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Full Length Research Paper

Brucellosis among ruminants in some districts of Bangladesh using four conventional serological assays

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Brucellosis causes a great economic loss to the livestock industries through abortion, infertility, birth of weak and dead offspring, increased calving interval and reduction of milk yield and it is endemic in Bangladesh. The present study was performed to know the seroprevalence of brucellosis for 1000 ruminants (135 Buffaloes, 465 cattle, 230 goats and 170 sheep) in five different districts of Bangladesh by four conventional serological tests such as: Rose Bengal Plate Test (RBT), tube agglutination test (TAT), competitive enzyme-linked immunosorbent assay (C-ELISA), and Fluorescent polarization assay (FPA). Sheep has the highest prevalence (8.24%) of brucellosis. The seroprevalence of brucellosis was significantly higher in animals with previous abortion record in case of buffaloes, cattle, goats and sheep than that with no abortion record. C-ELISA can be the most suitable choice for extensive use in many kinds of livestocks and accurate estimation of *Brucella* antibodies in ruminants in Bangladesh.

Key words: Brucellosis, ruminants, seroprevalence, competitive enzyme-linked immunosorbent assay (C-ELISA), Bangladesh.

INTRODUCTION

Brucellosis is a major zoonosis caused by the small, non-motile Gram-negative and intracellular coccobacilli belonging to the genus *Brucella*. It causes a great economic loss to the livestock industries through abortion, infertility, birth of weak and dead offspring, increased calving interval and reduction of milk yield (Roth et al., 2003). Brucellosis is mainly a disease of sexually matured animals and commonly transmitted to other animals by direct or indirect contact with infected animals or discharges such as: aborted fetuses, placental membranes or fluids. Infection to human results from direct contact with infected animals and consumption of contaminated milk and milk products (Diaz-Aparicio et al., 1994).

Brucellosis is endemic in Bangladesh (Amin et al., 2005; Rahman et al., 2006; Uddin and Rahman, 2007; Nahar and Ahmed, 2009; Rahman et al., 2009, 2010, 2011; Ahasan et al., 2010). In order to control and eradicate brucellosis from humans and livestock animals, it is very important to establish an appropriate serological method for diagnosis of brucellosis in the endemic areas.

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Table 1. Total number of tested animals by districts and the number of positive reactors based on RBT.

Districts	Total number of tested animals (positive reactors based on RBT)				
	Buffalo	Cattle	Goat	Sheep	Total
Bagherhatt	70 (2)	90 (1)	15 (1)	27 (3)	202 (7)
Bogra	20 (0)	60 (0)	30 (0)	30 (1)	140 (1)
Gaibandha	14 (0)	70 (0)	50 (2)	35 (2)	169 (4)
Mymensingh	12 (1)	135 (2)	100 (4)	40 (8)	287 (15)
Sirajgonj	19(1)	110(1)	35(1)	38(2)	202(5)

Although, isolation and identification of the causal agent is considered as gold standard but *Brucella* culture takes several days to weeks to grow. Diagnosis of brucellosis by serological study largely depends on the use of two or more tests. Single test is not recommended since this could not detect all positive reactors (Mahajan and Kulshreshtha, 1991; Radulescu et al., 2007). Agglutination tests such as: Rose Bengal Plate Test (RBT), tube agglutination test (TAT), slow agglutination test (SAT) and enzyme-linked immunosorbent assay (ELISA), are commonly used for detection of *Brucella* specific antibody response in livestock animals (Ferreira et al., 2003; Junaidu et al., 2008).

The classical serological like RBT, TAT and SAT are known to produce cross reactions with other Gram-negative bacteria having antigenic similarities with *Brucella* (Kittelberger et al., 1998) and therefore produce a lot of false positive reactions. Every year, a lot of undiagnosed cases of abortion, stillbirth and retained placenta are reported in sheep, goats, cattle and buffaloes of Bangladesh which might be caused by *Brucella*. Prevalence of brucellosis in small and large ruminants might constitute a significant hurdle for the development of livestock in Bangladesh. Early and accurate diagnosis is important for undertaking an effective control measure against brucellosis.

Fluorescent polarization assay (FPA) and competitive enzyme-linked immunosorbent assay (C-ELISA) is known to be more effective in detecting brucellosis as compared to traditional tests such as: RBT, SAT and Complement fixation test (CFT) (Nielsen et al., 2004, 2005, 2006; Abd El-Razik et al., 2007). FPA and C-ELISA could successfully detect antibodies against cross reacting *Yersinia enterocolitica* serotype O:9 and *B. abortus* with high sensitivity and accuracy (Nielsen et al., 2004, 2005, 2006). In order to perform accurate diagnosis of brucellosis in livestock in Bangladesh, it is important to find out the suitable tests. Therefore, four conventional serological tests of brucellosis were performed and compared with sera of ruminants in five different districts of Bangladesh.

MATERIALS AND METHODS

As serological sample, venous blood samples were randomly and aseptically obtained from sexually matured cattle, buffaloes, goats and sheep of both sexes. A total of 1000 blood samples were collected, 135 from buffaloes, 465 from cattle, 230 from goats and 170 from sheep of Bagherhatt, Bogra, Gaibandha, Mymensingh and sirajgonj districts of Bangladesh (Table 1). The study also recorded required clinical, epidemiological and reproductive information. During sampling, a questionnaire based data on age, sex, area, pregnancy status, disease history, reproductive problems such as abnormal uterine discharge, abortion and reproductive diseases were recorded.

The RBT was used as a screening test in order to identify the infected animal with brucellosis using *Brucella abortus* strain 1119-3 (Dae Sung Microbiological lab, South Korea) and the results were confirmed by TAT (QIA, South Korea), C-ELISA (Svanova Biotech AB, Uppsala, Sweden) and FPA (Diachemix LLC, Milwaukee, USA). RBT and TAT were performed according to the procedure described by OIE (2008). C-ELISA and FPA were performed according the protocol provided by the C-ELISA and FPA kits manufacturer company.

The questionnaire-based data was processed by Microsoft Excel and MSTATC and the results were statistically analyzed for interpretation by using Chi-square tests (χ^2). Probabilities associated with the observed values of chi-square were determined from relevant tables. Significance was determined at 1 and 5% level where applicable.

RESULTS

Overall seroprevalence

Serum samples were collected from 1000 ruminants (135 buffaloes, 465 cattle, 230 goats and 170 sheep) of five different districts, that is, Mymensingh, Gaibandha, Bogra, Bagherhatt, and Sirajgonj (Table 1). Overall seroprevalence for ruminants by four serological assays has been shown in the Table 2. The number of positive reactors by RBT was 4 out of 135 (2.96%) in buffaloes, 4 out of 465 (0.86%) in cattle, 8 out of 230 (3.48%) in goats

Table 2. Overall seroprevalence of brucellosis in buffaloes, cattle, goats and sheep based on RBT, TAT, C-ELISA and FPA.

Species	Total number of tested sera	Total number of positive reactors or suspected (%)			
		RBT	TAT	C-ELISA	FPA
Buffaloes	135	4 (2.96)	2 (1.48)	2 (1.48)	2 (1.48)
Cattle	465	4 (0.86)	0 (0.0)	1 (0.22)	0 (0.0)
Goats	230	8 (3.48)	6 (2.61)	5 (2.17)	5 (2.17)
Sheep	170	16 (9.41)	14 (8.24)	15 (8.82)	12 (7.06)

Table 3. Age related seroprevalence of brucellosis based on RBT, TAT, C-ELISA and FPA in buffaloes, cattle, goats and sheep.

Species	Age of animals (months; Ms)	Number of tested sera	Number of positive reactors or suspected (%)			
			RBT	TAT	C-ELISA	FPA
Buffaloes	12-24 Ms	8	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	25-48 Ms	48	2 (4.17)	1 (2.08)	1 (2.08)	1 (2.08)
	> 48 Ms	79	2 (2.53)	1 (1.27)	1 (1.27)	1 (1.27)
Cattle	12-24 Ms	8	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	25-48 Ms	173	2 (1.16)	0 (0.0)	0 (0.0)	0 (0.0)
	> 48 Ms	284	2 (0.70)	0 (0.0)	1 (0.35)	0 (0.0)
Goats	< 24 Ms	197	6 (3.05)	3 (1.52)	3 (1.52)	3 (1.52)
	> 24 Ms	33	2 (6.06)	3 (9.09)	2 (6.06)	2 (6.06)
Sheep	< 24 Ms	130	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	> 24 Ms*	40	16 (40.0)	14 (35.0)	15 (37.5)	12 (30.0)

* = Significant at 1% level of probability (*P<0.01*).

and 16 out of 170 (9.41%) in sheep. The number of positive reactors or suspects by TAT was 2 out of 135 (1.48%) in buffaloes, 0 out of 465 (0.00%) in cattle, 6 out of 230 (2.61%) in goats, and 14 out of 170 (8.24%) in sheep. The number of positive reactors or suspects by C-ELISA was 2 out of 135 (1.48%) in buffaloes, 1 out of 465 (0.22%) in cattle, 5 out of 230 (2.17%) in goats and 15 out of 170 (8.82%) in sheep. The number of positive reactors or suspects by FPA was 2 out of 135 (1.48%) in buffaloes, 0 out of 465 (0.00%) in cattle, 5 out of 230 (2.17%) in case of goats and 12 out of 170 (7.06%) in sheep.

Age related seroprevalence

Table 3 shows age versus seroprevalence of brucellosis. By TAT in case of buffaloes, 8 (comprising 12-24 month) showed no positive reaction but in 25-48 month age group, the seroprevalence was 2.08% (1 out of 48 samples) and in >48 months age group was 1.27% (1 out of 79). In case of cattle, no samples of any age group were found positive by TAT. The seroprevalence of brucellosis in goats of less than 24 month of age was 1.52% (3 out of 197). But in goats over 24 month of age, the prevalence of brucellosis was 9.09% (3 out of 33). In case of sheep of over 24 month, the prevalence of brucellosis was 35.0% (14 out of 33).

By C-ELISA in case of buffaloes, 8 buffaloes comprising of 12-24 month age group showed no positive reaction, but in 25-48 month age group, the seroprevalence was 2.08% (1 out of 48 samples) and was 1.27% in >48 months age group (1 out of 79). In case of cattle, no samples of 12-24 months and 25-48 months age group were found positive but in over 48 month age group, the seroprevalence was 0.35% (1 out of 284). The seroprevalence of brucellosis in goat of less than 24 month of age was 1.52% (3 out of 197) and in

Table 4. Sex related seroprevalence of brucellosis based on RBT, TAT, C- ELISA and FPA in buffaloes, cattle, goats and sheep.

Species	Sex of animals	Number of tested sera	Number of positive reactors or suspected (%)			
			RBT	TAT	C-ELISA	FPA
Buffaloes	Male	28	2 (7.14)	1 (3.57)	1 (3.57)	1 (3.57)
	Female	107	2 (1.87)	1 (0.93)	1 (0.93)	1 (0.93)
Cattle	Male	75	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Female	390	4 (1.03)	0 (0.0)	1 (0.26)	0 (0.0)
Goats	Male	38	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Female*	192	8 (4.17)	6 (3.13)	5 (2.60)	5 (2.60)
Sheep	Male	25	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Female*	145	16 (11.03)	14 (9.66)	15 (10.34)	12 (8.28)

* = Significant at 1% level of probability (*P<0.01*).

goats aged over 24 month, the prevalence of brucellosis was 6.06% (2 out of 33). In case of sheep over 24 month, the prevalence of brucellosis was 37.5 % (15 out of 33).

By FPA in case of buffaloes, 8 buffaloes comprising 12-24 month age group showed no positive reaction but in 25-48 month age group, the seroprevalence was 2.08% (1 out of 48 samples) and in >48 months age group was 1.27% (1 out of 79). The seroprevalence of brucellosis in goats of less than 24 month of age was 1.52% (3 out of 197). But in goats over 24 month of age, the prevalence of brucellosis was 6.06% (2 out of 33). In case of sheep over 24 month, the prevalence of brucellosis was 30.0 % (12 out of 40).

Sex related seroprevalence

Sex related seroprevalence of brucellosis is shown in Table 4. Relatively higher prevalence was found in female than in male cattle, goats, and sheep, whereas higher prevalence of brucellosis was found in male than in female in case of buffaloes.

In case of buffaloes, 28 males and 107 females were tested and the prevalence of brucellosis was 3.57% (1 out of 28) in case of males and 0.93% (1 out of 107) in case of females by TAT, C-ELISA and FPA, respectively. In case of females cattle its prevalence was 0.26% (1 out of 390) by C-ELISA, respectively. In goats, males sera were found positive reactor. In case of female goats, average seroprevalence were 3.13% (6 out of 192) by TAT, 2.60% (5 out of 192) by C-ELISA and FPA. In case of sheep, females only showed an average prevalence, 9.66% (14 out of 145) by TAT, 10.34% (15 out of 145) and 8.28% (12 out of 145) by C-ELISA, respectively.

Distribution of brucellosis related to abortion history

Distribution of brucellosis regarding to abortion history in animals is shown in Table 5. Among 834 female animals, 9 buffaloes, 21 cattle, 8 goats and 7 sheep have previous abortion record. The seroprevalence of brucellosis in aborted animal was 22.2% by TAT, C-ELISA and FPA in buffaloes, 4.76% by C-ELISA and 0.00% by TAT and FPA in cattle, 50.0% by TAT, C-ELISA and FPA in goats and 42.86% by TAT, 57.14% by C-ELISA and 71.43% by FPA in sheep. Among 834 female animals, 98 buffaloes, 369 cattle, 184 goats and 138 sheep have no previous abortion record. The seroprevalence of brucellosis in these animals was 0.00% by TAT, C-ELISA and FPA in buffaloes, 0.00% by C-ELISA, TAT and FPA in cattle, 1.09% by TAT, 0.54% by C-ELISA and FPA in goats and 7.97% by TAT and C-ELISA and 5.07% by FPA test in sheep.

DISCUSSION

Seroprevalence for *Brucella* exposure is essential for its control and many countries have eradication program to control brucellosis. Economic losses can be heavy due to abortion and infertility and subsequent culling, so that the herd should be monitored for the presence of infection. Despite eradication programs, vaccination, testing and

Table 5. Prevalence of brucellosis in previous aborted animal based on RBT, TAT, C- ELISA and FPA in buffaloes, cattle, goats and sheep.

Species	Previous abortion record (Yes/No)	Number of tested sera	Number of positive reactors and suspected (%)			
			RBT	TAT	C-ELISA	FPA
Buffaloes	Yes*	9	2 (22.2)	2 (22.2)	2 (22.2)	2 (22.2)
	No	98	2 (2.04)	0 (0.0)	0 (0.0)	0 (0.0)
Cattle	Yes*	21	3 (14.29)	0 (0.0)	1 (4.76)	0 (0.0)
	No	369	1 (0.27)	0 (0.0)	0 (0.0)	0 (0.0)
Goats	Yes*	8	5 (62.5)	4 (50.0)	4 (50.0)	4 (50.0)
	No	184	3 (1.63)	2 (1.09)	1 (0.54)	1 (0.54)
Sheep	Yes*	7	4 (57.14)	3 (42.86)	4 (57.14)	5 (71.43)
	No	138	12 (8.7)	11 (7.97)	11 (7.97)	7 (5.07)

* = Significant at 1% level of probability (*P<0.01*).

slaughter out, brucellosis remains a major zoonosis worldwide (WHO, 1986; Kakoma et al., 2003; Baek et al., 2003) and the disease has remained prevalent in many areas in the world. Each year half of a million cases of brucellosis are reported worldwide but according to WHO, these numbers are greatly under estimated. In recent years, many countries have eradicated brucellosis from their herd, and many other countries have significantly reduced the prevalence of the infection among their livestock populations. Even so, brucellosis is distributed throughout the world wherever livestock are being raised. Likewise, in many less developed countries and in developing countries brucellosis continues to cause major losses in livestock and poses a serious threat to people (Crawford et al., 1990). The distribution of the disease is geographically limited, but it nevertheless remains a major problem in parts of Africa and Latin America, Western and southern Asia including Bangladesh.

The objectives of this study were to make a sero-prevalence of brucellosis for ruminants in five different districts of Bangladesh by four conventional serological tests. The number of positive reactors by RBT were 4 out of 135 (2.96%) in buffaloes, 4 out of 465 (0.86%) in cattle, 8 out of 230 (3.48%) in case of goats, 16 out of 170 (9.41%) in sheep. The number of positive reactors or suspects by TAT were 2 out of 135 (1.48%) in buffaloes, 0 out of 465 (0.00%) in cattle, 6 out of 230 (2.61%) in case of goats, 14 out of 170 (8.24%) in sheep. The numbers of positive reactors or suspected by C-ELISA were 2 out of 135 (1.48%) in buffaloes, 1 out of 465 (0.22%) in cattle, 5 out of 230 (2.17%) in case of goats, 15 out of 170 (8.82%) in sheep. The numbers of positive reactors or suspected by FPA was 2 out of 135 (1.48%) in buffaloes, 0 out of 465 (0.00%) in cattle, 5 out of 230 (2.17%) in case of goats, 12 out of 170 (7.06%) in sheep. Rahman et al. (2006) reported an animal-level seroprevalence of brucellosis in cattle, 2.4-18.4% while the herd incidence in cattle was 62.5%. In case of goats, the prevalence was 3.15% by I-ELISA which was higher than 1.98%, reported by Ahasan et al. (2010) and 2.33% reported by Uddin and Rahman (2007) but it is lower than that of Rahman et al. (1988) who reported 14.57% positive cases of brucellosis in caprine in different areas of Bangladesh. In Bangladesh, there are ample opportunities for intermixing of species, grazing lands and composite small holdings of livestock maintained by nearly 80% of the rural population.

Recent study (Rahman et al., 2011) reported that among all the livestock species in Bangladesh, overall serological prevalence was 2.87% in buffaloes; 2.66% in cattle, 3.15% in goats and 2.31% in sheep, beside goats were found predominantly infected with brucellosis. In contrary, in this study we found the evidence of exposure to *Brucella spp*. is relatively high in sheep (8.24%). The prevalence and severity of the disease may vary with breed, geographic location, type of diagnostic test, husbandry and environmental factor (Amin et al., 2005).

In this study, there was a significant association among abortion and the prevalence of brucellosis (*P<0.01*). So, it can be concluded that the prevalence of brucellosis was significantly higher in animals with previous abortion record in buffaloes, cattle, goats and sheep than that with no abortion record. Ibrahim and Habiballa (1975) also

reported a prevalence of brucellosis of 14·2% in cows that had previously aborted record. Similar findings have reported other researchers (Sandoval et al., 1979; Shaw, 1986; Sandhu et al., 2001). Relatively higher prevalence was found in females than male in cattle, goats, sheep whereas higher prevalence of brucellosis was found in males than in females in case of buffaloes. Lavsen et al. (1988) found a higher prevalence of brucellosis among female cattle in Victoria, Canada. The higher rate of infection in females will be due to infection within the female's reproductive tract providing a potential reservoir for the organism to propagate.

RBT and TAT were developed for detection of antibodies to *Brucella* spp. in bovine serum. In these tests an acidified antigen preparation was used and therefore reduces the final antigen/serum mixture to approximately pH 3.65 (OIE, 2008). At this pH fibrinogen can be converted to an insoluble fibrin that could be interpreted by the investigator as agglutination, thereby giving rise to a false positive result. RBT and TAT are also unable to distinguish antibodies from cross-reacting organisms such as *Yersina enterocolitica* 0:9 which might be present in test sera and this would also lead to false positive results (Samartino et al., 1999). A further drawback of the RBT and TAT are that it requires the use of good quality serum, whereas whole blood and hemolysed serum do not interfere with the detection of serum antibodies in the FPA and C-ELISA test. Data of this study found that RBT showed the highest false positive reactions. Use of species independent competitive immunoassays such as C-ELISA would eliminate some false positives, and would therefore be more specific because production of fibrinogen is not an issue. Another advantage of the C-ELISA is that it is possible in most cases to distinguish between antibodies to *Brucella* spp. and antibodies from other cross-reacting Gram negative bacteria (Nielsen, 1990). The reactivity of the protein A/G HRPO conjugate with Bangladeshi livestock has not been reported. In this study, C-ELISA has been used for the first time to identify *Brucella* serologic reactors among a variety of livestock species in Bangladesh.

C-ELISA and FPA perform well with regards to sensitivity and specificity in sera from animals whereas RBT and TAT performed relatively poor (Gall et al., 2001). Therefore, it is assumed that the RBT and TAT tests overestimated the number of positive in this study. FPA and C-ELISA tests were detected the fewer number of *Brucella* reactors. Given the small number of animals tested, no one test can be judged superior to another and validation of each of these tests is impractical for the reasons stated above. In cases where an accurate estimation of *Brucella* antibodies is required, C-ELISA is qualitatively at least the most suitable choice. A disadvantage of C-ELISA is the considerable expertise and equipment required to perform the test in order to obtain reliable results. In this study, C-ELISA successfully detected the actual number of positive and negative reactors and there were no suspected cases with this test. Similar results of C-ELISA were also reported by Lucero et al. (1999). The FPA test is the diagnostic test of choice for detection of exposure to *Brucella* in animals (Gall et al., 2001). It has the ability, in some cases, to distinguish antibody from cross-reacting organisms (for example, *Y. enterocolitica* 0:9) from antibodies against *Brucella* spp. and is marginally better at it better than the C-ELISA test; it is technically simple to do; it is adaptable to field use even with hemolysed sera, milk and whole blood; and is relatively inexpensive (Gall et al., 2001). The results we obtained were based on cutoff values established for cattle (Nielsen et al., 2004), but they are probably sufficient for preliminary screening for evidence of *Brucella* exposure in other livestock. In this study, FPA could not detect *Brucella* specific antibody response as we have detected suspected/doubtful cases with this test.

Once reliable species-independent serologic testing is adopted for routine screening of livestock, identification of presumptively *Brucella*-positive stranded or sick livestock will assist in guiding treatment of affected animals and alert health care workers. Adopting FPA and C-ELISA for a more accurate determination of *Brucella* status would make this policy less error prone and will result in reintroduction of healthy livestock back into the others. This is the first time in Bangladesh that we used FPA for the diagnosis of brucellosis in Bangladesh.

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