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Seroprevalence of Brucellosis and its Associated Risk Factors in Sheep and Goat in the Farms and Slaughter House in Mymensingh, Bangladesh.

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ABSTRACT

A cross sectional study was performed to determine the seroprevalence and risk factors of brucellosis in sheep and goat on the farms, Veterinary Teaching Hospital of Bangladesh Agricultural University (BAU), and animal slaughter house of Mymensingh, Bangladesh. Sera were prepared after collecting blood samples from sheep (n=101) and goat (n=113). Risk factors relating to brucellosis were determined considering the variables generated from a questionnaire. These variables included animal's age, sex, pregnancy, and husbandry system. The sera were tested by Rose Bengal Plate Test (RBPT) for the detection of *Brucella abortus* specific antibodies in sheep and goat. The results revealed that 5.94% (n=6/101) sera of sheep, and 6.19% (n=7/113) sera of goat were positive for brucellosis. Higher prevalence of brucellosis was recorded in female sheep (7.54%) and goat (6.49%) as compared to male sheep (4.16%) and goat (5.50%), respectively. The sheep and goat above two years of age showed higher prevalence of brucellosis (8.69% and 6.45%) as compared to other ages. No risk factor was found to be statistically significant (p>0.05). Data of this study suggest that sheep and goat could be the reservoir hosts of brucellosis that might constitute a hurdle in the controlling of bovine and human brucellosis.

Key Words: Bangladesh, Brucellosis, Goat, Sheep, Rose Bengal plate test.

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Introduction

Brucellosis is a worldwide emerging zoonotic disease caused by Gram negative bacteria belongs to the genus *Brucella* (Islam *et al.*, 2013). It mainly affects the reproductive tract of animals and responsible for huge economic losses in livestock sector due to abortion, infertility, still birth, retention of placenta and decreased milk production (Radostits *et al*., 2007).

Brucellosis in cattle is primarily caused by *Brucella* (*B.*) *abortus*. In sheep and goat the infection is mainly caused by *B. melitensis*. Transmission of brucellosis in animals is occurred by direct contact with the infected animal and ingestion of contaminated aborted materials (Muflihanah *et al*., 2013). Humans acquire *B. abortus* infection through direct contact with the infected animals and consumption of unpasteurized milk and milk products (Young, 1995; Mohamand *et al*., 2014).

Brucellosis is endemic in humans and livestock population in Bangladesh (Islam *et al*., 2013a). This disease may constitute a considerable impact on human and animal health as well as on socioeconomic factors and it might be a significant drawback in the development of the livestock sector (Rahman *et al.*, 2011). Economic losses due to brucellosis results from abortion, loss of calf production, reduced milk yield and infertility (Rahman *et al.*, 2006).

Transmissions of *B. abortus* from cattle are known to occur in sheep and goat in the brucellosis endemic areas (Ogundipe *et al*., 1994). In the fiscal year 2011-12, sheep and goat populations were estimated as 3.08 and 25.11 millions, respectively which represent approximately 53.35% of the total livestock population in Bangladesh. (Bangladesh Economic Review, 2012). Farmers at village area rear sheep and goat along with cattle. They share the common house and pasture land with cattle. So, there is a chance of inter-species transmission of brucellosis from cattle to sheep and goat and vice versa is more likely in the context of Bangladesh (Akhter, 2012). Several investigations recorded the seroprevalence of brucellosis in sheep and goat in Bangladesh (Uddin *et al.*, 2007; Islam *et al.*, 2010; Rahman *et al.*, 2011; Rahman *et al.*, 2012). However, to our knowledge, there is no report on the seroprevalnce of brucellosis in sheep and goat on the farms and slaughter house in Mymensingh. Also, isolation of *Brucella* spp. from sheep and goat has not yet been performed in Bangladesh. Therefore, the study was designed to determine the seroprevalence of *B. abortus* specific antibody response in sheep and goat, and isolation of *Brucella* spp. from the seropositive reactor animals at the BAU sheep and goat farms, BAU Veterinary Teaching Hospital, and animal slaughter house in Mymensingh.

Materials and Methods

The study was conducted for a period of 10 months (July 2011 to May 2012) at the Department of Microbiology and Hygiene, BAU, Mymensingh.

Sample collection

Serum samples (n=214) were collected from sheep (n= 101) and goats (n= 113) from Veterinary Teaching Hospital and sheep and goat farms at BAU and municipal slaughter house, Mymensingh. Risk factors variables such as animal's age, sex, pregnancy status and husbandry practice were recorded (Table 1) by a questionnaire administered to the animals' attendants.

Table 1. Detail history of sheep and goats used in the study

Variables	Animal species	Category level	Number of observation
Gender	Sheep	Male	48
		Female	53
	Goat	Male	36
		Female	77
Age (year)	Sheep	Below I year	11
		1 year to 2 years	46
		Above 2 years	44
	Goat	Below I year	16
		1 year to 2 years	35
		Above 2 years	62
Pregnancy status	Sheep	Pregnant	07
		Non-pregnant	94
	Goat	Pregnant	11
		Non-pregnant	102
Floor type	Sheep	Earthen floor	78
		Cemented floor	23
	Goat	Earthen floor	60
		Cemented floor	17
		Slatted floor	43

Serological test

Rose Bengal Plate Test (RBPT) was used to detect *B. abortus* specific antibody in the serum samples. The antigen (*B. abortus* strain 119-3) was obtained from the Laboratory of Veterinary Public Health, College of Veterinary Medicine, Chonbuk National University, Republic of Korea. The test was performed according to the standard procedures of OIE (2008). The test and control sera were homogenized using a vortex and 10 µL of each serum was placed on a glass plate marked with circles of approximately 2 cm in diameter. After gentle shaking the antigen vial, 10 µL of antigen was placed beside the serum drop. The antigen and serum were mixed on the plate for 4 min. Definite clumping/ agglutination was considered as a positive reaction, while no clumping/agglutination was the indication of negative reaction (Fig. 1).

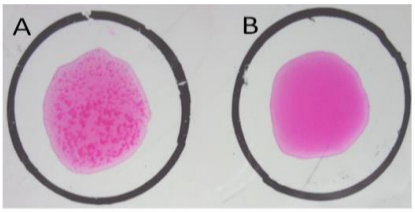

Fig. 1. Rose Bengal Plate Test. Definite clumping/ agglutination is considered as a positive reaction (A), while no clumping/agglutination is the indication of negative reaction (B).

Bacteriological study

Blood samples of seropositive sheep (n=6) and goat (n=7) were cultured on blood agar and brucella agar media for isolation of *Brucella* spp. Blood samples were processed by the lysis concentration method (Kolman *et al.,* 1991) with some modifications. Briefly, 100 µL blood sample was mixed with 900 µl distilled water in an Eppondorf tube. Hemolysed blood samples were centrifuged at 1500 rpm for 30 minutes at 4°C temperature. Supernatant was inoculated duplicate in blood agar and brucella agar media plates and incubated at 37°C for 5 days under 5% CO_2 atmosphere.

Statistical analysis

Statistical analysis was performed using 'Statistical package for the social sciences' (SPSS), version 17.0 (UK). The association between each risk factor and the outcome variable was assessed using the Chi-square (x^2) test. For all analysis a p value of ≤ 0.05 was considered to be statistically significant.

Results

The overall prevalence of brucellosis was 5.94% (n=6/101) in sheep and 6.19% (n=7/113) in goat. In the cases of sheep, the highest prevalence of brucellosis was recorded at the Veterinary Teaching Hospital (15.38%) followed by BAU farm (5.56%), and municipal slaughter house (4.28%). Prevalence of brucellosis in goat was the highest at the municipal slaughter house (7.5%) followed by Veterinary Teaching hospital (5.56%), and BAU farm (5.45%) (Table 2).

Table 2. Seroprevalence of brucellosis in sheep and goat at different study areas

Study areas	Animal species	No. of sera tested	No. of positive reactors (%)
Veterinary Teaching Hospital, BAU, Mymensingh	Sheep	13	2 (15.38)
	Goat	18	1 (5.56)
Sheep and Goat farms, BAU, Mymensingh	Sheep	18	1 (5.56)
	Goat	55	3 (5.45)
Municipal slaughter house, Mymensingh	Sheep	70	3 (4.28)
	Goat	40	3 (7.50)

BAU = Bangladesh Agricultural University

The prevalence of brucellosis in sheep and goat was found to be increased with the advancement of age (Table 3). The highest prevalence was recorded in sheep and goat over two years of age (8.69% and 6.45%, respectively).

Prevalence of brucellosis was higher in female as compared to male in both sheep and goat (Table 4). In female sheep, the prevalence of brucellosis was 7.54% against 4.16 % in male (p=0.445). Similarly, in female goat the prevalence was 6.49%, while in males, it was 5.5% (p=0.385).

Table 3. Prevalence of brucellosis in sheep and goat according to age

Animal species	Age of animals	No. of sera tested	No. of positive reactors (%)	p value
Sheep	6 months to 1 year	11	0 (0.00)	0.258
	1 year to 2 years	44	2 (4.54)	
	> 2 years	46	4 (8.69)	
Goat	6 months to 1 year	16	1 (6.25)	0.698
	1 year to 2 years	35	2 (5.71)	
	> 2 years	62	4 (6.45)	

Table 4. Seroprevalence of brucellosis in sheep and goat on the basis of sex

Animal species	Sex of animals	No. of sera tested	No. of positive reactors (%)	p value
Sheep	Male	48	2 (4.16)	0.445
	Female	53	4 (7.54)	
Goat	Male	36	2 (5.55)	0.385
	Female	77	5 (6.49)	

Out of 101 sheep, 7 were pregnant and 94 were non-pregnant. In case of goat 11 were pregnant and 102 were non-pregnant. Prevalence of brucellosis was higher in pregnant sheep and goat (7.6% and 9.09%) as compared to non-pregnant sheep and goat (5.6% and 6.52%) ($p > 0.05$).

Brucellosis prevalence was higher in sheep and goat reared on the earthen floor (6.41% and 6.66%) as compared to those of reared on cemented floor (4.34% and 5.88%) ($p > 0.05$). Goat reared on the slatted floor showed 4.65% prevalence of brucellosis.

Brucella organisms were not isolated from any of blood samples of seropositive sheep and goats.

Discussion

Brucellosis has been reported in human and animal populations in Bangladesh (Rahman *et al*., 2011; Islam *et al.,* 2013a). Prevalence of brucellosis varies from country to country, flock to flock and between different animal species and geographical areas. In this study prevalence of brucellosis was 5.94% in sheep and 6.19% in goats which indicates that goats are at higher risk of *Brucella* infection as compared to sheep. Unlike sheep, goat excretes the *Brucella* for a long period of time, which reduces the chance of spread of brucellosis among sheep flocks when compared to goat (Ashenafi *et al.,* 2007). Rahman *et al.* (2011) recorded 2.50% prevalence of brucellosis in goat and 1.25% prevalence of brucellosis in sheep in Bogra and Mymensingh districts. Uddin *et al.* (2007) reported 3.25% prevalence of brucellosis in sheep and 1.67% in goat at Mymensingh and Dhaka districts. A study conducted at Gaibandha district of Bangladesh reported 3.39% prevalence of brucellosis in sheep (Rahman *et al*., 2012). Islam *et al.* (2010) recorded 3.85% prevalence of brucellosis in black Bengal goat on the farms located at Savar and Rajshahi in Bangladesh. A study conducted in Saudi Arabia reported 11.6% prevalence of brucellosis in small ruminants (Radwan *et al*., 1983). In India, Prahlad *et al.* (1997) observed 50% prevalence of brucellosis in sheep and goat in Punjab and 32.73% in Rajasthan. Seroprevalence of brucellosis was 9.8% in goats at the public livestock farm in Pakistan (Arshad *et al.,* 2011). In Greece, Burriel *et al*. (2002) observed 16.8% prevalence of brucellosis in sheep.

This study recorded higher prevalence of brucellosis in sheep and goat in Mymensingh as compared to the prevalence results of Rahman *et al.* (2011) and Uddin *et al.* (2007) in the same area which could be due to differences in the sample size and the tests used (Ashenafi *et al.,* 2007). This variation of prevalence of brucellosis in sheep and goat might be associated with the difference of animal management and production systems between rural areas and farms. In rural area individual farmer rears few numbers of sheep and goats whereas in the farm large numbers of animals are raised together which might favour transmission of disease among farm animals.

Several epidemiological factors, such as: age, sex, breed, lactation number, herd size and living conditions influence the seroprevalence of brucellosis (Ghani *et al*., 1998). It is known that brucellosis is mainly a disease of sexually mature animals. Sexually mature and pregnant animals are more susceptible to *Brucella* infection than sexually immature animals (Quinn *et al*., 1999). On the contrary, younger animals are less susceptible to infection (Radostits *et al*., 2007). This may be due to the fact that sex hormones and erythritol, responsible for the growth and multiplication of *Brucella*, found in higher concentration in the sexually matured animals (Radostits *et al.,* 2007).

In this study a higher prevalence was found in adult sheep and goats. However, no statistically significant difference was observed between young and adults animal ($p > 0.05$). Ashenafi *et al.* (2007) recorded 5.3% prevalence of brucellosis in adult goats. Mudit *et al*. (2005) reported 1.63% prevalence of brucellosis in kids, 0.58% in young adults and 1.65% in adult goats, respectively.

In case of sheep, seroprevalence of brucellosis was increased with the increase of age of animals. However, Sergeant (1994) did not find any association between age and seroprevalence status of brucellosis in commercial ram flocks in New South Wales.

In the present study, the prevalence of brucellosis was higher in pregnant sheep (7.6%) and goat (9.09%) as compared to non-pregnant sheep (5.60%) and goat (6.52%). This result supports the findings of Islam *et al.* (2010). The present study reported higher prevalence of brucellosis in case of female sheep and goats than male but the difference was not statistically significant ($p > 0.05$). These results are in agreement with the findings of Ogundipe *et al.* (1994), Mirza *et al.* (1998), Mudit *et al.* (2005) and Rahman *et al.* (2011). Hirsh and Zee (1999) have stated that males are less susceptible to *Brucella* infection as compare to female animals, because of the absence of erythritol. However, in support of the current findings, Yibeltal (2005) did not find any observable difference in the prevalence of brucellosis between male and female sheep and goats.

Definite diagnosis of brucellosis can be accomplished only through the direct demonstration and identification of the causative agent by culture and isolation procedures (Orduna *et al.,* 2000). Accurate presumptive diagnosis can be achieved from serological techniques used in combination with epidemiological data. In this study, RBPT was used as a screening test for *Brucella* infection (MacMillan, 1990). The present study did not isolate *Brucella* spp. from any of the *Brucella* seropositive blood samples. Ganado and Bannister (1960) noticed suboptimal recovery rate of *Brucella* from blood samples. Seropositive animals sometimes yield negative culture results (Alton *et al.,* 1988).

Detection of *B. abortus* specific antibody response in sheep and goat in the study area indicate interspecies transmission. Mixing of sheep and goat with cattle at pasture lands, watering points and farms might be responsible for transmission of brucellosis among various animal species. Seropositivity of brucellosis in sheep and goat was considered to be due to natural infection because vaccination has not been practiced in Bangladesh.

Implementation of appropriate preventive strategies against brucellosis are important to minimize economic losses and safeguard public health (Ashenafi *et al*., 2007). Application of strict hygienic measures on the farm, proper disposal of aborted material, regular serological monitoring of brucellosis in animals on farms and use of vaccine are some of the important preventive measures against brucellosis in sheep and goat (Islam *et al.,* 2013). Handling of aborted materials using protective clothing and gloves, drinking of properly boiled milk and consumption of meat from brucellosis free sheep and goat could reduce the risk of transmission of brucellosis from sheep and goat to human (Corbel, 1997).

Conclusions

Data of this study suggest that brucellosis is endemic in sheep and goat populations in Mymensingh which underscore the need of implementation of control measures of brucellosis from these animal species.

Recommendation

The authors recommend more epidemiological investigation and characterization of *Brucella* infecting sheep and goat at species and biovar levels. Such investigations have important implications for undertaking effective control programmes of brucellosis in sheep and goat using appropriate vaccine and implementation of biosecurity measures.

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