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Research article

Molecular detection and epidemiology of *Brucella* in dairy cattle of Bangladesh

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A B S T R A C T

Brucella, a Gram – (ve) bacterium, causes late abortion in dairy cattle. The genus Brucella has six recognized species on the basis of host specificity but the greatest economic impact results from *Brucella abortus.* Brucellosis is considered as one of the most widespread zoonoses in the world by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Office International des Epizooties (The World Organization for Animal Health, OIE). It is an important zoonotic disease which causes significant losses in reproduction of sexually mature animals. In pregnant cows, brucellosis is characterized by late abortion, birth of weak calves, stillbirths and infertility. Therefore, a cross – sectional study was conducted in commercial dairy cattle in Bangladesh to describe the epidemiological scenario of *Brucella*. Nineteen upazillas (sub – districts) from 12 districts of 7 divisions were randomly chosen for the study. A total 44 dairy cattle farm, 1 – 6 farms per upazilla was recruited based on the presence of abortion history within past six months. A pretested questionnaire was used to collect epidemiological information through face to face interview and direct observation. Blood samples and aborted fetuses were collected for laboratory evaluation. Aborted fetuses were collected only from Chattogram Metro. Sero –positivity for *Brucella abortus* was evaluated on the samples obtained using OIE protocol based iELISA technique. Aborted fetus was evaluated through bacteriological culturing followed by PCR. The overall sero – prevalence of *Brucella* was 1.50 in dairy cattle of Bangladesh (Herd level and individual level). In this study, iELISA is more specific and sensitive for the detecting the *Brucella* antibody. PCR gives more accurate and precious results for detecting *Brucella abortus* .

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1. INTRODUCTION

Brucella is a Gram negative, facultative intracellular cocco – bacillus, or short rod bacteria, within the family of *Brucellaceae* (Baek et al., 2003; Kakoma et al., 2003). According to the host specificity, *Brucella* has six recognized species: *Brucella abortus, B. melitensis, B. suis, B. ovis, B. canis* and *B. Neotomae* (Foster et al., 2007). *B. abortus* has seven biovars *(*1– 6 and 9) and usually causes brucellosis in cattle, bison and buffalo (Acha and Szyfres, 2003). Wildlife reservoirs carry the largest risk to cattle herd re-infection. *B. abortus* causes abortion and reduces milk production in dairy cattle as well as decrease the survival rate of newborns (Bricker and Halling, 1994; Roth et al., 2003).

Brucella–prevalence and *Brucella* sero – prevalence was reported to be higher in dairy cattle, cross-bred cattle, adults, pregnant cattle and late stage fetuses in cattle living with other livestock or those supplied contaminated water or were serviced by infected bull (Mayer, 1980; Shuaibi, 1999; Amin et al., 2005; Gul and Khan, 2007; Jittapalapong et al., 2008; Dinka and Chala, 2009; Munir et al., 2011; Rahman et al., 2011; Abubakar et al., 2012).

Commonly used serological tests for *Brucella* spp. includes Rose Bengal Plate Test (RBPT) (Amin et al., 2005; Uzal et al., 1995), Indirect – Enzyme Linked Immuno Sorbent Assay (iELISA) (Rojas and Alonso, 1994; Shafee et al., 2012; Uzal et al., 1995), Complement Fixation Test (CFT) (Alton et al., 1975, 1988; MacMillan, 1990), Tube Agglutination Test (TAT) (Rahman, 2004). Frequently used diagnostic tests to isolate or identify *Brucella* are bacteriological culture ((Dehkordi and Taghizadeh, 2012) and Polymerase Chain Reaction (PCR) (Dehkordi and Taghizadeh, 2012; Moshkelani et al., 2011; Yazdi et al., 2008). As there are variable level of sensitivity and specificity of *Brucella* diagnostic tests, the present study therefore used iELISA to determine *Brucella* antibody and bacteriological culture and PCR to identify *Brucella*. The specific objectives of the present study were: to estimate sero – prevalence of *Brucella* in dairy cattle in Bangladesh, to determine *Brucella* prevalence in aborted fetuses of dairy cattle in Chattogram, Bangladesh, Molecular detection of *Brucella* through PCR.

2. MATERIALS AND METHODS

Study Period: June, 2013 to June, 2014

Study Area: 44 selected dairy farms from 73 sero – positive farms under the study periods by I –ELISA (OIE, 2009).

A cross-sectional study was carried out in commercial dairy cattle in Bangladesh during June – July 2013. The study covered 7 divisions of 7, 12 districts of 64 and 19 upazillas (sub–districts), or Thanas of 467. A total 44 dairy cattle farms were recruited with the consultation of local practicing cattle veterinarians of respective sub-districts based on anabortion history of farms within the past six months. Accordingly, 1 – 6 farms were randomly chosen from each upazilla, or Thana, for the study. A total of 592 cattle (4 – 66 lactating or non-lactating cattle or both per farm) were randomly selected for the study. The age of the selected animals ranged from 3 – 6 years.

Three interviewers (two MS students and one PhD student from Chattogram Veterinary and Animal Sciences University, CVASU, Bangladesh were thoroughly trained in surveying and interviewing techniques at CVASU. A questionnaire was developed in English. The questionnaire contained closed (yes/no/multiple choice) and open questions. It explored farm characteristics and individual animal level information i.e., farm location, farmers' education, number of employees, farm size, source of animals, source of semen, AI inseminator, abortion history, still birth history and age, breed, body condition score, parity, lactation stage and physiological status of individual animals. The questionnaire was pre – tested with three dairy cattle farms in Chattogram. None of these farms were included in the main study. Questions were updated following the pilot testing with ambiguous questions being eliminated or modified. Comments and suggestions made by the respondents in the pilot-testing were also addressed to improve the questionnaire. Farmers were interviewed on their own premises. Immediately before the interview and verbal consent of the respondent was acquired. Interviewers interviewed 2 – 3 farmers per day. Interview length lasted approximately 45 minutes.

Age was assessed based on farm records or dentition (Degefu et al., 2011). Breed was confirmed by phenotypic characteristics (Amin *et* al., 2005). Body condition score was evaluated as per criteria described by Abubakar et al. (2012).

Samples: Sera (592) from 7 Divisions; and aborted fetuses (25) from Chittagong Division

Experimental Design: Samples collected from aborted fetuses (lungs, kidneys, liver and eyes) were inoculated on Columbia Agar Base and *Brucella* Selective Supplement plus Horse Serum specific to *Brucella* for growing and isolating. The inoculated plates were then incubated at 37^0C and 5% CO_2 for 7 days. After growth, several colonies were transferred into 50% glycerin and kept at – 20^0C for molecular testing. DNA from cultured samples of *Brucella* spp. was extracted using the FABGK001 (50 preps) DNA extraction kit and its extraction protocol

(FABGK 001). A published PCR protocol was used for *Brucella* evaluation (Dehkordi et al., 2012). The primers derived from the rrs (16S) gene of *B. abortus*, primer A, 5'-GCGCTCAGGCTGCCGACGCAA-3' and primer B, 5'-ACCAGCCATTGCGGTCGGTA-3' (Dehkordi et al., 2012) and 5 ml of template sample DNA.

An iELISA developed by SVANOVAR Brucella– Ab I – ELISA (Article Number 10 – 2700 – 10) was used to detect IgG antibodies against acute infection of *Brucella abortus*. The OIE protocol was followed for testing serum samples (OIE, 2009). The ratio of the Percent Positivity (PP) value of test sample and positive control of equal or more than 40 was considered as *Brucella abortus* sero – positive.

The detailed iELISA procedure is given as below:

1. Conjugate was prepared by adding 11.5ml sample diluent buffer to each one of five vials. For preparation of sample and control, a dummy plate was taken.
2. A total of 196µl sample diluents were added to each well of this dummy plate. Then 4µl negative control (NC) was added in 1st well (A_1) and 2nd well (B_1,) and 4µl positive control (PC) was added in 3rd well (C_1) and 4th well (D_1). A total of 4µl sample was added in rest of the wells and mixed them properly.
3. Then an ELISA plate was taken. A total of 100µl diluted sample was loaded from the dummy plate. This was incubated at 37^0 C for 1 hour in shaking incubator. It was then washed 4 times by 200µl washing buffer in each well.
4. A total of 100µl conjugate was added in each well and then incubated at 37^0 C for 1 hour in shaking incubator. It was washed 4 times by 200µl washing buffer in each well.
5. A total of 100µl substrate was added in each well and incubated 15 minutes at 37^0 C. A total of 100µl of stop solution was added to each well.
6. Then it was read at 405 nano – meter (nm) with a corrected optical density (OD) using a reference filter. Calculation of results was done as described below:

$$PP = \frac{OD_{\text{Sample or NC}}}{OD_{PC}} \times 100$$

(Here, OD = Optical Density; NC = Negative Control; PC = Positive Control)

PP of ≥40 was considered as positive.

Data analysis: Descriptive and summative statics were used on the results of Bacteriological and PCR test results. Statistical analysis was carried out on field and laboratory data as required by using STATA Software. A p – value of <0.05 was considered statistically significant.

3. RESULTS

Sero-prevalence of Brucella

The overall sero – prevalence of *Brucella abortus* 1.5% in dairy cattle of Bangladesh (Table 1)

Table 1: Overall sero – prevalence of *Brucella* in dairy cattle of Bangladesh accounting different cluster variables (N=592)

Cluster variable	Prevalence	SE	95% CI
Farm	0.015	0.005	0.008 – 0.029
Sub-district (Upazilla)	0.015	0.006	0.006 – 0.036
District	0.015	0.006	0.006 – 0.038
Division	0.015	0.008	0.004 – 0.051

Univariate association

Univariate association between factors and the sero – prevalence of Brucella

Table 2: Univariate analysis of the bacteriological result of *Brucella* in Cattle, Chattogram

Variable	Category	N	Positive	%	p-value
Cow age	≤4.5 years	7	1	14.3	0.74
	≥4.6 years	18	4	22.2	
Fetus age	≤120 days	7	1	28.6	0.69
	>120-180	12	2	16.7	
	>180	6	2	33.3	

Cattle of older age group (≥4.6 years) had higher sero– prevalence than cattle of younger age

group (≤4.5 years) (p=0.74). Fetus age group had similar findings as cow age group (p=0.69).

Laboratory identification and prevalence of Brucella

Bacteriological identification of Brucella in aborted fetus

Five (20%, all specimens of individual fetus were positive) of aborted fetuses (N=25) were determined as *Brucella abortus* positive on bacterial evaluation. Out of 100 individual specimens (4 specimens per fetus: eye – ball, liver, lung and kidney), 20 specimens were turned out as the positive of *Brucella abortus.* The distribution of *Brucella abortus* by specimens as follows: 5 in eye – ball, 5 in liver, 5 in lung and 5 in kidney. Age of cow and age of abortion of fetuses had no effect on the occurrence of *Brucella abortus*.

Molecular identification of Brucella

All 20 individual specimens of 5 *Brucella abortus* positive aborted fetuses (based on bacterial culture) were also PCR *Brucella abortus* positive. The results of PCR of *Brucella abortus* are presented in Table 3 and Figure 1.

4. DISCUSSION

Brucellosis is known to be an economically important highly contagious and zoonotic disease of animals and human beings globally (OIE, 2000; Mathur, 1971). In Bangladesh, brucellosis was first identified in cattle at 1967 (Mia and Islam, 1967). Therefore, the present study was carried out to estimate the dairy cattle farm characteristics; *Brucella* antibody and *Brucella* prevalence with associated risk factors, and identified the *Brucella abortus* by microbiology and PCR.

The sero – prevalence of brucellosis in dairy farms were 1.5% which agreed with the results of Amin et al. (2004), Dey et al. (2013) and Rahman et al. (2013). However, a bit higher sero – prevalence was reported by Shamim et al. (2009) 3.3% and Nahar and Ahmed (2009) 4.5%. This variation might be due to tests applied, sample size, age, breed, sex, pregnancy status of the animal, study area, breeding techniques, herd size and reproductive diseases (Gul and Khan, 2007). There are variable levels of sensitivity and specificity of *Brucella* diagnostic tests, the present study therefore used iELISA to determine *Brucella* antibody rather than other tests like RBPT, CFT AND TAT (Munir et al., 2011; Rojas and Alonso, 2000; Shafee et al., 2012; Uzal et al., 1995). Molecular studies like PCR gave precise, rapid and accurate test use for detecting *Brucella abortus* in clinical samples (Bricker and Halling, 1994). This study agreed with the results of Dehkordi and Taghizadeh, 2012; Moshkelani et al., 2011; Yazdi et al., 2008 which gave higher sensitivity and specificity for iELISA (Both 100%).

Table 3: PCR result for *Brucella abortus* from aborted fetuses

Fetus ID	Eyeball	Liver	Lung	Kidney	Pooled sample	Remarks
Fetus 1	+ve	+ve	+ve	+ve	+ve	+ve (113bp)
Fetus 11	+ve	+ve	-ve	+ve	-ve	+ve (113bp)
Fetus 14	+ve	+ve	-ve	-ve	+ve	+ve (113bp)
Fetus 24	+ve	-ve	+ve	+ve	-ve	+ve (113bp)
Fetus 25	+ve	+ve	+ve	-ve	+ve	+ve (113bp)

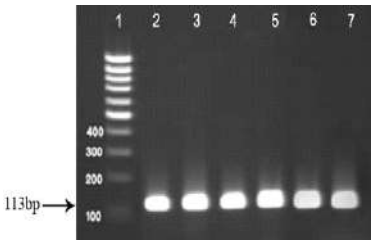

Figure 1: An ethidium bromide-stained agarose gel of PCR products was showing the sensitivity of the assay where DNA marker 50bp and band at 113bp

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REFERENCES

Abubakar, M., Mansoor, M. and Arshed, M.J. 2012. Bovine Brucellosis: Old and New Concepts with Pakistan Perpective. Pakistan Veterinary Journal, 32(2): 147 – 155.

Acha, N. P. and Szyfres, B. 2003. Brucellosis. In: Zoonoses and Communicable Diseases Common to Man and Animals. Pan American Health Organization (PAHO), Washington,DC, USA, 3(1): 24 – 45.

Alemu, F., Admasu, P., Feyera, T. and Niguse, A. 2014. Seroprevalence of bovine Brucellosis in

Eastern Showa, Ethopia.Academic Journal of Animal Diseases, 3(3): 27 – 32.

Alton, G. G., Jones, L. M., Angus, R. D., Verger, J. M. 1988.Techniques for the Brucellosis Laboratory. Institut de Recherche Agronomique, Paris, pp. 17 – 136.

Alton, G.G., Maw, J., Rogerson, B.A. and McPherson, G.G. 1975. The serological diagnosis of bovine brucellosis: An evaluation of the complement fixation, serum agglutination and rose Bengal tests. Australian Veterinary Journal, 51(2), 57 – 63.

Amin, M. R. K., Rahman, M. B., Rahman, M. S., Han, J. C., Park, J. H.and Chae, J. S. 2005. Prevalence of *Brucella* antibodies in sera of cows in Bangladesh. Journal of Veterinary Science, 6: 223 – 226.

Amin, M.R.K., Rahman, M.B., Sarkar, S.K., Kabir, S.M.L. and Akand, M.S.I. 2004.Serological Epidemiology of Brucellosis in Cattle of Mymensingh District of Bangladesh. Journal of Animal and Veterinary Advances, 3(12), 898 – 900.

Baek, B. K., Lim, C. W., Rahman, M. S., Kim, C. H., Oluoch, A. and Kakoma, I. 2003.Brucella abortus infection in indigenous Korean dogs. Canadian Journal of Veterinary Research, 67: 312 – 314.

Bangladesh Bureau of Statistics (BBS). 2006. Preliminary report on household income and expenditure survey 2005. Date of Publication: January, 2006.

Bricker, B.J. and Halling, S.M. 1994.Differentiation of *Brucella abortus*, bv 1, 2 and 4, *Brucella melitensis*, *Brucella ovis*, and *Brucella suis* bv1by PCR. Journal of Clinical Microbiology, 32, 2660 – 2666.

Degefu, H., Mohamud, M., Hailemelekot, M. and Yohannes, M. 2011.Seroprevalance of bovine Brucellosis in agro pastoral areas of Jijjiga zone of Somali National Regional State, Eastern Ethiopia. Ethoipian Veterinary Journal, 15(1), 37 – 47.

Dehkordi, F. S., Saberian, S. and Momtaz, H. 2012**.** Detection and segregation of the *Brucella abortus* and *Brucella melitensis* in aborted bovine, ovine, caprine, buffaloes and camelid fetuses by application of Conventional and Real – time Polymerase Chain Reaction. Thailand Veterinary Medicine, 42(1): 13 – 20.

Dehkordi, F.S. and Taghizadeh, F. 2012. Prevalence and some risk factors with Brucellosis and Leptospirosis in aborted fetuses of ruminant species. Research Opinions in Animal and Veterinary Sciences, 2(4), 275 – 281.

Dey, S.K., Rahman, M.S., Rima, U.K., Hossai, M.Z., Chowdhury, G.A., Pervin, M., Habib, M.A. and Khan, M.A.H.N.A. 2013.Serological and pathological investigation of Brucellosis in dairy cows of Mymensingh District, Bangladesh. Bangladesh Journal of Veterinary Medicine, 11(2), 107 – 112.

Dinka, H. and Chala, R. 2009. Seroprevalence study of bovine Brucellosis in pastoral and agro – pastoral areas of East Showa Zone, Oromia Regional State, Ethiopia. American – Eurasian Journal of Agricultural and Environmental Sciences, 6 (5), 508 – 512.

Forbes, L. B. and Tessaro, S. V. 1996. Infection of cattle with *Brucella abortus*biovar I isolated from a bison in Wood Buffalo National Park. Canadian Veterinary Journal, 37: 415 – 419.

Foster, G., Osterman, B. S., Godfroid, J., Jacques, I. and Cloeckaert, A. 2007. *Brucella ceti* sp. nov.and *Brucella pinnipediali s*sp. nov.for *Brucella* strains with cetaceans and seals as their preferred hosts. Int J Syst Evol Microbiol., 57:2688 – 2693.

Gul, S. T. and Khan, A. 2007. Epidemiology and epizootology of brucellosis. Pakistan Veterinary Journal, 27: 145 – 151.

Jittapalapong, S., Inpankaew, T., Sangwaranond, A., Phasuk, C., Pinyopanuwat, N., Chimnoi, W., Kengradomkij, C., Sunutta, C. and Arunwipat, P. 2008. Current status of Brucellosis in dairy cows of Chiag Rai Province, Thailand. Kasetsart Journal of Natural Science, 42, 67 – 70.

Kakoma, I., Oluoch, A. O., Baek, B. K., Rahman, M. S. and Kiku, M. 2003. More attention warranted on *Brucella aboetus* in animals. Journal of American Veterinary Medical Association, 222, 284.

MacMillan, A. 1990. Conventional serological test. In: Animal Brucellosis, eds. Nielsen, K., Duncan, J.R.. CRC Press, Boca Raton, FL, 153 – 197.

Mahajan, V., Banga, H. S., Filia, G., Gupta. M. P. and Gupta, K. 2017. Comparison of diagnostic tests for the detection of bovine Brucellosis in the natural cases of abortion. Iranian Journal of Veterinary Resaerch, 18(3): 183 – 189.

Mathur, T. N. M. 1971. Brucellosis as a world problem. Dev Biol Stand, 48: 219 – 228.

Mayer, C.E. 1980.Report on veterinary activity. Institute of Agricultural Research, Ethopia.

Mia, A. S. and Islam, H. 1967. A preliminary study on the incidence of the bovine fertility and economic loss caused by it. Pakistan Veterinary Journal, 1: 12 – 15.

Moshkelani, S., Javaheri – Koupaeil, M., Rabiee, S. and Moazeni, M. 2011. Detection of *Brucella* spp. and *Leptospira*spp. by multiplex polymerase chain reaction (PCR) from aborted bovine, ovine and caprine fetuses in Iran. African Journal of Microbiological Research, 5(26): 4527 – 4630.

Munir, R., Farooq, U., Fatima, Z., Afzal, M., Anwar, Z. and Jahangir, M. 2011.Sero – prevalence of Brucellosis in Bovines at Farms under Different Management Conditions. British Journal of Dairy Sciences, 2(3): 35 – 39.

Nahar, A. and Ahmed, M. U. 2009. Seroprevalence study of brucellosis in cattle and contact human in Mymensingh district. Bangladesh Veterinary Medicine, 7: 269 – 274.

OIE. 2009. OIE Terrestrial Manual. Chapter 2.4.3.: Bovine Brucellosis: pp. 07 – 14.

OIE. 2000. Bovine brucellosis. In: Manual of standards for diagnostic tests and vaccines. 4th Edition, OIE, Paris, pp. 328 – 345.

Rahman, M. S. 2004. Plate and tube agglutination tests for diagnosis of *Brucella abortus* Biotype 1 infection in Sprague – Dawley rats. Bangladesh Journal of Veterinary Medicine, 2(1): 63 – 67.

Rahman, A.K.M.A., Saegerman, C., Berkvens, D., Fretin, D., Gani, M.O., Ershaduzzaman, M., Ahmed, M.U. and Emmanuel, A. 2013. Bayesian estimation of of true prevalence, sensitivity and specificity of indirect ELISA, Rose Bengal test and Slow Agglutination Test for the diagnosis of Brucellosis in sheep and goats in Bangladesh. Pakistan Veterinary Medicine, 110, 242 – 252.

Rahman, M.S., Faruk, M.O., Her, M., Kim, J.Y., Kang, S.I. and Jung, S.C. 2011. Prevalence of Brucellosis in ruminants in Bangladesh. Veterinary Medicine, 56 (8), 379 – 385.

Rojas, Z. and Alonso, O. 2000. ELISA for the diagnosis and epidemiology of *Brucella abortus* infection in cattle in Chile. Institute de Microbiologia, Universidad Austral de Chile, Valdivia, Chile.

Roth, E., Zinsstag, J., Orkhon, D., Ochir, G.C., Hutton, G., Cosivi, O., Carrin, G. and Otte, J. 2003. Human health benefits from livestock vaccination for Brucellosis: case study. Bulletin of World Health Organization, 81, 867 – 876.

Shafee, M., Rabbani, M., Ahmad, M. U. D., Muhammud, K., Sheikh, A. A., Awan, M. A. and Shabbir, M. Z. 2012. Seroprevalence of bovine Brucellosis using i – ELISA in Quetta, Balochistan, Pakistan. The Journal of Animal and Plant Sciences, 22(Supl. 3), 125 – 127.

Shamim, A., Alam, N., Rahman, M.S., Rahman, M.A., Haque, F. and Hee – Jong, S. 2009.Serological and pathological investigation of Brucellosis in dairy cows in Korea. Korean Journal of Veterinary Service, 32(3), 219 – 225.

Shuaibi, A. 1999. Palestinian Brucellosis control programme. Country Reports, Brucellosis Information Workshop, Ramallah, Palestine.

Uzal, F.A., Carrasco, A.E., Echaide, S., Nielsen, K. and Robles, C.A. 1995. Evaluation of an indirect ELISA for the diagnosis of bovine Brucellosis. Journal of Veterinary Diagnostic Investigation, 7, 473 – 475.

Yazdi, S. H., Khazraiinia, P., Salehi, Z. T., Behroozikhah, A. M. 2008. Development of a multiplex polymerase chain reaction assay for differentiation of field strain isolated and vaccine strain S19 and RB51 of *Brucella* in Iran. Iranian Journal of Veterinary Research, 9(1), Ser. No. 22: 19 – 24.