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Prevalence of *Brucella* antibodies in sera of cows in Bangladesh

Kazi M. R. Amin[1], M. Bahanur Rahman[1], M. Siddiqur Rahman[2], Jae-cheol Han[2,3], Jin-ho Park[2], Joon-seok Chae[2,*]

[1]*Department of Microbiology & Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*
[2]*Bio-Safety Research Institute and College of Veterinary Medicine, Chonbuk National University, Jeonju 561-756, Korea*
[3]*Livestock Development and Research Institute, Iksan Branch, Iksan 570-390, Korea*

The study was carried out to investigate the prevalence of *Brucella* antibodies in sera of 120 cows in Bangladesh Agricultural University Dairy Farm and adjacent villages, Bangladesh. The epidemiological history and blood was collected from the cows. The serum samples were subjected to Rose Bengal Test (RBT) and plate agglutination test (PAT) for initial screening of *Brucella* antibodies and the positive sera samples were then subjected to tube agglutination test (TAT) for further confirmation. The higher rate of *Brucella* antibody was recorded in rural farm (5.0%) than organized farm (2.5%) and in pregnant cows (5.9%) than non-pregnant cows (4.7%). A total of 3 (4%) *Brucella* positive antibody cases were recorded in cows of above four years of age whereas, 1 (2.3%) positive case was found in cows of less than 4 years of age. The study revealed that number of Red Shindi was the highest and the prevalence of brucellosis in Bangladesh cow population is not negligible and it is worthwhile to consider adoption of preventive measures.

Key words: Bangladesh, brucellosis, plate agglutination test, Rose Bengal test, tube agglutination test

Introduction

Brucellosis is a zoonotic disease caused by gram-negative bacteria *Brucella* that are pathogenic for a wide variety of animals and human beings [19]. It is an emerging disease since the discovery of *B. melitensis* as the cause of Malta Fever by Bruce in 1887 and the isolation of *B. abortus* from aborted cattle by Bang in 1897 [24]. The importance of brucellosis is not known precisely, but it can have a considerable impact on human and animal health, as well as socioeconomic impacts, especially in which rural income relies largely on livestock breeding and dairy products [31]. Human brucellosis is caused by exposure to livestock and livestock products. Infection can result from direct contact with infected animals and can be transmitted to consumers through raw milk and milk products. In humans, the symptoms of disease are weakness, joint and muscle pain, headache, undulant fever, hepatomegaly, splenomegaly, and night sweats [17]. Recently, it has been reported that brucellosis can affect the central and peripheral nervous system of human [2]. In animals, brucellosis mainly affects reproduction and fertility, reduces survival of newborns, and reduce milk yield. Mortality of adult animals is insignificant [34]. In Bangladesh, about 80% of total population live in villages and the rural income relies largely on livestock breeding and dairy products and the people has every day close contact with the livestock. The economic importance and the prevalence of brucellosis in man and animals have reported from some parts of Bangladesh [1,13,27,28,29]. The present investigation was carried out to investigate the prevalence of *Brucella* antibodies through Rose Bengal test (RBT), plate agglutination test (PAT) and tube agglutination test (TAT) in sera of cows in Bangladesh Agricultural University (BAU) Dairy Farm and adjacent villages, Bangladesh.

Materials and Methods

Experimental design

Cows housed in the dairy farm of BAU and the villages adjacent to BAU were included in this study. A total of 120 cows of different age groups, either pregnant or non-pregnant were examined during the period from March 2003 to February 2004.

Epidemiological study

The epidemiological study regarding the age, status of pregnancy and the type of breeds were investigated from the records available in the dairy farms.

*Corresponding author
Tel: +82-63-270-3881; Fax: +82-63-278-3884
E-mail: jschae@chonbuk.ac.kr

Collection of blood and preparation of sera

About 5-7 ml of blood was collected from the jugular vein of cows using a sterile disposable syringe and needle. Then the sera was prepared by centrifugation as per standard procedure and stored in vials at –20°C until used.

Serological tests

The serum samples were subjected to RBT and PAT for initial screening of *Brucella* antibodies and the positive sera samples were then subjected to TAT for further confirmation. For TAT, PAT, RBT, the *B. abortus* strain 1119-3 was used as antigen and the antigen was purchased from Dae Sung Microbiological Labs. Co., Ltd (Uiwang, Korea).

For PAT, the procedure of Ryu *et al.* [32] was followed. Briefly, 0.03 ml of antigen solution was added to 0.08, 0.04, 0.02, 0.01, 0.005, 0.00125 ml of each sample serum on a glass plate and then incubated for 8 min at room temperature. 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 [4].

For RBT, the procedure of Baek *et al.* [5] was followed. Briefly 30 µl of serum was mixed with equal volume of antigen on a white enamel 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 [22].

For TAT, the procedure of Hur *et al.* [12] was followed. Briefly, quantities of 0.08, 0.04, 0.02, 0.01, 0.005, 0.00125 ml of serum samples were placed in different tubes and mixed with 2 ml of diluted antigen. The results were read after incubation at 37°C for 48 hours. A positive reaction was one in which the serum-antigen mixture was clear and gentle shaking did not disrupt the flocculi. A negative reaction was one in which the serum-antigen mixture was not clear and gentle shaking revealed no flocculi.

Statistical analysis

The results of the tests were statistically analyzed for interpretation by using Chi-square (x^2) tests. Probabilities associated with the observed values of Chi-square were determined from relevant. Significance was determined at 5% level.

Results

Four different cross breeds cows were recorded in epidemiological study namely Jersey cross, Holstein cross, Sahiwal cross, and Red Shindi cross (Table 1). The number of Red Shindi cross breed cows was the highest.

The prevalence of *Brucella* antibodies in sera of cows in Bangladesh Agricultural University Dairy Farm and adjacent villages has shown in Table 2. Eighty cows were examined from organized farm and forty cows were examined from rural areas. The prevalence of *Brucella* antibodies were recorded 2.5%, and 2.5% by RBT and PAT, and TAT, respectively in farm cows and the prevalence of *Brucella* antibodies were recorded 7.5%, and 5.0% by RBT and PAT, and TAT, respectively in rural cows. Two positive confirmed cases were observed both in farm and rural areas. The higher rate of *Brucella* antibody was recorded in rural farm than organized farm. The difference between these two areas was not statistically significant.

In this study eighty-six cows were non pregnant and thirty-four cows were pregnant. The prevalence of *Brucella* antibodies was found to be 5.8% and 4.7% by RBT and PAT, and TAT, respectively in non-pregnant cows and 5.9%, and 5.9% by RBT and PAT, and TAT, respectively in pregnant cows. The prevalence of *Brucella* antibodies was higher in pregnant cows (5.9%) than non-pregnant cows (4.7%) but it was not statistically significant. Four positive confirmed cases were found in non-pregnant cows and 2 positive confirmed cases were found in pregnant cows.

Table 1. Breeds of cows examined for *Brucella* antibodies in sera of Bangladesh Agricultural University Dairy Farm and adjacent villages

Breeds	No. of cows
Jersey cross*	25
Holstein cross	27
Sahiwal cross	31
Red Shindi cross	37
Total	120

*Cross bred cows are the progenies of local cows inseminated with exotic semen.

Table 2. *Brucella* antibodies diagnosed by Rose Bengal test (RBT), plate agglutination test (PAT) and tube agglutination test (TAT) in sera of cows in Bangladesh Agricultural University Dairy Farm and adjacent villages

Group of cows	No. of cows	Positive reactors (%) by RBT and PAT	Positive reactors (%) by TAT
Farm cows	80	2 (2.5%)	2 (2.5%)
Rural cows	40	3 (7.5%)	2 (5.0%)
Non-pregnant cows	86	5 (5.8%)	4 (4.7%)
Pregnant cows	34	2 (5.9%)	2 (5.9%)
2.5-4 year old cows	45	1 (2.2%)	1 (2.2%)
>4 year old cows	75	4 (5.3%)	3 (4.0%)

Forty-five cows were within the age of 2.5-4 years and seventy-five cows were more than four years old. The prevalence of *Brucella* antibodies were 2.2%, and 2.3% by RBT and PAT, and TAT, respectively in cows having 2.5-4 years age and 5.3%, and 4.0% by RBT and PAT, and TAT, respectively in cows having more than 4 years of age. The maximum prevalence rate of brucellosis was recorded in cows having more than 4 years of age (5.3%) than the cows having less than 4 years (2.3%) of age. The difference was not statistically significant. Only one case was found as positive confirmed at the age of 2.5-4 years and 3 positive confirmed cases were found at more than 4 years of age.

Discussion

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 [25]. The diagnosis of brucellosis based exclusively on *Brucella* isolation presents several drawbacks. The slow growth of *Brucella* may delay diagnosis for more than 7 days and 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 employed [10]. Hence, the serological tests are important for diagnosis of brucellosis. The main serological test used for diagnosis of *Brucella* infection is the RBT as a screening test and sometimes RBT is more sensitive than the complement fixation test [4]. The TAT has become the standard method, is the test recommended for collection of quantitative information on immune responses, and is the most frequently used confirmatory serological test [16]. The PAT was originally developed to provide a rapid test and it would approximate the results of the TAT. In many countries, the PAT, which may give false-negative results, is the routine test and is sometimes the only one used [16]. TAT was the first test used for the diagnosis of brucellosis in people and was soon adapted for use in animals [21]. In this study, we used PAT and RBT as screening test and TAT was used as confirmatory test.

In this study, the higher rate of *Brucella* antibody was recorded in rural farm (5%) than organized farm (2.5%). This result is more or less similar to the findings of Ahmed *et al.* [1], who detected 5.0% *Brucella* positive reactors in indigenous zebu cattle at Bangladesh Agricultural University Dairy Farm and 2.76% positive reactors among rural cows. Mehra *et al.* [20] reported 6.3% positive cases of brucellosis in Madhya Pradesh, India in organized farm. Gray and Martin [11] also recorded considerably higher prevalence of *Brucella* infection (29.5%) in organized herds and lower prevalence of brucellosis (3.9%) in rural dairy cows. Similar results were also obtained by Mathur [19], Sarker *et al.* [33], Rahman *et al.* [27], Rahman and Rahman [28]. However, the transmission of brucellosis in organized farm may be due to the introduction of infected animals into a susceptible herd and may be spread by the dairy attendants infected with this diseases and vice-versa [14,29].

The higher rate of *Brucella* antibody was recorded in pregnant cows (5.9%) than non-pregnant cows (4.7%). Similar results were also reported by Ahmed *et al.* [1] and they found 3.23% in pregnant indigenous zebu cows and 3.13% in non-pregnant indigenous zebu cows. However, Lavsen *et al.* [15] found the higher prevalence rate of brucellosis among pregnant cows than the non-pregnant cows. This findings correlate with the observation of Plommet [26]. The high rate of infection in pregnant cows might be due to the infected reproductive tract of cows, which could act as a potential reservoir for the organism to propagate and later become active to infection exhibiting clinical symptoms of diseases.

The prevalence and severity of disease may vary with the breed, geographic location, type of diagnostic test, husbandry and environmental factor. The earlier reports of brucellosis from Bangladesh was mostly from the cows of Dhaka and Tangail district using milk ring test, PAT and RBT [27,28]. The economic loss caused by brucellosis and the sero-prevalence of brucellosis in human and in indigenous zebu cattle were studied [1,13,29]. The present study was carried out in Bangladesh Agricultural University Dairy Farm and adjacent rural areas using PAT and RBT as screening test and TAT was used as confirmatory test where the cows were mostly cross bred. In Bangladesh, cows were maintained in tying-stalls and the poor health management may be responsible for higher prevalence of brucellosis in rural area in this study.

A total of 3 (4.0%) *Brucella* positive antibody cases were recorded in cows of above four years of age whereas, 1 (2.3%) positive cases were found in cows of less than 4 years of age. This findings correlate with the observation of Chantal and Thomas [7], who found the high prevalence rate (8.7%) of brucellosis in cattle of 5-10 years old. Similar reports were also recorded by other investigators [6,8,9,23]. So, it may be considered that the high prevalence rate of brucellosis among older cows might be related to maturity with the advance age and therefore the organism found there way to propagate to remain either as latent infection or it may cause clinical manifestation of disease [30]. However, the older animals supposed to be infected, because of more contact with infectious agents and sometimes from malnutrition during pregnancy. The fact that that number of Red Shindi was the highest and the prevalence of brucellosis in Bangladesh cow population is not negligible and it is worthwhile to consider adoption of preventive measures.

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