Predominance of Leptospira wolffii in north-central Bangladesh, 2019

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Abstract

Leptospira was detected in 48.9% of blood samples from 182 febrile patients in north-central Bangladesh in 2019. Most *Leptospira* were classified as *L. wolffii* (93%) on the basis of phylogenetic analysis of 16S ribosomal RNA genes, while others were assigned to *L. borgpetersenii* and *L. meyeri*.

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Leptospirosis is the most common anthropozoonosis worldwide. It is caused by a pathogenic species of the genus *Leptospira*, with an estimated 1.03 million cases and 58 900 deaths annually [1]. In developing countries, this disease remains largely underestimated as a result of the variability of its clinical manifestation and the unavailability of appropriate laboratory diagnostic facilities. In Bangladesh, the prevalence of leptospirosis among febrile illnesses has been described mostly by using serologic tests [2–4]. In our previous study, *L. interrogans* was first genetically identified as a major *Leptospira* species in northcentral Bangladesh [5]. On the basis of those results, we conducted an extended epidemiologic study of *Leptospira*.

Blood samples were collected from 182 febrile patients in Mymensingh Medical College hospital, located in north-central Bangladesh, between April and December 2019. The enrolled patients had fever (temperature $>38.5^{\circ}$ C) for more than 5 days with headache, myalgia and general prostration associated with additional symptoms (e.g. jaundice) (Supplementary Table SI). Leptospira-specific IgM was detected in 60 samples (33.0%) by an enzyme-linked immunosorbent assay (ELISA) kit (DRG International, Springfield Township, NJ, USA), and the leptospiral 16S ribosomal RNA (rRNA) gene was identified in 65 samples (35.7%) by the nested PCR method [6]. A total of 89 samples (48.9%) tested positive for Leptospira by ELISA, PCR or both, while 24 and 29 samples were positive by solely ELISA or PCR respectively. Among the 89 positive cases, 65 occurred in male subjects, and 67 occurred in patients who lived in rural areas. Although positive cases were found in any month during the study period, more cases occurred in July, September and October. The overall detection rate of Leptospira was higher than that in our previous study in Bangladesh [5] but was comparable to that in Malaysia [7]. All patients were cured by administration of doxycycline and ceftriaxone for a 7- to 10-day period along with treatment of symptoms.

Nucleotide sequences of partial 16S rRNA gene were directly determined with PCR products of 29 samples. Phylogenetic analysis revealed that 27 samples (93%) clustered with *L. wolffii*, while one sample each was assigned to *L. borgpetersenii* (pathogenic group) and *L. meyeri* (nonpathogenic group) (Fig. 1). Nucleotide sequences were deposited in GenBank under accession numbers MT611935 to MT611940.

Our previous study in 2018 demonstrated the dominance of *L. interrogans*, while *L. wolffii* was identified in only a single sample [5]. In contrast, in the present study, *L. wolffii* was predominant and we found no *L. interrogans*. *L. wolffii* was detected sporadically from April to November, which may suggest that this species is persistently transmitted among the local population. *L. wolffii* has been classified as an intermediate species and is found in a wide area from South-East Asia to the Middle East as a minor pathogen of human leptospirosis which is also detected in animals and the environment [7–11]. Our study is the first to note the predominance of this species in human infection.

By whole genome-based phylogenetic analysis of *Leptospira*, the formerly described 'intermediate group' was classified into subclade P2, one of the pathogenic clades, as well as P1, representing the former 'pathogenic group' [12]. Such genetic evidence may also underscore the importance of *L. wolffii* as a



FIG. I. Phylogenetic dendrogram based on partial 16S ribosomal RNA gene sequences of *Leptospira* constructed by maximum likelihood method using MEGA6 programme, following alignment with Clustal W algorithm. Tree was statistically supported by bootstrapping with 1000 replicates; phylogenetic distances were measured by Kimura two-parameter model with uniform rates among sites. Samples we analysed are indicated by solid circles; previous study in Mymensingh, Bangladesh [5], uses diamonds. Bootstrap values more than 80% are shown. Scale bar represents genetic distance, i.e. number of substitution per site. Subclusters are shown at right using designation in parentheses (P1, P2, S1/S2) by Guglielmini et al. [12] along with former designations (pathogenic, intermediate, nonpathogenic). Cluster of *L. wolffii* and closely related species to *L. wolffii* in P2 subclade is shown by vertical line.

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cause of human leptospirosis. *L. wolffii* has been reported to be a dominant species in dogs in Iran, suggesting its putative transmission to humans [10]. Further surveillance is necessary to monitor the prevalence and species of *Leptospira* in Bangladesh, particularly to determine the epidemiologic trend of *L. wolffii* and its prevalence in dogs as a potential reservoir.

Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https:// doi.org/10.1016/j.nmni.2020.100765.

References

- [I] Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, Martinez-Silveira MS, et al. Global morbidity and mortality of leptospirosis: a systematic review. PLoS Negl Trop Dis 2015;9:e0003898.
- [2] Morshed MG, Konishi H, Terada Y, Arimitsu Y, Nakazawa T. Seroprevalence of leptospirosis in a rural flood prone district of Bangladesh. Epidemiol Infect 1994;112:527–31.
- [3] LaRocque RC, Breiman RF, Ari MD, Morey RE, Janan FA, Hayes JM, et al. Leptospirosis during dengue outbreak, Bangladesh. Emerg Infect Dis 2005;11:766-9.

- [4] Kendall EA, LaRocque RC, Bui DM, Galloway R, Ari MD, Goswami D, et al. Leptospirosis as a cause of fever in urban Bangladesh. Am J Trop Med Hyg 2010;82:1127–30.
- [5] Aziz MA, Aung MS, Paul SK, Ahmed S, Haque N, Roy S, et al. First molecular identification of two Leptospira species (Leptospira interrogans and Leptospira wolffii) in Bangladesh. New Microbe. New Infect 2019;31:100570.
- [6] Djadid ND, Ganji ZF, Gouya MM, Rezvani M, Zakeri S. A simple and rapid nested polymerase chain reaction-restriction fragment length polymorphism technique for differentiation of pathogenic and nonpathogenic *Leptospira* spp. Diagn Microbiol Infect Dis 2009;63: 251–6.
- [7] Philip N, Bahtiar Affendy N, Ramli SNA, Arif M, Raja P, Nagandran E, et al. Leptospira interrogans and Leptospira kirschneri are the dominant Leptospira species causing human leptospirosis in Central Malaysia. PLoS Negl Trop Dis 2020;14:e0008197.
- [8] Slack AT, Kalambaheti T, Symonds ML, Dohnt MF, Galloway RL, Steigerwalt AG, et al. *Leptospira wolffii* sp. nov., isolated from a human with suspected leptospirosis in Thailand. Int J Syst Evol Microbiol 2008;58:2305-8.
- [9] Zakeri S, Sepahian N, Afsharpad M, Esfandiari B, Ziapour P, Djadid ND. Molecular epidemiology of leptospirosis in northern Iran by nested polymerase chain reaction/restriction fragment length polymorphism and sequencing methods. Am J Trop Med Hyg 2010;82:899–903.
- [10] Zakeri S, Khorami N, Ganji ZF, Sepahian N, Malmasi AA, Gouya MM, et al. *Leptospira wolffii*, a potential new pathogenic *Leptospira* species detected in human, sheep and dog. Infect Genet Evol 2010;10:273-7.
- [11] Balamurugan V, Gangadhar NL, Mohandoss N, Thirumalesh SR, Dhar M, Shome R, et al. Characterization of *Leptospira* isolates from animals and humans: phylogenetic analysis identifies the prevalence of intermediate species in India. Springerplus 2013;2:362.
- [12] Guglielmini J, Bourhy P, Schiettekatte O, Zinini F, Brisse S, Picardeau M. Genus-wide *Leptospira* core genome multilocus sequence typing for strain taxonomy and global surveillance. PLoS Negl Trop Dis 2019;13:e0007374.