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Research Article

Leptospira Epidemiology in Dairy Cattle of Bangladesh

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Abstract

Bovine Leptospirosis causes enormous economic loss due to reproduction failure and production loss. It has been perceived as a rising global public health concern. Bovine species acts as career or vectors, whereas human are the dead end host. No scientific attempt has previously been taken to investigate epidemiological diversity of *Leptospira* in commercial dairy cattle in Bangladesh. Hence, a cross -sectional study was conducted in commercial dairy cattle in Bangladesh to describe epidemiological scenario of *Leptospira*. Nineteen upazillas or sub - districts from 12 districts of 7 divisions were randomly chosen for the study. A total 43 dairy cattle farm, 1-6 farms per upazilla was recruited based on the presence of increasing abortion history within past six months. A pre-tested questionnaire was used to collect epidemiological information through face-to-face interview and direct observation. Blood and urine samples and aborted fetuses were collected for laboratory evaluation. Sero-positivity for *Leptospira* hardjo was evaluated on the samples obtained using OIE protocol-based Enzyme Linked Immuno Sorbent Aaasay (ELISA) technique. Dark Field Microscopy (DFM) Examination was carried on urine samples. Aborted fetus was evaluated through bacteriological culturing followed by Polymerase Chain Reaction (PCR). The PCR positive samples were further sequenced for phylogenetic analysis. The overall sero-prevalence of *Leptospira* was 17.9% (95% CI: 9.4%-31.4%) in dairy cattle in Bangladesh. The proportionate *Leptospira* prevalence was 55.6% in cattle (n = 45 urine samples) and 32% in fetuses (n = 25) and 32% in specimens obtained from fetuses (n = 100) were estimated from urine and fetal sample, respectively. Breed (Exotic vs Cross: OR = 3.4) and age (≤ 4.5 vs ≥ 4.6: OR = 5.0) were identified as potential risk factors for *Leptospira* sero-prevalence in dairy cattle. One of the sequences of Leptospira isolate in the present study had a close congener (78%) of the sequence of *Leptospira* isolated from cattle in Brazil. Overall results suggest *Leptospira* is commonly circulating in dairy cattle in Bangladesh. Preventive measures including breed selection, vaccination status, quarantine of new animals, and exclusion of wildlife vectors, and farm superintendence should be practiced. Field veterinarians should also be properly educated in handling abortion cases and treating animals for prophylactic measures..

Keywords: *Leptospirosis*; Sero-Prevalence; Dairy Cattle; Bangladesh

Introduction

Leptospira is a Gram-negative bacterium (*S*pirochete*)* and detected by Enzyme Linked Immuno Sorbent Assay, Micro Agglutination Test, Dark Field Microscopy, Polymerase Chain Reaction. In dairy cattle, *Leptospira* causes abortion in rate up to 30% when it occurs during the final third of the pregnancy [27]. The causative organisms are shed in urine and survive in surface water, streams, or moist, alkaline soil. There are more than 100 serotypes of *Leptospira* but only seven serotypes have been recognized in dairy cattle, it is a worldwide zoonotic disease and having global public health problem, with its increased morbidity and mortality [2,47]. A study determined that rural people in Bangladesh are at high risk to leptospiral infection [31]. When *Leptospira interrogens* serovar *hardjo* infection becomes endemic within in a herd or region, it is common to have 30-40% of the animals infected and shedding the organisms in their urine at any one time [9]. There is no specified information on actual load of bovine leptospirosis in Bangladesh with a negative impact of 750-1000 US$/Cow/Year [7].

Leptospira colonizes the kidneys of carrier animals such as cattle, goat, and sheep [44] and is shed in urine, which is the primary source of environmental contamination [19]. Animals and humans become infected by exposure of a skin cut or mucous membrane abrasion to contaminated urine, mud, soil, or surface water (rivers, lakes, or ponds) [29,41].

Evaluation of the prevalence of Leptospirosis in cattle has been performed in a variety of countries using urine samples and aborted fetuses. The prevalence of Leptospirosis in urine ranged from 4.0% in Turkey [10] to 90% in the Netherlands due to seasonal variations and types of soil, and the prevalence in aborted fetuses ranged from 6.1% in Canada [38] to 12.8% - 20.9% in Iran [5,30] due to geographical location and seasonal variation.

Leptospira diagnosis is difficult and relies on a wide variety of laboratory techniques including molecular tests, and microscopic examination. For urine samples, DFM examination (Sensitivity: 10^4 bacteria/ml with very low specificity) [24] is used to directly visualize the bacteria. For both urine and aborted fetal tissue samples, bacterial culture (Sensitivity: 5%-50% and specificity 100%) can detect the presence of Leptospirosis. Overall, PCR is the most sensitive diagnostic test for *Leptospira* detection (Sensitivity: 100% and specificity: 93%) [24] and is the preferred technique.

Although varieties of research have previously been carried out on the presence of *Leptospira hardjo* or other *Leptospira* serovars in aforementioned countries, very few studies have been attempted to investigate Leptospirosis using urine and aborted fetus samples in cattle in Bangladesh as well as by phylogenetic analysis [21,30,41]. Therefore, the present study increases our understanding on the presence of Leptospirosis in cattle in Chattogram, Bangladesh. The objectives of the present study were to estimate proportionate prevalence of *Leptospira* and identify the similar sequence of *Leptospira* through phylogeny.

Materials and Methods

- **Study Period:** June, 2013 to June, 2014
- **Study Area:** 43 selected dairy farms from 73 sero-positive farms under the study periods by ELISA (OIE, 2008)
- **Samples:** Sera (464) from 7 Divisions; urine [45] and aborted fetuses [25] from Chattogram Division
- **Experimental Design:**

- Preparation of inoculum from samples → Culture on medium (*Leptospir*a Based EMJH Medium and *Leotpspira* Enrichment Medium) → Multiplication of *Leptospira* → Examination under DFM for isolation → DNA extraction → Molecular characterization by conventional PCR → Gene sequencing and phylogenies
- Oligonucleotide primers used in PCR to detect *Leptospira hardjo*: rrs (16S) gene of *L. interrogens* (10)
- Forward Primer 5'-GGCGGCGCGTCTTAAACATG-3'
- Reverse Primer 5'-TTCCCCCCATTGAGCAAGATT-3'

- **Data analysis:** Descriptive and summative statics were used on the results of EMJH and DFM test results. Statistical analysis was carried out on field and laboratory data as required by using STATA Software. A p-value of < 0.05 ($p < 0.05$) was considered statistically significant.

Results

The overall sero-prevalence of *Leptospira hardjo* was 17.9% in dairy cattle of Bangladesh. The cluster variables had no significant effect on the prevalence (Table 1).

Both on microscopic and bacterial evaluation, 55.6% of samples (N = 45) were identified as positive for *Leptospira hardjo*. Older cattle had a higher proportion of *Leptospira* infection (73.3%) than younger cattle (46.7%) ($p = 0.09$, which is statistically significant). However, the proportion of *Leptospira* positive samples did not vary among different breeds (Table 2). The finding of thread-like *Leptospira hardjo* bacteria under DFM is presented in figure 1.

Cluster variable	Prevalence	SE	95% CI
Farm	0.179	0.054	0.094-0.314
Sub-district (Upazilla/Thana)	0.179	0.059	0.085-0.337
District	0.179	0.062	0.080-0.354
Division	0.179	0.066	0.068-0.393

Table 1: Overall sero-prevalence of *Leptospira* in the dairy cattle of Bangladesh (N = 464) accounting for different cluster variables.

Figure 1: Thread-like structures of *Leptospira hardjo* under Dark Field Microscopy.

		Dark Field Microscopy		
Factor	**Category**	**+ (%)**	**-(%)**	***P* (Fisher's exact test)**
Breed	Fresian and Shahiwal	8 (61.5%)	5	0.775
	Holstein Fresian	15 (51.7%)	14	
	Holstein Fresianand Local/ Shahiwal and Local	2 (66.7)	1	
Age (Year)	6-6.5	14 (46.7%)	16	0.09
	7	11 (73.3%)	4	

Table 2: Univariate analysis of DFM results for *Leptospira* infections in cattle, Chattogram.

Upon bacterial evaluation on pooled tissue samples (Samples of eye ball, liver, lung and kidney per fetus), 32% of aborted fetuses (N = 25) were positive for *Leptospira hardjo*. Out of 100 individual specimens 32 were positive for *Leptospira hardjo*. The distribution of *L. hardjo* by specimen was as follows: 8 eyeball, 8-liver, 8-lung, and 8-kidney from 8 different fetuses. The fetuses of older cows had a significantly higher *Leptospira* prevalence (22.5%) than in the fetuses of younger cows (17.7%) (p = 0.025). Age of fetal abortion had no effect on *Leptospira* prevalence (Table 3).

All fetal specimens that were *Leptospira* positive on bacterial culture were also *Leptospira* positive on PCR. The PCR results are presented in table 4 and figure 2.

Only two PCR positive samples (Fetus 20: eyeball and fetus 21: eyeball) were successfully sequenced. These two sequences were compared with Leptospira sequences available in the GeneBank. One of the fetal sample *Leptospira* sequences (Cattle_Bangladesh_F/1-825) (Fetus 20) had a close congener to a *Leptospira* sequence isolated from cattle in Brazil (Figure 5.3). The other fetal sample sequence (Cattle_Bangladesh_F/1-862) (Fetus 21) had poor similarity to sequences of *Leptospira* isolated from humans and water in the United Kingdom (Figure 3).

Discussion

Leptospirosis is an emerging, infectious, zoonotic disease. It has high morbidity and mortality and is currently causing a global pub-

Variable	Category	N	Positive	%	*P (Fisher's Exact test)*
Breed	Friesian	5	2	40.0	
	Holstein Friesian	18	6	33.3	
	Holstein Friesian× Local	1	0	100	
	Shahiwal × Local	1	0	100	
Breed	Holstein Friesian	18	6	33.3	0.82
	FS/Shahiwal × Holstein Friesian and Local	7	2	28.6	
Cow age	≤ 75 months	17	3	17.7	0.025
	≥ 75 months	8	5	22.5	
Fetus age	≤ 120 days	7	1	14.3	0.38
	> 120-180	12	4	33.3	
	> 180	6	3	50.0	

Table 3: Univariate analysis of the bacteriological results for *Leptospira* in Cattle, Chattogram.

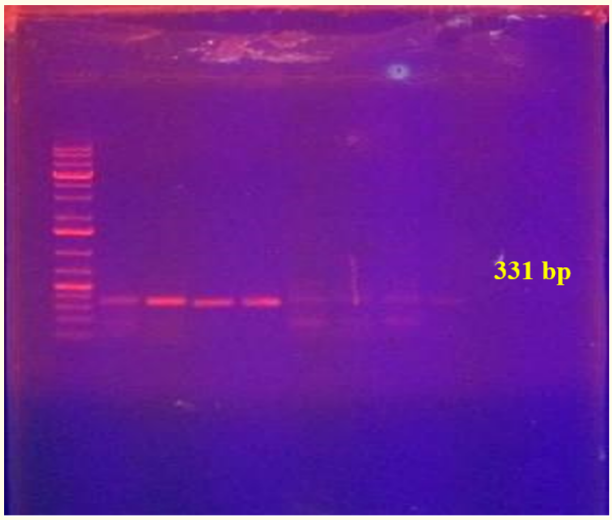

Figure 2: An ethidium bromide-stained agarose gel of PCR products that shows the sensitivity of the assay. DNA marker (100bp); band at 331bp.

Fetus ID	Eyeball	Liver	Lung	Kidney	Pooled sample
Fetus 18	+	+	+	+	+
Fetus 19	+	+	+	+	+
Fetus 20	+	+	+	+	+
Fetus 21	+	+	+	+	+
Fetus 22	+	+	+	+	+
Fetus 23	+	+	+	+	+
Fetus 24	+	+	+	+	+
Fetus 25	+	+	+	+	+

Table 4: PCR results for *Leptospira hardjo* by fetus and specimens.

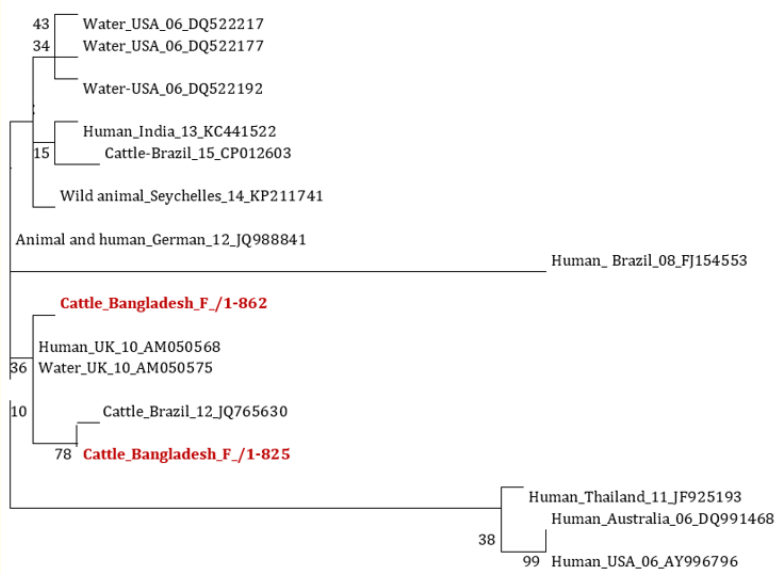

Figure 3: Phylogram of two successfully isolated sequences (red) of Leptospira compared to similar and dissimilar GeneBank sequences of Leptospira isolated from animals, humans, and water sources.

lic health problem [26]. This section discusses the important findings of the present study on Leptospirosis in dairy cattle in Bangladesh, their implications, and the study's limitations.

This study was the first to estimate the sero-prevalence of *Leptospira* in dairy cattle in Bangladesh. The prevalence was 17.9%, which corresponds with the results of studies conducted in countries with similar dairy cattle production system. The reported sero-prevalence of *Leptospira* in dairy cattle in neighboring countries was reported to be 12.8% in India (South Gujarat) [37], 19.4% in Bhutan [46], 20.3% in Sri Lanka [20], 32% in Nepal [39], and 63.3% in Pakistan [3]. Variable sero-prevalence of *Leptospira* has also been documented in dairy cattle in different countries across the world, for example 3% in Germany [16], 10.4% in Spain [17], 27.4% in Australia [45], 30.3% in Tanzania [43], 31.3-47.6% in Brazil [35,15], 87% in India [32], 88.2% in Mexico [25] and 89.9% in Poland [12]. These discrepancies in *Leptospira* sero-prevalence in dairy cattle might be due to the type of study, different geographical locations, management and farm husbandry practices, disease resistant patterns, levels of natural immunity, and use of vaccines [4,35,45].

The Random Effect Logistic regression model determined cattle breed and age as significant risk factors associated with *Leptospira* sero-positivity in the current study. Cross breed cattle had around three and half times higher odds of *Leptospira* sero-positivity than exotic breeds. This result is supported by some researchers [36] who found that temperate or cross breed cattle had a significantly higher prevalence of infection ($p = 0.001$) than local breeds. Younger animals were five times more likely to be sero-positive than older dairy cattle which is a surprising result and difficult to explain. Some researchers [33] found that cattle over five years old were more likely to be sero-positive (12.5%) when compared to other age groups (0-1.8%). This may be due to the long duration and persistence of antibodies in the animals after a longer period of exposure.

Cattle with more than 1 parities were 3.1 times more likely to exhibit *Leptospira* infection than cattle with one or no parity ($p = 0.15$; 95% CI 0.7-14.8) which was similar to one research [33].

The prevalence of *Leptospira* in aborted fetuses of dairy cattle was 32% in the current study. This result is similar to an earlier study [13] which reported a 28.6-31.9% prevalence in dairy cattle in Iran. However, other studies in Iran have reported variable estimates of *Leptospira* prevalence in aborted fetuses of dairy cattle such as 12.8% [5], 14.6% [14] and 20.9% (30). The sero-prevalence of Leptospirosis in neighboring countries have been reported

as 19.4% in Bhutan (46), 12.8% in India (South Gujarat) [37], 32% in Nepal [39], 63.3% in Pakistan [3] and 20.3% in Sri Lanka [20]. The prevalence of *Leptospira* in each sampled organ of aborted fetus of dairy cattle was 25% in the present study. This finding is also similar to a study in Iran [13] (30.9%). However, a research in Iran [30] again found a lower prevalence (11.6%).

The prevalence of *Leptospira* in dairy cattle urine samples was 55.6%, which is closely aligned with the result of some researches [22,23] who found 53.8-56.2% in dairy cattle in Iran. However, one research [42] found 12.1% in Iran, and one research [27] detected 68.3% in Kerala, India. Older cattle had a higher proportion of *Leptospira* infection (73.3%) than younger cattle (46.7%) (p = 0.09), which is very likely the case and explained earlier that older cattle have more exposure time and are weaker immunologically [14,27,36] which support the higher prevalence in older cattle. Similar age specific finding of *Leptospira* in urine of dairy cattle was reported by many studies [6,8,18,19,21].

Of the two PCR positive samples successfully sequenced, one (Cattle_Bangladesh_F_/1-825) was significantly similar (78%) to the sequence of *Leptospira hardjo* isolated from cattle in Brazil [11] (Figure 4.3). This is a new finding. However, it is difficult to explain how the Brazilian *Leptospira* strain was introduced to Bangladesh when there is no trading of cattle between the two countries. This *Leptospira* strain could have existed long in Bangladesh as suitable environment conditions to grow and survive for the organism are present [18,14,41], but no exploration was attempted earlier.

The other sequence in this study (Cattle_Bangladesh_F_/1-862) had poor similarity (36%) to sequences of *Leptospira* isolated from humans and water in UK. This is a preliminary indication of sequence similarities between cattle and human *Leptospira*. Longer versions of the isolated sequences are needed to explore a genuine relationship in future.

Conclusion

The proportionate *Leptospira* prevalence (*Leptospira hardjo)* was 55.6% in cattle (urine samples) and 32% in fetuses. One of the sequences of *Leptospira* in the present study (Cattle Bangladesh F/1-825) had a close congener to a sequence of *Leptospira* isolated from cattle in Brazil. Overall findings suggest that a substantial amount of *Leptospira* infection is circulating in the commercial cattle population of Chattogram. Therefore, both preventive and prophylactic measures including breed selection, vaccination, quarantine of new animals, and careful herd management should be practiced. To prevent potential transmission, occupational groups such as field veterinarians should handle all cases of abortion according to OIE protocol [34].

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Bibliography

1. Adler B. "Vaccines against leptospirosis". *Current Tropical Microbiology and Immunology*, 387 (2015): 251-272.

2. Ahmed N., *et al*. "Multilocus sequence typing method for identification and genotypic classification of pathogenic *Leptospira* species". *Annals of Clinical Microbiology and Antimicrobials* 5 (20036): 28.

3. Anwar K., *et al*. "Seroprevalence of leptospirosis in aborted dairy cattle in Peshawar district suburb, Khyber Pakhtunkhwa, Pakistan". *International Journal of Current Microbiology and Applied Sciences* 2.8 (2013): 73-78.

4. Avant S. "Vaccine protects against leptospirosis in cattle". *USDA Agricultural Research Service*.

5. Badiee A., *et al*. "Prevalence of *Leptospira*spp. in bovine aborted fetuses of dairy cattle herds by PCR in Tehran province". *Journal of Veterinary Clinical Research* 1.3 (2010): 153-160.

6. Bal AE., *et al*. "Detection of *Leptospires* in urine by PCR for early diagnosis of Leptospirosis". *Journal of Clinical Microbiology* 32.8 (1994): 1894-1898.

7. Bangladesh Bureau of Statistics (BBS). Preliminary report on household income and expenditure survey 2005. Date of Publication: (2006).

8. Baquero MI., *et al*. "Evaluation of a Polymerase Chain Reaction for the Diagnosis of Leptospirosis in Cattle". *The Open Veterinary Science Journal* 4 (2010): 31-35.

9. Bolin CA., *et al*. "Diagnosis and control of bovine leptospirosis". Proceedings of the 6th Western Dairy Management Conference (2003): 155-159.

10. Cetinkaya B., *et al*. "Detection of *Leptospira* Species by polymerase Chain Reaction (PCR) in urine of cattle". *Turkish Journal of Veterinary and Animal Sciences* 24 (2000): 123-130.

11. Cosate MRV., *et al*. "Molecular typing of *Leptospira* interrogans serovar Hardjo isolates from Leptospirosis outbreaks in Brazilian livestock". *BMC Veterinary Research* 13 (2017): 177-200.

12. Czopowwicz MJK., *et al*. "*Leptospira*l antibodies in the breeding goat population of Poland". *Veterinary Records* 169 (2011): 230.

13. Dehkordi FS and Taghizadeh F. "Prevalence and some risk factors with brucellosis and leptospirosis in aborted fetuses of ruminant species". *Research Opinions in Animal and Veterinary Sciences* 2.4 (2012): 275-281.

14. Doosti A and Tamimian NH. "Diagnosis of *Leptospira*l Abortion in Bovine by Polymerase Chain Reaction". *Global Veterinaria* 7.1 (2011): 79-82.

15. Dos-Santos JP., *et al*. "Seroprevalence and risk factors for leptospirosis in goats in Uberlandia, minas Gerais, Brazil". *Tropical Animal Health Production* 44 (2012): 101-106.

16. Drager KG and Jonas D. "Serological prevalence of leptospirosis: a survey in pigs and cattle in Reihland-Pfaiz covering several years". *Tierartzliche-Umschau* 45 (1990): 483-486.

17. Espi A., *et al*. "Bovine leptospirosis: microbiological and serological findings in aborted fetuses". *Veterinary Record* 110 (1982): 147-150.

18. Eys GGMV., *et al*. "Detection of *Leptospires* in Urine by Polymerase Chain Reaction". *Journal of Clinical Microbiology* 27.10 (1989): 2258-2262.

19. Faine S., *et al*. "*Leptospira* and Leptospirosis". 2nd Edition, MediSci. Melbourne, Vic. Australia (1999): 301-304.

20. Gamage CD., *et al*. "Prevalence and carrier status of leptospirosis in smallholder dairy cattle and peridomestic rodents in Kandy, Sri Lanka". *Vector-Borne and Zoonotic Diseases* 11.8 (2011): 1041-1047.

21. Gerritsen MJ., *et al*. "Sample Preparation Method for Polymerase Chain Reaction-Based Semiquantitative Detection of *Leptospira interrogans* Serovar Hardjo Subtype Hardjobovis in Bovine Urine". *Journal of Clinical Microbiology* 29.12 (1991): 2805-2808.

22. Hajikolaei MRH., *et al*. "Seroprevalence of *Leptospira*l infection in buffalo (*Bubalus bubalis*)". *Bulletin- Veterinary Institute in Pulawy* 50 (2006): 341-344.

23. Hajikolaei MRH., *et al*. "Exiatence of *Leptospira interrogans* in kidney and shedding from urine and relationship with histopathological and serological findings in water buffaloes (*Bubalus bubalis*)". *Revue de Médecine Vétérinaire* 167 (2016): 9-10.

24. Hartskeerl RA., *et al*. "Emergence, control and re-emerging leptospirosis: Dynamics of infection in the changing world". *Clinical Microbiology and Infectious Diseases* 17 (2011): 494-501.

25. Joel NE., *et al*. "*Leptospira* prevalence in a population of Yucatan, Mexico". *Journal of Pathology* (2011): 408604.

26. Ko AI., *et al*. "*Leptospira*: the dawn of the molecular genetics era for an emerging zoonotic pathogen". *Nature Reviews Microbiology* 7 (2009): 736-747.

27. Krishna VS., *et al*. "Evaluation of dark field microscopy, isolation and microscopic agglutination test for the diagnosis of canine leptospirosis". *International Journal of Pharmacy and Biological Sciences* 29.3 (2012): 85-89.

28. Laras K., *et al*. "The importance of leptospirosis in South-east Asia". *American Journal of Tropical Medical Hygiene* 67.3 (2002): 278-286.

29. Levett P. "Leptospirosis". *Clinical Microbiology Reviews* 14.2 (2001): 296-326.

30. Momtaz H and Moshkelani S. "Detection and characterization of *Leptospira* spp. isolated from aborted bovine clinical samples". *Acta Veterinaria Brno* 81.1 (2012): 21-25.

31. Morshed MG., *et al*. "Seroprevalence of leptospirosis in a rural flood prone district of Bangladesh". *Epidemiol Infect* 112.3 (1994): 527-531.

32. Natarajaseenivasan KKV., *et al*. "Seroprevalence of *Leptospira* infection in dairy cattle, rats and humans in the Cauvery river valley of Southern India". *Southeast Asian Journal of Tropical Medical Public Health* 42 (2011): 679-686.

33. Ngbede EO., *et al*. "Serological prevalence of leptospirosis in cattle slaughtered in the Zango abattoir in Zaria, Kaduna State, Nigeria". *Veterinaria Italiana* 48.2 (2012): 179-184.

34. OIE. "OIE Terrestrial Manual". Chapter 2.1.9.: Leptospirosis (2008): 255.

35. Oliviera TS., *et al*. "Evaluation of the *Leptospira* interrogans Outer membrane protein OmpL37 as a vaccine candidate". *Plos One* 10 (2015): 1371.

36. Parvez A., *et al*. "Seroprevalence and Associated Risk Factors of *Leptospira Interrogans* Serovar *Hardjo* in Dairy Cattle of Chittagong, Bangladesh". *Pakistan Veteterinary Journal* 35.3 (2015): 350-354.

37. Patel JM., *et al*. "Seroepidemiological pattern of leptospirosis in bovine of South Gujarat, India". *Veterinary World* 7.11 (2014): 999-1003.

38. Prescott JF., *et al*. "Seroprevalence and Association with Abortion of Leptospirosis in Cattle in Ontario". *Canadian Journal of Veterinary Research* 52 (1988): 210-215.

39. Rai SK., *et al*. "Serilogical Study of *Leptospira* Infection in Nepal by One - point MCA Method". *Journal of Infectious Diseases and Antimicrobial Agents* (2000): 29-32.

40. Rao AM. "Preventive measures for leptospirosis: rodent control". *Indian Journal of Medical Microbiology* 24 (2006): 325-328.

41. Saito M., *et al*. "PCR and Culture Identification of Pathogenic *Leptospira* spp. from Coastal Soil in Leyte, Philippines, after a Storm Surge during Super Typhoon Haiyan (Yolanda)". *Applied and Environmental Microbiology* 80.22 (2014): 6926-6932.

42. Sakhaee E., *et al*. "Serological and bacteriologic diagnosis of bovine leotpspirosis in Tehran suburb dairy farms". *Iranian Journal of Veterinary Research*, University of Shiraz 8.4 (2007): 325-332.

43. Schoonman L and Swai ES. "Herd-and animal-level risk factors for bovine leptospirosis in Tanga region of Tanzania". *Tropical animal Health Production* 42 (2010): 1565-1572.

44. Smythe L., *et al*. "Review of leptospirosis notifications in Queensland and Australia: January 1998-June 1999". *Communicable Diseases Intelligence* 24.6 (2000): 153-157.

45. Subharat S., *et al*. "Serosurvey of leptospirosis and investigation of a possible novel serovar Arborea in farmed deer in New Zealand". *New Zealand Veterinary Journal* 59.1 (2011): 139-142.

46. Tenzin J. "Risk based surveillance of Leptospirosis in cross-species domestic animals in Bhutan". 2nd National One Health Workshop (2015): 4-6.

47. World Health Organization (WHO). "Human Leptospirosis: Guidance for diagnosis, surveillance and control". Geneva: International *Leptospira*l Society (2003).