First molecular identification of two *Leptospira* species (*Leptospira interrogans* and *Leptospira wolffii*) in Bangladesh

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Abstract

Leptospiral 16S rRNA genes were detected in 13 blood samples from 74 febrile patients in north-central Bangladesh, and their sequences phylogenetically clustered with those of *Leptospira interrogans* or *Leptospira wolffii*. Genetic diversity in O-antigen polymerase (*wzy*) was found in an *L. interrogans* sample.
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Leptospirosis is a zoonotic disease caused by pathogenic species of the genus *Leptospira*, and exhibits a wide spectrum of clinical manifestations. Human infection with *Leptospira* usually results from exposure of skin or mucous membranes to water or soil contaminated with the urine of infected animals. Although leptospirosis is distributed worldwide, it is most common in developing countries in tropical and subtropical regions, associated with conditions of poor sanitation, flooding and overcrowding. In Bangladesh, a high prevalence of leptospirosis has been described in rural areas (38%) [1], but relatively lower prevalence (<10%) among patients with febrile illness in urban areas before 2010 [2–4]. These studies employed mostly serological tests to diagnose leptospirosis and assign leptospiral serogroups. Although PCR-based detection was attempted in one study [2], genetic confirmation and identification of leptospiral species has never been performed in Bangladesh.

In the present study, blood samples were collected from 74 febrile patients in Mymensingh Medical College hospital, located in north-central Bangladesh, during a period between January and October 2018. The enrolled patients had fever (>38.5°C) for more than 5 days with malaise, headache, rash and conjunctival suffusion. By using nested PCR targeting 16S rRNA [5] (see Supplementary material, Table S1), *Leptospira* was detected in 13 samples (17.6%). Nested PCR was attempted also for the flagellin gene (*flaB*) [6] and O-antigen polymerase gene (*wzy*) [7]. Nucleotide sequences were directly determined with PCR products. Phylogenetic analysis of the partial 16S rRNA gene sequences revealed that six and one (LepMMC36) samples clustered with *Leptospira interrogans* serovar Copenhageni and *Leptospira wolffii* (strain Khorat-H2^T), respectively (Fig. 1), showing identical sequences to these species (see Supplementary material, Figs S1, S2). Sequences for *flaB* and *wzy* were obtained from only a *L. interrogans* sample LepMMC8; the *flaB* sequence was identical to that of *L. interrogans* serovar Copenhageni, while *wzy* showed 99% identity to *L. interrogans* associated with a two-amino-acid difference in O-antigen polymerase (see Supplementary material, Fig. S3), including a unique amino acid at position 42. Nucleotide sequences obtained in the present study were deposited in GenBank under Accession numbers MK567966–MK567972, MK569049, MK569050.

Two previous studies of leptospirosis in Bangladesh suggested that infections in patients involved a broad range of *Leptospira* serogroups, including those of *L. interrogans* by serological tests [1,3]. Our present study genetically confirmed the presence of *L. interrogans* as a putative dominant species, and *L. wolffii* for the first time in Bangladesh. *Leptospira wolffii*, classified into intermediate species, was first reported in Thailand [8], and has been found in Southeast Asia, India [9] and Iran [10]. A presumptive wide distribution of *L. wolffii* in Asia may be supported by our study. Sequence diversity detected in *wzy* of *L. interrogans* sample LepMMC8, compared with previously reported strains, suggests the occurrence of potential genetic variants among *L. interrogans*.

The present study first demonstrated the presence of leptospiral genes in febrile patients in Bangladesh, revealing two *Leptospira* species. Further epidemiological studies are

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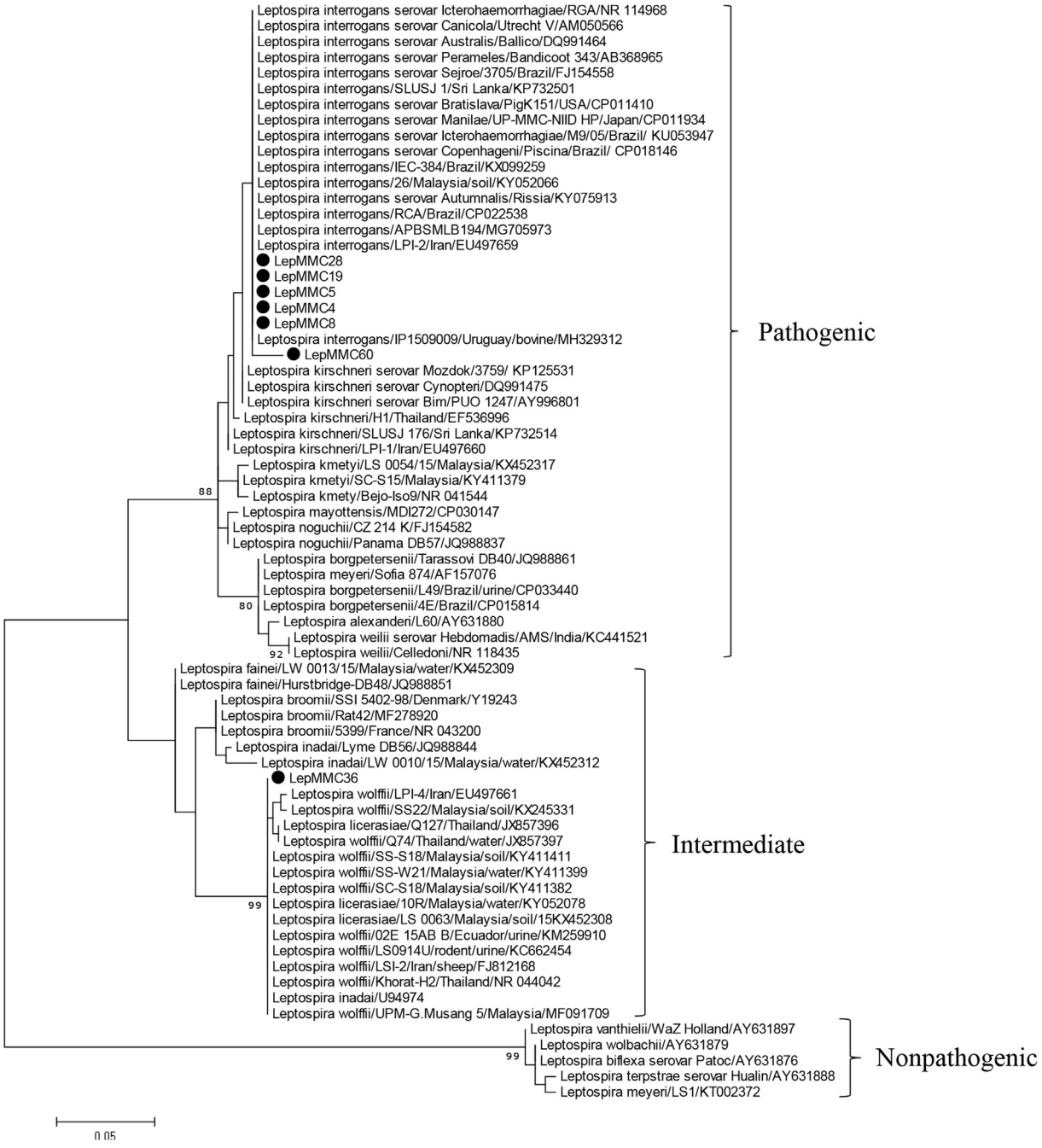

FIG. 1. Phylogenetic dendrogram based on partial 16S rRNA gene sequences (70 sequence data, 199–350 nucleotides) of *Leptospira* constructed by maximum likelihood method using MEGA6 program, following alignment with ClustalW algorithm. The trees were statistically supported by bootstrapping with 1000 replicates, and phylogenetic distances were measured by a Kimura two-parameter model with uniform rates among sites. Samples analysed in the present study are marked with closed circles. Three groups (pathogenic, intermediate, non-pathogenic) are shown on the right. Bootstrap values more than 80% are shown. Scale bar represents the genetic distance, i.e. number of substitutions per site.

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necessary to monitor the prevalence of *Leptospira* and its genetic diversity.

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.2019.100570.

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