Asian J. Med. Biol. Res. 2016, 2 (1), 13-18; doi: 10.3329/ajmbr.v2i1.27563

Asian Journal of Medical and Biological Research
ISSN 2411-4472 (Print) 2412-5571 (Online)
www.ebupress.com/journal/ajmbr

Article

Seroprevalence of ovine brucellosis in Bangladesh

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Received: 11 January 2016/Accepted: 14 February 2016/ Published: 31 March 2016

Abstract: An investigation was carried out to study the seroprevalence and risk factors for Brucella seropositivity in sheep in Bangladesh. For this, highly sheep populated ten different districts including all seven divisions of Bangladesh were selected where sheep of Panchagarh (151), Naogaon (101), Tangail (83), Noakhali (56), Sunamganj (52), Patuakhali (119) and Dhaka (75) were tested but sheep of Chapai Nawabganj, Khulna, Noakhali and Feni has under tested for Brucellosis. In the present study, seroprevalence of ovine brucellosis was tested by Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Enzyme-Linked Immunosorbent Assay (ELISA). About 5% sheep might have got Brucella infection. The highest Brucella antibody was observed in sheep of Tangail district (8.4%) followed by the sheep of Savar (8.0%), where as sheep of Subarna Char and Kala Para were free from Brucella antibody. In relation to age and sex, adults (6.5%) were more positive than young (3.6%) and female (5.3%) were more susceptible than male (3.8%).

Keywords: seroprevalence; ovine brucellosis; agglutination test for Brucella

1. Introduction

Brucellosis has a considerable impact on human and animal health as well as a socioeconomic impact, and especially in rural areas that largely rely on livestock. In developing countries, brucellosis is still considered the most serious and devastating zoonotic disease (Quinn *et al*., 1994; Al-Majali, 2005; Al-Majali *et al*., 2007; Rahman *et al*., 2015). Brucellosis in sheep and goats is primarily caused by *Brucella melitensis*, and rarely by *Brucella abortus* or *Brucella ovis* (Luchsinger and Anderson, 1979). However, sero-epidemiological data of this disease in sheep are lacking in Bangladesh. *Brucella* spp., the gram negative, non-motile and non-capsulated coccobacilli, is quite detrimental to the sheep health. The disease produced by this organism is known as brucellosis. It is also an important zoonotic disease leading to debilitating disease in human. Because of the major economic impact on animal health and the risk of human disease, most countries have attempted to provide the resources to eradicate the disease from the domestic animal population (Radostits *et al*., 2000). The Brucella organism especially *Brucella melitensis, Brucella ovis and Brucella abortus* produce infections in sheep. *Brucella melitensis* causes abortion in ewes and does, while *Brucella ovis* causes epididymitis and orchitis in rams only. Occasionally *Brucella ovis* causes abortion in ewes and neonatal mortality in lambs (Radostits *et al*., 2000). All these occurrences interfere with the better reproductive performance of a flock. On the other hand, *Brucella melitensis* is the most invasive and pathogenic for humans of the three classical species of the genus, and is the cause of 'Malta' or 'Mediterranean" fever in humans (Radostits *et al*., 2000). So, it is essential to rear *Brucella* free sheep in the country.

Serological tests using the RBPT, SAT, TAT, mercaptoethanol test and ELISA are generally used for the detection of *Brucella* infection in livestock. ELISAs have been evaluated for many years for their ability to detect serum antibodies to brucellosis in domestic animals. ELISA for the diagnosis of brucellosis has several advantages when compared with other tests. First, it directly identifies a specific antibody. Second, it is more sensitive than other agglutination tests and thus has the potential to detect infected animals. Third, ELISA results provide an epidemiological tool for investigating the infection status of flocks in places where vaccination has never been practiced, like Bangladesh (Rahman, 2005).

For these reasons, the research programme has been taken into a great consideration. The objectives of the present study were- (i) to investigate the seroprevalence of ovine brucellosis, and (ii) to develop prevention and control strategy against brucellosis.

2. Materials and Methods

To determine the seroprevalence of ovine brucellosis in Bangladesh, a survey plan was designed in both longitudinal and cross-sectional dimension covering all divisions of Bangladesh. The research was carried out during the period from July 2009 to May 2010. Samples were collected from randomly selected native sheep of Panchagarh (151), Naogaon (101), Tangail (83), Noakhali (56), Sunamganj (52), Patuakhali (119) and Dhaka (75) districts. Blood (about 4.0 ml/animal) was collected from jugular vein of each of the selected 637 sheep in separate sterilized test tubes and kept in refrigerator overnight. Sera were separated as per conventional method by centrifugation at 3000 rpm for 10 minutes. Sera were transferred to the eppendorf tubes and stored at –20°C until tested. The three different serological tests, Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Enzyme-Linked Immunosorbent Assay (ELISA), were performed to determine the true prevalence of brucellosis.

2.1. Rose Bengal Plate Test (RBPT)

Sufficient antigen, test sera, positive and negative control sera for a day's testing were removed from refrigeration and brought to room temperature (22±4°C). The test was validated at the start of each day by using known positive and negative control sera obtained from the Veterinary and Agrochemical Research Centre (VAR, officially accredited for Brucella serology, Brussels, Belgium). Equal volumes (30µl) of serum and antigen (concentrated suspension of *B. abortus*, Weybridgestrain 99; Institut Pourquier, France) were mixed and rotated on a glass plate for 4 min. The result was considered positive when there was any degree of agglutination noticeable.

2.2. Serum Agglutination Test (SAT)

The antigen used was *B abortus* biotype 1 Weybridge 99 (Synbiotics Europe, France). Sera were serially diluted at 1/12.5, 1/25, 1/50, 1/100 in 96-well microtiter plates. The plates were agitated and incubated at 37°C for 20-24 h. Reading was done on the basis of degree of agglutination and expressed in international units (IU). Any serum with an antibody titer greater than or equal to 30 IU/ml, as prescribed by the EU, was considered positive.

2.3. Indirect Enzyme-Linked Immunosorbent Assay (iELISA)

iELISA was performed using *B. abortus* biotype 1(Weybridge 99) as antigen. Protein G-horseradish peroxidise (G-HRP) was used as conjugate. For the standard curve, 6 dilutions (1/270 to 1/8640) of the positive reference serum (No. 1121) were prepared. Reading of optical densities (OD) was done at 492 nm and 620 nm using an automatic ELISA reader (VMAX). The results ($OD_{492} – OD_{620}$) were expressed as antibody units in comparison with a reference serum. The conversion of ODs into units (U/ml) was done using six dilutions of the reference serum to establish a standard curve. The cut-off value for a positive result was defined at 2 U/ml of test serum.

2.4. Data analysis

The data were analysed by using SPSS software version 12 (SPSS, Inc., Chicago, IL, USA).

3. Results and Discussion

The highest Brucella antibody was observed in sheep of Tangail district (8.4%) followed by the sheep of Savar (8.0%), where as sheep of Subarna Char and Kala Para were free from Brucella antibody. The overall seroprevalence was 5% (Table 1) which is higher than the findings of Abdala, 1966; Omer *et al*., 2000; Ei-Ansary *et al*., 2001and Ayele, 1991 but lower than that of Waghela, 1976; Falade and Hussein, 1997 and Shehu *et al*.,1999.

Table 1. Upazila wise distribution of sheep-level prevalence (at least one test positive) of ovine brucellosis.

Upazila Name	District Name	Negative	Positive	Total	Prevalence (%)
Tetulia	Panchagarh	76	4	**80**	5.0
Panchagarh Sadar		69	2	**71**	2.8
Naogaon Sadar	Naogaon	94	7	**101**	6.9
Tangail Sadar	Tangail	76	7	**83**	8.4
Subarna Char	Noakhali	15	0	**15**	0.0
Companiganj		39	2	**41**	4.9
Derai	Sunamganj	51	1	**52**	1.9
Patuakhali Sadar		69	2	**71**	2.8
Galachipa	Patuakhali	26	1	**27**	3.7
Kalapara		21	0	**21**	0.0
Savar	Dhaka	69	6	**75**	8.0
	Grand Total	**605**	**32**	**637**	**5.0**

Table 2. Upazila wise distribution of flock-level prevalence of ovine brucellosis.

Upazila Name	District Name	Negative	Positive	Total	Prevalence (%)
Tetulia	Panchagarh	9	2	11	18.2
Panchagarh Sadar		5	3	8	37.5
Naogaon Sadar	Naogaon	4	9	13	69.2
Tangail Sadar	Tangail	10	7	17	41.2
Subarna Char	Noakhali	1	0	1	0.0
Companiganj		1	1	2	50.0
Derai	Sunamganj	6	2	8	25.0
Patuakhali Sadar		8	2	10	20.0
Galachipa	Patuakhali	5	1	6	16.7
Kala Para		1	0	1	0.0
Savar	Dhaka	0	1	1	100.0
Grand Total		**57**	**21**	**78**	**35.4**

Table 3. Flock and sheep-level prevalence of ovine bruceloosis according to different tests.

Test	Flock		Sheep		Prevalence (%)	
	Tested	Positive	Tested	Positive	Flock	Sheep
RBT	78	9	637	11	11.5	1.7
SAT		11		11	14.1	1.7
ELISA		19		22	24.4	3.5
Three test		2		4	2.6	0.6

Figure 1. Collection of blood from jugular vein.

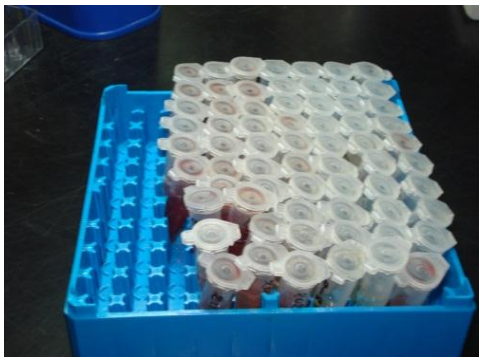

Figure 2. Serum in eppendorf tube.

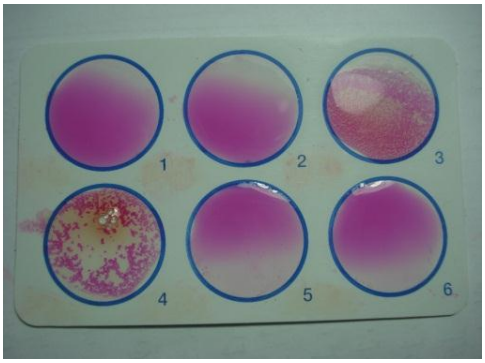

Figure 3. Rose Bengal Plate Test; Positive test showing agglutination no.3 and 4.

Figure 4. Serum Agglutination Test; Positive test showing agglutination left lower tube.

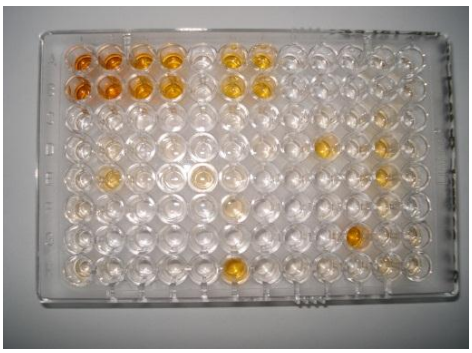

Figure 5. Enzyme Linked Immuno-sorbent Assay;
Positive test showing reddish color no. F10 and G6.

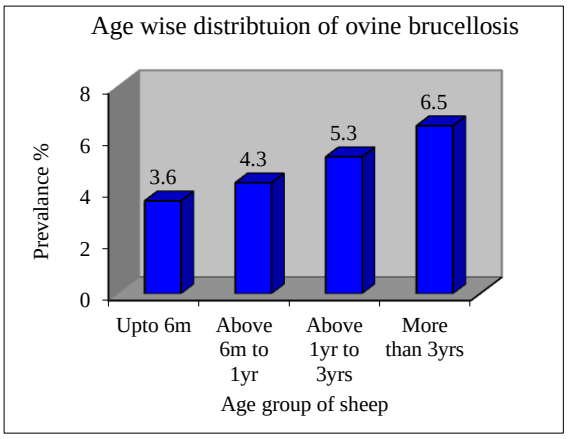

Figure 6. Age wise prevalence of ovine brucellosis.

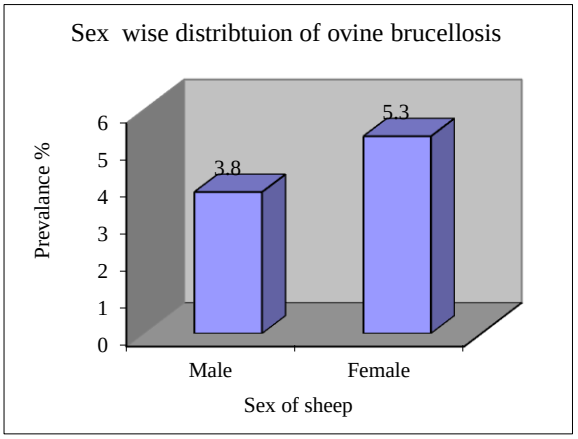

Figure 7. Sex-wise prevalence of ovine brucellosis.

The overall seroprevalence in flock level was 35.4% in native sheep. The highest Brucella antibody seroprevalence was observed in flock of Savar (100%) followed by the sheep of Naogaon (69.2%), where as the flocks of Subarna Char and Kala Para were free from Brucella antibody (Table 2).
Among the three tests maximum positive was found in ELISA test in both flock (24.4%) and sheep (3.5%) level. Only 2 flock and 4 sheep was positive in all the three tests used (Table 3).
In relation to age groups, adults (6.5%) were found to be more positive than young (3.6%) and showed a linear increasing rate along with increasing age (Figure 6). On the other hand female (5.3%) are more susceptible than male (3.8%) (Figure 7).
However, the effects of ovine brucellosis are often insidious and unrecognized, especially in areas where marked fluctuations in fertility occur due to variation in ewe nutrition, clover disease, predation by foxes, or other circumstances (Doug and Swan, 1998).

4. Conclusions

It could be said that about 5% sheep might have got Brucella infection; and it might be spread at a higher percentage in all the surrounding animals including human being. The highest Brucella antibody was observed in sheep of Tangail district (8.4%) followed by the sheep of Savar (8.0%), where as sheep of Subarna Char and Kala Para were free of Brucella antibody. In relation to age and sex, adults (6.5%) were more positive than young (3.6%) and female (5.3%) were more susceptible than male (3.8%). Serosurveillance across the country has to be performed properly in order to take necessary action for eradicating this disease from the domestic animal population. Culling of the true seropositive sheep from the flock has been suggested. Thus sheep population of Bangladesh may be kept apart from this detrimental zoonotic disease. Further research is needed for isolation and molecular characterization of the Brucella organism.

Conflict of interest

None to declare.

References

Abdala AED, 1966. Sudan Veterinary Science, Animal Health and Husbandry. 7: 28.

Al-Majali AM, 2005. Seroepidemiology of caprine brucellosis in Jordan. Small Rumin. Res., 58: 13-18.

Al-Majali AM, A Majok, N Amarin and O Al-Rawashdeh, 2007. Prevalence of, and risk factors for, brucellosis in Awassi sheep in Southern Jordan. Small Rumin. Res., 73: 300-303.

Ayele G, 1991. Livestock and economic differentiation in northeast Ethiopia : the Afar case. Nomadic People, 29: 10-20.

Doug H and H Swan, 1998. Ovine brucellosis. AG0365. ISSN 1329-8062.

Ei-Ansary EH, A Mohammed, ARA Hamad and AGO Karom 2001. Brucellosis among animals and human contacts in eastern Sudan. Saudi Med. J., 22: 557-579.

Falade S and AH Hussein, 1997. *Brucella* sero – activity in Somali goats. Trop. Anim. Hlth. Prod., 17: 93-99.

Luchsinger DW and RK Anderson, 1979. Longitudinal studies of naturally acquired *Brucella abortus* infection in sheep. Am. J. Vet. Res., 40: 1307-1312.

Omer MK, E Skjerve, G Holstad, Z Woldehiwot and AP Macmillan, 2000. Prevalence of antibodies to *Brucella* spp in cattle, sheep, goats, horses and camels in the state of Eritrea; influence of husbandry systems. Epidemiol. Infect., 125: 447-453.

Quinn PJ, ME Carter, B Markey and GR Carter, 1994. Clinical Veterinary Microbiology. pp. 261-267, Wolfe Publishing, London.

Radostits OM, IGJ Mayhew and DM Houston, 2000. Veterinary Clinical Examination and Diagnosis. London WB Saunders. ISBN 0702024767.

Rahman MS, 2005. Serological and bacteriological diagnosis of Brucella abortus biotype 1 infection in Sprague-Dawley rats. Indian J. Anim. Sci., 75: 610-616.

Rahman MS, SML Kabir and MS Rahman, 2015. Seroprevalence of canine brucellosis in Dhaka city corporation area, Bangladesh. Asian J. Med. Biol. Res., 1: 17-21.

Shehu LM, H Yusuf, AC Kudi and DU Kalla, 1999. Seroprevalence of brucellosis in ruminants in Bauunchi and environs. Nig. Vet. J., 20: 67-74.

Waghela S, 1976. Bull Anim. Hlth. Prod. Afr., 1: 53.