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Article

Seroprevalence of canine brucellosis in Dhaka city corporation area, Bangladesh

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Abstract: An investigation was conducted to determine the seroprevalence of brucellosis in pet dogs of Dhaka City Corporation area. A total of 50 sera samples from pet dogs were collected. Additional data on risk factors (breed, age, sex, any abnormality during sample collection etc.) were recorded during sample collection by using standard questionnaire. Sera were separated from blood samples and tested with the Rose Bengal Plate Test (RBPT) and Indirect Enzyme linked Immunosorbent Assay (IELISA) parallely. The overall seroprevalence of brucellosis in pet dogs was found 4.00% by RBPT & IELISA. The results of both serological methods were equal in terms of ability to detect Canine Brucellosis. Statistically higher seroprevalence of brucellosis (6.06%) was found in pet dog aged 1.5 to 2.5 years. Higher seroprevalence (15.38%) was found also in female pet dogs and no response in male pet dogs.

Keywords: sero-prevalence; brucellosis; Pet dogs; RBPT; IELISA

1. Introduction

Brucellosis is one of the world's major zoonotic problems. Though it has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand (Geering*et al*, 1995), but it remains an uncontrolled problem in regions of high endemicity such as the Africa, Mediterranean, Middle East, parts of Asia and Latin America (Refai, 2002). Almost all domestic species can be affected with brucellosis except cats which are resistant to *Brucella* infection. Though its distribution is worldwide; yet brucellosis is more common in countries with poorly standardized animal and public health programme (Capasso, 2002). The disease has been reported in cattle, buffaloes, sheep, goats, pigs, dogs and humans (Sharma *et al.* 1979).The disease also called as "Undulant Fever" and "Mediterranean Fever" (WHO, 2006).

Brucellosis is essentially a disease of sexually matured animals and have predilection for ungulates placentae, foetal fluids, joints and testes of bulls, rams, boars and male dogs. The disease is manifested by reproductive failure, which includes abortion of dead pups or live and stillborn pups in bitches at late gestation; infertility is a problem as early embryonic death may occur at twenty days or fetal resorption. In male, infertility as well as epididymitis, orchitis and scrotal dermatitis. Infertility because of abnormal sperm and poor sperm motility. This disease can be transmitted to human through the direct or indirect contact with dogs. Once the acute period of the disease is over, symptoms of brucellosis are mostly not

pathognomonic, and the organism can be chronically located in the supramammary lymphatic nodes and mammary glands of 80% of infected animals. Recent serosurveys for brucellosis in Bangladesh indicate low level seropositivity for brucellosis, however, seropositivity often indicates the presence of a larger problem than immediately shown by the absolute number of serologic reactors detected. Also, animals were tested with *Brucella abortus* antigen that can detect *Brucella abortus*, *Brucella melitensis*, *Brucellasuis* may have been detected. In Bangladesh, seroprevalence of brucellosis was first detected in cattle in 1967 (Mia and Islam 1967), in buffalo in 1997 (Rahman *et al*. 1997) and human brucellosis was first reported in 1983 (Rahman *et al.* 1983). However, some reports on the seroprevalence of human brucellosis, bovine brucellosis, caprine brucellosis and ovine brucellosis are available (Rahman *et al*. 1978; Rahman and Rahman 1981; Mustafa 1984; Rahman *et al*. 1983; Amin *et al.* 2005; Rahman *et al.* 2006). But there is only one report on canine brucellosis from Bangladesh (Talukder *et al.*, 2011). Therefore, the present study was conducted to determine the seroprevalence of brucellosis in pet dogs of Dhaka City Corporation area, Bangladesh.

2. Materials and Methods

For serological study a total of 50 pet dog's serum samples were collected during the period of January-June, 2014 from Central Veterinary Hospital, 48, Kazi Alauddin Road, Dhaka-1000. The study recorded some clinical, epidemiological and reproductive data in the sample collection sheet including species, breed, age, sex, any genital lesion during sample collection etc. The animals were controlled by mechanical device and about 3-5 ml of blood was drawn directly from the fore arm with the help of sterile syringe and needle. During collection of blood all precaution were taken to avoid hemolysis. The collected blood was immediately transferred to the collecting test tubes and allowed to clot undisturbed at the room temperature for half an hour in the laboratory and later were kept overnight at 4 °C in refrigerator. Then the sera were separated in the next day by centrifugation at 3000 rpm for 10 minutes and the sera were stored at - 20 °C until tested. Each of the collected serum samples was tested with Rose Bengal Plate Test (RBPT) and Indirect Enzyme linked Immunosorbent Assay (IELISA) as per the manufacturer's instruction. The RBPT antigen (V2001) obtained from the Manufacturer Company named Lillidale Diagnostics, Badbury View, Bothenwod, Wimborne, Dorset, BH21 4HU, UK, prepared from *B. abortus* (Strain 99) stained with Rose Bengal dye and suspended in acid buffer (pH 3.65).

Test results and potential association with age, sex and among tests were performed by SPSS 11.5 for Chi-square analysis.

3. Results

The overall seroprevalence of brucellosis in pet dog as shown in Table 1 was 2 (4.00%) by the two serological methods and both serological methods had equal results.

Table 1. Overall seroprevalence of brucellosis in pet dogs.

Species	Total number of pet dog tested	Sero-positive No. (%)		
		Rose Bengal Plate Test (RBPT)	Indirect Enzyme Linked Immunosorbent Assay (IELISA)	Overall seroprevalence
Dog	50	2 (4.00%)	2 (4.00%)	2 (4.00%)

Sero-prevalence of canine brucellosis based on Rose Bengal Test (RBT) is shown in Table 2. It appears from the Table 2 that out of 50 pet dog sera tested with RBPT, of which only 2 (4.00%) had positive reaction to canine brucellosis.

Table 2. Sero-results of brucellosis in pet dog based on Rose Bengal Plate Test (RBPT).

Species	Total no. of pet dog tested	Positive No. (%)	Negative No. (%)
Dog	50	2 (4.00%)	48 (96.00%)

Sero-prevalence of canine brucellosis based on indirect enzyme linked immunosorbent assay (IELISA) is shown in Table 3. It appears from the Table 3 that out of 50 pet dog sera tested with IELISA, of which only 2 (4.00%) had positive titre to canine brucellosis.

Table 3. Seropositive rate of brucellosis in pet dog based on Indirect Enzyme linked Immunosorbent Assay (IELISA).

Species	Total no. of pet dog tested	Positive no. (%)	Negative no. (%)
Dog	50	2 (4.00%)	48(96.00%)

Sero-prevalance of brucellosis is compaired among different test including Rose Bengal Plate Test (RBPT) and Indirect Enzyme Linked Immunosorbent Assay (IELISA) and the result are shown in Table no. 4. The seroprevalance of canine brucellosis was detected by using RBPT and I ELISA respectively. From the comparison, it is revealed that the prevalence of canine brucellosis was found same between the two methods.

Table 4. Comparison of seropositivity results of two serological methods used for the detection of canine brucellosis.

Test used	Total no. of pet dog	Sero-result No. (%)	
		Positive	Negative
Rose Bengal Plate Test (RBPT)	50	2 (4.00%)	48 (96%)
Indirect Enzyme Linked Immunosorbent Assay (IELISA)		2 (4.00%)	48 (96%)

Age-wise sero-prevalence of canine brucellosis is presented in the Table 5. It appears from the Table 6 that the sera of all of the 17 pet dogs aged up to 1 year old showed negative reaction with the two sero-tests. Out of 33 pet dogs aged between 1.5-2.5 years old tested with RBPT and IELISA, of which 2 (6.06%) and 2 (6.06%) dogs showed positive reaction to brucellosis respectively. These results indicate that the prevalence of brucellosis is mainly in adult dogs.

Table 5. Age-wise sero-prevalence of canine brucellosis detected by using two different serotests.

Species	Age	Total no. of pet dog	Test-wise seropositive No. (%)		Level of significance (χ2-test)
			Rose Bengal Plate Test(RBPT)	Indirect Enzyme Linked Immunosorbent Assay(I ELISA)	
Dog	1 year	17	0 (00.00%)	0 (00.00%)	0.046*
	1.5-2.5 year	33	2 (6.06%)	2 (6.06%)	

S*=Significant at 5% ($p<0.05$) level

Sex-wise sero-prevalence of canine brucellosis is presented in the Table 6. The numbers of male and female pet dogs are 37 and 13 respectively. Male have no seropositivity while female have seropositivity with a prevalence of 15.38% by RBPT and IELISA. Higher prevalence was found only in female pet dog.

Table 6. Sex-wise sero-prevalence of canine brucellosis detected by using two different sero-tests.

Sex	Total no. of pet dog tested	Test-wise sero-positive No. (%)	
		Rose Bengal Plate Test (RBPT)	Indirect Enzyme Linked Immunosorbent Assay (IELISA)
Male	37	0 (00.00%)	0 (0.00%)
Female	13	2 (15.38%)	2 (15.38%)

4. Discussion

Brucellosis is a widespread and an important reproductive disease and remains a major zoonosis (Kakoma *et al.*, 2003; Baek *et al.*, 2003; WHO, 1986). Adequate reports on the sero-prevalence of brucellosis in human and food animals are available in the world literature including Bangladesh (Rahman *et al.*, 1997; Rahman *et al.*, 1988; Rahman *et al*, 1983; Rahman and Rahman, 1982; Rahman and Rahman, 1981; Rahman and Mia, 1970). In addition, the sero-prevalence of canine brucellosis in both the pet and stray (Radojicic *et al.*, 2006) dogs have been reported but there seems to be no such published reports from Bangladesh (Samad, 2008). However, there is much pet dog population in Bangladesh. Many people are interested to rear pet dogs as their companion and this is the most common way to spread-out the infection from dog to human population. Each year half of a million new cases of brucellosis are reported worldwide, but according to the World Health Organization (WHO), these numbers greatly underestimate the true prevalence. The objectives of the study were to implement of IELISA and RBPT to know the status of sero-prevalence of canine brucellosis and to provide information for disease control in pet dog and transmission of brucellosis to cow and human being. Indirect Enzyme linked immunosorbent assay (IELISA) has been evaluated for many years for their diagnostic performance to detect serum antibody to brucellosis in dogs (Srinivasan *et al.*, 1992).The present investigation revealed that the overall sero-prevalence 4.00% was found in RBPT and ELISA, Which is closely similar to Radojicic *et al.* (2006) found 4.27% sero-positivity in pet dog in the territory of Belgrade, but Brown *et al.* (1976) found only 1% sero-prevalence found in pet dog Atlanta-Athens, Georgia. The results suggest that, although RBPT could be used as a screening test for brucellosis due to its low cost and easy execution. IELISA provided better estimates of the actual prevalence of the infection. In case of age wise sero-prevalence in pet dog, between the age groups, the prevalence of brucellosis was found 6.06% in RBPT and IELISA in the age between 1.5-2.5 years, but in 1 year age pet dog there is no prevalence. There was significant association statistically ($p< 0.05$) between age group. Sergeant (1994) also found that there was no apparent association between age and serological status, or age and the prevalence, but Ghani *et al.* (1998) stated that several epidemiological factors, such as age, sex, breed, lactation number, herd size and living conditions influence the sero-prevalence of brucellosis. The prevalence of brucellosis in pet dog was found to be higher in female (15.38%) than male (0%) by among RBPT and IELISA tests respectively.

5. Conclusions

The result of this study would provide a baseline data for further study on canine brucellosis in pet dog population. Pet dog may be considered as a carrier of *Brucella abortus* infection and indicate a risk (source) for human and food animals. This study suggests that the findings of RBPT and IELISA were same. The finding of this study is suggested also that pet dogs should be routinely included in the brucellosis surveillance and control program in human and food animals.

Conflict of interest

None to declare.

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