Original Article

Sero-prevalence of visceral leishmaniasis (VL) among dogs in VL endemic areas of Mymensingh district, Bangladesh

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ABSTRACT

Objective: The present study was conducted to determine the sero-prevalence of canine visceral leishmaniasis (VL) among street and owned dogs at Trishal Upazila of Mymensingh district, Bangladesh.

Material and methods: Blood was collected aseptically from targeted dogs and serum was separated out using standard centrifugation method. The rK39-antigen-based dipstick test was used to detect anti-leishmania antibodies in serum.

Results: The study revealed that 35% of the dogs in the study area were sero-positive for *L. donovani*. Living status of the dogs (street or owned) was a potential risk factor and sero-prevalence was significantly higher in free roaming street dogs ($P=0.009$) and dogs with skin lesions and enlarged lymph nodes ($P<0.05$). The female and adult dogs were more susceptible.

Conclusion: VL is an important zoonotic disease wich is transmissible to humans by the bite of phlebotomine sand fly. Dogs are the main reservoir. The higher sero-prevalence of VL indicates the potential rule of dogs to maintain the zoonosis wich need to be explored more specifically by isolation and typing of the parasite.

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INTRODUCTION

A well known protozoal infection in numerous mammallian species including human is leishmaniasis and it is caused by the different organisms of the genus Leishmania. It is a vector born disease which is transmitted by sand flies belongs to the genus Phlebotomus when they bite on host animals. There observed a constant prevalence of VL all over the world. This disease is exposed to 88 countries of the world either a localized form characterized by stigmatizing disease or a fatal visceral form (visceral leishmaniasis: VL) which is called kala-azar and the later is fatal almost 90% cases if untreated. The remarkable clinical signs are irregularity in fever, unthiftiness, anemia and liver and spleen enlargement. Worldwide incidence of VL is about 200000 to 400000 per year and World Health Organization (WHO) estimated in 2014 that the majority of the cases (about 90%) occured in some selected countries viz. India, Brazil, Ethiopia, Sudan and Somalia. The three major endemic countries for this disease are Bangladesh, India and Nepal (WHO, 2016). In these reasons the VL responsible for the condemnation of about 400,000 disability-adjusted life-years per year. Every year in Bangladesh 40,000 to 45,000 people are affected with VL where the average population at risk is about 20 millions. VL is endemic in 45 districts of Bangladesh where more than 50% cases occurs in Mymensingh district. In near future it is estimated that approximatetly 65 million people may be at risk of having this dieease (Rahman et al., 2015).

The important reservoir both for *L. infanturn* and *L. donovani* are dogs, jackals, foxes and various types of rodents. The leishmania vector also fed on cattle, horse or others livestock, but it has not investigated yet the role of these animals to maintain the infection of VL. In South Asia this disease had existance since 1990. Actually VL is transmitted by the bite of female fly *Phlebotomus argentipes*. VL is a fatal disease and in untreated cases case fatality rate is almost 100% although if treated it is as lower as 10%. In Bangladesh few studies were conducted to trace the potentiality of domestic animals in the transpmission of VL to human but the findings are not conclusive. It is evidenced that VL affected person may be a source of infection for new population (Picado et al., 2014). An important tool for the diagnosis of VL infection among the animals is serological investigation for antibody. Previous studies revealed a large number of asymptomatioc sero-positive dogs (Bizhga et al., 2013). A WHO recommended test the rK-39 strip test is a popular test protocol in the South Asia for the detection of VL affected patients. This test is based on cromatography, basically an immune chemistry.

A recombinant antigen is identified in this test (Vaish et al., 2012). The first kala-azar (visceral leishmaniasis) patient was recorded in Jessore District of Bangladesh in 1824 (Ahluwalia et al., 2003). There are so many researches conducted in Bangladesh regarding VL but most of them for better non-invasive diagnostic methods in human (Alam et al., 1996). A significant number of human clinical case reports had also published (Sarker et al., 2003). Only a limited number of epidemiological studies were noticed (Talukder et al., 2003). Although the dog is the principal reservoir of VL the studies commencing risk factor analysis of VL in dog are severely lacking in Bangladesh. So far known, none of the study was based on antigenic serological investigation as well as risk factor analysis in Bangladesh. Considering the above facts the present study was conducted to determine the sero-prevalence of canine visceral leishmaniasis among stray and owned dogs at Trishal Upazila of Mymensingh district of Bangladesh.

MATERIALS AND METHODS

Ethical approval: The supportive hands like- personnel for animal restraining and sample collection were previously immunized against rabies. The dog owners were asked to sign a written consent form. This study had prior approval from the American International University Bangladesh (AIUB) and CVASU Animal Experimentation Ethics Committee (AEEC), Bangladesh.

Location of study area and study population: This study was conducted at six selected villages of Trishal upazila under Mymensingh district of Bnagladesh. A cross setion of population were considered for this study among stray dogs and owned dogs. The period of the current research was from October 2013 to March 2014. **Figure 1** showing the map of study areas.

Sample size calculation: We calculated sample size based on assumption considering 10% prevalance. The presumptive precession and confidence interval were 5% and 95%, respectively. All together we found our sample size was 138 dogs. The size of the sample was estimated by using Epi-info version 3.2.2 software. Dog populations were hard to reach and it was difficult to select them by random sampling method. Therefore purposive sampling was done. Due to constrain of available resources, budget and time, the samples were collected from 103 dogs.

Data collection: A standard questionaire was prepared and tested for accuracy. This prepared questionnaire was implemented for the collection of demographic data of dogs. The researchers observed the sex and physical
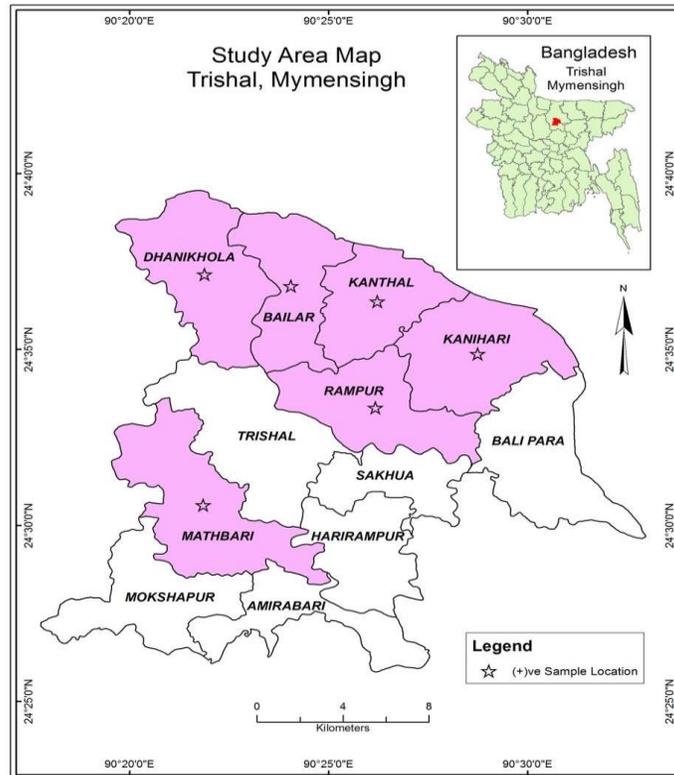
Figure 1: Figure shows sampling locations of six villages in Trishal Upazila of Mymensingh district in Bangladesh. The map was made using latitude and logitude data of sampling places by using software ArcGIS.

status of animals while restraining the dogs and filled up the field data sheet. In case of street dogs, age was estimated by dentition, observing physical growth and external genital organs. In case of pet dog, the owners were asked for age. The pups below six months of age and the dog owners who were not willing to provide samples and data to us were excluded from the research.

Physical Examination: The dogs were subjected to physical examinations to findout fever, skin lesion, enlargement of peripheral lymphnode and mucous membrane color. Raising of core body temperature atleast one degree from the normal average value (102.5°F) was considered as fever. Enlargement of lymphnode and abnormal color of mucous membrane were identified by comperison with normal.

Blood sample collection: Three ml blood were collected from the dogs via radial venipuncture and stored at 4°C until serum separation. The location wise enrolled dog for sample collection **(Table 2)** was Bailor (n=16), Dhanikhola (24), Kanthal (n=14), Mathbaria (n=19), Rampur (n=12) and Kanaihari (n=18).

Laboratory investigation: Centrifugation method was used to collect serum from the blood samples. For convenient in the field setup the rK39 strip test (an immune-chromatographic test) was used which is the ideal format for use under field conditions. Readily available commercial kits (Kalazar Detect TM®; In Bios International, Inc., Seattle, WA) were used to detect the presence of Kalazar antibody in the form of dipstick test according to manufacturer's guideline. 20 µl serum sample was taken into the wells of trips. 100 µl of buffer solution was added on it. After 10 minutes the results were obatined. The test was considered positive when both control and test sample visualized two pink lines.

Data compilation and frequency expression: The collected data were transported to analytical software STATA/IC-13. Descriptive statistics were calculated to express sero-positivity status of dogs and different factors. Categorical variables were summarized as frequency and percentages.

Analysis of risk factors: To workout the association between serologically positive dogs and different categories of risk factors Chi Square test and or Fisher's exact test were implemented. Uni-variable random effects (RE) logistic regression model was fitted to assess associations of predictors with the dependent variable

(sero-positivity) to select the variables. In case of multivariable logistic regression model, we considered the associated sero-positivity in the univariate analysis where *P*<0.1. The relative contributions of various predictors were assessed according to method descrobed by Dohoo et al. (2003). To investigate the confounding variables and effects the parameters were estimated both in and out from the model. If the estimates varies ≥15% between the two then the explanatory variables were considered to have the confounding effects.

RESULTS

The main aim of this research was to investgate the potentiality of dog as reservoir host for VL in the VL endemic regions of Bangladesh. Sampling was done from 103 dogs belong to six different villages of Mymensingh district. Out of 103 dogs, 45% were male and 55% were female. The adults and juvenile dogs were 86% and 14%, respectively. The health condition of most of the dogs (70%) were poor indicate poor nutritional status.

Eight (14%) dogs were pregnant, seven (12%) were lactating and four (7%) were with pups among the female dogs. Twenty seven percent dogs were found with fever, 65% with skin lesion, and 66% with enlarged lymph node in clinical examination **(Table 1)**.

Location wise sero-prevalence of canine leishmaniasis

The status of canine Leishmania infection in different geographic areas is shown in **Table 2**. The results showed that 35% of the dogs in the study area were serologically positive for canine *L. donovani* infection. Highest number of dogs were CnL positive from Dhanikhola (n=9), where as the highest rate of CnL positive recorded from Kanthal (43%). On other hand, lowest number of dogs were positive in Ramour (n=4), lowest prevalence were in Kanaihari (28%). However, the status of canine Leishmania infection in different locations did not varied significantly. This result indicate that Leishmania infection among domestic dogs was prevalent in the six studied villages of Trishal Upazila and it is a VL endemic focus in Bangladesh.

We investigated different variables to identify the potential risk factors among them dog ownership, age of dog, health condition, skin lesion, enlarged lymph node and VL infection were significantly related as risk factors (*P*<0.05) with the sero-prevalence of *L. donobani* **(Table 3)**. Street dogs were 3.8 times more prevalent (44%) to seropositive than pet dogs. In according to age categories,

adult were 8.44 times more susceptible to become seropositive of *L. donobani*. The nutritional status of the dogs were categorized on the basis of health condition and the dogs with poor health condition were about 13 times more prevalent to *L. donobani* than the dogs with good health condition. The dogs had skin lesion and enlarged lymph node were 4.05 and 3.8 times respectively, more susceptible to *L. donobani*.

In the fited model interaction or confounding was not found. The test for goodness of fit indicated a significant predictive ability to differentiate positive cases from negatives.

Table 1: Overview of the of dog sample population at Trishal, Mymenshingh

Factors	Categories	Frequency	Percentage (%)
Ownership (N=103)	Pet dogs	35	34
	Street dogs	68	66
Sex (N=103)	Male	46	45
	Female	57	55
Age (N=103)	Juvenile	14	14
	Adult	89	86
Health condition (N=103)	Good	31	30
	Poor	72	70
Pregnancy (N=57)	Yes	8	14
	No	49	86
Lactating (N=57)	Yes	7	12
	No	50	88
With pup (N=57)	Yes	4	7
	No	53	93
Fever (N=103)	Yes	28	27
	No	75	73
Skin lesions (N=103)	Yes	67	65
	No	36	35
Enlarged lymph node (N=103)	Yes	68	66
	No	35	34
Pale mucus membrane (N=103)	Yes	24	23
	No	79	77

Table 2: Sero-prevalence of canine Leishmania in different selected location

Sampling sites	Total dog sampled (n)	Seropositive (%)	95% CI
Kanaihari	18	5 (28)	10-53
Bailor	16	5 (31)	11-59
Rampur	12	4 (33)	10-65
Mathbaria	19	7 (37)	16-62
Dhanikhola	24	9 (37)	19-59
Kanthal	14	6 (43)	18-71
Total	**103**	**36 (35)**	**29-45**

N = 103; CI= Confidence Interval

Table 3: Univariate associations between potential risk factors and sero-prevalence of Leishmania in dogs

Variables	Category	χ2 test			Univariate logistic regression		
		+ (%)	-	P	OR	95% CI	P
Ownership	Pet dogs	6 (17)	29	0.007	3.8	1.4-10.38	0.009
	Street dogs	30 (44)	38				
Sex	Male	12 (26)	34	0.09	2.06	0.89-4.78	0.093
	Female	24 (42)	33				
Age	Juvenile	1 (7)	13	0.02	8.42	1.05-67.30	0.044
	Adult	35 (39)	54				
Health condition	Good	2 (6)	29	0.001	12.97	2.88-58.48	0.001
	Poor	34 (47)	38				
Pregnancy	Yes	2 (25)	6	0.54	1.67	0.32-8.5	0.542
	No	34 (36)	61				
Lactating	No	32 (33)	64	0.20	2.67	0.22-12.64	0.217
	Yes	4 (57)	3				
With pup	No	34 (34)	65	0.52	1.91	0.26-14.17	0.526
	Yes	2 (50)	2				
Fever	No	24 (32)	51	0.30	1.6	0.65-3.89	0.306
	Yes	12 (43)	16				
Skin lesions	No	6 (17)	30	0.004	4.05	1.5-11.02	0.006
	Yes	30 (45)	37				
Enlarged lymph node	No	6 (17)	29	0.007	3.8	1.40-10.38	0.009
	Yes	30 (44)	38				
Pale mucus membrane	Yes	8 (33)	16	0.85	1.1	0.35-2.39	0.849
	No	28 (35)	51				

N=103; CI=Confidence Interval

Table 4: Multivariable logistic regression

Type of dog Street	Odds Ratio	P>z	95% Confidence Interval	
Sex_Female	1.66	0.276	0.67	4.16
Age_Adult	4.87	0.148	0.57	41.60
Skin lesion_Yes	3.45	0.020	1.24	9.81
Enlarged lymphnode_Yes	2.95	0.046	1.02	8.54

After adjustment of factors only skin lesion and enlarged lymph nodes were found significant. The odds ratio was 3.45 in dogs with skin lesions compared with no skin lesions dogs (P=0.02). The odds ratios indicated 2.95 times higher likelihood of VL in dogs with enlarged lymphnodes **(Table 4)**.

DISCUSSION

Canine VL is equally important for veterinary as well as public health sector because it causes disease both in animals and human and it is inter-communicable between two through fly, hence it is utmost important to know the actual prevalence for effective control measures. Serology is well suited to determine the prevalence because it represents the affected percentage both in clinical and subclinical patients. The exact numbers of stray dogs in Bnagladesh is unknown but supportive estimates suggested it is around 2.5 million (Alam et al., 2013). As stray dogs and foxes sometimes have some common territories so the foxes might contain VL in their body. Thus dogs and foxes may potential sources for VL in human. There is an extreme crisis on data of prevalance of VL in canine species in Bnagladesh.

In Bangladesh, the main reservoir host for VL is yet unknown. The findings of the current study suggest that dogs might be the reservoir host as other researchers from India and Sri Lanka have similar findings (Sharma et al., 2009; Goswami et al., 2003). The number of infections in human has been increased since 1970 in these regions with a higher percentages of case fatality rates (up to 18%). In the endemic areas the prevalence of VL in dogs also higher but most of them are asymptomatic (Ready, 2014). The dog may carry asymptomatic infection for months or years. Worldwide dogs are reservoir for *L. infantum*. In Africa and Asia the main causative agent of VL is *L. donovani* and canine may act as reservoir host (Haddadzade et al., 2013).

In Eastern Sudan, it was recorded that dogs are main reservoir for *L. donovani* (Hassan et al., 2009). In the current study, it seems that there is a general tendency for older animals to have had more exposure to *Leishmania*

infection. Research findings also suggested that the sero-prevalance is linked with age of the animal ([Hosseininejad et al., 2012](#)). In current study, the females dogs were more likely to be infected by leishmaniasis compared to male, this is may be due to higher exposure levels of female dogs to mosquito bites ([Kumthekar et al., 2014](#)). A relative high proportaion of infected street dogs compares to owned dogs suggest the conclusion that they might be the potential source for human infection. Street dogs are likely to be 4 times more susceptible to infection than owned dogs. It is due to more exposure to mosquito bites than the owned dogs. The free roaming is an important factor for achieving infection ([Hosseininejad et al., 2012](#); [Kumthekar et al., 2014](#)). [Gálvez et al. (2010)](#) reported that dogs those are living in the street are more likely to be infected with parasitic diseases and similar findings also reported in Granada and Spain where they claimed higher density of vactors in the outside environment ([Martin-Sanchez et al., 2009](#)).

The clinical menifestations of VL infected dogs are variable. Most of the sero-positive dogs remain asymptomatic. Some most common clinical features are: presence of interrupted fever or absence of fever, weakness, unthriftiness, reduced feed intake, enlargement of spleen and peripheral lymphnodes etc. ([Criado et al., 2000](#); [Silva et al., 2000](#)). Some less common symptoms are: lesions in eye, mucous membrane and skin, coughing, sneezing, vomiting etc. ([Khan et al., 2012](#)). Canine infection with *L. donovani* may be symptomatic or asymptomatic but in both cases they are equally potential to spread this disease because both of the groups are infectious to the Phlebotomine flies ([Mohebali et al., 2005](#); [Lima et al., 2014](#)). Moreover, canine infection is much a more concerning issue than other hosts. A proportionately higher percentages of asymptomatic infection may be due to the fact the animals have already developed immunity against infection. ([Gálvez et al., 2010](#)). The highest percentages of sero-positive cases were found among the dogs with enlarged lymph node and it is supported by previous study conducted by [Lima et al. (2014)](#). Many of the previous researches declared the most obvious clinical signs among the VL positive dogs are skin lesions ([Silva et al., 2000](#); [Lima et al., 2014](#)). The rk39 strip test is convenient field test for Bnagladesh because of its easy use, economic and sensetivity although some cross reaction may occurs with *Trypanosoma cruzi* ([Mettler et al., 2005](#)).

So, the findings of the current study sugest the potentiality of street dogs as reservoir of VL in the VL endemic reagions of Bnagladesh. We investigated the serum antibody. The presence of antibody does not always indicate the presence of organism because antibody may present due to the previous exposure. So more robast dignostic test should be implemented to explore the actual scenarios of VL in these selected regions.

CONCLUSION

The current study documented the presence of sero-positive dogs infected with VL in a selected human VL endemic region of Bangladesh. High percentage of sero-positive dogs indicates potential role of street dogs and they act as reservior host in maintaining the VL by regular cycling the infection with fly. However, sero-prevalance study is not enough to draw the conclusion because presence of antibody does not always indicate the presence of infection. Antibody may develop from previous exposure. So a comprehensive study is required where specific identification of VL will be done and molecular typing may be done. Necessary action should be taken to increase awareness among dog owners as well as among general people about visceral leishmaniasis. Routine examination is necessary for dogs in VL endemic areas of Bangladesh.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

AI went to the field to collect the samples and carried out the research. MMH and LR supervised the overall research work. AI, MMH, PD, SI and MA wrote the manuscript and revised. All the authors approved the manuscript for submission.

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