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SEROLOGICAL AND PATHOLOGICAL INVESTIGATION OF BRUCELLOSIS IN DAIRY COWS OF MYMENSINGH DISTRICT, BANGLADESH

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ABSTRACT

Brucellosis is a widespread and economically important infectious disease of animals and humans caused by the members of the genus *Brucella*. The disease is manifested by abortion, birth of unthrifty calves and retained placentae in female animals. The correct and prompt diagnosis is important for controlling and eradicating the disease from animals. This experiment was carried out in the Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh to detect *Brucella* antibody in dairy cows using Rose Bengal Plate Test (RBPT), indirect Enzyme Linked Immunosorbent Assay (i-ELISA) and histopathology. The study was carried out between the periods of January 2012 to September 2012. Placental tissue obtained from aborted cow and internal organs of aborted fetus were used in histopathological study. A total of 190 sera sample were collected with the history of reproductive disorders, like late abortion, retained placenta and anestrous from Veterinary Hospital, Bangladesh Agricultural University (BAU) and meat market Kewatkhali, Mymensingh. The seroprevalance of brucellosis using RBPT and iELISA were 2.63% and 1.05% respectively. Histopathological study revealed multifocal necrosis in hepatic parenchyma, depletion of lymphocytes in spleen and lymphnodes and distended trabeculi in spleen and lymphnodes in aborted calf, diffuse fibrosis around the placental epithelia and in placental tissues. Distinct histopathological lesions in the liver and spleen of *Brucella* suspected calf were not evident but the retained fetal membrane showed characteristics fibrosis and reactive cellular infiltration. It needs to adopt nucleic acid based technologies in order to confirm the species of *Brucella* involved and as well as to avoid cross reactivity of antibodies as seen in RBPT and iELISA.

Key words: Rose Bengal Plate test, Indirect ELISA, Brucellosis, Histopathology, Dairy cows

INTRODUCTION

Bovine brucellosis, caused by *Brucella abortus* is a bacterial zoonosis manifested by reproductive disorders like abortion, infertility, retained placenta, stillbirth and low productivity in animals resulted in economic loss to the dairy farmers (Nicoletti, 1980). Each *Brucella spp* has a preferred natural host that serves as a reservoir (Quinn *et al.,* 1994). Brucellosis have a considerable impact on human and animal health, as well as a socioeconomic impact especially in rural areas that largely rely on livestock breeding and dairy products for their livelihood. In developing countries, brucellosis is still considered the most common zoonotic disease (Al-Majali 2005; Quinn *et al.,* 1994). Brucellosis is the second most important zoonotic disease in the world after rabies (FAO, 2006). *Brucella spp* is hazardous infectious bacteria listed among CDC's category B bioterrorism agents (Franz *et al.,* 1997; Sidell *et al.,* 1997; FAO, 2006; CDC, 2008). Irrespective of the route of infection, the organism provokes a regional lymphadenitis which is characterized by infiltration of reticuloendothelial cell neutrophils, few eosinophils and plasma cells (Bishop *et al.,* 1994). Microscopically, the endometrium is infiltrated by lymphocytes, plasma cells and neutrophils. Microgranulomas may be scattered in the endometrium. The affected cotyledons or parts of them are covered by sticky, odourless, brownish exudates and are yellowish-grey as a result of necrosis. Parts of the intercotyledonary placenta are thickened, oedematous, yellowish-grey and may contain exudates on the surface. The chorion is not uniformly affected and large parts may appear quite normal (Radostits *et al.,* 2000). The udder in infected ruminants does not show any gross lesions, although supramammary lymph nodes may be enlarged (Godfroid *et al.,* 2004). In Bangladesh, approximately 80 percent of people living in villages, and rural income are largely dependent on livestock. There are undiagnosed cases of abortion, stillbirth and retained placenta in dairy cattle which is suspected to be due to brucellosis. Conventionally, serological tests are used to screen for, or to confirm the disease (Navarro *et al.,* 2004). These screening tests are inexpensive, fast and highly sensitive but not necessarily highly specific. The most widely used serologicals tests for diagnosis of brucellosis in animals are Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT) and indirect Enzyme Linked Immunosorbent Assay (iELISA). The diagnostic value may be questionable on individual basis because of cross-reacting antibodies. However, for screening of the herd these tests remain ideal (Corbel *et al.,* 1983; Nielsen *et al.,* 2004; Nielsen *et al.,* 2006).

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There are lots of undiagnosed cases of abortion, stillbirth and retained placenta in farm animals, which are thought to be due to brucellosis and could have significant impact on the development of livestock in Bangladesh (Rahman *et al.,* 2010). Literature available indicated that systemic study on to the histopathologic changes of suspected tissues of dairy animals are lacking in Bangladesh. The present research work was, therefore, undertaken to detect the *Brucella* antibody in dairy cows and observed histopathological changes in aborted fetal membrane and fetal tissues.

MATERIALS AND METHODS

Study area

The study was conducted for a period of nine months from January 2012 to September 2012 in the Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh to diagnose brucellosis in dairy cows by using Rose Bengal Plate Test (RBPT), Indirect ELISA (iELISA) and histopathology.

Selection of suspected animal

One dairy cow reported to have a history of late abortion (Figure 1) and retained placenta (Figure 2) were selected in this study. Moreover, randomly selected animals sera were also used from the slaughterhouse (N=120) to identify the presence of seropositivity in apparently healthy stock.

Collection of blood and sera samples

Blood samples were collected without any anticoagulant and kept at least 1 hour at room temperature in a slightly inclined position to facilitate clotting and separation of serum. After this period, the clotted blood samples with sera were transferred to refrigerator at 4^{o}C and kept overnight. 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 2,500 rpm for 10 minutes. After centrifugation a clear sera were aspirated, collected in microcentrifuge tube and stored at -20^{o}C until used. Out of 190 cow's sera, 70 were collected from the cattle brought to the Veterinary Hospital, Bangladesh Agricultural University for treatment. Sera sample, 120 were collected randomly from the slaughter house to identify the seropositivity of brucellosis in dairy cows destined for slaughter.

Gram's staining of impression smears

Impression smears from the placental tissues of aborted cow, liver and spleen from the aborted calf was made onto clean slides and stained with Gram's staining to identify the presence of Gram –ve bacteria.

RBPT, iELISA and histopathology

The RBPT antigen used was prepared from a stained suspension of heat killed *Brucella abortus* strain 1119-3 and obtained from Dae Sung Microbiological Lab, South Korea. The RBPT was performed as per manufacturer's instructions. Briefly, 75 µl of serum was taken on to a clean Rose Bengal glass plate by using micropipette. The rose Bengal colored antigen bottle was shaken well to ensure homogenous suspension and then 25µl of the Rose Bengal colored antigen was added to the serum. The antigen and serum onto the slides were mixed thoroughly and left for 5 min. The +ve result was marked by clumping of the RBPT antigen as seen by naked eye compared to a test –ve sera. The iELISA was carried out according to manufacturer instruction (Svanova Biotech AB, art. No. 10-2700-10, SE-751 83 Sweden). In brief, all reagents supplied by the manufacturer company were equilibrated to room temperature before use. The ready to use ELISA plate used were coated with *Brucella abortus* antigen. Using micropipette, 100µl of suspected serum sample in dilution buffer (1:500 dilution) was added to each well. As known +ve and –ve, three wells for each were added with 4 µl of positive control serum (Reagent A) and 4 µl of negative control serum (Reagent B) and toped up with 96µl of dilution buffer. The plates was shaken thoroughly and incubated at 37°C for 1 hour. The plates was rinsed 3 times with PBS-Tween-20 by filled up the wells at each rinse, emptied the plate and tapped hard to remove all remains of fluid. Then 100µl of 1:2000 dilution of horse radish peroxidase (HRP) conjugate was added to each well and incubated at 37°C for 1hour. Again rinsed the plate with PBS at pH 7.4. Then 100µl TMB substrate solutions was added to each well and incubated for 10minutes at room temperature. The reaction was terminated by adding 50µl stop solution (10% H_2SO_4) to each well. The optical density (OD) of the controls and test samples was measured at 450nm in a microplate photometer.

For convenient of each study sample was run in triplicate in the well and average value read was measured. The baseline OD value 0.234±0.104 was considered negative. The histopathological study with the representative tissues were carried out according to the protocol of Luna, 1968.

The study was conducted to determine the seroprevelence of brucellosis in dairy cows and investigate pathological lesions in internal organs of the fetus and placenta of a aborted cow.

RESULTS AND DISCUSSION

Table 1. Prevalence of brucellosis in cattle based on RBPT and i-ELISA

Species	Sample size	RBPT		i-ELISA	
		Positive	Prevalence (%)	Positive	Prevalence (%)
Cattle	190	5	2.63	2	1.05%

Figure 1. Aborted fetus from a cow. The fetus delivered dead at 8 months of pregnancy

Figure 2. The placenta of the cow failed to discharge after 24 hours of abortion

Brucellosis is an endemic disease of dairy industry throughout the globe and an important cause of abortion (Rahman *et al*., 2010). This study was carried out with randomly selected serum sample to measure serum anti *Brucella abortus* antibodies reactivity and changes in tissues of suspected dead calf. Out of 190 randomly sera sample tested in RBPT, five (2.63%) sera found to clump *Brucella* antigen on to Rose Bengal Plate in contrast to negative control. Similarly out of 190 cattle sera tested in iELISA, two had shown positive reaction (OD value 0.645±0.102) with a prevalence of 1.05%. This finding is in agreement with the reports of Amin *et al.* (2004); Rahman *et al.* (2010, 2011) and Kadir (2010), who reported 2%, 2.4%, 2.66% and 2.72% prevalence of *Brucellosis* in dairy cattle respectively. However a bit higher reactivity of Brucellosis in dairy animals was reported by Shamim (2009), Nahar and Ahmed (2009) were 3.30% and 4.5%, respectively. This variation in the prevalence may be due to variation in the 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).

Similarly, a low seroprevalence of brucellosis in small ruminants in Bangladesh was also reported by Rahman *et al*. (2013).

Histopathology

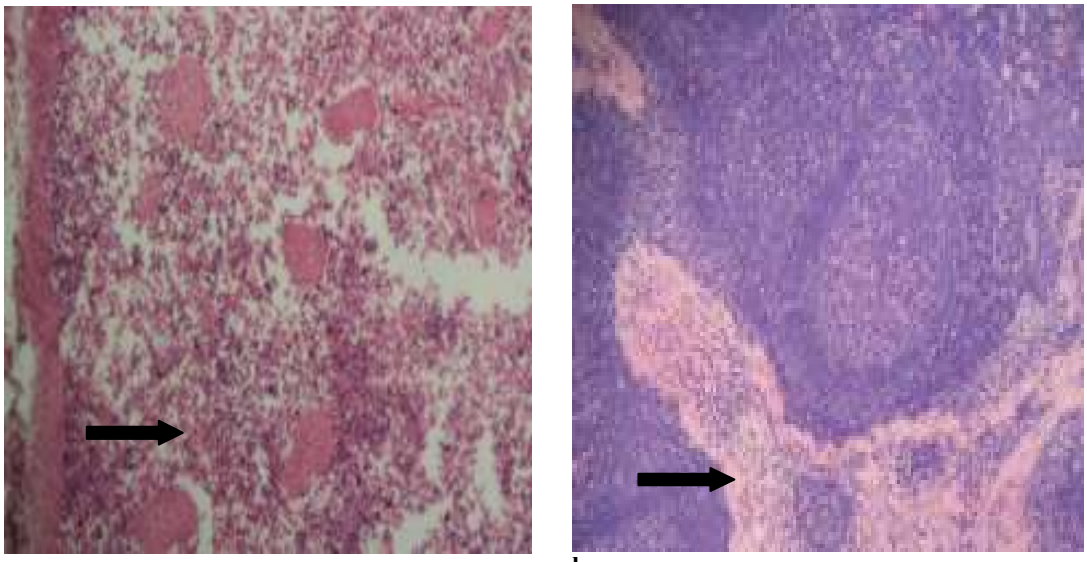
For histopathological study placenta from an aborted cow and spleen and lymphnode from an aborted fetus was collected and stained with H&E. The cow yielded positive reaction both in RBPT and iELISA. During histopathological study there was depletion of lymphocytes in spleen and lymphnodes which was characterized by reducing densities of lymphocytes (Figire 3).

The smooth muscular trabeculi in spleen (Figure 3a, black arrow) and fibromuscular trabeculi in lymphnode (Figure 3b, arrow) were distended. In placenta there was diffuse fibrosis around the placental epithelium (Figure 4).

Whereas Godfroid *et al. (*2004) recorded multifocal small foci of necrosis or microgranulomas in the liver. However gram negative coccobacilli (*Brucella* sp) was not seen in these organs following Gram's staining which is normally present in *Brucella* spp induced aborted fetus and placenta.

Brucellosis in cows is best known as one of the main reproductive disease, capable of causing abortion storms in the breeding season during the last third of pregnancy, retention of the fetal membrane, still births and reduction in milk yield which resulting in great economic loss (Refai, 2003). The liver of aborted fetus showed multifocal necroses in hepatic parenchyma and necrosed tissue was replaced by fibrous connective tissue and reactive cells (Figure 5).

Multiple granulomatus nodule characteristic of brucellosis was not seen in liver. However, it needs to adopt nucleic acid based technology (Al-Attas *et al.,* 2000) and competitive ELISA (Nielsen *et al*., 1995) as well as to use large number of sample to identify Brucellosis in dairy cattle and design prevention and control strategies accordingly.



a **b**

Figure 3. Spleen (left) and lymph node (right) obtained from a dead calf delivered from brucella seropositive cow and stained with H&E. There were depletion of lymphocytes (black arrow) in spleen and lymphnode and distended trabeculi (black arrow, 10x).

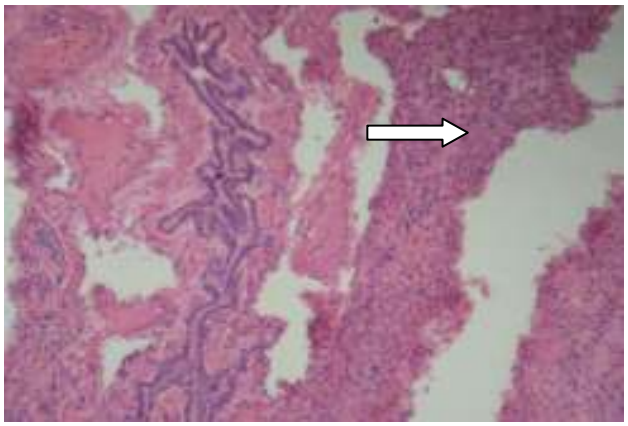


Figure 4. A piece of placenta obtained from the cow with a history of abortion and seropositivity to RBPT and i-ELISA. There was diffuse fibrosis (arrow) around the placental epithelium and in placental tissues (H&E, 10x).

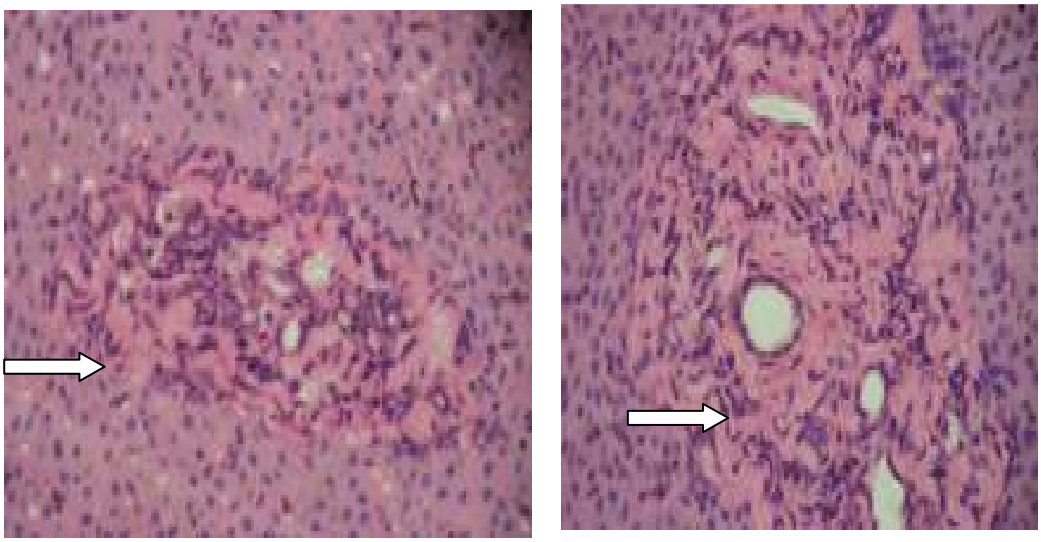


Figure 5**.** Section of a liver collected from a dead calf suspected to infect with *Brucella*. There were multifocal necrosis in hepatic parenchyma and necrosed tissue was replaced by fibrous connective tissue (In both plates). Distinct granuloma characteristics to typical brucellosis were not seen (H& E, 40x).

REFERENCES

1. Al-Attas RA, Al-Khalifa M and Alqurashi AR (2000). Evaluation of PCR, culture and serology for the diagnosis of acute human brucellosis. *Annual Saudi Medicine* 20: 224 - 228.
2. Al-Majali AM (2005). Seroepidemiology of caprine brucellosis in Jordan. *Small Ruminant Research* 58: 13 – 18.
3. Amin KMR, Rahman MB, Sarkar SK, Kabir SML and Akand MSI (2004). Serological epidemiology of brucellosis in cattle of the Mymensingh district of Bangladesh. *Journal of Animal and Veterinary Advances* 3: 898 - 900.

4. Bishop GC, Bosman PP and Herr S (1994). Bovine brucellosis. Infectious diseases of livestock with Special Reference to Southern Africa. *Vol* 2. Oxford University Press, Cape Town, 1053 - 1066.
5. CDC: Centres' for Disease Control and Prevention (2008). Laboratory-Acquired Brucellosis Indiana and Minnesota. *Emergency Infectious Diseases* 57: 39 - 42.
6. Corbel MJ, Stuart FA and Brewer RA (1983). Observations on serological cross-reactions between smooth *Brucella* species and organisms of other genera. *3th international Symposium on Brucelosis; in Develop Biology Standard* 56: 341- 348.
7. FAO (2006). Progress in understanding brucellosis. *Vet Record* 641.
8. Franz DR, Jahrling PB and Friedlander AM (1997). Clinical recognition and management of patients exposed to biological warfare agents. *Journal of American Medical Association* 278: 399 - 411.
9. Godfroid J, Bosman PP, Herr S and Bishop GC (2004). Bovine brucellosis. *Infectious Disease of Livestock* 3: 1510 - 1527.
10. Gul ST and Khan A (2007). Epidemiology and epizootology of brucellosis. *Pakistan Veterinary Journal* 27: 145 - 151.
11. Kadir (2010). Seroprevalence of Brucellosis in cattle in some of the selected areas of Naogaon District, M.S Thesis, Department of Medicine, Bangladesh Agricultural University, Mymensingh.
12. Nahar A and Ahmed MU (2009). Seroprevalence study of brucellosis in cattle and contact human in Mymensingh district. *Bangladesh Veterinary Medicine* 7: 269-274.
13. Navarro E, Casao MA and Solera J (2004). Diagnosis of human brucellosis using PCR single nucleotide polymorphisms for rapid identification of Brucella isolates at the species level. *Applied Environmental Microbiology* 73: 7331 - 7337.
14. Nicoletti P (1980). The epidemiology of bovine brucellosis. *Advances in Veterinary Science and Comparative Medicine* 24: 69 - 98.
15. Nielsen K, Kelly L, Gall D, Nicoletti P, and Kelly W (1995). Improved competitive enzyme immunoassay for the diagnosis of bovine brucellosis. *Veterinary Immunology and Immunopathology* 46: 285 – 291.
16. Nielsen K, Smith P, Widdison J, Gall D, Kelly L, Kelly W and Nicoletti P (2004). Serological relationship between cattle exposed to *Brucella abortus*, *Yersinia enterocolitica* O:9 and *Escherichia coli* O157:H7. *Veterinary Microbiology* 100: 25 - 30.
17. Nielsen K, Smith PYW, Nicolett P, Jungersen G, Stac, J and Godfroid J (2006). Serological discrimination by indirect enzyme immunoassay between the antibody response to *Brucella sp*. and *Yersinia enterocolitica* O: 9 in cattle and pigs. *Veterinary Immunology and Immunopathology* 109: 69 – 78.
18. Quinn PJ, Carter ME, Markey B and Carter GH (1994). *Brucella* species In: *Clinical Veterinary Microbiology* pp. 261-267.
19. Radostits OM, Blood DC and Hinchcliff KW (2000). *Veterinary Medicine:* A textbook of the diseases of cattle, sheep, pigs, goats and horses. 9th edn. Saunders Co. Ltd., London, 877 - 889.
20. Rahman MS, Han JC, Park J, Lee JH, Eo SK and Chae JS (2011). Prevalence of brucellosis and its association with reproductive problems in cows in Bangladesh. *Veterinary Record* 159: 432–445.
21. Rahman MS, Huque MF, Ahasan, MS, Song HJ (2010). Indirect enzyme linked immunosorbent assay for the diagnosis of brucellosis in cattle. *Korean Journal of Veterinary Service* 33: 113–119.
22. Rahman AKMA, Saegerman C, Berkvens D, Fretin D, Gani MO, Ershaduzzaman M, Ahmed MU and Emmanuel A (2013). Bayesian estimation 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. *Preventive Veterinary Medicine* 110: 242-252.
23. Refai MK (2003). Brucellosis in animals and man in Egypt. *Egypt Journal of Veterinary Science* 37: 1-31.
24. Shamim A, Alam N, Rahman MS, Rahman MA, Haque F and Hee-Jong S (2009). *Korean Journal of Veterinary Service* 32(3): 219-225.
25. Sidell FR, Takafuji ET and Franz DR (1997). Medical aspects of chemical and biological warfare. Washington, DC: US Department of Health and Human Services, Office of the Surgeon General 42: 514–521.