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Review

Brucellosis in human and domestic animals in Bangladesh: A review

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According to the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organization of Animal Health (OIE), brucellosis is considered to be the most widespread zoonosis throughout the world. It is a neglected bacterial zoonotic disease in many countries including Bangladesh. The aim of this study was to review published reports of the brucellosis in humans and domestic animals (cattle, buffalo, sheep, goats, pigs and dogs) in Bangladesh. The prevalence studies are based primarily on the following serological tests: Rose Bengal test (RBT), plate agglutination test (PAT), tube agglutination test (TAT), mercaptoethanol agglutination test (MET), standard tube agglutination test (STAT), slow agglutination test (SAT), milk ring test (MRT), indirect enzyme-linked immunosorbant assay (I-ELISA), competitive ELISA (C-ELISA), complement fixation test (CFT), fluorescent polarization assay (FPA); genus specific and species specific real time PCR. Seroprevalences of brucellosis were found to be affected by the sensitivity and specificity of serological tests employed. Brucellosis prevalence varied based on occupations of people (2.5-18.6%) and species of domestic animals (3.7% in cattle, 4.0% in buffalo, 3.6% in goats and 7.3% in sheep, 4.8% in pigs, 4% in dogs). The prevalence of brucellosis in humans was reported in farmers (2.6-21.6%), milkers (18.6%), butchers (2.5%) and veterinarians (5.3-11.1%) who have direct contact with domestic animals and their products or who consume raw milk. According to published reports, brucellosis does affect people and domestic animals of Bangladesh and there is only one published reports available on the characterization of the *Brucella* isolates of animals in Bangladesh at the species level. There is an immediate need for a concerted effort to control and eradicate brucellosis from domesticated animals in Bangladesh.

Key words: Bangladesh, brucellosis, domestic animals, prevalence.

INTRODUCTION

Early indications of brucellosis date back to the Crimean War (1853-1856) in which *Brucella* spp. was shown as the causative agent of human disease. It was first described in 1859 on the island of Malta by Marston. The first identification of *Brucella* spp. was performed by Dr. Bruce in 1887 and in 1897 Dr. Bang identified *Brucella abortus*. Because of its global expansion, *B. abortus* infection takes different names as Bang's disease, Malta

Figure 1. Aborted fetus from a cow in Bangladesh. The fetus delivered dead at 8 months of pregnancy (c.f. Dey et al., 2013).

fever or undulant fever (OIE, 2014).

According to the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organization of Animal Health (OIE), brucellosis is considered to be the most widespread zoonosis throughout the world (Mustafa and Nicoletti, 1995). This highly contagious zoonotic disease is caused by different species of the genus *Brucella*. These small, Gram negative, non-motile, facultative intracellular, non-spore forming, rod shaped coccobacilli (Baek et al., 2003; Kakoma et al., 2003) are pathogenic for a wide variety of domestic animals including cattle, buffalo, sheep, goat, dog, pig and man (Mathur, 1971). Species of *Brucella* that cause disease in domesticated animals are: *B. abortus* (cattle and buffalo), *Brucella ovis* (sheep), *Brucella melitensis* (sheep and goats), *Brucella suis* (swine) and *Brucella canis* (dog). Brucellosis is primarily a disease of the reproductive tract of domestic animals. The mammary gland is a very important source for transmission of *Brucella* because of its predilection for supramammary lymph nodes and associated shedding in milk. In animals, brucellosis mainly affects reproduction and fertility, with abortion or birth of weak offspring, retention of placenta (Figures 1, 2, 3 and 4) and reduced milk yield. Clinical signs of mastitis are seldom detectable in naturally infected cattle and goats. In man, the clinical picture resembles many other febrile diseases, but sacroiliitis and hepato-splenomegaly are the most prominent symptoms. Severe complications are endocarditis and neurological disorders (Colmenero et al., 1996).

Transmission of *Brucella* to humans results from direct contact with the infected domestic animal, consumption of unpasteurized milk and milk products (Corbel, 2006). Human brucellosis is mainly an occupational disease affecting animal caretakers, farmers, artificial inseminators, abattoir workers, meat inspectors and veterinarians due to frequent exposure to infected domestic animals (Corbel, 2006). Close contact with domestic animals may occur when humans assist animals during parturition or abortion or handling of stillbirth. Farmers and people working in abattoirs frequently have small lesions on their hands that could be the entry point for *Brucella* from infected tissues. Inhalation of *Brucella* has been reported in slaughterhouse workers where the concentration of *Brucella* can be high due to aerosol generation (Sammartino et al., 2005). Dairy farmers who milk animals by hand have a greater chance of becoming infected by the *Brucella* infected animals (Sammartino et al., 2005). Meat inspectors and artificial inseminators who do not take adequate biosafety precautions while performing their jobs are at risk of contracting *Brucella* from the infected animals (Sammartino et al., 2005). Transmission of brucellosis in domestic animal results from ingestion of contaminated feeds and water, inhalation of aerosolized bacteria, sexual intercourse and direct contact with infected placenta and uterine discharges (Corbel, 2006; Radostits et al., 2007). Vertical transmission of *Brucella* is also reported from infected cattle or dam to calf, lambs or kids and other animals (Rahman, 2004; Baek et al., 2005, Rahman and Baek, 2008a). There are two main factors associated with an animal's susceptibility to *Brucella* infection. First, brucellosis primarily affects sexually mature animals (Sammartino et al., 2005). Second, suscep-tibility dramatically increases during pregnancy (Sammartino et al., 2005). Uterine discharge and placenta expelled from infected animals are the main sources of transmission of *Brucella* to humans and animals. Understanding the mode of transmission of *Brucella* is important because it plays a key role in the disease epidemiology. Major risk factors of animal infection are the husbandry practices, local habits and management of the herd/flock. Environmental factors that affect the ability of *Brucella* to survive outside the mammalian hosts are to be considered in the epidemiology of brucellosis. High humidity, low temperature and absence of direct sun light may favor survival of

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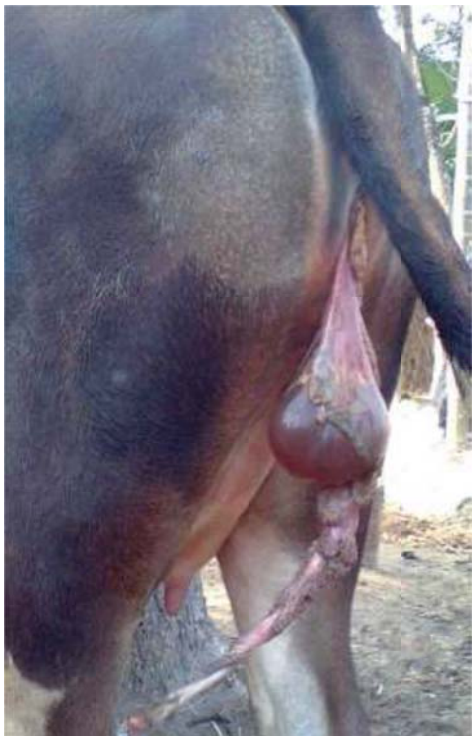

Figure 2. The placenta of the cow failed to discharge after 24 hours of abortion in Bangladesh (c.f. Dey et al., 2013).

Figure 3. Abortion in black Bengal goat from Bangladesh.

Brucella for several months in water, aborted fetuses, placental membranes, liquid manure, hay, buildings, equipment and clothes (Sammartino et al., 2005).

Figure 4. Abortion in sheep from Bangladesh.

Human brucellosis poses major economic and public health challenges in affected countries especially in the Mediterranean countries of Europe, northern and eastern Africa, Near East countries, India, Central Asia, Mexico, and Central and South America (Pappas et al., 2006). Human brucellosis remains the most common zoonotic disease worldwide, with more than 500,000 new cases reported annually (Pappas et al., 2006). Globally this disease is woefully under-reported because of its vague clinical flu like symptoms, difficulty in laboratory diagnosis and lack of familiarity by medical professionals (Corbel, 2006). Therefore, the true incidence of human brucellosis is unknown for most developing countries of the world including Bangladesh. Data documenting human brucellosis are very meager in Bangladesh. Published reports indicate that it is an occupational disease among farmers, milkers, butcher and veterinary practitioners in Bangladesh (Nahar and Ahmed, 2009; Muhammad et al., 2010; Rahman 2011a).

Diagnosis of brucellosis in humans and domestic animals is mainly based on detection of *Brucella* lipopolysaccharide (LPS) specific antibodies in milk and serum samples using serological tests. Serological tests are commonly used for *Brucella* diagnosis in cattle and small ruminants at herd level. The sensitivity of RBT fulfills the requirements for surveillance at flock level (European Commission Regulation, 2002). The SAT is an easy to use screening method with a good sensitivity but lower specificity. CFT is an OIE mandatory test for international trade (Nielsen, 2002). None of the above

mentioned tests can distinguish between antibodies produced after vaccination and those due to field infection (Nielsen et al., 1989). Different enzyme-linked immunesorbent assays (ELISA) have been developed to overcome these problems and are capable of detecting *Brucella* carriers being seronegative by RBT, SAT and CFT (Van Aert et al., 1984). Nowadays, real time (RT) PCR methods are used to amend serological diagnostics. DNA of *Brucellae* can readily be detected from serum of infected animals even if blood culture fails. Additionally, species differentiation out of serum using IS711 species specific RT PCR is possible (Rahman et al., 2013a). The genomes of several *Brucella* species have been sequenced and different molecular methods including multiple locus variable number repeat analysis (MLVA) has been developed for species identification and subspecies genotyping (Le Fleche et al., 2006). Genotyping may be used to study the diversity of genotypes and to trace source of infection (Kattar et al., 2008).

In this review, we summarized the published literatures on human and domestic animal brucellosis in Bangladesh and we recommended strategies to control brucellosis in Bangladesh.

BRUCELLOSIS IN HUMAN

Approximately 2.4 billion people are at risk of getting brucellosis every year in the world. Bangladesh is situated in the northern part of south Asia between 20°38′ and 26°38′ north latitude and between 88°01′ and 92°41′ east longitude. Bangladesh has one of the highest popu-lation densities in the world (1015 per sq km). It has 147,570 km^2 area of land with seven divisions.

Rahman (1983) conducted the first sero-prevalence study of brucellosis in humans in Bangladesh. This study recorded 12.8% prevalence of brucellosis in dairy and agricultural workers and 21.6% prevalence among goat farmers.

Nahar and Ahmed (2009) carried out a seroprevalence study using RBT and STAT on 50 human sera. The study recorded *Brucella* positive specimen in animal owners (1 of 7), animal attendants (1 of 13) and veterinary students (1 of 26). Muhammad et al. (2010) analyzed 210 human sera of people at risk in the Mymensingh district using a variety of *Brucella* serological tests. Seroprevalences among occupational groups were 11.1% in veterinary personnel, 6.5% in dairy workers and 4.7% in animal farmers.

Rahman et al. (2012a) conducted a study to determine the seroprevalence of brucellosis in a high-risk exposure group of individuals (n = 500). The prevalence of brucellosis was 2.6% in farmers, 18.6% in milkers, 2.5% in butchers and 5.3% in veterinary practitioners. The prevalence was higher in males (5.6%) than females (0.8%). The highest prevalence was recorded in Dhaka district (24%) followed by Mymensingh district (2.9%). Higher prevalence was recorded in farmers handling goats (8.5%) as compared to farmers handling cattle and goats (4.7%) or cattle only (3.5%). The prevalence was higher in individuals with the history of drinking raw milk (11.4%) than individuals not drinking raw milk (3.9%). The highest prevalence was recorded in individuals (16.2%) having contact with animals for more than 26 years. The prevalence was higher in 41-80 years age group (6.2%) followed by 21-40 years group (3%) and 14-20 years age group (2.3%), respectively. The study emphasized that contact especially with goats, is a significant risk factor for infection with *Brucella* of individuals in high-risk group.

The results of all seroprevalence studies indicated that brucellosis is an occupational health hazards in Bangladesh among milkers, farmers and veterinarians. The type of animals owned or handled, and duration of contact with domestic animals and consumption of raw milk are the risk factors associated with brucellosis in humans in Bangladesh (Rahman et al., 2012a).

BRUCELLOSIS IN CATTLE

Cattle constitute the major domestic animal in Bangladesh. Most of the households in the villages of Bangladesh rear cattle and Bangladesh has 23.4 million cattle. Cattle reared in Bangladesh are mainly indigenous zebu, some exotic breeds and their crosses predomi-nantly Holstein-Friesian, Jersey, Sahiwal and Sindhi. Dairying is part of the mixed farming systems and a predominant source of income, nutrition and jobs and a strong tool to develop a village micro economy of Bangladesh in order to improve rural livelihoods and to alleviate rural poverty. One of the infectious diseases, which are a major constraint for dairy animal productivity, is brucellosis. Brucellosis in dairy cattle is caused by *B. abortus* (Rahman, 2011a, b)

Brucellosis in cattle in Bangladesh was first reported by Mia and Islam (1967). Prevalence of brucellosis in cattle was demonstrated as 18.4% (Rahman and Mia, 1970). Prevalence was also reported from milk samples in dairy farms as 11.4, 11.7 and 4.2% in Savar, Tangail and BAU dairy farms, respectively (Rahman et al., 1978). Milk samples of cattle provided 5.5 and 11.4% prevalence rates of brucellosis in BAU dairy farm and central cattle breeding and dairy farm (CCBDF) Savar, respectively (Rahman and Rahman, 1981).

Prevalence of brucellosis in cows on dairy farms of Pabna, Faridpur and Bogra districts were 11.5, 2.9 and 2.0%, respectively (Rahman and Rahman, 1982). The annual economic loss in Bangladesh due to bovine brucellosis in indigenous cows was 720,000 EUR (total) and 12000 EUR per 1000 cross-bred cows and a total of 276000000 EUR in cross-bred cows in Bangladesh (Islam et al., 1983).

Islam et al. (1992) recorded 15% prevalence of brucellosis in exotic breed of cows and 9% in local cattle breed after screening 760 sera of cows from Avoynagar, Puthia, Hazirhat, Comilla, Manikgonj and Moshurikhola of

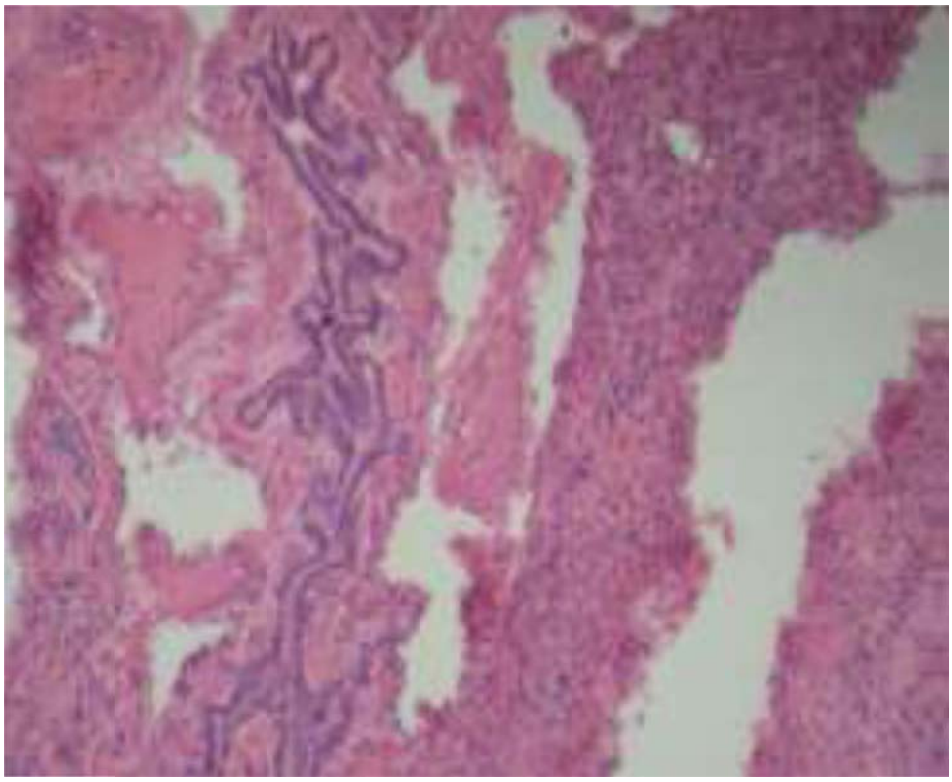

Figure 5. A piece of placenta obtained from the cow with a history of abortion and seropositivity to RBPT and I-ELISA. There was diffuse fibrosis around the placental epithelium and in placental tissues (H & E, 10x) (c.f. Dey et al., 2013).

Bangladesh by rapid screening test and tube agglutination test (TAT). Ahmed et al. (1992) reported 5% prevalence of brucellosis in dairy farms and 2.8% prevalence in rural cows by plate agglutination test (PAT) and TAT. This study recorded 3.2% prevalence of brucellosis in pregnant cow and 3.1% in non-pregnant cows. Prevalence of brucellosis was higher in cows above 3 years age (4.8%) than cows less than 3 years (0.7%). The prevalence of brucellosis was 9.1% in cows with a history of previous abortion.

Rahman et al. (2006) reported the prevalence of brucellosis and its association with reproductive problems in cows in Bangladesh. The prevalence of the disease among the 260 rural cows was 3·08% by the RBT and the PAT, and 1·92% by the TAT. The difference between the two groups was not statistically significant. In cows with a history of retained placenta, the prevalence of brucellosis was 13·04% by the RBT and PAT, and 8·70% by the TAT. The prevalence of brucellosis in repeat breeding cases was 1·45% by all three serological tests. There was a statistically significant difference in the prevalence of the disease between cows with a history of retained placenta and repeat breeding cases ($P<0·05$). The prevalence of brucellosis in cows that had mastitis was 4·76% by the RBT and PAT, but was not demonstrated by the TAT.

During the period of 2004-2012, a total of 1487 serum or milk samples were obtained from cattle in six districts of Bangladesh and overall prevalence of brucellosis 4.2% was found in Mymensingh, 8% in Dinajpur, 1.1% in Bagherhat, 5% in Chittagong and 0% both in Bogra and Gaibandha districts (Amin et al., 2004; Nahar and Ahmed, 2009; Ahasan et al., 2010; Rahman et al., 2012b; Sikder et al., 2012). Dey et al. (2013) recorded serological and pathological investigations of brucellosis in dairy cows in Bangladesh. Out of 190 randomly sera sample tested, prevalence was 2.63% by RBPT and 1.05% by I-ELISA. Histopathological study of placenta from an aborted cow and spleen and lymphnode and liver from an aborted fetus were performed. During histopathological study there was depletion of lymphocytes in spleen and lymphnodes which was characterized by reducing densities of lymphocytes. The smooth muscular trabeculi in spleen and fibromuscular trabeculi in lymphnode were distended. In placenta, there was diffuse fibrosis around the placental epithelium. The liver of aborted fetus showed multifocal necroses in hepatic parenchyma and necrosed tissue was replaced by fibrous connective tissue and reactive cells (Figures 5, 6, 7 and 8).

Rahman et al. (2013a) recorded 5.29% prevalence of brucellosis in 700 cattle sera by RBT. RBT positive samples were retested by CFT, SAT, ELISA and real

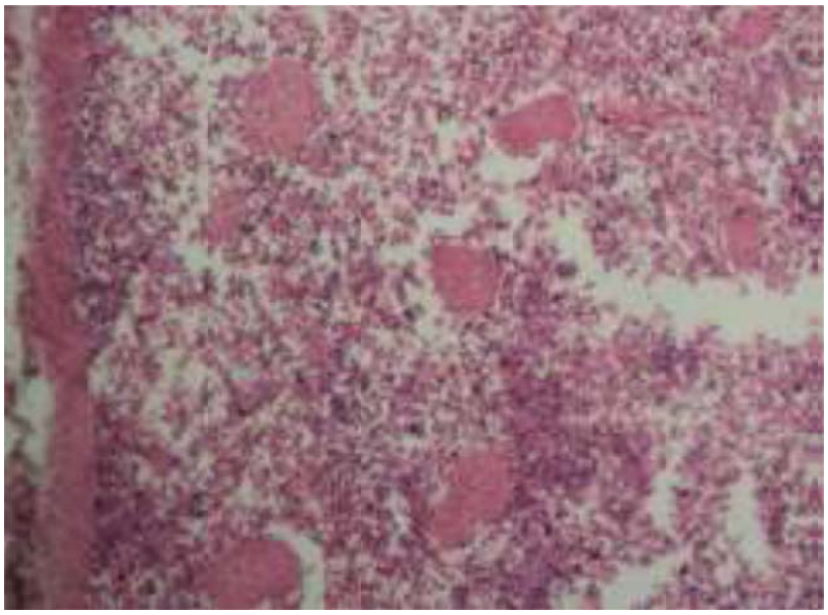

Figure 6. Spleen obtained from a dead calf delivered from *Brucella* seropositive cow and stained with H & E. There were depletion of lymphocytes in spleen and distended trabeculi (10x) (c.f. Dey et al., 2013).

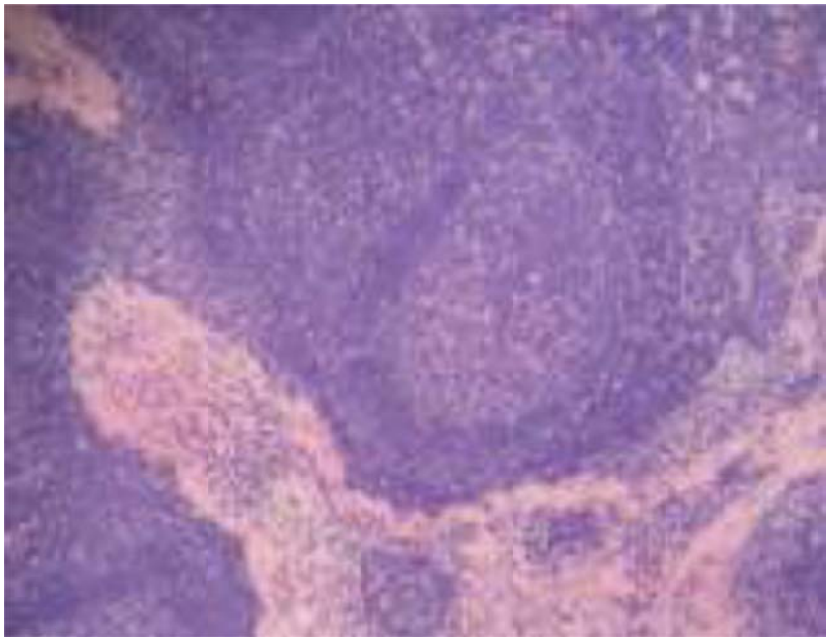

Figure 7. Lymph node obtained from a dead calf delivered from *Brucella* seropositive cow and stained with H&E. There were depletion of lymphocytes in lymphnode and distended trabeculi (10x) (c.f. Dey et al., 2013).

time PCR. *Brucella* DNA was found in 4.43 % of the cattle by genus specific real time PCR of which *B. abortus* DNA was found in 1.9% of cattle by species specific real time PCR in Kurigram and Mymensingh districts of Bangladesh (Figure 9) (for amplification of real time PCR). Rahman et al. (2013b) reported the prevalence and diagnostic test comparison of brucellosis in cattle in Pabna and Mymensingh districts of Bangladesh. The seroprevalence of brucellosis in cattle was estimated to be 4.23, 3.07 and 2.31% by RBT, SAT and I-ELISA,

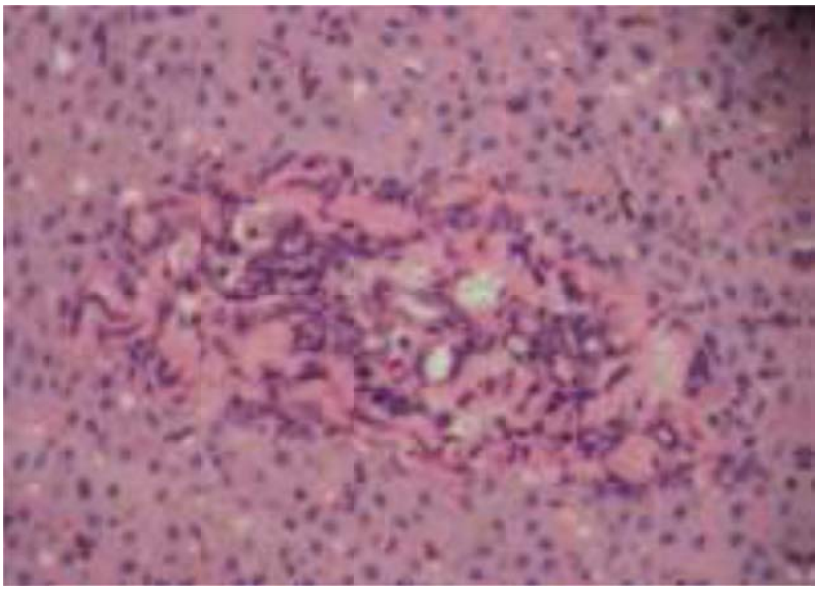

Figure 8. Section of a liver collected from a dead calf suspected to BE infectED with *Brucella*. There were multifocal necrosis in hepatic parenchyma and necrosed tissue was replaced by fibrous connective tissue (H & E, 40x) (c.f. Dey et al., 2013).

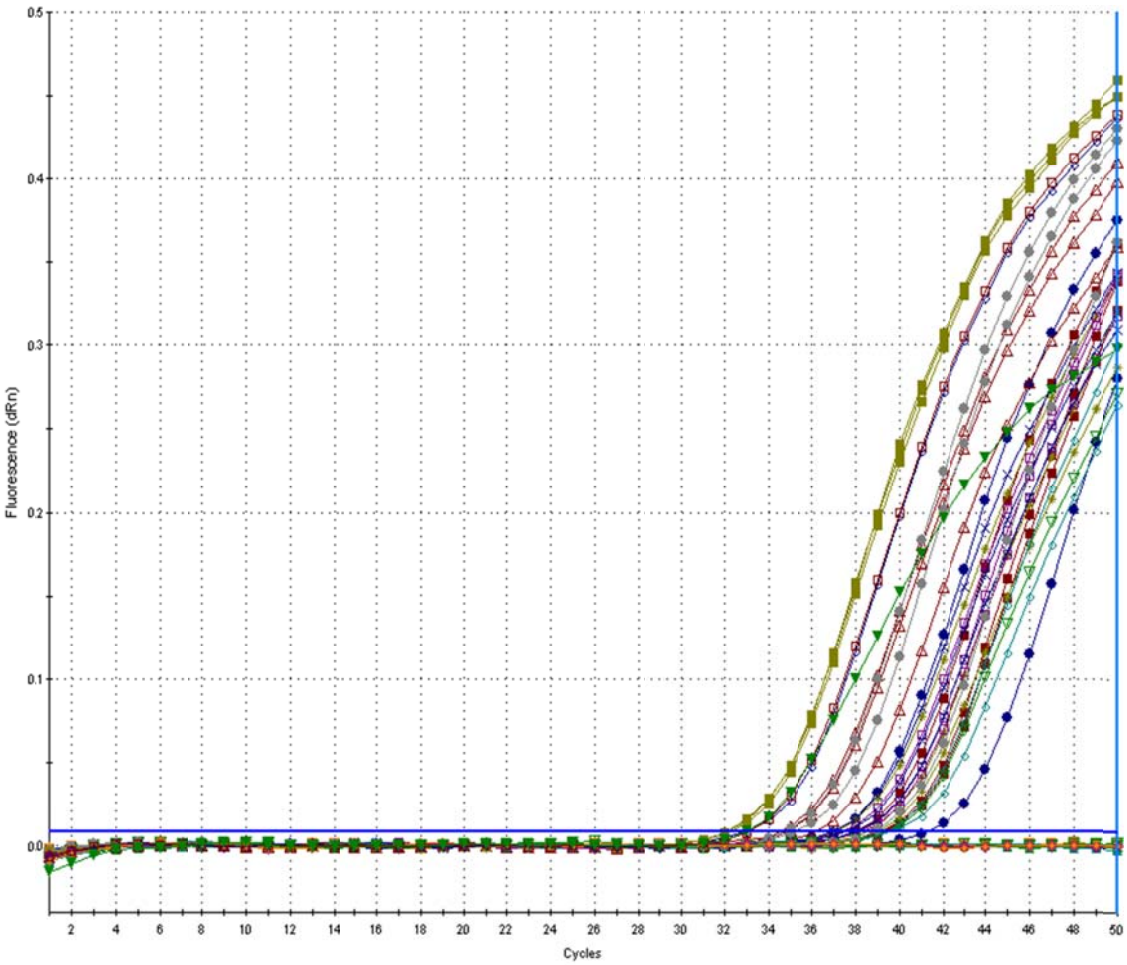

Figure 9. Amplification plots in real time PCR with the cattle and buffalo sera of Bangladesh.

Table 1. Prevalence of brucellosis in cattle in seven districts of Bangladesh.

District	No. tested	No. positive	Positive (%)	Reference
Mymensingh	250	5	2.00	Amin et al. (2004)
Mymensingh	200	9	4.50	Nahar and Ahmed (2009)
Mymensingh	132	14	10.60	Ahasan et al. (2010)
Mymensingh	100	7	7.00	Rahman et al., (2013a)
Mymensingh	190	5	2.63	Dey et al. (2013)
Dinajpur	50	4	8.00	Ahasan et al. (2010)
Mymensingh	135	2	1.50	Rahman et al. (2012b)
Bogra	60	0	0.00	Rahman et al. (2012b)
Gaibhandha	70	0	0.00	Rahman et al. (2012b)
Bagherhat	90	1	1.10	Rahman et al. (2012b)
Chittagong	500	25	5.00	Sikder et al. (2012)
Kurigram	600	30	5.00	Rahman et al., (2013a)

respectively. The comparison of the serological tests result revealed the highest prevalence in RBT than SAT and I-ELISA. The prevalence of *Brucella* was 2.5% in Pabna and 2.14% in Mymensingh. It was observed that, a higher prevalence of *Brucella* was found in female (2.67%) than in male (1.82%), natural breeding (2.67%) than artificial breeding (1.81%), in aged animals (3.33%) than young (1.25%). But these differences were not statistically significant. There exists significant difference between prevalence of brucellosis in cattle with history of abortion than without history of abortion. See Table 1 for prevalence of brucellosis in cattle in different districts of Bangladesh.

BRUCELLOSIS IN BUFFALO

Asia is the native home of the water buffalo, with 95% of the world population, with about half of the total in India and Bangladesh. It is valuable for its meat and milk, as well as the labour it performs. It is often referred to as “the living tractor of the East”, as it is relied upon for plowing and transportation in many parts of Asia including Bangladesh (Rahman, 2012a).

Buffaloes are known to be affected with *B. abortus* and less frequently with *B. melitensis* (Munir et al., 2008; Ahmed et al., 2010). Similar to cattle, *Brucella* infections are known to result in late gestation (6-9 months) abortions (Sanjrani et al., 2013), infertility (Sukumar et al., 2012) and latent infection of mammary gland lymph nodes with shedding of organisms in the milk (Ahmed et al., 2010), yet abortions are less common in buffaloes (The Center for Food Security and Public Health Iowa State University, 2009) with the disease being endemic in most buffalo raising countries. Shedding of *Brucella* in milk creates a potential threat to human health particularly for consumers using unpasteurized milk and milk products (Ahmed et al., 2010). A slightly lower incidence of brucellosis has been recorded in buffaloes as compared to cattle in studies that simultaneously evaluated the serologic presence of brucellosis in these two species (Hussain et al., 2008), however, in other studies, a higher incidence of the disease was recorded in buffaloes as compared to cattle (Nasir et al., 2004). Thus, it can be presumed that buffaloes are differentially affected with *B. abortus*.

The first report on the occurrence of brucellosis in buffaloes appears to have originated in India in 1918 at the Indian Veterinary Research Institute, Mukteshwar (Anonymous, 1918). The first seroprevalence study of brucellosis in buffalo in Bangladesh was conducted by Rahman et al. (1997) in selected areas of Bangladesh. The overall seroprevalence in buffalo was 6.9% by PAT and 2.4% by TAT. The prevalence of brucellosis was 7.1 and 1.2% in buffalo with history of retained placenta and repeat breeding, respectively.

Rahman et al. (2012b) screened 135 sera of buffaloes from five districts of Bangladesh and found prevalence of brucellosis in Bagherhat, Mymensingh and Sirajgonj districts as 2.9, 8.3 and 5.3%, respectively. No prevalence of brucellosis was recorded in buffalo both in Bogra and Gaibandha districts. Age and sex as potential risk factors for brucellosis in buffalo was analyzed by Rahman et al. (2011a). Recently Rahman et al. (2013a) recorded prevalence of brucellosis in 99 buffaloes sera of Bagerhat and Mymensingh using RBT, SAT, CFT, I-ELISA, genus specific and species specific real time PCR (see Figure 9 for amplification of real time PCR). The presence of *Brucella* DNA was found in 7.1 % of the buffaloes investigated and *B. abortus* DNA was found in 6.1% of the buffaloes. Prevalence of brucellosis in buffaloes in different districts of Bangladesh is shown in Table 2.

BRUCELLOSIS IN GOAT

Economically and culturally, the goat has played an important role in traditional Bengali society. Among the Asiatic countries, Bangladesh, a tropical agro-based developing country, possess the third largest repository

Table 2. Prevalence of brucellosis in buffalo in five districts of Bangladesh.

District	No. tested	No. positive	Positive (%)	Reference
Bagherhat	70	2	2.85	Rahman et al. (2012b)
Bagherhat	80	5	6.50	Rahman et al. (2013b)
Bogra	20	0	0.00	Rahman et al. (2012b)
Gaibandha	14	0	0.00	Rahman et al. (2012b)
Mymensingh	12	1	8.33	Rahman et al. (2012b)
Mymensingh	19	2	10.52	Rahman et al. (2013a)
Sirajgonj	19	1	5.26	Rahman et al. (2012b)

Table 3. Prevalence of brucellosis in goats in eight districts of Bangladesh.

District	No. tested	No. positive	Positive (%)	Reference
Mymensingh and Dhaka	300	5	1.67	Uddin et al. (2007b)
Dhaka and Lalmonirhat	20	0	0.00	Das et al. (2008)
Mymensingh	208	8	3.85	Islam et al. (2010)
Bogra and Mymensingh	120	7	5.83	Rahman et al. (2011b)
Bagherhat	15	1	6.67	Rahman et al. (2012b)
Bogra	30	0	0.00	Rahman et al. (2012b)
Gaibandha	50	2	4.00	Rahman et al. (2012b)
Mymensingh	100	4	4.00	Rahman et al. (2012b)
Sirajgonj	35	1	2.86	Rahman et al. (2012b)
Nilphamari	154	5	3.24	Rahman et al. (2012c)

of goats, with a population of more than 34 million heads, according to the FAO (WHO, 2006). This figure represents more than 57% of total livestock in Bangladesh. More than 90% of the goats of the country are of the Black Bengal breed. Each year goat production provides 127,000 MT meat, which accounts for 25% of total red meat in Bangladesh (Bangladesh Economic Review, 2012). As goats come in very close contact with humans, the risk of transmitting this zoonosis is very high (Rahman, 2012b).

Serological prevalence (14.5%) of brucellosis in goats in Bangladesh was first reported by Rahman (1983). A higher incidence of the disease was observed in goats with reproductive disorders (Rahman et al., 1988). Overall prevalence of brucellosis in goats were 1.7% in Mymensingh and Dhaka districts, 0% in Dhaka and Lalmonirhat districts, 3.9% in Mymensingh district, 5.8% in Bogra and Mymensingh districts, 6.7% in Bagherhat districts, 0% in Bogra district, 4% in Gaibandha district and 2.9% in Sirajgonj district (Uddin et al., 2007a, b; Das et al., 2008; Islam et al., 2010; Rahman et al., 2011b, 2012b). Potential risk factors for brucellosis in goats included age, sex, pregnancy status, management system (concrete floor versus mud floor; flock rearing vs. individual rearing; non grazing versus free grazing; mixed with cattle versus without cattle; and rural versus farm goats) and reproductive disorders (abortion vs. retained placenta). Prevalence odds of brucellosis in goats that are pregnant were 7 times greater than the prevalence odds of brucellosis for goats that are not pregnant. Rahman et al. (2012c) found overall seroprevalence of brucellosis 59% in Black Bengal goats in Nilphamari district of Bangladesh. A significantly ($p<0.01$) higher prevalence of brucellosis was found in Black Bengal goats with the history of previous abortion (33.33%). An insignificant ($p>0.05$) but higher prevalence of brucellosis was found in adult Black Bengal goats (>24 months) than young ones. The prevalence was relatively higher in cross-bred than pure Black Bengal goats, in female than male and in pregnant than non-pregnant Black Bengal goats. Prevalence of brucellosis in goats in different districts of Bangladesh is shown in Table 3.

BRUCELLOSIS IN SHEEP

Among the livestock populations, sheep still occupies the third position and about 80% sheep is reared by rural farmers in Bangladesh. The sheep in Bangladesh are mainly indigenous and utilized for meat purposes but also important for good quality leathers and source of income to rural people. In Bangladesh, sheep and goats are a very valuable asset especially for poor people. Most cases of brucellosis infection in sheep are inapparent and lack

Table 4. Prevalence of brucellosis in sheep in six districts of Bangladesh.

District	No. tested	No. positive	Positive (%)	Reference
Mymensingh and Dhaka	62	3	4.84	Uddin et al. (2007a)
Bogra and Mymensingh	80	3	3.75	Rahman et al. (2011b)
Bagherhat	27	3	11.11	Rahman et al. (2012b)
Bogra	30	1	3.33	Rahman et al. (2012b)
Gaibandha	35	2	5.71	Rahman et al. (2012b)
Gaibandha	206	7	3.39	Rahman et al. (2012d)
Mymensingh	40	8	20.00	Rahman et al. (2012b)
Sirajgonj	38	2	5.26	Rahman et al. (2012b)

Table 5. Prevalence of brucellosis in pigs in 2 districts of Bangladesh.

District	No. tested	No. positive	Positive (%)	Reference
Bogra	62	3	4.80	Rahman (2011c)
Bogra	63	4	6.60	Rahman et al. (2012e)
Sirajgonj	41	2	4.80	Rahman (2011c)
Sirajgonj	42	3	7.10	Rahman et al. (2012e)

clinical signs. Serological evidence of brucellosis in sheep in Bangladesh was first reported by Uddin et al. (2007b). *Brucella* antibodies were prevalent in 8.84% sheep.

The overall prevalence of brucellosis in sheep (n = 312) reported by Rahman et al. (2011b, 2012b) from Mymensingh and Dhaka, Bogra and Mymensingh, Bagherhat, Bogra, Gaibandha, Mymensingh and Sirajgonj districts was 4.8, 3.8, 11.1, 3.3, 5.7, 20 and 5.3%, respectively. Prevalence odds of brucellosis in sheep that are greater than 2 year of age were 90 times greater than the prevalence odds of brucellosis for sheep that are less than or equal to 2 years (Rahman et al., 2011a, b). Further investigation by Rahman et al. (2012d) recorded seroprevalence of brucellosis in sheep in the Gaibandha districts of Bangladesh as 3.39% by RBPT and 2.91% by i-ELISA. The prevalence of brucellosis was higher in female sheep (3.41%) than male (3.33%) and in sheep with history of abortion (4.34%) than without history of abortion (3.08%). The higher rate (4.59%) of *Brucella* antibody was recorded in sheep of 1-2 years of age. Prevalence of brucellosis in goats in different districts of Bangladesh is shown in Table 4.

BRUCELLOSIS IN PIGS

Brucellosis in pigs is caused by *B. suis*. The capability of *B. suis* to colonize the bovine udder with subsequent shedding in milk means that it has the potential to be a serious human health risk. Outbreaks in slaughter houses have been caused by inhalation of *B. suis*. Most cases occur in people employed in meat processing industry and animal rearing (Radostits et al., 2007). Though, out of 590 million pigs in the world, about 34% are raised in tropical countries. From to the religious point of view and for the limited number of pork consumers, the pig population is not large as compared to other ruminants and birds in Bangladesh. Furthermore, it is difficult to get the exact number of pigs in Bangladesh. But the pig population is increasing in the tribal areas. The pig rearing continues to be primitive scavenging in nature because they are raised by certain rural people who are educationally, economically and socially most backward. Serological evidence of brucellosis in pig in Bangladesh was first reported by Rahman (2011c).

Further serological status of brucellosis in pigs was diagnosed by Rahman et al. (2012e) in Bangladesh using RBT and SAT. Overall seroprevalence was 6.7 and 4.8% by RBT and SAT, respectively. It was observed that, insignificantly higher prevalence of brucellosis based on SAT was found in female (5.6%) than male (2.9%) in aged animal (8.1%) than young (0.0%) and in pregnant animal (12.5%) than non pregnant animal (2.1%) ($p>0.05$). Prevalence of brucellosis was 42.9% in aborted pigs and 1.6% in non aborted pigs. The association between abortion status and prevalence of brucellosis was statistically highly significant ($p<0.01$). Prevalence of brucellosis in pigs in different districts of Bangladesh is shown in Table 5

BRUCELLOSIS IN DOGS

Dogs fill a variety of roles in human society and are often trained as working dogs. The most important role of dogs is as companion. Dogs have lived with and worked with humans in so many roles that their loyalty has earned

Table 6. Prevalence of brucellosis in dogs in 2 districts of Bangladesh.

District	No. tested	No. positive	Positive (%)	Reference
Mymensingh	30	4	13.33	Talukder et al. (2011)
Dhaka	50	2	4.00	Rahman (2014b)

them the sobriquet man's best friend. Dog population in Bangladesh may be considered as a carrier of *Brucella* infection and might act as a risk for food animal and human health (Rahman, 2014a). Dogs may become infected through ingestion of infected bovine placental tissue. *Brucella* infected dogs may abort and vaginal discharges have a potential for transmitting *Brucella* to susceptible animals. Both *B. abortus* and *B. melitensis* infection have been reported in dogs kept on farms (Baek et al., 2003).

The first report on the sero-prevalence of brucellosis in stray dogs of Bangladesh by using four commercial sero-diagnostic kits was conducted by Talukder et al. (2011). The overall sero-prevalence of canine brucellosis was recorded as 13.33, 6.67, 6.67 and 10.0% with RBPT, SAT, STAT and ELISA, respectively. Significantly ($p<0.01$) higher sero-prevalence rate of canine brucellosis was recorded in stray dogs aged between 7 and 36 months (14.81, 7.40, 7.40 and 11.11%) in comparison with aged group up to 6 months (0, 0, 0 and 0%) with RBPT, SAT, STAT and ELISA, respectively. The sero-prevalence rate of canine brucellosis was found significantly ($p <0.01$) higher in female dogs (15.78, 10.52, 10.52 and 15.78%) in comparison with male (9.09, 0, 0 and 0%) with RBPT, SAT, STAT and ELISA, respectively.

Rahman (2014b) conducted a serological study for a total of 50 pet dog's serum samples collected from Dhaka, Bangladesh. The overall seroprevalence of brucellosis in pet dogs was found to be 4.00%. Statistically significant higher seroprevalence of brucellosis (RBPT and ELISA, 6.06% respectively) was found in dog aged 1.5 to 2.5 years. Higher seroprevalence (15.38%) was found in female pet dogs and no response in male pet dogs. Prevalence of brucellosis in dogs in different districts of Bangladesh is shown in Table 6.

Recommended strategy to control brucellosis in Bangladesh

It is important to remember that brucellosis is an important zoonosis and nearly every case of human brucellosis has an animal origin and, therefore, control is primarily a veterinary responsibility (Nicoletti, 1992). The *Brucellae* are 'survivors' in both extracellular and intracellular environments. Compatible relationships with the hosts including variable incubation periods, asymptomatic carriers and resistance to treatments are the important problems. The animal husbandry factors such as commerce, nomadism, commingling and increasing population sizes assure difficulties in control of diseases.

The serosurveillance studies of brucellosis in humans and animals suggest that brucellosis is endemic in the surveyed areas of Bangladesh. Without control measures, the infected domestic animals will continue to serve as reservoirs for the spread of the disease to uninfected domestic animals and humans.

CONTROL OF BRUCELLOSIS IN HUMANS

Public health education

Efforts should be focused on the public health education regarding the disease and its risk factors. The duration of contact with animals and the type of animal handled appeared to be the most significant risk factors for human brucellosis in Bangladesh (Rahman et al., 2012a). Exposure could be minimized by educating individuals within the high risk group (Rahman et al., 2012a).

Food safety

Brucella spp. are readily killed by pasteurization or heating of raw milk. Pasteurization process is not available in all parts of Bangladesh. Boiling or heating of milk at 80-85°C (176-185 8°F) for several minutes will kill the *Brucella* (Corbel, 2006).

Personal hygiene

Protective clothes such as overalls, rubber gloves and rubber boot should be used during handling of domestic animals. If gloves are not available, washing of hands with soap and water immediately after examination is recommended. Consuming of food and smoking must be forbidden in the abattoirs while handling domestic animals (Sammartino et al., 2005).

Improved diagnostic and treatment facilities

Brucellosis in humans is under-reported globally (Corbel, 2006) and likely under-reported in Bangladesh as well. Due to the scarcity of diagnostic and medical tools, treatment of brucellosis is often not possible. Appropriate test facilities for early and accurate diagnosis of brucellosis and prescription of effective antimicrobial treatment regimen must be included in the human health care system of Bangladesh.

Collaboration between human and veterinary medicine

Control of brucellosis in domestic animals is the key to decreasing human cases since it is transmitted to humans from infected domestic animals and their products (Jiang and Baldwin, 1993). Collaboration between the department of health and department of livestock services are important to control brucellosis in domestic animals and thereby eliminate transmission to humans. Veterinary medicine must implement methods to control/eradicate brucellosis in domestic animals while human medicine must develop complementary methods to prevent transmission and develop effective treatment of human patients. So it is critical that physicians and veterinarians cooperate in these efforts.

CONTROL OF BRUCELLOSIS IN DOMESTIC ANIMALS

Surveillance program

Surveillance is important for determining prevalence and thereby allow for the development of preventive and control measures and eventual eradication of brucellosis in domestic animals. Brucellosis is primarily diagnosed by serological tests and rapid screening tests can be done by either RBT or PAT in the field. Conventional serological tests like rivanol, 2-MET and complement fixation tests (CFT), I-ELISA, C-ELISA and FPA are used as confirmatory tests. An excellent surveillance option is testing bulk tank milk samples among dairy herds by MRT (Sarker et al., 2014). The appropriate places for testing animals are slaughterhouses, livestock markets or any livestock sale station. This surveillance will help trace-back the infected animals to the herd or flock of origin. The polymerase chain reaction (PCR) can be used for identification of *Brucella* species or biovars and would be useful for epidemiological trace-back in a brucellosis control program (Rahman et al., 2013a).

Control of unrestricted animal movements

The initial introduction of disease into a herd or flock is often due to replacement animals introduced from an infected herd or flock of unknown disease status (Crawford et al., 1990). Implementation of quarantine and serosurveillance of the new replacement animals before they enter the farms and checking the imported animals at border check points before entering into the country are required to ensure that these animals are free from brucellosis.

Epidemiological investigations

Animal age, sex, gestation stage, virulence of the pathogen, environmental conditions affect exposure to infection (Nicoletti, 1984). A detailed epidemiological investigation focusing on host, agent and environment factors needs to be performed throughout the country in order to identify the risk factors associated with transmission and maintenance of brucellosis in animals.

Investigation of causes of abortion

Making animal abortion notifiable and investigations into the causes of abortion help identify not only *Brucella* but also allow for the identification of other causative agents. This method of detection relies on compliance by farmers and veterinarians provided enough resources are available to conduct investigations following a report of an abortion (Crawford et al., 1990).

Improved animal management practices

The practice of mixing of cattle, either through grazing or sharing of watering points, is a significant risk factor for brucellosis (Crawford et al., 1990). Avoiding mixing of replacement cattle without screening for brucellosis and promoting the use self-contained units instead of shared facilities could help control brucellosis. In case of abortion, the aborted fetus must be properly disposed under biosafety precautions. Avoid burying infected fetuses because dogs and other wild animals may dig them up and disseminate the disease. Any entrance where the animals are located must use step in tanks on the floor filled with disinfectant.

Training of farmers

Training of the livestock farmers on the effective implementation of sanitary and hygienic livestock management practice following abortion helps reduce spreading the disease amongst animals as well as to the humans. Education of the farmers and animal care workers on the basic hygiene and sanitary procedures and techniques as well as practical demonstration on the use of disinfection and personal protection methods are important (Sammartino et al., 2005).

Use of vaccines

The use of vaccines is one of the important measures for prevention and control of brucellosis. In areas with endemic brucellosis only vaccination will control brucellosis. *Brucella* vaccines in use for livestock are the *B. melitensis* Rev 1, live *B. abortus* strain 19, and *B. abortus* strain RB51. The Rev 1 vaccine is a modified live *B. melitensis* vaccine used in small ruminants between the ages of three and four months that confers immunity for three to five years

(Blasco and Molina-Flores, 2011). Use of strain RB51 vaccine in cattle could be a good choice for control of brucellosis in Bangladesh. The use of strain RB51 has been shown to help prevent, control and eradicate cattle brucellosis in the countries where it has been adapted (Luna-Martinez and Mejia-Teran, 2002; Rahman and Baek, 2008b; Rahman 2011d).

Test and slaughter

In order to be a cost-effective disease control measure, test and slaughter is best implemented in areas where there is a less than two percent prevalence of brucellosis in the flocks and herds (Corbel, 2006). In developing countries, test and slaughter can be difficult to carry out due to enormous cost involved in the indemnification paid to the farmers for slaughtered animals (Blasco and Molina-Flores, 2011).

Enhanced biomedical research

Biomedical research focusing on epidemiology, isolation and characterization of field isolates, development of the best diagnostic method and more effective vaccines against brucellosis in non-bovine species should be undertaken. To date, only one published reports is available on the characterization of the *Brucella* isolates of animals in Bangladesh at the species level (Rahman et al., 2013a).

Government commitment

Regulations and adequate monetary support (political will) from the local and national government organizations strengthen the collaboration among farmers, veterinarians and regulators that are essential for effective implementtation of a country-wide brucellosis control and eradication program.

CONCLUSIONS

Brucellosis is considered as a neglected bacterial zoonotic disease in Bangladesh and it is present in the domesticcated animal species and humans in surveyed regions of Bangladesh. The differences in seroprevalence of brucellosis in the study areas may be linked to ecological factors, differences of animal's density and husbandry practices and type of serological tests. The variation of prevalence of brucellosis between animals on farms and domestic holding are likely to be attributed to certain risk factors such as cattle management practices, population dynamics and biological features (for example, host immunity) that largely influence the epidemiology of *Brucella* spp. Prevalence of brucellosis is higher in sexually mature and pregnant animals, in female than male, in animals with history of abortion than without history of abortion. A detailed and statistically valid surveillance study of brucellosis in high-risk group of people and domesticated animals throughout the country is necessary to know actual disease burden. Public health education for target groups of people, understanding the risk factors of brucellosis, hygienic animal management practice (bio-safety), early diagnosis, collaboration among veterinarians, medical doctors and farmers and vaccination of animals are necessary for control of brucellosis in Bangladesh.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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