information. The questionnaire based data on age, gender, area, client's complaint, number of animals in herds, disease history, reproductive problems such as abnormal uterine discharge, abortion or previous abortion in sheep and goat and their reproductive diseases were recorded. All blood samples were processed for sera preparation and were tested with slow agglutination test (SAT) and Rose Bengal test (RBT) as screening test. I-ELISA was used for confirmatory diagnosis.

RBT was performed according to the procedure as described by Office International des Epizooties, France [15]. The test serum samples and Rose-Bengal antigen were kept 1 h in room temperature before beginning of the test. The result was considered positive when there was any degree of agglutination noticeable and absence of agglutination considered as negative.

SAT was carried out with EDTA as described by Garin *et al*. [7]. Briefly the slow agglutination test was performed in "U" bottom 96 well microplate and tested to duplicate well by 100 μL serial dilution (initial dilution 1/6.25) and then 100 μL antigen was added. The plate was incubated at 37°C for 24 h (± 4 h) for reading. Agglutination reaction was observed by using a magnifying mirror against illumination source. Reading was taken on the basis of this protocol and the standardization we performed (75% agglutination of the OIEISS).

I-ELISA was performed according the protocol and suppliance provided by the ELISA kit (Svanova Biotech AB, Sweden) as per manufacturer instruction. The questionnaire-based data was processed by Microsoft Excel and Static, and results were statistically analyzed for interpretation.

Results

As seroprevalence of brucellosis was compared among different tests including RBT, SAT, I-ELISA, the results has been shown in Table 1. The seroprevalence of brucellosis according to RBT, SAT, I-ELISA, was in goats 5.83%, 4.17%, 2.50% respectively and in sheep 3.75%, 2.50%,1.25% respectively. Not significant but the study also represented the brucellosis seroprevalence of a goat was 2 times more than that of a sheep. In female, 100 among 120 sera of goat and, 3 goats (3.00%) had abortion or previously abortion record and they showed positive reactions by all tests. In 60 female of 80 sheep, 1 case (1.66%) of abortion was found with

Table 1. Seroprevalence of brucellosis based on Rose Bengal test (RBT), slow agglutination test (SAT) and indirect enzyme linked immunosorbent assay (I-ELISA)

Species	Sex of animals	No. of sera tested	No. of positive reactors (%)		
			RBT	SAT	I-ELISA
Goat	Male	20	2 (10.00)	1 (6.25)	0 (0.00)
	Female	100	5 (5.00)	4 (4.00)	3 (3.00)
	Total	120	7 (5.83)	5 (4.17)	3 (2.50)
Sheep	Male	20	1 (5.00)	0 (0.00)	0 (0.00)
	Female	60	2 (3.33)	2 (3.33)	1 (1.66)
	Total	80	3 (3.75)	2 (2.50)	1 (1.25)

Table 2. Prevalence of brucellosis in aborted /previously aborted sheep and goats

Species	Condition of animals	No. of sera tested	No. of positive reactors (%)		
			RBT	SAT	I-ELISA
Goat	Previous abortion record	3	3 (100)	3 (100)	3 (100)*
	No record	117	4 (3.41)	2 (1.70)	0 (0.00)
Sheep	Previous abortion record	1	1 (100)	1 (100)	1 (100)*
	No record	79	2 (2.53)	1 (1.26)	0 (0.00)

*Significant at 1% level of probability ($p < 0.01$).

positive reaction. In 20 male of goat and 20 male of sheep, any positive reactions were not detected by I-ELISA (Table 2). In this study, in case of goats and sheep, there existed a significant ($p < 0.01$) association among abortion and the prevalence of brucellosis. So based on this it can be concluded that the prevalence of brucellosis was significantly higher in aborted or previously aborted goats and sheep than that with no abortion record.

Discussion

Brucellosis remains a major zoonosis worldwide [5, 10, 13, 25]. In some areas *Brucella melitensis* has emerged as a cause of infection in goat as well as in sheep and goats. Each year half of a million new cases of brucellosis are reported worldwide, but according to the World Health Organization (WHO), these numbers greatly underestimate the true prevalence.

The objectives of the study were to implement serological methods and to improve the understanding the epidemiology of *Brucella* in goat and sheep and to provide information for disease control in livestock and human being. Seropositivity was considered to be due to natural infection because vaccination has never been practiced in Bangladesh. Serological testing using the RBT, SAT, and ELISA is generally used for the detection of *Brucella* infections in livestock. The serological assay allows the detection of *Brucella* specific antibodies in a whole blood sample collected from animal directly at a farm or in the field. It is widely accepted that agglutination tests (SAT and to lesser extent RBT) are not recommended for the diagnosis of chronic brucellosis since these tests mainly detect IgM and IgG. The amount of IgM found in the sera will decline with time and become undetectable in agglutination tests in most chronic cases [15]. However, in experimental conditions agglutination tests are able to detect infections as early as two weeks post infection and thus remain excellent tools to use in order to detect early infections [8].

ELISA has been evaluated for many years for their diagnosis performance to detect serum antibody to brucellosis in domestic animals. ELISA for diagnosis of brucellosis has several advantages when compared with other tests. First, it has a direct method of identification of specific antibody. Second, it is more sensitive than other the agglutination test and thus has the potential to detect infected animals. Third, ELISA results provide an epidemiological tool for investigation of the infective status of herds [20].

The present investigations revealed that the overall seroprevalence of brucellosis in goat was 2.50% which is similar to or lower than 2.33~14.57% reported by others [18, 21]. Sex, Age, location and living condition influence the sero prevalence of brucellosis. The prevalence of brucellosis in sheep is similar to the findings recorded by other study [21]. The prevalence of brucellosis in goat and sheep was found to be higher in female 2.50% (4 out of 160) in I-ELISA than male 0.00%. The higher rate of infection in female will be due to infection within the female reproductive tract providing a potential reservoir for the organism to propagate. This finding is similar to the findings recorded by other study [21]. The study suggested warranting immediate preventive measures in these areas. To prevent expansion of brucellosis, it is an important to take care of aborting animals and materials related to abortion. Further study for isolation, identification and typing of *Brucella* spp. epidemiological relatedness of among animals or between animal and human in Bangladesh is recommended.

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References

1. **Ahasan MS, Rahman MS, Song HJ.** A sero-surveillance of *Brucella* spp. antibodies and individual risk factors of infection in cattle of Bangladesh. Korean J Vet Serv 2010, **33**, 121-128.
2. **Amin KMR, Rahman MB, Kabir SML, Sarkar SK, Akand MSI.** Serological epidemiology of brucellosis in cattle of Mymensingh districts of Bangladesh. J Anim Vet Adv 2004, **3**, 773-775.
3. **Amin KMR, Rahman MB, Rahman MS, Han JC, Park JH, Chae JS.** Prevalence of *Brucella* antibodies in sera of cows in Bangladesh. J Vet Sci 2005, **6**, 223-226.
4. **Arda M, Bisping W, Aydn N, İstanbulluoğlu E, Akay Ö, Izgür M, Diker S, Karaer Z.** An etiologic and serologic investigation of ovine abortion in central Anatolia Region. Vet J Ankara Univ 1987, **34**, 195-206.
5. **Baek BK, Lim CW, Rahman MS, Kim C-Hyun, Oluoch A, Kakoma I.** Brucella abortus *infection in indigenous Korean dogs.* Can J Vet Res 2003, **67**, 312-314.
6. **Cordes DO, Carter ME.** Persistence of *Brucella abortus* infection in six herds of cattle under brucellosis eradication. N Z Vet J 1979, **27**, 255-259.
7. **Garin B, Trap D, Gaumont R.** Assessment of the EDTA seroagglutination test for the diagnosis of bovine brucellosis. Vet Rec 1985, **117**, 444-445.
8. **Godfroid J, Käsborer A.** Brucellosis in the European Union and Norway at the turn of the twenty-first century. Vet Microbiol 2002, **90**, 135-145.
9. **Jacques I, Olivier-Bernardin V, Dubray G.** Efficacy of ELISA compared to conventional tests (RBPT and CFT) for the diagnosis of *Brucella melitensis* infection in sheep. Vet Microbiol 1998, **64**, 61-73.
10. **Kakoma I, Oluoch AO, Baek BK, Rahman MS, Matsuda K.** *More attention warranted on* Brucella abortus *in animals.* J Am Vet Med Assoc 2003, **222,** 284.
11. **Leyla G, Kadri G, Ümran O.** Comparison of polymerase chain reaction and bacteriological culture for the diagnosis of sheep brucellosis using aborted fetus samples. Vet Microbiol 2003, **93**, 53-61.
12. **Mathur TN.** Brucellosis and Farm management. Indian Vet J 1971, **48**, 219-228.
13. **Matyas Z, Fujikura T.** Brucellosis as a world problem. Dev Biol Stand 1984, **56**, 3-20.
14. **Mia AS, Islam H.** A preliminary study on the incidence of bovine infertility and economic loss caused by it. Pak Vet J 1967, **1**, 5-10.
15. **Office International des Epizooties (OIE).** O.I.E. Manual of Standards for Diagnostic Tests and Vaccines. 4th ed. OIE, Paris, 2008.
16. **Rahman MA, Islam MS, Alam MGS, Shamsuddin M.** Seroprevalence of brucellosis in the buffalo (*Bubalus bubalis*) of a selected area in Bangladesh. Buffalo J 1997, **13**, 209-214.
17. **Rahman MM, Chowdhury TIMFR, Rahman A, Haque F.** Seroprevalence of human and animal *Brucellosis* in Bangladesh. Indian Vet J 1983, **60**, 165-168.
18. **Rahman MM, Haque M, Rahman MA.** Sero-prevalence of caprine and human brucellosis in some selected areas of Bangladesh. Bangladesh Vet J 1988, **22**, 85-93.
19. **Rahman MM, Rahman MA.** Incidence of *Brucella* infection in sub-clinical mastitic udder. Bangladesh Vet J 1981, **15**, 39-42.
20. **Rahman MS.** Experimental infection and protective immunity of Sprague-Dawley rats with *Brucella abortus.* Ph.D dissertation. Chonbuk National University, Jeonju, 2003.
21. **Rahman MS, Faruk MO, Her M, Kim JY, Kang SI, Jung SC.** Prevalence of brucellosis in ruminants in Bangladesh. Vet Med (Praha) 2011, **56**, 379-385.
22. **Rahman MS, Han JC, Park J, Lee JH, EO SK, Chae JS.** Prevalence of brucellosis and its association with reproductive problems in cows in Bangladesh. Vet Rec 2006, **159**, 180-182.
23. **Rahman MS, Huque MF, Ahasan MS, Song HJ.** Indirect enzyme linked immunosorbent assay for the diagnosis of brucellosis in cattle. Korean J Vet Serv 2010, **33**, 113-119.
24. **Redkar R, Rose S, Bricker B, DelVecchio V.** Real-time detection of *Brucella abortus*, *Brucella melitensis* and *Brucella suis*. Mol Cell Probes 2001, **15**, 43-52.
25. **World Health Organization (WHO).** Joint FAO/WHO Expert Committee on Brucellosis. 6th report. WHO Technical Report Series No. 740. 1986.