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RESEARCH ARTICLE

Seroprevalence and Associated Risk Factors of *Leptospira interrogans* Serovar *Hardjo* in Dairy Cattle of Chittagong, Bangladesh

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ABSTRACT

A serological survey was conducted to determine the seroprevalence and risk factors of leptospirosis in commercial dairy cattle in Chittagong, Bangladesh during the period of April 2011 to September 2012. This study was carried out by randomly selected six farms having 206 dairy cows. A total of 110 serum samples were collected for the detection of *Leptospira* (L.) *interrogans* serovar *Hardjo* antibody by ELISA. The results showed that a total of 52 sera were positive (seroprevalence 47.27%). Urine samples were collected from seropositive animal for the detection of *Leptospira* organisms under dark field microscopy but none were found positive. The univariate analysis revealed that the prevalence of leptospirosis was significantly higher in lactating animals and pregnant cows ($P<0.05$). Among farm level exposures; owner's educational qualification, source of semen, size of farm, farm and number of employees were potential factors of leptospirosis ($P<0.05$). A multivariate analysis showed that the higher educational qualification of farm owners (OR=1.35), farms having more than 15 employee (OR=13) and farms located in peri-urban areas (OR=1.14) had higher risk of leptospirosis. However, the study concluded that leptospirosis is prevalent and distributed among dairy farms in Chittagong, Bangladesh. The seropositive dairy cows did not show any evidence of disease except abortion, stillbirth and death of weak calves. Further studies need to be carried out to prove the infectivity, serovar determination and implementation of preventive measures among dairy farm and people at risk.

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INTRODUCTION

Leptospirosis is considered to be worldwide zoonotic, emerging infectious disease and having global public health problem with high morbidity and mortality (Ko *et al.*, 2009). The infection is common in the developing countries, since there are favorable conditions for its transmission (Bharti *et al.*, 2003). Bovine leptospirosis is a cause of mastitis, abortion, stillbirth, or birth of weak calves. Leptospirosis is a well known cause of reproductive losses in cattle with relatively mild acute clinical signs. Abortion, stillbirth, or birth of weak calf occurs as a result of Leptospira infection. Abortion may occur several weeks after infection of the dam and is usually not associated with any obvious illness in the cow (Bahari *et al.*, 2011). Leptospirosis has major economic concern when it is involved in the reproductive failure of food animals (Bomfim and Koury, 2006; Saglam *et al.*, 2008). Infection of the reproductive system could result in a "storm of abortions" causing considerable economic losses from meat and milk reductions (Tooloei *et al.*, 2008). The infection among cattle can occur directly via infected urine, post abortion uterine discharges, infected placenta or by sexual contact. Indirect transmission plays a much greater role in the spread of infection. It occurs through exposure to an environment contaminated with infective material from farm animals. The disease in human beings is mostly an occupational hazard in farming community, veterinarian, butchers and laboratory workers. The organisms enter into the host-body when they come in contact with abraded skin or mucus membranes (Bhatti, 2008).

Laboratory tests are needed for the confirmatory diagnosis of leptospirosis. The organism may be demonstrated in the cerebrospinal fluid, blood or urine by dark-field microscopy. The ELISA is a fundamental tool of clinical immunology and being employed as initial screening test. Other tests like microscopic agglutination test, fluorescent antibody test, radial immunoassay, indirect hemagglutination test, complement fixation test and PCR are used for the diagnosis of leptospirosis. Recovery of Leptospires from clinical samples by culture is one of the definitive diagnostic tests of Leptospirosis (Bharti *et al.,* 2003). Most cases of leptospirosis are diagnosed by serology. ELISA is most widely used laboratory method and also commercially available but PCR is not a commonly used method for leptospira diagnosis. It has greater sensitivity and specificity over the microscopic agglutination test ELISA can detect antibody from 2nd weeks to onward infection (Ahmad *et al*., 2005).

The reported prevalence values of animal leptospirosis across the world are between 2% and 46% depending on the animal species (Leal-Castellanos *et al*., 2003; Faria *et al*., 2007). This wide variation might be related to several factors such as climate, animal species, time of the year, and method of investigation. A serological survey in a rural flood prone district of Bangladesh showed 38% sero-positivity of tested human sera, indicating that the rural population is at high risk of leptospiral infections (Morshed *et al.,* 1994). *Leptospira* was detected by PCR in 18% (63/359) dengue-negative patients in Dhaka, Bangladesh where poverty and poor education were implicated as conditions leading to rodent-borne transmissions (LaRocque *et al.,* 2005). But there is no Chittagong based study on dairy cow which is considered as the milk pocket area located in south east part of Bangladesh. Therefore, the presented study was carried out, for the first time, to estimate the sero-prevalence and associated risk factors of leptospirosis in dairy cows in Chittagong, Bangladesh.

MATERIALS AND METHODS

Study area, period and population: The study was conducted on commercial dairy farms in Chittagong during the period of April 2011 to September 2012. Six dairy farms (206 cows), registered under the Department of Livestock Services (Chittagong), were considered as the source of population from for epidemiological survey, of which 110 cows were selected randomly for this study by generating random number table using Microsoft Excel 2007® for sero-samplings. The study area consisted of rural, urban and peri-urban regions located at Potenga, Baddarhat, Panchlaish, Khulshi, Fatikchari and Hathazari in Chittagong (Fig. 1).

Preset questionnaire and data collection: A structured questionnaire was constructed to acquire farm and cow level production and reproduction information including management, demography and health. The questionnaire was designed to comprise mostly closed and open ended (categorical) questions to easy data processing, minimize variation, and improve precision of responses (Thrusfield, 2005). The questionnaire was backed up by repeated questioning to capture intended information from the

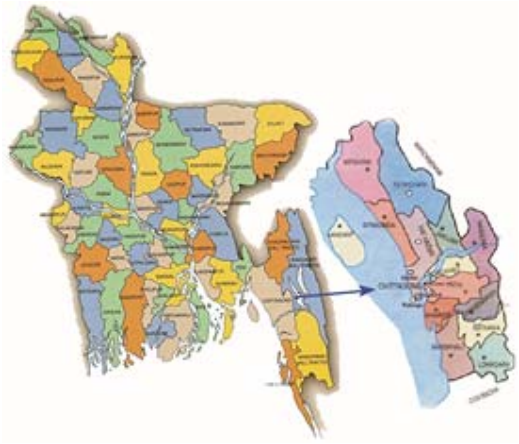

Fig. 1: Different study area in dairy cattle in Chittagong district of Bangladesh (Small white circle indicates the study areas).

farmers, managers and attendants, and complemented with taking records from farm register, artificial insemination cards, visit log books etc. Important data includes total population at farm, breed, pregnancy status and age determined from birth records and dentition characteristics and body condition scores given by observing the animal. Farm level information like types of roughage, concentrate, mixed or ready feed supplied to the cow, source of roughages mainly cultivated or purchase from market, open or close confinement housing pattern, system of offering ration for two or three times in a day, history of deworming for every six monthly or yearly or not and previously vaccine given against Foot and Mouth Disease (FMD) and Black quarter (BQ) was done or not, history of early or late abortion and other reproductive disorders, disposal of aborted materials either buried or trough away in field, herd owner socio-economic status either education in primary, higher secondary or graduate level, any knowledge on leptospirosis too as given for other factors.

Sample collection and processing: Approximately 10 ml of blood samples were collected from jugular vein of each cow by using disposable sterile syringe (10 ml) after using antiseptic from the cattle. Then sera were separated and stored in 1.5 ml Eppendorf tube at -20°C until laboratory tests were performed. The test procedure was performed according to the manufacturers protocol (Bovine Leptospira Hardjo antibody test, 5-LIN-SO, Linnodee Animal Care, Oakmount, Holestone Road, Ballyclare, Northern Ireland BT39OTJ, UK. Lot: 010411). The results were interpreted according to the manufacturer's instructions. Negative and positive controls were kept with each test run. Urine was collected from sero-positive cows. Freshly collected urine samples were examined under the dark field microscopy.

Data analysis: Descriptive univariate and multivariate analysis were performed using STATA 11.2®. All potential cow and farm level exposure variables were examined individually for their effects on the occurrence of leptospira seropositivity (1=yes or no) using chi- square test. Factors identified in univariate analysis at the significance level of $P<0.05$ were forwarded for

multivariable logistic regression model. The logistic regression model was developed and tested its validity. The model outputs were expressed in Odds ratio, standard error (SE) and 95% of confidence interval (CI).

RESULTS

The seroprevalence of leptospirosis was found 52/110 (47.27%) in dairy cattle of Chittagong in Bangladesh, but no urine samples were found positive to leptospirosis under Dark Field Microscopic test. Cow level exposures revealed that; lactation status (Lactating cow) (P=0.02) and physiological status at survey (pregnant cow) (P=0.05) were evident to be potential factors of leptospirosis. On the other hand age, lactation number, Body Condition Score and breed were not significant factors in sero-positivity of leptospirosis. Farm level exposures like Owner educational qualification (higher educational level) (P=0.07), Source of semen (Department of Livestock Services) (P=0.07), size of farm (farm having 50-80 cows) (P=0.05), farm location (farm located in peri-urban area) (P=0.01), total number of employee at farm (farm having more than 25 employee) (P=0.05) were evident to be potential factors of leptospirosis at univariate analysis (Table 1). Dry cows have nearly half less risk (OR=0.42) compared to cows in milk, cyclic/served (OR=0.33) and fresh cows (OR=0.20) have relatively less risk of being sero-positive than pregnant cows. Farms having owner with higher educational qualification (higher secondary-post graduate) (OR=1.36) were 1.36 times higher at risk than lower educational qualification, Farm employee more than 15 (OR=13) were 13 times higher at risk than less number of employee (<15), farm located in peri-urban areas (OR=1.14) were 1.14 times higher at risk than farms located in local and urban areas. Farms having less than 50 cows were of less risk (0R=0.29) than farms of larger size (>50 cows/farm), private sourced semen were less risk (OR=0.05) than used semen from other sources at multivariate analysis (Table 2).

DISCUSSION

The sero-prevalence of leptospirosis in dairy farms were 47.27%, agreed with the results of Zhou *et al.* (2009), Jung *et al*. (2010) and Roberts *et al.* (2010). However higher prevalence of bovine leptospirosis has been reported in some countries like 87% in India (Natarajaseenivasan *et al*., 2011), 89.9% in Poland (Czopowicz *et al*., 2011) and 88.2% in Mexico (Joel *et al.*,

Table 1: Seroprevalence of *Leptospira interrogans* serovar *Hardjo* in randomly selected dairy cattle in Chittagong, Bangladesh

Cow level risk factors	Tested sample	Positive sample	%	Farm level risk factors	Tested sample	Positive sample	%
Age group (n=110)				Size of farm (number of cows/farm)			
Up to 3year	27	12	44.4	Up to 10 cows	16	3	18.7
>3.5-5 year	25	12	48.0	>10-50 cows	35	17	48.5
>5-7 year	36	14	38.8	>50-80 cows	35	21	60.0
>7 year	22	14	63.6	>80 cows	24	11	45.8
Lactation Number (n=110)				Total number of employee in farm			
1	32	15	46.8	Less than 5	16	3	18.7
2	38	16	42.1	>5-15	35	17	48.5
3	30	16	53.3	>15-25	24	11	60.0
>4	10	5	50.0	>25	35	21	45.8
Breed Group (n=110) (F=Friesian, L=Local, S=Sahiwal)				Farm Location			
FXL Cross	52	23	44.2	Isolated	24	11	45.8
FXLXF Cross	31	17	54.8	Urban area	31	10	32.2
SXFXL Cross	27	12	44.4	Peri-urban area	55	31	56.3
Body Condition Score Group (n=110)				Sources of Semen(DLS=Department of Livestock Services, NGO=Non Government Organizations)			
2.75-3.24	48	25	52.0	DLS	75	31	41.3
3.25-3.49	20	8	40.0	NGO/Private	35	21	60.0
>3.5-3.74	24	14	58.3	Stock Type			
>3.75	18	5	27.7	Own stock	86	42	48.8
				Replacement	24	10	41.6
Days in Milk (n=110)				Total farm area (in acres)			
Up to 100	36	20	55.5	Up to 0.3	51	20	39.2
>100-200	26	10	38.4	>0.3-3	35	21	60.0
>200	14	4	28.5	>3	24	11	43.8
Physiologic Status at Survey (n=107)				Who does the insemination(FA= Field Assistant, Govt.=Government)			
Fresh cow	14	11	78.5	Govt. FA	36	13	36.1
Cyclic/served	23	13	56.5	Private FA	24	11	45.8
Pregnant	70	28	40.0	Farm Owner	50	28	56.0
Lactation Status (n=107)				Owner Qualification			
In milking cow	84	45	53.5	Primary-SSC	35	21	60.0
Dry cow	23	7	30.4	HSC-post graduate	75	31	29.4

Table 2: Risk factors of *Leptospira interrogans* serovar *Hardjo* in dairy cattle tested by ELISA in multivariate analysis

Variables/ parameter	Risk factors	Odds ratio	Standard error	z	P>|z|	95% Confidence interval	
Cow level exposures	Dry cows	0.43	0.23	-1.62	0.106	0.15	1.19
	Cyclic/served	0.33	0.26	-1.41	0.158	0.071	1.53
	Fresh cows	0.19	0.14	-2.33	0.020	0.049	0.77
	Owner education	1.36	0.75	0.55	0.584	0.45	4.03
	Total employee	13.00	32.63	1.02	0.306	0.09	1777.45
Farm level exposures	Farm location	1.14	0.78	0.20	0.845	0.30	4.36
	Farm size	0.29	0.52	-0.69	0.490	0.008	9.65
	Source of semen	0.05	0.13	-1.22	0.221	0.0005	5.64

2011). On the other hand, in some countries lower prevalence has been recorded such as 20.3% in Sri Lanka (Gamage *et al.*, 2011), 19.1% in Iran (Tabatabaeizadeh *et al.*,2011), 30.3% in Tanzania (Schoonman and Swai, 2010), 31.3% in Brazil (Dos-Santos *et al.*, 2012) 31.3% and 27.4% in Australia (Subharat *et al.*, 2011). This variation might be due to different geographical locations, management and husbandry practices, disease resistance among different breeds and levels of natural immunity.

The majority of leptospira infections are asymptomatic and the presence of antibodies in the absence of infection indicates exposure to the organism in these animals which were approved by Hassanpour *et al*. (2011). Cattle are the common hosts of *Leptospira interrogans* serovar *Hardjo* observed by Ellis *et al.* (2000)**.** Distinct variations in maintenance hosts and the serovars occur throughout the world, but are particularly common in tropical and subtropical regions where environmental conditions favor the survival and transmission of leptospira (Hartskeerl, 2006). The apparent geographical variation in the sero-prevalence may reflect differences in the levels of natural immunity, management and husbandry practices employed, and sensitivities and specificities of the diagnostic methods used among researchers as well as genetic variation in disease resistance among the breeds (Swai and Schoonman, 2010).

This study also revealed that no *Leptospira* organisms detected in urine under dark field microscopy similar with the findings of Chandrasekharan *et al*. (2004) who reported that *Leptospira* organisms visualized during the first few days of the acute illness, while leptospiremia occurs, by dark field microscopic examination of body fluids such as blood, urine, cerebrospinal fluid and dialysate fluid, approximately 10^9 *leptospires*/ml are necessary for one cell/field to be visible. A variety of clinical specimens may be used for isolation of leptospira from blood or cerebrospinal fluid, or urine sample may be used during the first 7-10 days of infection during symptomatic illness as reported by Bharti *et al*. (2003).

The sero-prevalence for *hardjo* and *pomona* tended to increase with age of the animals reported by Dos Santos *et al.* (2012) contrasting the results of the present study. In this study no significant effect were found among breed on contrary to the findings of Bahaman *et al.* (1987) who showed the drought masters had the highest prevalence whilst the Kedah-Kelantan (an indigenous breed) had the lowest prevalence of leptospiral infection. In general, the temperate breeds of cattle had a significantly (P=0.001) higher prevalence of infection than local breeds. There was no significant effect of body condition score on sero-prevalence of bovine leptospirosis near to the findings of Roberts *et al*. (2010). The intensity of production was a factor which favored the occurrence of Leptospiral infection at the farm level consistent with the result of Dos Santos *et al*. (2012). Higher educational qualification of the owner had significant factor of leptospirosis which is counteracts the results by Dias *et al.* (2007). The higher prevalence of leptospirosis in the farms of owners having higher educational qualification might be due to less attention of the owner to the farm because of their official jobs and other business. Leptospirosis was widely prevalent in urban areas described by Platts-Mills *et al.* (2011) and Dias *et al*. (2007) but present study showed that leptospirosis is also significantly prevalent in peri-urban areas. While we cannot account for the different findings of Platts-Mills *et al*. (2011) but the availability of the rodents in the peri-urban areas might be one of the reasons for high prevalence because rodents act as a reservoir of leptospirosis. Large farm size and number of employee also evident potential risk factors of leptospirosis and both are positively correlated consistence with the observation of Dos Santos *et al.* (2012) and Tabatabaeizadeh *et al.* (2011). A majority of the large cattle and buffalo farms demonstrated a high prevalence of leptospira infection reported by Bahaman *et al.* (1987). The hygienic measurement and sanitation facilities in large scale dairy farm are poor in compare to small scale dairy farm and overcrowded population helps to spreading the infection rapidly and these might be potential risk factors for higher prevalence of leptospirosis.

Conclusion: Leptospirosis was found prevalent and widely distributed in Chittagong. Commercial dairy farms were at the higher risk of leptospirosis. The allied risk factors for the occurrence of leptospirosis in the study area are usually overlooked. Further studies are needed to identify species and biovars, to understand the dynamics of transmission cycles and institution of preventive and control measures (either by vaccination or bio-security policy) particularly among dairy cows, and to identify alternative management practices to replace those that are risk factors for animal and human infections.

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Authors' contribution: MAMP, MAR and MAP implemented the study design. MAP and MRF carried out the laboratory experimentation. MAP drafted and revised the manuscript.

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