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PREVALENCE OF BRUCELLOSIS IN PIGS: THE FIRST REPORT IN BANGLADESH

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ABSTRACT

Brucellosis, a bacterial zoonotic disease, has been reported in ruminants but still no report in pigs in Bangladesh. The aim of this study was to describe seroprevalence of brucellosis in swine in Bangladesh. Blood from a total of 105 pigs was collected from selected areas of Bangladesh. All samples were screened using Rose Bengal Test (RBT) and further confirmed by using Slow Agglutination Test (SAT). A structured questionnaire was used to collect the epidemiological data related to the animals and husbandry practices. Out of the 105 sera analyzed, 7 (6.7%) and 5 (4.8%) were found to be positive 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 ($p<0.01$). This report of prevalence of brucellosis in pigs is very important with regards to the human health and other livestock and might help Government and NGOs to design preventive measurement and establish livestock health policy.

Key words: Prevalence, Swine, Brucellosis, Bangladesh

INTRODUCTION

Brucellosis in pigs caused by *Brucella suis*, a bacterial infection causes bacteraemia and chronic inflammation in the reproductive organs of both sexes. Five different biovars of *B. suis* cause infection in animals other than pigs such as rain deer, caribou, hares and various murine species and occasionally in cattle and dogs (Godfroid *et al.,* 2002). 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 animals rearing while sources include the domestic cattle, pig, sheep, goat and unpasteurized dairy products (Radostits, 2000).

Though, out of 590 million pigs in the world, about 34% are raised in tropical countries. Due to the religious point of view and for the limited number of pork consumers, the pig population is not large 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. Due to the high number of piglet born, easy rearing with available natural resources, high disease resistance and low production cost, pig rearing is getting importance in the tribal regions to eliminate poverty. Such as in study areas, there are some tribal communities are rearing pigs by receiving asset grant from the FSUP (Food Security for the Ultra Poor) project funded by European Union. These pigs are predominantly belongs to the native and indigenous dwarf type producing low quality pork. 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. Therefore, it has a great value to identify brucellosis in pigs because of socioeconomic impacts of rural ultra poor people.

In Bangladesh, brucellosis in cattle (Rahman *et al*., 2011a, 2010, 2009, 2006; Amin *et al.,* 2005), buffalo (Rahman *et al*., 2006), sheep (Rahman *et al.,* 2011b; Rahman *et al.*, 2008; Uddin *et al*., 2007b), goat (Rahman *et al.*, 2011b; 2008; Uddin *et al.*, 2007b) have been reported that are causing huge economic losses to the livestock industry. The diagnosis of brucellosis is confirmed by isolation of *Brucella* by bacteriological culture or by the detection of an immune response by serological test to its antigens (Orduna *et al*., 2000). But the diagnosis of brucellosis based exclusively on *Brucella* isolation presents several drawbacks. Like the slow growth of *Brucella* may delay diagnosis for more than 7 days and also, the sensitivity is often low ranging from 50 to 90% depending on disease stage, culture medium, quantity of bacteria and culture technique employed (Gotuzzo *et al*., 1986).

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Hence, the serological tests are important for diagnosis of brucellosis. Serological test like the Rose Bengal Test (RBT), Slow Agglutination Test (SAT), Mercaptoethanol test and Enzyme Linked Immunosorbent Assay (ELISA) are generally used for the detection of *Brucella* infection in animal. To the best of knowledge, there is no published report of brucellosis of pig in Bangladesh. Therefore, the aim of this study was to determine the seroprevalence of brucellosis in pigs for the first time in Bangladesh by Rose Bengal Test (RBT) as screening test and later by Slow Agglutination Test (SAT) as confirmatory test.

MATERIALS AND METHODS

Blood and sera samples collection

A total of 105 blood samples (Table 1) were collected from two different districts of Bangladesh. Where there was availability of chute, pig was entered into the chute and restrained but where chute was not available, the pig was controlled by the owner and attendant. The site of blood collection at ear vein or tail vein was soaked with tincture of iodine or alcohol before collecting blood. About 5-7 ml of blood was collected with the help of sterile disposable syringe and needle and was kept undisturbed on a tray for at least 1 hour at room temperature in a slightly inclined position to facilitate clotting. The clotted blood samples with sera were transferred to refrigerator at 4°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. Clear sera were collected following centrifugation at 3000 rpm for 10 minutes and were transferred to the sterilized labeled eppendorf tube and stored at 20°C until used. A structured questionnaire was used to collect epidemiological data of the animals. Before analyzing the data, the questionnaire data was rechecked.

Table 1. Sera samples collected from pigs in Sirajgang and Bogra district

Area / location	Sex	No. of pig samples
Sirajganj district	Male	15
	Female	27
Bogra district	Male	19
	Female	44
	Total	105

Serological tests

Rose Bengal Test (RBT)

The RBT was performed according to the procedure as described by Uddin *et al*. (2007a, 2007b). The test serum samples and *Brucella abortus* antigen (William James House, Cowley Rd. Cambridge, CB4 0WX, UK) were kept 1 hour in room temperature before beginning the test. 30 µl of each serum to be tested was placed on a glass plate circled approximately 2 cm in diameter. Then the vial of antigens was shacked gently and 30µl of antigen was put beside each of the sera. The antigens and the serum were mixed on the plate with a stirrer and spread over the entire area enclosed by the circle. Then the plate was placed on a mechanical rotator at 80-100 rpm for 4 minutes and the reading was taken immediately. Any agglutination or precipitation was considered as positive, whereas no reaction (negative) indicated the absence of *Brucella* antigen in the sera.

Slow Agglutination Test (SAT)

SAT was carried out with EDTA. The SAW (Synbiotics, concentrated suspension of *B. abortus*, Weybridge, stain 99) antigen was diluted @1ml antigen with 19 ml SAT buffer solution. The SAT buffer was prepared by adding 0.93gm EDTA (5mM, Triplex®) with 500 ml PBS, where PBS was prepared by adding 5 tablets of PBS (Dulbecco-A, Oxoid, UK) in 500 ml distilled water. Briefly, the slow agglutination test was performed in flat bottom 96 well micro plates. At first for each test serum, a row of 3 wells of the 96 well micro plates was selected to make double dilution of the sera. 168µl of SAW buffer was pipette in first well and 100 µl in the2nd well and 3rd well of the micro plate, respectively Then 32 µl of serum was added in 1st well (dilution 1/6.25) after well mixing of the serum and PBS EDTA in the 1st well and 100µl was taken from this well and was placed in the second well (1/12.5).

One hundred microliter (100 µl) from the 2nd well was transferred into the 3rd well and finally 100 µl of liquid in excess was discarded from 3rd well. Note that, all wells contained 100µl. Then in each well 100 µl of standardized SAW antigen was added. This gives the serial serum dilution of 1/12.5, 1/25, 1/50. The plate was then incubated at 37ºC for 24 hours (+/- 4hrs) for reading. After 24 hours, the agglutination reaction was observed by using a magnifying mirror against illumination source. Notably, for every group of samples tested, a positive control serum was included. Reading was taken on the basis of this protocol and the standardization was performed (75% agglutination of the OIEISS). The results were interpreted according to instruction of Veterinary Agrochemical Research Center (Groeselenberg 99, 1180 Brussels, Belgium).

Statistical analysis

The results were statistically analyzed by z-test for proportions based on the result of both tests in terms of age, sex, abortion, pregnancy status and study area. A probability associated with the observed values was determined from relevant tables and significance was determined at 5% level.

RESULTS AND DISCUSSION

Brucellosis is an important zoonosis and serological surveillance is essential for its control (Erdenebaatar *et al*., 2004). The importance of brucellosis was primarily due to its public health significance and economic loss. Additionally, pigs infected with brucellosis can serve as a source of infection to other domestic animals. Bangladesh is an endemic area for brucellosis. This study investigates the serological status of brucellosis of pigs in selected areas of Sirajganj and Bogra districts of Bangladesh using RBT and SAT. This study helps to understand the epidemiology of *Brucella* in pig, to buildup awareness and to provide information for disease control in animals and human being. Seropositivity to be considered due to natural infection occurred because vaccination for brucellosis in pigs has never been practiced in Bangladesh.

Out of 105 pigs sera examined using Rose Bengal Test (RBT), 7 pigs showed positive reaction with a prevalence of brucellosis 6.7%. It was shown that 5 pigs were sensitive to brucellosis in SAT with a prevalence of 4.8%. Seroprevalence of brucellosis was higher in RBT (6.7%) than SAT (4.8%) (Table 2). This finding is little higher than a previous report of prevalence (3%) of brucellosis in pigs by Meng *et al*. (2009) and lower than the seroprevalence (7.5%) of brucellosis determined by Watarai *et al*. (2006). The prevalence and severity of disease may vary with the breed, geographic location, type of diagnostic test, husbandry and environmental factor as well as the biovar of the organism (Amin *et al*., 2005). Since in Bangladesh, pigs are usually reared in backyard or in the field as free range system, they are getting slow dose of infection to several infectious agents regularly and are thought to be resistant to most disease due to the presence of antibodies. However, this hypothesis difficult to prove since there is no study has been conducted with pigs in Bangladesh.

Table 2. Seropositive rate of brucellosis in Pigs based on RBT and SAT

Total No. of samples tested	Number of positive reactors		Percentage of positive reactors	
	by RBT	by SAT	by RBT	by SAT
105	7	5	7 (6.7%)	5 (4.8%)

RBT= Rose Bengal Test SAT= Slow Agglutination Test

With regards to the age, the results of this study showed a higher prevalence within the 3-4 years age group compared to the age group of 2-3 years age . Whereas no positive sera were detected in 1-2 years old pigs in both tests. Additionally, RBT showed more reactivity than SAT at 3-4 years age group (Table 3). The finding is coincided with the observation of Ruiz-Fons *et al*. (2006). The older animals supposed to be more infected, because of more contact with infectious agents and sometimes become susceptible from malnutrition during pregnancy. But there was no significant statistical association between age group and the prevalence ($p > 0.05$). The antibody titer against *B. abortus* is associated with low prevalence in young stock than the adults (Ahmed and Munir, 1995). Kazi *et al*. (2005) reported higher prevalence of infection in animals more than 4 years of age compared to younger animals. It appears that the higher prevalence of brucellosis among older pigs might be related to maturity with the advancing age. Thereby, the organism may have propagated to remain either as latent infection or it may cause clinical manifestation of the disease (Kazi *et al*., 2005).

Notably, it is difficult to compare our results since no such study was conducted in pigs in Bangladesh. Out of 105 pigs, 71 were female and 34 were male. 5 female showed positive reaction in RBT with a prevalence of 7 % and 4 female showed positive reaction in SAT with a prevalence of 5.6%. The male showed positive reactivity with a prevalence of 5.9% by RBT and 2.9% by SAT. Higher prevalence of brucellosis was found in female than male but the association was non-significant ($p > 0.05$) (Table 3). The prevalence of brucellosis in relation to the sex of animals was reported by some researchers and found significantly higher prevalence in females than in males (Hussein *et al.*, 2005), whereas MacMillan *et al.* (1982) showed that *B. abortus* causes intermittent bacterimea in the mares but not in the stallions. Uddin *et al.* (2007a, 2007b) found relationship between brucellosis and sex. Muma *et al.* (2006) could not find any association between *Brucella* antibody titers and sex. In this study, total 24 sows were pregnant. Higher prevalence of brucellosis was found in pregnant sows than non pregnant sows that was 12.5% and 4.3% by RBT, and 12.5% and 2.1% by SAT, respectively (Table 3). But this relationship is not statistically significant ($p < 0.05$). Brucellosis is essentially a disease of the sexually mature animals. The predilection site is the reproductive tract, especially the gravid uterus. Allantoic factors, including erythritol possibly steroid hormones and other substances stimulate the growth of most of the *Brucellae* (Radolf, 1994). Erythritol, a sugar alcohol synthesized in the ungulate placenta and stimulates the growth of virulent strains of *B. abortus*, has been credited with the preferential localization of this bacterium within the placenta of ruminants (Smith *et al*., 1962).

Among 71 sows, 7 sows had previous record of abortion. The prevalence of brucellosis in aborted sows was 42.9% in both RBT and SAT, while the non-aborted sows showed the prevalence of brucellosis 3.1% in RBT and 1.6% in SAT (Table 3). It was found that the prevalence of brucellosis was significantly ($p < 0.01$) higher in aborted or previously aborted sows than the sows having no record of abortion when the sera samples tested by RBT and SAT. Ibrahim and Habiballa (1975) reported a prevalence of brucellosis 14.2% in cows that had aborted previously. Other researchers had reported similar findings (Shaw, 1986; Barman *et al*., 1989; Sandhu *et al*., 2001). Rahman *et al*. (2006) reported cows with a history of retained placenta, had a prevalence of brucellosis 8.70% and there was a statistically significant difference in the prevalence of brucellosis and history of retained placenta.

Table 3. Demographic related seroprevalence of brucellosis in pigs based on RBT and SAT

	Sera tested	Positive (%) by RBT	Positive (%) by SAT
Age			
1 to 2 years	40	0 (0.0%)	0 (0.0%)
2 to 3 years	28	2 (7.1%)	2 (7.1%)
3 to 4 years	37	5 (13.5%)	3 (8.1%)
Sex			
Male	34	2 (5.9%)	1 (2.9%)
Female	71	5 (7%)	4 (5.6%)
History of abortion*			
Yes	7	3 (42.9%)	3 (42.9%)
No	64	2 (3.1%)	1 (1.6%)
History of pregnancy			
Yes	24	3 (12.5%)	3 (12.5%)
No	47	2 (4.3%)	1 (2.1%)

* Significant at 1% level ($p < 0.01$) RBT= Rose Bengal Test SAT= Slow Agglutination Test

In Sirajganj district, the prevalence was 6.7% in male and 7.4 % in female and overall prevalence was 7.1% by RBT. There was no positive reaction to SAT in male but the prevalence in female was 4.76% with a overall prevalence 4.8% in Sirajganj district by SAT (Table 4). In Bogra district, the prevalence was 5.3% and 6.8% by RBT and 5.3% & 4.5% by SAT in case of male and female animal, respectively. The overall prevalence in Bogra district was 6.6% by RBT and 4.8% by SAT (Table 4). In this study, statistically insignificant higher prevalence of brucellosis was found in Bogra (6.6%) district than Sirajganj district (7.1%) by RBT ($p >0.05$). But the prevalence was same (4.8%) in both district determined by SAT.

Table 4. Area/location related seroprevalence of brucellosis in pigs based on RBT and SAT

Location	Sex	Sera tested	No. (%) of positive by RBT	No (%) of positive by RBT	No.(%) of positive by SAT	No. (%) of positive by SAT
Sirajganj	Male	15	1 (6.7%)	3 (7.1%)	0 (0.0%)	2 (4.8%)
	Female	27	2 (7.4%)		2 (4.8%)	
Bogra	Male	19	1 (5.3%)	4 (6.6%)	1 (5.3%)	3 (4.8%)
	Female	44	3 (6.8%)		2 (4.5%)	

RBT= Rose Bengal Test SAT= Slow Agglutination Test

Ghani *et al.* (1998) and Uddin *et al*. (2007a, 2007b) stated that several factors such as age, sex, breed, location, herd size and living condition influence the seroprevalence of brucellosis. 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. To the best of our knowledge, this is the first report of seroprevalence of brucellosis in pigs in Bangladesh. Regular sero-monitoring of the pigs, culling of positive reactors from breeding program are important to eradicate or control of this zoonotic disease. Further studies for isolation, identification and typing of specific *Brucella sp*. are recommended.

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