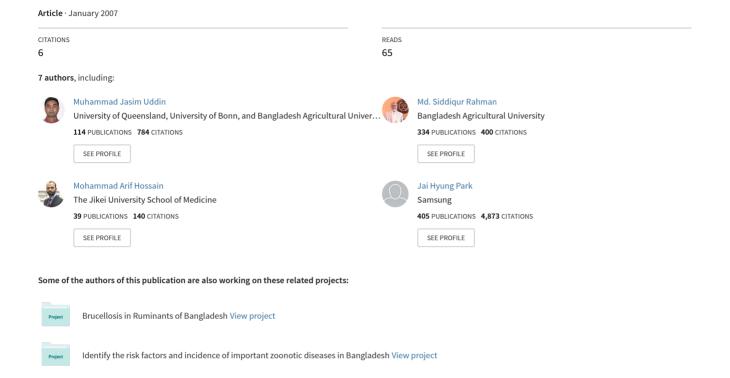
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# Relation between brucellosis and husbandry practices in goats in Bangladesh

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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 of the hindrances for 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 ME test (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  $30\mu\ell$  was added to  $40\mu\ell$  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,  $40\mu\ell$  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-mercatoethanol, 7.14ml) freshly and stored at  $4^{\circ}$ C. Test sera with a volume of  $40\mu\ell$  of each sam-ple 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\*

abbotiatea	With I car	mg by beem				
Rearing type	No of	Numb	er of sera	positive (	(%) by	Level of
	sera	RBT	PAT	TAT	MET	significance
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	Numb	Number of sera positive (%) by					
	sera	RBT	PAT	TAT	MET	significance		
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	Num	Level of			
	sera	RBT	PAT	TAT	MET	significance
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

<sup>\*</sup>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	Num	Level of significance			
Criteria	sera	RBT	PAT	TAT	МЕТ	
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

<sup>\*</sup> Z: No grazing or stall feeding, F: Free range grazing

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

<sup>\*\*:</sup> NS= Not significant

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 bacterical culyure. 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 bonds in proteins. In the disulfide 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 slaughthe abattoir. Darwish tered at 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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# References

- 1. Abdalla AED. 1966. Animal health and husbandry. Sudan Vet Sci 7:28.
- 2. Waghela S. 1976. Bulletin of animal health and production for Africa. 1:53.
- 3. Falade S, Hussein AH. 1979. Brucella seroactivity in Somali goats. *Trop Anim Health Prod* 11(4): 211-212.
- 4. Omer MK, Skjerve E, Holstad G, et al. 2000. Prevalence of antibodies to *Brucella* spp. in cattle, sheep, goats, horses and camels in the State of Eritrea: Influence of husbandry systems. *Epidemiol Infect* 125(2):447–453.
- 5. Shehu LM, Yusuf H, Kudi AC, et al. 1999. Seroprevalence of brucellosis in ruminants in Bauunchi and environs. *Nigerian Vet J* 20(1):67-74.
- 6. Singh SV, Gupta VK, Singh N. 2000. Comparative evaluation of a field-based dot-ELISA kit with three other serological tests for the detection of Brucella antibodies in goats. *Trop Anim Health Prod* 32(3):155-163.
- 7. Rahamn MM, Haque M, Rahman MA.

- 1988. Seroprevalence of caprine and human brucellosis in some selected areas of Bangladesh. Bangladesh Vet J 22(1-2):85-92.
- 8. Rahman MS. 2003. Experimental infection and protective immunity of Sprague-Dawley rats with *Brucella abortus*. Ph. D. thesis. College of Veterinary Medicine, Chonbuk National University, Republic of Korea.
- 9. Baek BK, Lim KH, Hur J, et al. 2000. Immunological responses in pigs immunized with inactivated *Brucella suis*. *Kor J Vet Publ Hlth* 24(2):133-142.
- 10. O.I.E. 2000. OIE manual of standards for diagnostic tests and vaccines, 4 eds, 12 rue de Prony, 75017 Paris, France.
- 11. Morgan WJ, MacKinnon DJ, Lawson JR, et al. 1969. The rose bengal plate agglutination test in the diagnosis of brucellosis. *Vet Rec* 85(23): 636-641.
- 12. Ryu YS, Park CK, Chang CH. 1997. Diagnostic manual for animal disease. Mong World, Seoul: 193-206.
- 13. Alton GG, Jones LM, Angus RD, et al. 1988. *Techniques for the Brucellosis Laboratory*. Institute National de Resear che Agronomique, Paris: 17-136.
- 14. Hur J. 2001. Studies on immunological responses in dogs inoculated with *Brucella* spp. Ph. D. thesis, College of Veterinary Medicine, Chonbuk National University, Republic of Korea.
- 15. Alton GG, Jones LM, Pietz DE. 1975. Laboratory techniques in brucellosis. 2 eds, World Health Organization, Geneva: 150-156.
- 16. Thrusfield M. 1995. *Veterinary epidemiology*. 2 eds, Blackwell Science Ltd,

- USA: 251-281.
- 17. WHO. 1986. Technical report series no. 740. Joint FAO/WHO Expert Committee on Brucellosis. 6th report.
- 18. Memish Z, Mah MW, Al Mahmoud S, et al. 2000. Brucella bacteremia: clinical and laboratory observations in 160 patients. *J Infect* 40(1): 59-63.
- 19. Orduna A, Almaraz A, Prado A, et al. 2000. Evaluation of an immunocapture agglutination test (*Brucella* capt) for serodiagnosis of human brucellosis. *J Clin Microbiol* 38(11): 4000-4005.
- 20. Yagupsky P. 1999. Detection of brucellae in blood culture. *J Clin Microbiol* 37:3437-3442.
- 21. Gotuzzo E, Carrillo C, Guerra J, et al. 1986. An evaluation of diagnostic methods for brucellosis. the value of bone marrow culture. *J Infect Dis* 153(1):122-125.
- 22. MacMillan, A. 1990. *Animal brucellosis*. (Eds) Nielsen K and Duncan JR. Boca Ratan, CRC Press, Florida, USA: 153.
- 23. Lucero NE, Bolpe JE. 1998. Buffered plate antigen test as a screening test for diagnosis of human brucellosis. *J Clin Microbiol* 36:1425-1427.
- 24. George WB. 1994. Hand book of Zoonoses. 2 eds, Section. A: Bacterial, rickettsial, chlamydial, and mycotic, CRC Press, Inc. 2000 Corporate Blvd, Boca Raton, Florida, USA, 33431.
- 25. Samartino L, Gall D, Gregoret R, et al. 1999. Validation of enzyme linked immunosorbent assays for the diagnosis of bovine brucellosis. *Vet Microbiol* 70(3-4):193-200.
- 26. Rahman MM, Chowdhury TIMF, Rah-

- man A, et al. 1993. Seroprevalence of human and animal brucellosis in Bangladesh. *Indian Vet J* 60:165-168.
- 27. Rahman MM, Chowdhury TIAF, Chowdhury MUA. 1978. Investigation of brucellosis among cattle. *Bangladesh Vet J* 12 (14): 12–15.
- 28. Sharma VD, Sethi MS, Yadav MP, et al. 1979. Sero-epidemiologic investigations on brucellosis in the states of Uttar Pradesh (U.P.) and Delhi (India). *Int J Zoonoses* 6(2):75-81.
- 29. Lord VR, Nieto S, Sandoval E, et al. 1987. Brucellosis in goats: serological and bacteriological studies in Venezuela. *Vet Trop* 12:27-37.
- 30. Bekele T, Kasali OB, Tekelye B. 1990. Brucellosis in sheep and goats in central Ethiopia. *Bull Anim Health Product Africa* 38(1): 23-25.
- 31. Ahmad R. 1997. Incidence of brucellosis in goats. *Pakistan Vet J* 11(2): 94.
- 32. Rao PB, Madhubala K, Rao MR. 1998. Prevalence of viral and bacterial diseases among goats in Andhra Pradesh. *Indian Vet J* 75(10): 924-925.
- 33. Sandhu KS, Filia G, Sharma DR, et al. 2001. Prevalence of brucellosis among dairy animals of Punjab. *Indian J Comp Microbiol Immunol Infect Dis* 22(2):160-161.
- 34. Burriel AR, Vougiouka OM, Butsini S, et al. 2002. A serologic investigation of some causes of reproductive failure among small ruminants in Greece.

- *Online J Vet Res* 6:57-63.
- 35. Al-Majali AM. 2005. Seroepidemiology of caprine brucellosis in Jordan. *Small Ruminant Res* 58(1):13-18.
- 36. Singh SV, Singh N, Goupta VK, et al. 1998. Seroprevalence of brucellosis in a few important Indian goat breeds. *Small Ruminant Res* 30(2):93-98.
- 37. Darwish M, Benkirane A. 2001. Field investigations of brucellosis in cattle and small ruminants in Syria, 1990–1996. *Rev Sci Tech* 20(3): 769–775.
- 38. McDermott JJ, Arimi SM. 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Vet Microbiol* 90(1-4):111-134.
- 39. Saini SS, Sharma JK, Kwatra MS. 1992. Assessment of some management factors responsible for prevalence of brucellosis among traditionally managed animal population of Punjab. *Indian J Anim Sci* 62(91): 832-834.
- 40. Meador VP, Hagemoser WA, Deyoe BL. 1988. Histopathologic findings in *Brucella abortus*—infected, pregnant goats. *Am J Vet Res* 49(2): 274-280.
- 41. Ocholi RA, Kwaga JK, Ajogi I, et al. 2004. Phenotypic characterization of Brucella strains isolated from livestock in Nigeria. *Vet Microbiol* 104 (3-4): 229-230.
- 42. Reviriego FJ, Moreno MA, Dominguez L. 2000. Risk factors for brucellosis seroprevalence of sheep and goat flocks in Spain. *Prev Vet Med* 44 (3-4):167-173.