

ORIGINAL ARTICLE

Seroprevalence and risk factors of leptospirosis in dairy cattle at some selected coastal areas in Barishal district, Bangladesh

M. Rahman, S. Rahman, M.S. Ahmed*

Department of Medicine, Surgery and Obstetrics, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University, Barisal-8210, Bangladesh

Abstract

Background: Leptospirosis is a worldwide distributed zoonotic disease which affects humans and animals in countries with humid, tropical and subtropical climates. It is a well known cause for abortion, stillbirth or birth of weak calf. The present study was performed to investigate the seroprevalence, risk factors and biochemical values of bovine leptospirosis in dairy cattle at the mentioned area.

Methods: A cross sectional study was performed from February 2021 to January 2022. A total of 240 serum samples of dairy cattle were investigated by rapid test (Genomix Bovine LeptospiraAb Rapid Detection Test Kit^R). The serum samples were further assessed for biochemical analysis. Risk factors were identified by multivariable logistic regression analysis.

Results: The overall prevalence of bovine leptospirosis in dairy cattle was 10%. The odds of leptospirosis was 3.21 (95% CI: 1.20; 8.54) higher in crossbred cows than local cows. In addition, pregnant cows were at 3.66 times higher risk for leptospirosis than non-pregnant cows. The serum creatinine level was significantly higher (0.94 ± 0.06) in disease positive cows than healthy ones (0.73 ± 0.05).

Conclusions: Female crossbred cows should be targeted for future control and surveillance programs in the study area.

Keywords: Cross breed, BCS, Poor hygiene, serum creatinine

*Correspondence: selimpstu476@pstu.ac.bd

Introduction

Livestock is an integral component of the complex farming system in Bangladesh as it not only serves as a source of meat protein but also a major source of farm power services as well as employment. Human life is highly associated with livestock populations in the different livestock production systems (Bekele *et al.*, 2010). But the sector is continuously facing some threats; bovine leptospirosis is one of them. The disease is caused by spirochetes bacteria of the genus *Leptospira*, which is classified into pathogenic and nonpathogenic species with more than 250 pathogenic serovars (Chadsuthi *et al.*, 2018). The infection usually occurs in humans and animals through direct exposure of infected animals or indirect exposure to the soil or water contaminated with urine of infected animals through skin abrasions or mucus membranes and consumption of contaminated feed and water (Haake and Levett, 2015). Rodents are considered as main reservoirs; other animals can also be reservoirs and contribute to the dissemination of infection (Andersen-Ranberg *et al.*, 2016).

Leptospirosis is a well-known cause for abortion, stillbirth or birth of weak calf. It has major economic importance due to the reproductive failure of farm animals. The disease in humans is mostly an occupational hazard in farming communities, veterinarian, butchers and laboratory workers (Parvez *et al.*, 2015). They also reported 47.27% seroprevalence of leptospirosis in cattle. Moreover, they revealed that the disease was significantly higher in lactating and pregnant cows. Denipitiya *et al.* (2017) found 9% and 20% leptospirosis in cattle and buffaloes, respectively. About 6.44% cattle were reported to be infected with leptospirosis in Brazil (Fávero *et al.*, 2017). Morshed *et al.*, (1994) showed that 38% rural peoples of Bangladesh were sero-positive with leptospirosis. Climate change effects like flooding is an important risk factor facilitating the transmission of *Leptospira* from livestock to humans and other animals by spread of bacteria in wet soils and

surface water, where the bacteria can survive for several weeks or months (Saito *et al.*, 2013).

Dark field microscopy is a one of the diagnostic tools for diagnosis of Leptospirosis. Most cases of the disease were diagnosed by serological test. The ELISA is a fundamental screening test of the disease. It is widely used for the diagnosis of the disease. Other tests like microscopic agglutination test (MAT), fluorescent antibody test, radio immunoassay, indirect hemagglutination test, complement fixation test and PCR are used for the diagnosis of leptospirosis (Ahmed *et al.*, 2005). Leptospirosis was diagnosed by PCR in 18% dengue-negative patients in Dhaka, Bangladesh where rodents were an important risk factor for the transmission of disease in poor communities (LaRocque *et al.*, 2005). Biochemical analyses of blood serum are very useful to get insight into the metabolic and health status of animals. Therefore, biochemical parameters could be a good indicator for diagnosis of diseases in animals (Bari *et al.*, 2018). During diagnostic procedure, it is very useful to compare the values obtained from ill animals with normal values in healthy animals (Jezek *et al.*, 2006). The literature available so far revealed limited studies on the prevalence of bovine leptospirosis in Bangladesh. Considering the above facts, the study was performed to determine the seroprevalence and serum biochemical values of bovine leptospirosis in cattle at the mentioned area.

Materials and methods

Study area and duration

This study was performed at the different coastal belt areas where climate change effects were visible at some selected areas of Barishal district, Bangladesh to determine the seroprevalence, risk factors and biochemical parameters examination of bovine leptospirosis in dairy cattle from February/2021 to January, 2022.

Diagnosis of the disease

The presumptive diagnosis of bovine leptospirosis in cattle were performed on the basis of owner's complaints, anamnesis, general clinical examination, clinical findings and field screening test (Rapid test kit for bovine leptospirosis)

History taking

Disease history was recorded carefully by asking questions to the owner, farmer or animal attendant.

General clinical examination

Physical condition of health, behavior, abortion, still birth, weak calf and other systemic abnormalities were noted by visual examination of the patient.

Clinical examination

Clinical examination of all cattle was conducted on the basis of disease history and owner's complaint, clinical findings and techniques used to diagnose bovine leptospirosis in cattle.

Serological test

Rapid diagnostic test (Immuno chromatographic assay): The serum samples were subjected to rapid diagnostic test using Genomix Bovine LeptospiraAb Rapid Detection Test Kit (GENOMIX CARL PVT.LTD) according to manufacturer instructions.

Analysis of biochemical parameters

About 10 ml of whole blood sample was collected from the jugular vein, using plain vacutainer tubes and needles from selected healthy cattle. The tubes were tilted and serum was collected either passively by decanting or after centrifugation of the blood samples at 2500 rpm for 5 minutes. The serum was stored at -20°C until tested serologically. The biochemical parameters (Calcium, Phosphorus, Creatinine, Glucose and Cholesterol) were tested according to manufacturer instructions (Crescent

Diagnostics ®) and reading was taken using EMP Biochemical Analyser®.

Statistical analysis

All of the field and laboratory data were imported to the Microsoft Office Excel-2007. Univariable association between independent variables and leptospirosis sero status was checked by Chi-square test. Variables associated at $P < 0.20$ in the univariable screening were entered in the multivariable logistic regression model to identify risk factors. The methods of model selection, checking collinearity and confounding were described according to Noman *et al.* (2021).

Results and discussion

Seroprevalence

The overall prevalence of bovine leptospirosis in cattle was recorded as 10%. Parvez *et al.*, (2015) reported a higher seroprevalence of 47.27% in dairy cattle. The researchers from other countries also reported various prevalence of leptospirosis in cattle such as 20.3 % in Sri Lanka (Gamage *et al.*, 2011), 19.1% in Iran (Tabatabaeizadeh *et al.*, 2011) and 27.4% in New Zealand (Subharat *et al.*, 2011). The variation might be due to different geographical locations, management practices, disease resistance and levels of natural immunity.

Risk factors

Table 1 shows the results of univariable association between leptospirosis serostatus and explanatory variables. Breed, lactation status and farm location were associated with a P-value < 0.20 and included in the multivariable logistic regression model. The demographic risk factors for bovine leptospirosis are shown in Table 2. The odds of leptospirosis was 3.21 (96% CI: 1.20; 8.54) higher in crossbred cows than local cows. In addition, pregnant cows were at 3.66 times higher risk for leptospirosis than non-pregnant cows.

According to breed wise prevalence, significantly higher prevalence was recorded in cross bred cattle (14.44%) than local breed (5.2%) (Table1).

Rahman and others

Parvez *et al.*, (2015) found that there was no significant effect on breed wise prevalence of leptospirosis in cattle. On the other hand, Bahaman *et al.*, (1987) reported that local wereless susceptible than cross breed cattle which support the present study.

The present study also showed that pregnant cows (20%) were more susceptible than non-pregnant cows (6.67%). According to the physiological status of dairy cattle, it has been reported that fresh cows (78.5%) were more infected with bovine leptospirosis than cyclic

(56.5%) and pregnant cows (40%) (Parvez *et al.*, 2015). However, Gompo *et al.*, (2020) reported that there was no significant effect of the physiological status of dairy cows on the seroprevalence of leptospirosis in dairy cows.

Leptospirosis according to management factor

Table 3 shows the distribution of leptospirosis according to the management factors. However, none of the variation was statistically significant.

Table 1. Univariable association between bovine leptospirosis and explanatory variables in dairy cattle

Demographic determinants	Categories	Number tested	Positive sample	Percentage (%)	P value
Age	<3 years	52	5	9.61	0.911
	3-5 years	76	8	10.52	
	>5-7 years	72	6	8.33	
	>7 years	40	5	12.5	
Breed	Local	115	6	5.2	0.017
	Cross breed	125	18	14.4	
Parity	1	57	3	5.26	0.414
	2	68	7	10.29	
	3	77	8	10.38	
	>4	38	6	15.79	
BCS	>3-4	65	3	4.61	0.080
	>2-3	91	8	8.79	
	>1-2	84	13	15.48	
Lactation status	Milking cows	168	20	11.9	0.132
	Dry cows	72	4	5.56	
Physiological status	Non pregnant	180	12	6.67	0.002
	Pregnant	60	12	20	
Total		240	24	10	

Table 2. Demographic risk factors for leptospirosis identified in the multivariable logistic regression model

Demographic determinants	Categories	Estimate	SE	Odds ratio (95 % CI)	P value
Breed	Local	-	-	Reference	-
	Cross breed	1.17	0.49	3.21 (1.20; 8.54)	0.02
Physiological status	Non pregnant	-	-	Reference	-
	Pregnant	1.29	0.45	3.66 (1.52; 8.82)	0.004

Table 3. Seroprevalence of bovine leptospirosis in dairy cattle based on management factor

Management factor	Categories	Number tested	Positive sample	Percentage (%)	P value
Hygienic management of the farm	Good	65	4	6.15	0.406
	Medium	80	8	10	
	Poor	95	12	12.63	
Farm location	Urban	51	3	5.89	0.144
	Semi urban	84	6	7.14	
	Rural	105	15	14.29	
Farm size (animal numbers)	<10	58	6	10.34	0.944
	>10-50	70	8	11.42	
	>50-80	74	7	9.46	
	>80	38	3	7.89	
Housing	Intensive	90	6	6.67	0.314
	Semi intensive	25	2	8	
	Open	125	16	12.8	

Biochemical parameters in bovine leptospirosis of dairy cattle

Among the tested biochemical parameter, calcium, glucose and cholesterol level was significantly lower in diseased positive dairy cattle as 7.62 ± 0.44 , 56.11 ± 2.70 and 96.76 ± 9.84 than healthy control animals as 9.20 ± 0.29 , 65.01 ± 3.35 and 122.12 ± 1.59 , respectively. The value of phosphorus (6.22 ± 0.41) levels was comparatively higher in disease negative cattle than infected individuals (5.41 ± 0.29).

The creatinine level was significantly increased (0.94 ± 0.06) in disease positive cattle than healthy ones (0.73 ± 0.05) (Table 4). Leptospirosis causes impaired renal function and increases blood creatinine level which supports the present study (Budihal and Perwez, 2014). Chronic leptospirosis in cattle causes mild fever, lethargy and body weight loss (Aqib *et al.*, 2019) which might significantly reduce the calcium, glucose and cholesterol level in infected animals.

Table 4. Changes of biochemical parameters due to bovine leptospirosis in dairy cattle

Parameters	Leptospirosis	Mean±SEM	p-value
Calcium (mg/ml)	Yes	7.62±0.44	0.006*
	No	9.20±0.29	
Phosphorus (mg/ml)	Yes	5.41±0.29	0.051
	No	6.22±0.41	
Creatinine (mg/dl)	Yes	0.94±0.06	0.019*
	No	0.73±0.05	
Glucose (mg/dl)	Yes	56.11±2.70	0.023*
	No	65.01±3.35	
Cholesterol (mg/dl)	Yes	96.76±9.84	0.011*
	No	122.12±1.59	

* Significant at 5% level

Conclusion

Leptospirosis is endemic in the dairy cattle of the study area. Female crossbred cows should be targeted for future control and surveillance programs in the study area.

Competing Interest

The authors declare that they have no competing interests.

Acknowledgement

The authors are grateful to the University Grants Commission (UGC) of Bangladesh for funding.

Reference

- Ahmed SN, Shah S, Ahmad FMH. Laboratory diagnosis of leptospirosis. *Journal of Postgraduate Medicine*. 2005; 51(3):195-200.
- Andersen-Ranberg EU, Pipper C, Jensen PM. Global patterns of leptospira prevalence in vertebrate reservoir hosts. *Journal of Wildlife Diseases*. 2016; 52:468-477. doi: [10.7589/2014-10-245](https://doi.org/10.7589/2014-10-245).
- Aqib AI, Ijaz M, Farooqi SH, Shoaib M, Kulyar MFA, Yasmeen K. Leptospirosis: Rising Nuisance for Cattle and Threat to Public Health, *Bacterial Cattle Diseases*, Hussein Abdel hay El-SayedKaoud. *Intech Open*, 2019; doi: [10.5772/intechopen.82211](https://doi.org/10.5772/intechopen.82211).
- Bahaman AR, Ibrahim AL, Adam H. Serological prevalence of leptospiral infection in domestic animals in West Malaysia. *Epidemiology and Infection*. 1987; 99: 379-392.
- Bari MS, Rana EA, Ahaduzzaman M, Masud AA, Das T, Hasan T. Hemato-biochemical parameters of Peste des Petits Ruminants (PPR) affected goats in Chittagong, Bangladesh. *Journal of Advanced Veterinary and Animal Research*. 2018; 5:211-217. <http://doi.org/10.5455/javar.2018.e270>.
- Bekele B, Demelash A, Fufa A, Regassa J, Godfroid, Skjerve E. Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana. *Tropical Animal Health and Production*. 2010; 43: 651-656. doi: [10.1007/s11250-010-9748-2](https://doi.org/10.1007/s11250-010-9748-2).
- Budihal SV, Perwez K. Leptospirosis Diagnosis: Competency of Various Laboratory Tests. *Journal of Clinical and Diagnostic Research*. 2014; 8(1):199-202. doi: [10.7860/JCDR/2014/6593.3950](https://doi.org/10.7860/JCDR/2014/6593.3950).
- Chadsuthi S, Chalvet-Monfray K, Wiratsudakul A, Suwancharoen D, Cappelle J. A remotely sensed flooding indicator

- associated with cattle and buffalo leptospirosis cases in Thailand 2011-2013. BMC Infectious Diseases. 2018; 18:602. doi:10.1186/s12879-018-3537-3.
9. Denipitiya DTH, Chandrasekharan NV, Abeyewickreme W, Hartskeerl RA, Hapugoda MD. Identification of cattle, buffaloes and rodents as reservoir animals of *Leptospira* in the District of Gampaha, Sri Lanka. BMC Research Notes. 2017; 10:134. <https://doi.org/10.1186/s13104-017-2457-4>.
 10. Fávero JF, de Araújo HL, Lilenbaum W, Machado G, Tonin AA, Baldissera MD, Stefani LM, Da Silva AS. Bovine leptospirosis: Prevalence, associated risk factors for infection and their cause-effect relation. Microbial pathogenesis. 2017; 107:149-54. <https://doi.org/10.1016/j.micpath.2017.03.032>
 11. Gamage CD, Koizumi N, Muto M, Nwafor-Okoli C, Kurukurusuriya K, Rajapakse JRPV, Kularatne SAM, Kanda K, Lee RB, Obayashi Y, Watanabe H, Tamashiro H. Prevalence and carrier status of leptospirosis in smallholder dairy cattle and peridomestic rodents in Kandy, Sri Lanka. Vector Borne Zoonotic Diseases. 2011; 11:1041-1047. doi: [10.1089/vbz.2010.0153](https://doi.org/10.1089/vbz.2010.0153).
 12. Gompo TR, Jyoti S, Pandit S, Sapkota RC, Pandey A. Sero-prevalence and risk factors of leptospirosis in commercial cattle herds of Rupandehi district, Nepal. bioRxiv. 2020; doi: <https://doi.org/10.1101/2020.07.29.226464>.
 13. Haake DA, Levett PN. Leptospirosis in human. In: *Leptospira and Leptospirosis* edn: Springer. 2015; pp. 65-67.
 14. Jezek J, Klopcic M, Klinkon M. Influence of age on biochemical parameters in calves. Bulletin of the Veterinary Institute in Pulawy. 2006; 50: 211-214.
 15. LaRocque RC, Breiman RF, Ari MD, Morey, FA Jahan, Hayes JM, Hossain MA, Brooks WA and Levett PN. Leptospirosis during dengue outbreak, Bangladesh. Emerging Infectious Diseases. 2005; 11:766-769. doi: 10.3201/eid1105.041212.
 16. Morshed MG, Konishif H, Terada Y, Arimitsu Y and Nakazawa T. Seroprevalence of leptospirosis in a rural flood prone district of Bangladesh. Epidemiology Infection. 1994;112:527-531. doi: [10.1017/S0950268800051220](https://doi.org/10.1017/S0950268800051220).
 17. Noman Z, Anika TT, Haque ZF, Rahman AK, Ward MP, Martinez-López B. Risk factors for rabid animal bites: a study in domestic ruminants in Mymensingh district, Bangladesh. Epidemiology & Infection. 2021; 149.
 18. Parvez MA, Prodhan MAM, Rahman MA, Faruque MR. Seroprevalence and associated risk factors of *Leptospira interrogans* serovar Hardjo in dairy cattle of Chittagong, Bangladesh. Pakistan Veterinary Journal. 2015; 35(3):350-354.
 19. Saito M, Villanueva SY, Chakraborty A, Miyahara S, Segawa T, Asoh T, Ozuru R, Goriani NG, Yangihara Y, Yoshida S. Comparative analysis of *Leptospira* strains isolated from environmental soil and water in the Philippines and Japan. Applied Environmental Microbiology. 2013;79(2):601-609. doi:10.1128/AEM.02728-12.
 20. Subharat S, Wilson PR, Heuer C, Collins-Emerson JM, Smythe LD, Dohnt MF, Craig SB and Burns MA. Serosurvey of leptospirosis and investigation of possible novel serovar Arborea in farmed deer in New Zealand. New Zealand Veterinary Journal. 2011; 59:139-142. doi: [10.1080/00480169.2011.561780](https://doi.org/10.1080/00480169.2011.561780).
 21. Tabatabaeizadeh E, Tabar GH, Farzaneh N, Seifi HA. Prevalence of *Leptospira hardjo* antibody in bulk tank milk in some dairy herds in Mashhad suburb. African Journal of Microbiology Research. 2011; 5:1768-1772.