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Evaluation of four Serological Tests for the Detection of Brucellosis in Goats and Cattle under the Field Condition of Bangladesh

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ABSTRACT

As the prompt and accurate diagnosis is important for undertaking an effective control measure, the present study was undertaken to evaluate four serological tests for diagnosis of *Brucella* infection in goats and cattle in some selected areas of Bangladesh. Sera were collected from goats (n = 108) and cattle (n = 60). All sera were screened for *Brucella* specific antibody response by the rose bengal plate test (RBT), slow agglutination test (SAT), indirect enzyme-linked immunosorbent assay (i-ELISA) and competitive ELISA (c-ELISA). Isolation of *Brucella* organisms was performed by culturing of blood samples onto bacteriological media and identification of the organism was conducted by Gram's staining and biochemical tests. The sensitivity and specificity of the serological tests were estimated as follows: in goats, RBT: 100 and 93.40%, SAT: 100 and 96.22%, i-ELISA: 66.67 and 92.38%, c-ELISA: 100%, respectively and in cattle, RBT: 100 and 94.92%, SAT: 100 and 96.61%, i-ELISA: 50 and 93.10%, c-ELISA: 100%, respectively. RBT was found as a suitable screening test and c-ELISA could be used as a confirmatory test for the diagnosis of *Brucella* infection.

Key words: Brucellosis, rose bengal plate test , slow agglutination test , indirect enzyme-linked immunosorbent assay, competitive enzyme-linked immunosorbent assay

INTRODUCTION

Brucellosis is a major emerging zoonosis caused by the small, non-motile gram-negative and intracellular coccobacilli belonged to the genus *Brucella* (Kakoma *et al*., 2003; Baek *et al*., 2003; Shapoury *et al*., 2006). 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; Franco *et al*., 2007; Islam *et al*., 2009). 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; Das *et al*., 2008). Every year, a lot of undiagnosed cases of abortion, stillbirth and retained placenta are reported in goat and cattle 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.

Currently, diagnosis of brucellosis is mainly performed by classical serological tests such as: Rose Bengal Plate Test (RBT), Tube Agglutination Test (TAT) and Slow Agglutination Test (SAT). However, these tests are known to produce cross reactions with other Gram negative bacteria having antigenic similarities with *Brucella* (Kittelberger *et al*., 1998). Enzyme-Linked Immunosorbent Assay (ELISA) could successfully detect antibodies against cross reacting *Yersinia enterocolitica* serotype O:9 and *B. abortus* with high sensitivity and accuracy (Nielsen *et al*., 2006) In order to perform accurate diagnosis of brucellosis in livestock in Bangladesh it is important to find out the suitable tests having high sensitivity and specificity. The present study was performed to compare the sensitivity and specificity of four serological tests such as: RBT, SAT, indirect ELISA (i-ELISA) and competitive ELISA (c-ELISA) for diagnosis of brucellosis in goats and cattle in Bangladesh.

MATERIALS AND METHODS

Collection of samples: Goat (n = 108) and cattle (n = 60) sera were randomly collected from some selected areas of Mymensingh district in Bangladesh. All sera were taken from the sexually matured animals.

Serological tests: The RBT was performed as per standard procedure (Alton *et al*., 1975) with little modifications. Briefly, 75 μL of serum was taken on a rose bengal plate or glass plate by micropipette. The rose bengal coloured antigen bottle was shaken well to ensure homogenous suspension and then 25 μL of the Rose Bengal coloured antigen was added to the serum. The antigen and serum were mixed thoroughly and waited for 5 min. The result was observed immediately after 5 min. Definite clumping/ agglutination was considered as positive reaction, whereas no clumping/agglutination was regarded as negative.

Slow Agglutination Test (SAT) was carried out with EDTA as described by Garin *et al*. (1985) with slight modifications. Briefly 168 μL of Serum Agglutination de Wright (SAW) buffer was pipetted in the first well and 100 μL in the second and third wells of a 96 wells microplate. Then 32 μL of serum was added in the first well (dilution 1/6.25). After mixing 100 μL was transferred to the second well (1/12.5). Then 100 μL of diluted serum was transferred to the third well and finally, 100 μL of liquid was discarded from the third well. Thus, all wells contained 100 μL of diluted serum samples. 100 μL of standardized SAW antigen was added to all wells. The plate was then incubated at 37°C for 24 h (+/- 4 h). After 24 h agglutination reaction was recorded by using a magnifying mirror against illumination source.

The i-ELISA and c-ELISA were performed using commercial kits according the manufacturer's protocol (Svanova Biotech AB, art. No. 10-2700-10, SE-751 83 and No. 10-2701-02, SE-751 83, Uppsala, Sweden).

Bacteriological study: Blood samples of goat and cattle tested positive in the RBT were cultured onto nutrient agar, blood agar and brucella agar media (Becton, Dickinson and company, Sparks, MD, USA). The media were inoculated with 100 μL of processed blood sample. The inoculated media were incubated at 37°C for 3-5 days in presence of 5% CO_2. The media were examined daily. Colony morphology characteristic of *Brucella* were subcultured onto brucella agar to obtain pure culture.

Brucella suspected pure culture was stained with Gram's staining method. Biochemical characterization of the *Brucella* suspected isolates was performed by catalase, oxidase and urease tests.

RESULTS

Screening of sera by RBT: Goat and cattle sera were screened with RBT for the detection of *Brucella* specific antibody response. Out of 108 sera of goat 9 were tested positive for brucellosis. In case of cattle 4 of 60 sera were found to be positive for *Brucella* specific antibody response by RBT. The results of RBT screening of sera of goat and cattle are shown in Table 1.

Detection of *Brucella* specific antibody response by SAT: Out of 108 sera of goat 6 were tested positive for brucellosis by the SAT. In case of cattle 2 of 60 sera showed *Brucella* specific antibody response in SAT. The results of SAT to detect *Brucella* specific antibody response in the sera of goat and cattle are shown in Table 1.

Detection of *Brucella* specific antibody response by i-ELISA: In goat, 1 serum sample was tested positive for Brucella by i-ELISA. On the other hand, all sera of cattle were found to be negative for Brucella by i-ELISA. The results of i-ELISA for detection of *Brucella* specific antibody response in the sera of goat and cattle is given in Table 2.

Detection of *Brucella* specific antibody response by c-ELISA: Two sera of goat showed *Brucella* specific antibody response by c-ELISA. In cattle 1 serum sample was tested positive by c-ELISA. The results of c-ELISA for detection of *Brucella* specific antibody response in the sera of goat and cattle is given in Table 2.

Sensitivity and specificity of RBT, SAT and i-ELISA and c-ELISA: Sensitivity of RBT and SAT for diagnosis of brucellosis was found to be 100% in both goats and cattle when compared to gold standard test (c-ELISA). A relatively lower level of sensitivity was recorded by i-ELISA: 66.67% in goats and 50% in cattle. On the other hand, specificity of RBT, SAT and i-ELISA was found to be 93.94, 96.22 and 92.38% in goats and 94.92, 96.61 and 93.10% in cattle, respectively, (Table 3) In both goats and cattle, specificity of c-ELISA was found to be 100%.

Bacteriological findings: Two out of 9 goat blood samples and 1 out of 4 cattle blood samples yielded bacterial growth characterized by small pinpoint, smooth and honey colored colony. Gram's staining of the pure culture revealed Gram negative, small cocco-bacilli arranged in single or chain. All isolates gave positive reaction to catalase, oxidase and urease tests.

Table 1: Detection of *Brucella* specific antibody response in the sera of goats and cattle by rose bengal plate and slow agglutination tests

Animal species	No. of sera tested	No. of positive cases by RBT (%)	No. of positive cases by SAT (%)
Goat	108	9 (8.33)	6 (5.56)
Cattle	60	4 (6.67)	2 (3.33)

Table 2: Detection of *Brucella* specific antibody response in the sera of goats and cattle by i-ELISA and c-ELISA

Animal species	No. of sera tested	No. of positive cases by i-ELISA (%)	No. of positive cases by c-ELISA (%)
Goat	108	1 (0.93%)	2 (1.85%)
Cattle	60	0 (0.00%)	1 (1.67%)

Table 3: Sensitivity and specificity of RBT, SAT and i-ELISA compared to gold standard test (c-ELISA) for the detection of *Brucella* antibodies in goats and cattle

Sera of animals	Serological tests	Sensitivity (%)	Specificity (%)
Goat	RBT	100.00	93.94
	SAT	100.00	96.22
	i-ELISA	66.67	92.38
Cattle	RBT	100.00	94.92
	SAT	100.00	96.61
	i-ELISA	50.00	93.10

DISCUSSION

Brucellosis remains a major emerging zoonosis worldwide (WHO, 1986; Kakoma *et al*., 2003; Baek *et al*., 2003). 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. 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: RBT, SAT, STAT and ELISA are commonly used for detection of *Brucella* specific antibody response in livestock animals (Blasco *et al*., 1994; Ferreira *et al*., 2003; Jain and Tilak, 2008; Junaidu *et al*., 2008). The ELISA is known to be more effective in detecting brucellosis as compared to the traditional tests such as: RBT, SAT and CFT (Jacques *et al*., 1998; Abd El-Razik *et al*., 2007). The c-ELISA is a highly sensitive and specific diagnostic assay since it directly detects antibody and has no or minimal false positive reactions than the i-ELISA and agglutination tests (Nielsen *et al*., 2004).

In this study, sensitivity and specificity of RBT, SAT and i-ELISA were determined in comparison to the gold standard test c-ELISA, for the diagnosis of brucellosis in goats and cattle under the field condition of Bangladesh. In the present study RBT detected 13 seropositive cases after screening of 168 sera. Seropositive samples when cultured onto bacteriological media only 3 samples (2 from goat and 1 from cattle) were found to be culture positive. The i-ELISA could not detect *Brucella* specific antibody response in case of cattle. On the contrary, c-ELISA successfully detected *Brucella* specific antibody response in two goats and one cattle which were culture positive.

Data of this study found that RBT showed the highest false positive reactions and i-ELISA exhibited higher false negative reactions as compared to the c-ELISA. Similar results were also recorded by Nielsen *et al*. (2004).

In this experiment, SAT was found to be more specific than RBT for detecting brucellosis in both goats and cattle which is in agreement with the findings of Sulima and Venkataraman (2008). In the present study, c-ELISA successfully detected the actual number of positive and negative reactors. Similar results of c-ELISA were also reported by Lucero *et al*. (1999).

CONCLUSION

In this study, RBT detected the highest positive reactors for *Brucella* specific antibody response compared to other serological tests. Thus, RBT could be used as a suitable screening test for diagnosis of brucellosis in goat and cattle. All culture positive samples were tested positive by the c-ELISA indicating that this test could be used for confirmatory diagnosis of brucellosis.

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REFERENCES

Abd El-Razik, K.A., H.M. Desoukey and W.M. Ahmed, 2007. Investigations on brucellosis in Egyptian baladi does with emphasis on evaluation of diagnostic techniques. Pak. J. Biol. Sci., 10: 342-348.

Alton, G.G., L.M. Jones and D.E. Pietz, 1975. Laboratory Techniques in Brucellosis. 2nd Edn., World Health Organization (WHO), Geneva, Switzerland.

Amin, K.M., M.B. Rahman, M.S. Rahman, J.C. Han, J.H. Park and J.S. Chae, 2005. Prevalence of brucella antibodies in sera of cows in Bangladesh. J. Vet. Sci., 6: 223-226.

Baek, B.K., C.W. Lim, M.S. Rahman, C.H. Kim, A. Oluoch and I. Kakoma, 2003. *Brucella abortus* infection in indigenous Korean dogs. Can. J. Vet. Res., 67: 312-314.

Blasco, J.M., B. Garin-Bastuji, C.M. Marin, G. Gerbier, J. Fanlo, M.P.J. de Bagues and C. Cau, 1994. Efficiency of different rose Bengal and complement fixation agents for the diagnosis of *Brucella melitensis* infection in sheep and goats. Vet. Rec., 134: 415-420.

Das, T., M. Ershaduzzaman, K.K. Islam, M.M. Haque, M.M. Rahman and K.B.M. Sailful Islam, 2008. Surveillance of *Brucella melitensis* and *Brucella abortus* from aborted bengal goats in Bangladesh. Res. J. Vet. Sci., 1: 28-36.

Diaz-Aparicio, E., C. Marin, B. Alonso-Urmeneta, V. Aragon and S. Perez-Ortiz *et al*., 1994. Evaluation of serological tests for diagnosis of *Brwcella melitensis* infection of goats. J. Clin. Microbiol., 32: 1159-1165.

Ferreira, A.C., R. Cardoso, I.T. Dias, I. Mariano and A. Belo *et al*., 2003. Evaluation of a modified Rose Bengal test and an indirect enzyme-linked immunosorbent assay for the diagnosis of *Brucella melitensis* infection in sheep. Vet. Res., 34: 297-305.

Franco, M.P., M. Mulder, R.H. Gilman and H.L. Smits, 2007. Human brucellosis. Lancent Infect. Dis., 7: 775-786.

Garin, B., D. Trap and R. Gaumont, 1985. Assessment of the EDTA seroagglutination test for diagnosis of bovine brucellosis. Vet. Rec., 117: 444-445.

Islam, M.A., M.M. Khatun, B.K. Beak and S.I. Lee, 2009. Effects of *Brucella abortus* biotype 1 infection on the reproductive performance of sprague-dawley rats. Pak. J. Biol. Sci., 12: 353-359.

Jacques, I., V. Olivier-Bernadin and G. Dubray, 1998. Efficacy of ELISA compared to conventional tests (RBPT and CFT) for diagnosis of *Brucella melitensis* infection in sheep. Vet. Microbiol., 64: 61-73.

Jain, R. and V. Tilak, 2008. Evaluation of different serological techniques in laboratory diagnosis of brucellosis. J. Indian Med. Assoc., 106: 522-524.

Junaidu, A.U., S.I. Oboegbulem and M.D. Salihu, 2008. Seroprevalence of brucellosis in prison farm in Sokoto, Nigeria. Asian J. Epidemiol., 1: 24-28.

Kakoma, I., A.O. Oluoch, B.K. Baek, M.S. Rahman and K. Matsuda, 2003. More attention warranted on *Brucella abortus* in animals. J. Am. Vet. Med. Assoc., 222: 284-284.

Kittelberger, R., P.G. Bundesen, A. Cloeckaert, I. Greiser-Wilke and J.J. Letesson, 1998. Serological cross-reactivity between *Brucella abortus* and *Yersinia enterocolitica* 0:9: IV. Evaluation of the M- and C-epitope antibody response for the specific detection of *B. abortus* infections. Vet. Microbiol., 60: 45-57.

Lucero, N.E., L. Foglia, S.M. Ayala, D. Gall and K. Nielsen, 1999. Competitive enzyme immunoassay for diagnosis of human brucellosis. J. Clin. Microbiol., 37: 3245-3248.

Mahajan, N.K. and R.C. Kulshreshtha, 1991. Comparison of serological tests for *Brucella melitensis* infection in sheep. Trop. Anim. Health Prod., 23: 11-16.

Nielsen, K., D. Gall, P. Smith, S. Balsevicius and F. Garrido *et al.*, 2004. Comparison of serological tests for the detection of ovine and caprine antibody to *Brucella melitensis*. Rev. Sci. Tech., 23: 979-987.

Nielsen, K., P. Smith, W. Yu, P. Nicoletti, G. Jungersen, J. Stack and J. Godfroid, 2006. Serological discrimination by indirect enzyme immunoassay between the antibody response to *Brucella* sp. and *Yersinia enterocolitica* O:9 in cattle and pigs. Vet. Immunol. Immunopathol., 109: 69-78.

Radulescu, R.A., G. Petriceanu, A. Ragalie and E. Gutu, 2007. Comparative evaluation of serological assays for brucellosis diagnosis. Rev. Rom. Med. Vet., 17: 83-92.

Roth, F., J. Zinsstag, D. Orkhon, G. Chimed-Ochir and G. Hutton *et al.*, 2003. Human health benefits from livestock vaccination for brucellosis: Case study. Bull. World Health Organ., 81: 867-876.

Shapoury, R., M. Sattari and Z.M. Hassan, 2006. Studies on the antimicrobial effect of allicin on the intra macrophages *Brucella*. Pak. J. Biol. Sci., 9: 1935-1939.

Sulima, M. and K.S. Venkataraman, 2008. Comparative evaluation of three serological tests for diagnosis of ovine brucellosis. Indian J. Vet. Med., 28: 37-38.

WHO, 1986. Joint FAO/WHO Expert Committee on Brucellosis. World Health Organization, Geneva, ISBN-10: 924120740X.