

## Relation between brucellosis and husbandry practices in goats in Bangladesh

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### Abstract

A study on the relation between some husbandry practices and brucellosis in goats in Bangladesh was conducted at selected areas of Mymensingh and Dhaka district, Bangladesh, from March 2005 to May 2006. Sera from 300 goats were tested by Rose bengal test (RBT), plate agglutination test (PAT), tube agglutination test (TAT) and mercaptoethanol test (MET). Out of the 300 goats, 1.67% (n=5) were positive to RBT and PAT respectively, and 2.0% (n=6) were positive to TAT and 2.33% (n=7) were positive to MET. The prevalence of brucellosis was bigger in goats reared collectively (n=2, 4%) than reared individually (n=5, 2%), and bigger in goats housed with concrete floor (n=2, 4%) than that of bare floor (n=5, 2%). The rate of brucellosis was higher in goats keep separately (n=6, 2.61%) than that of kept with other animals (n=1, 1.43%) especially with cattle. Out of 290 goats from free grazing, 7 were positive but no positive reactor (n=10) was found in non grazing goats. In conclusion, however, seroprevalence of brucellosis had no statistically significant association with rearing type, housing type and grazing or not.

Key words : Brucellosis, Rearing system, Floor type, Keeping system and grazing type.

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## Introduction

There are about 33.55 million goats in Bangladesh<sup>1)</sup>. These goats can significantly play an important role in the economic well being of the resource-poor farmer. Moreover, the goats enterprise becomes more popular due to socioeconomic condition and their ability to survive on poor quality pastures and forage that is unsuitable for other species of ruminants. Besides, goats require relatively small investment and can therefore, be a source of cash income for small-scale farmers.

The goats in Bangladesh are mainly utilized for meat purposes, goat milk is used for human consumption, goats are also important for good quality leathers. The goat rank second in terms of meat, milk and skin production representing about 28.0, 23.0 and 28.0 % among the total contribution of livestock, respectively, in Bangladesh (FAO)<sup>2)</sup>. The disease is one of the hindrances for the development of goat industry and there is a lot of report for abortion but there is no report whether the abortion of goats was due to brucellosis. In spite of the presence of huge goats population, Bangladesh fails to optimally utilize this resource as the sectors suffering from lower productivity. Among many factors that limit the economic return from goats production diseases stand in the front line. One of such diseases that hamper the productivity of goats is brucellosis. Brucellosis is an important zoonosis threatening the public health in many countries of the world.

A lot of papers for goat brucellosis

have been reported from the different of the world<sup>3-8)</sup>, but only one report on brucellosis of goats was available in Bangladesh<sup>9)</sup>. As no precise reports were practical in Bangladesh, present study was undertaken to determine the relation between impact of some husbandry practices and brucellosis of goats.

## Materials and Methods

The study was conducted for a period of 15 months from March 2005 to May 2006 in the Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

### Experimental animals

Blood samples were collected from 300 goats of different areas of Bangladesh. The sexually matured female goat populations were randomly selected for this study. All of the study animals were indigenous breeds. No *Brucella* vaccine has been used in the study areas. The study recorded some husbandry information. All samples were processed for sera preparation. RBT and PAT were used as screening test, and the result was confirmed by TAT and MET.

### Blood and sera samples collection

About 3–5ml of blood was collected from jugular vein of each goat with the help of sterile disposable syringe and needle. Later on, the sera were poured into the separate test tube from each labeled syringe and the test tube was

marked with same number by permanent marker. Then the sera were centrifuged at 2,000 rpm for 10 min. After centrifugation a clear sera were found and then the sera were transferred to the sterilized labeled eppendorf tubes, which were wrapped with parafilm. The eppendorf tubes were stored in ice chamber at 20°C for future use<sup>8)</sup>.

### Serological tests

RBT, PAT, TAT and MET were used for the diagnosis of brucellosis. Both animals negative and positive by RBT and PAT were further confirmed by the TAT and MET.

**RBT :** The preparation of diagnostic antigen and procedure were conducted according to the procedure of Baek et al<sup>9)</sup>. The prepared antigen was standardized according to the procedure of OIE<sup>10)</sup>. Sera samples and the antigen were brought to room temperature. Then thirty micro liters of serum was mixed with the equal volume of antigen on a clear glass plate circled approximately 2 cm in diameter with manicure. The mixture was rocked gently for 4 min. at room temperature, and then observed. Any sign of agglutination was considered positive<sup>11)</sup>.

**PAT :** The preparation of diagnostic antigen and procedure were conducted according to the procedure of Ryu et al<sup>12)</sup>. The prepared antigen was standardized according to the procedure of OIE<sup>10)</sup>, Sera samples and the antigen were brought to room temperature. Anti-

gen solution 30 $\mu$ l was added to 40 $\mu$ l of each sample in a glass plate and then incubated for 8 min at room temperature. Then the plate was hand rotated three times, at 4 and 8 min after mixing and just before reading. Any sign of agglutination was considered positive<sup>13)</sup>.

**TAT :** The preparation of diagnostic antigen and procedure were conducted as described by Hur<sup>14)</sup>. The prepared antigen was standardized according to the procedure of OIE, 2000. Serum samples and the antigen were brought to room temperature. Thereafter, 40 $\mu$ l of serum samples were placed in different tubes and mixed with 2ml of diluted antigen. The results were read after incubation at 37°C for 48 hours. A positive reaction was one read when the serum antigen mixture was clear and gentle shaking did not disrupt the flocculi. The reaction was negative if the serum antigen mixture was not clear and gentle shaking revealed on flocculi.

**MET :** The MET was performed as described by Alton et al<sup>15)</sup>. Briefly, 0.1M 2-ME solution in normal saline was made (one liter of distilled water include sodium chloride, 8.5g 2-mercapto-ethanol, 7.14ml) freshly and stored at 4°C. Test sera with a volume of 40 $\mu$ l of each sample were placed in different test tubes and 1ml of 0.1M 2-ME in saline and 1ml of concentrated TAT antigen diluted 1:50 in normal saline solution were added to each tube. The tubes were then shaken and incubated as described in TAT. The procedure of

TAT was followed to interpret the titers obtained in the MET.

### Statistical analysis

The seroprevalence was determined by considering the total number of animals tested and positive reactors using the formula given by Thrusfield<sup>16)</sup>. The results were statistically analyzed for interpretation by using Chitests ( $\chi^2$ ). Probabilities were determined from relevant Tables. Significance determined at 5% and 1% level.

## Results

The overall prevalence of brucellosis in goat was 2.33% (n=300). Prevalence of brucellosis on the basis of rearing system of goats was presented in Table 1. Out of 300 goats, the prevalence was 2% in RBT and PAT respectively. The positive rate was 4% in TAT and also in MET among 50 collectively reared goats. While it was 1.6 in TAT and 2% in MET among 250 individually reared goats. In this study, there was no significant relation between rearing system and

Table 1. Brucella antibodies diagnosed by RBT, PAT, TAT and MET in goats associated with rearing system\*

Rearing type	No of sera	Number of sera positive (%) by				Level of significance
		RBT	PAT	TAT	MET	
Collective	50	1 (2.0)	1 (2.0)	2 (4.0)	2 (4.0)	NS**
Individual	250	4 (1.6)	4 (1.6)	4 (1.6)	5 (2.0)	NS

\*: RBT: Rose bengal test, PAT: plate agglutination test, TAT: tube agglutination test, MET: mercaptoethanol test. \*\*: NS= Not significant

Table 2. Brucella antibodies diagnosed by RBT, PAT, TAT and MET in goats associated with floor types of goats house

Floor type	No of sera	Number of sera positive (%) by				Level of significance
		RBT	PAT	TAT	MET	
Concrete	50	1 (2.0)	1 (2.0)	2 (4.0)	2 (4.0)	NS*
Uncovered	250	4 (1.6)	4 (1.6)	4 (1.6)	5 (2.0)	NS

\*NS= Not significant

the prevalence brucellosis.

In respect of floor types of goat house, the prevalence of brucellosis showed in Table 2. Among 50 goats housed in concrete floor, one (2%) and two (4%) were seropositive in RBT and in TAT, respectively. Out of 250 goats kept in earthen floor house, the prevalence

were 1.6% in RBT, and 2% in MET. In this study, floor type had no significant association with the prevalence of brucellosis.

On the basis of keeping system of the goats, the prevalence of brucellosis was presented in Table 3. Among 300 goats, 230 were kept in separate house and 70

goats were found to be housed with other species especially with domesticated cattle, even some shared the same house with human. In case of separately housed goats, the prevalence was 2.17%

in RBT and TAT, 1.74% in PAT and 2.61% in MET. On the other hand, in case of goats kept with other species, the prevalence was found 0.0% in RBT, and 1.43% in PAT, TAT and MET.

Table 3. Brucella antibodies diagnosed by RBT, PAT, TAT and MET in animals associated with keeping system of goats

Keeping type	No of sera	Number of sera positive (%) by				Level of significance
		RBT	PAT	TAT	MET	
Separately	230	5 (2.17)	4 (1.74)	5 (2.17)	6 (2.61)	NS*
With other species	70	0 (0.00)	1 (1.43)	1 (1.43)	1 (1.43)	NS

\* NS= Not significant

Table 4. Brucella antibodies diagnosed by RBT, PAT, TAT and MET in goats associated with types of grazing

Grazing criteria	No of sera	Number of sera positive (%) by				Level of significance
		RBT	PAT	TAT	MET	
Z*	10	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	NS**
F	290	5 (1.72)	5 (1.72)	6 (2.07)	7 (2.41)	NS

\* Z: No grazing or stall feeding, F: Free range grazing

\*\* NS= Not significant

Keeping system had also no significant association with the prevalence brucellosis. The prevalence of brucellosis on the basis of grazing system of the goats presented in Table 4. Among 300 goats only 10 goats were found to feed in stall or no grazing but no positive reactor was found in this group. On the other hand, 290 goats of free range feeding showed the prevalence of 1.72% in RBT and PAT, 2.07% in TAT, and 2.41% in MET. In this study, there existed no significant association among grazing types and the prevalence of brucellosis when the sera samples tested by RBT, TAT and MET.

## Discussion

Brucellosis is a zoonotic disease, and about half million of new cases have been reported in the world wide each year, but according to the WHO<sup>17)</sup>, however, these numbers greatly underestimate the true prevalence. The bacteria initially localize in the regional lymph node, and then were disseminated hematogenously to the organ of the reticuloendothelial system to multiply within phagocytic cells<sup>18)</sup>. The release of bacterial endotoxin from phagocytic cells, produce the constitutional symptoms and

sign of disease.

The diagnosis of brucellosis is confirmed by isolation of *Brucella* by bacteriological culture or by the detection of an immune response by serological test to its antigens<sup>19)</sup>. There are several drawbacks in the diagnosis of brucellosis exclusively based on *Brucella* isolation. For example, the slow growth of *Brucella* may delay diagnosis for more than 7 days<sup>20)</sup>. Also, the sensitivity is often low, ranging from 50 to 90% depending on disease stage, *Brucella* species, culture medium, quantity of bacteria and culture technique used<sup>21)</sup>.

The RBT can be used as a screening test for serological diagnosis of *Brucella* infection<sup>22)</sup> and more sensitive than the CFT when the animals were positive in bacterial culture. The TAT is recommended for collection of quantitative information on immune responses, and is most frequently used for confirmatory serological test. PAT is the routine test tool and is sometimes the only one used in many countries even though it may showed false-negative results<sup>23)</sup>. PAT was originally developed to provide a rapid test and it would approximate to the results of TAT. TAT was the first test used for diagnosis of brucellosis in human and was soon adapted for use in animals<sup>24)</sup>. In some countries *Brucella* positive serum samples are subjected to MET as confirmatory test<sup>25)</sup>. The MET depends on ability of 2-ME to split the disulfide bonds in proteins. In the absence of urea the chemical selectively inactivate IgM, leaving the IgG intact.

Bangladesh has been reported as an endemic area for brucellosis because a

considerable number of human and animal populations are exposed to the infection each year<sup>26,27)</sup>. The present investigation revealed that the overall seroprevalence of brucellosis is 2.33% in goats. Sharma et al<sup>28)</sup> reported prevalence of brucellosis in goat was 5.53%. Rahman et al<sup>7)</sup> reported 14.57% positive cases of brucellosis in caprine in different areas of Bangladesh. The difference of that might be due to the time passed, variation in methodology, sanitation and rearing system, keeping pattern, hygienic management, awareness of people, treatment of animals, improvement of veterinary services and reducing the number of goats. Lord et al<sup>29)</sup> reported 12.4% positive in goats but Bekele et al<sup>30)</sup> reported the overall prevalence rates were 1.38% in goats, and Ahmad<sup>31)</sup> found 1.85% positive goats. Rao et al<sup>32)</sup> recorded the prevalence of brucellosis was 7% in goats of Andhra Pradesh. Sandhu et al<sup>33)</sup> found 1.18% of goats were positive for brucellosis but Burriel et al<sup>34)</sup> found 13.1% of goats were positive to *Brucella* infection in Greece. Al-Majali<sup>35)</sup> investigated the seroprevalence of brucellosis in goats in Jordan and reported 27.7% goats had antibodies against *Brucella*.

In case of collective rearing the prevalence of brucellosis was higher than that of individual rearing but brucellosis has no significant association with collective rearing and individual rearing. In case of collective rearing there was more chance to be contacted between each other and there was more density of the collectively rearing animals than individual animals. Singh et al<sup>36)</sup> reported

that prevalence of brucellosis was 0.8% in the village flocks, 4.9% in the organized farms and 7.1% in the goats slaughtered at the abattoir. Darwish and Benkirane<sup>37)</sup> reported that in goats, seroprevalence fluctuated in the two sectors, but was higher in the private sector where husbandry is principally extensive. Omer et al<sup>4)</sup> reported that the highest individual seroprevalence was in dairy herds kept under the intensive husbandry system, with an individual prevalence of 8.2% and unit (herd) seroprevalence of 35.9%. Individual prevalence of 3.8% (goats) and unit prevalence of 33.3% (goats) were found. McDermott and Arimi<sup>38)</sup> reported that the prevalence is the highest in pastoral production systems and decreases as herd size and size of landholding decreases. Saini et al<sup>39)</sup> described that the provision of floor space, running space, lighting, ventilation and sanitation had significant relation with positive reactors.

In this study, the higher prevalence of brucellosis (2.61%) was found in goat that were kept separately but it has been reported that in southern Europe and Western Asia, where cattle are kept in close association with sheep or goats, infection in cattle can also be caused by *B melitensis*<sup>10)</sup>. On the other hand, *B ovis* can infect goats<sup>40)</sup> and *B abortus* biovar 1 had been isolated from sheep and goats<sup>41)</sup>.

In this study, the prevalence of brucellosis was higher in free ranging goats than no grazing or stall feeding goats. This may be due to the use of common pasture by the goats. Reviriego et al<sup>42)</sup> mention several risk factors at the

group level: contact with goats and grazing in communal pastures as risk factors, and frequency of disinfecting practices as a protective factor etc which has significant relation with the prevalence of brucellosis.

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