

Isolation and characterization of *Leptospira interrogans* from two patients with leptospirosis in Western Province, Sri Lanka

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Abstract

Leptospirosis is an endemic infectious disease causing considerable morbidity and mortality in Sri Lanka; however, reports on the isolation of *Leptospira* from infected patients in Sri Lanka have been largely unavailable since the 1970s. Two isolates were obtained and characterized from 100 blood cultures from leptospirosis-suspected patients. Phylogenetic analysis of partial *flaB* gene sequences identified the isolates as *Leptospira interrogans*. The patient serum samples from which *Leptospira* was isolated reacted with the *Leptospira* serogroups Sejroe and Canicola at a titre of 1:200. Exposure to domestic sewage and gutters filled with muddy water was suspected to be the source of infection in these two culture-positive patients. This study reports the successful isolation of pathogenic *Leptospira* from two patients in Western Province, Sri Lanka.

Leptospirosis is a zoonotic disease that is recognized as a significant public health threat in Sri Lanka due to its high morbidity and mortality [1–3]. Although several diagnostic methods, including the microscopic agglutination test (MAT), PCR and antibody detection assays, are available at reference centres, these methods have several limitations that may yield false negatives due to low detectable bacterial load or antibodies. Culture isolation, on the other hand, is a confirmatory test for leptospirosis that can overcome these limitations. In recent years, several studies have identified pathogenic *Leptospira* species (including *L. interrogans*, *L. kirschneri*, *L. borgpetersenii*, *L. santarosai* and *L. weilli*) from human patients using molecular methods in Sri Lanka [1, 4, 5]. *Leptospira* is a fastidious organism with specialized culture requirements and thus is not routinely cultured [6]. However, the isolation of infecting *Leptospira* strains is important from an epidemiological perspectives and can also provide information on the mammalian maintenance hosts involved in transmission [7, 8]. The first isolation of *Leptospira* from a human in Sri Lanka was reported in 1962, and reports of successful isolation have not been published since 1973 in Sri Lanka [5, 9]. In the present study, we succeeded in isolating *Leptospira* strains from two human patients in Western Province, Sri Lanka.

This was a prospective hospital-based study carried out at a tertiary care hospital in the southern part of Colombo District, Western Province, Sri Lanka between June and December 2017. Patients who were clinically suspected of leptospirosis according to the World Health Organization (WHO) guidelines [10] and had been admitted to the medical wards were included. A 5 ml venous blood sample was collected on admission and transported at 4°C to the Department of Microbiology, University of Sri Jayewardenepura, Sri Lanka within an hour of collection. One hundred microlitres of whole blood was inoculated into semi-solid Ellinghausen–McCullough–Johnson–Harris (EMJH) medium, which was incubated at room temperature in the dark and examined for the presence of spirochetes by dark-field microscopy weekly for 4 months. Once motile live spirochetes were observed, an aliquot of culture was subjected to DNA extraction using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) followed by nested PCR targeting the *flaB* gene as described previously [11]. The positive amplicons were purified and sequenced using the Big Dye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster city, CA, USA). The *flaB* sequences were aligned together with 25 known reference *flaB* sequences in MEGA7 using ClustalW, and phylogenetic distance was calculated in

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Keywords: culture; isolation; leptospirosis; Sri Lanka.

Abbreviations: EMJH, Ellinghausen–McCullough–Johnson–Harris; MAT, microscopic agglutination test.

The GenBank accession numbers for the [*flaB* gene sequence] sequences of *Leptospira* are MH243436 and MH243437.

Table 1. *Leptospira* strains used for MAT

No.	Serovar	Strain	Serogroup	Species
1	Patoc	Patoc I	Semaranga	<i>L. biflexa</i>
2	Javanica	Veldrat Batavia 46	Javanica	<i>L. borgpetersenii</i>
3	Tarassovi	Perepelitsin	Tarassovi	<i>L. borgpetersenii</i>
4	Weerasinghe	Weerasinghe	Automnalis	<i>L. interrogans</i>
5	Hardjo	Hardjoprajitno	Sejroe	<i>L. interrogans</i>
6	Canicola	Hond Utrecht IV	Canicola	<i>L. interrogans</i>
7	Icterohaemorrhagiae	RGA	Icterohaemorrhagiae	<i>L. interrogans</i>
8	Pomona	Pomona	Pomona	<i>L. interrogans</i>
9	Hebdomadis	Hebdomadis	Hebdomadis	<i>L. interrogans</i>
10	Rathnapura	Wimalasena	Grippotyphosa	<i>L. interrogans</i>

MEGA7 using the neighbour-joining method [12]. The *flaB* sequences were deposited in GenBank and accession

numbers were obtained (SLUSJ_182, MH243436; SLUSJ_183, MH243437). Furthermore, a quantitative PCR

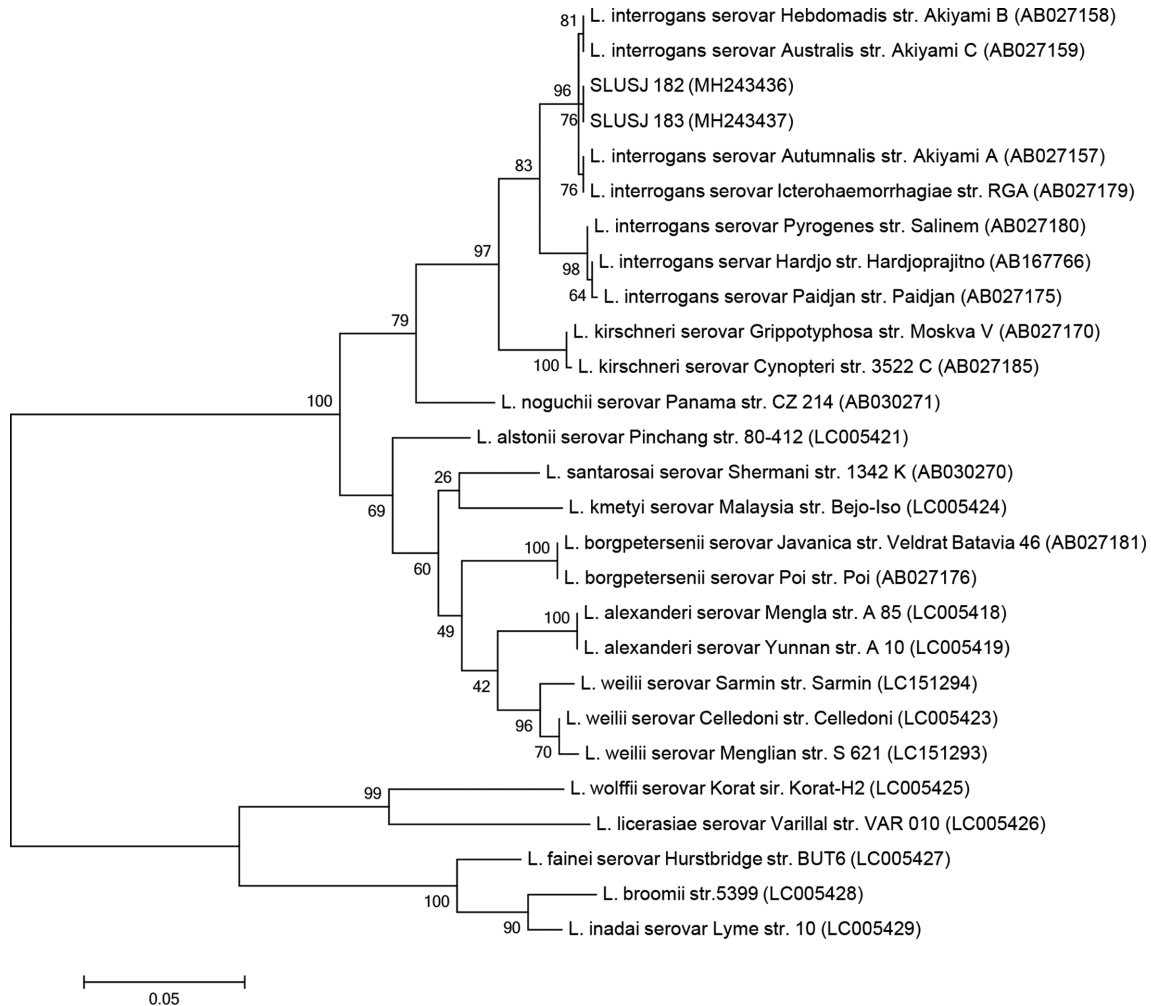


Fig. 1. Phylogenetic tree based on the *Leptospira flaB* gene sequence. The sequences were aligned in MEGA7 using ClustalW, and phylogenetic distances were calculated in MEGA7 using maximum likelihood. The numbers of nodes were bootstrap supported after 500 replicates.

(qPCR) assay using the EvaGreen technology was applied to quantify the *Leptospira* load in the blood of culture-positive patients [13]. Serum samples from culture-positive patients were subjected to MAT to identify the infective serogroup. A panel of 10 *Leptospira* serogroup strains were used for MAT (Table 1).

Among 100 blood cultures from clinically suspected leptospirosis patients, 2 cultures turned out to be positive (SLUSJ_182 and SLUSJ_183). The phylogenetic distance of the *flaB* sequences of the isolates suggested that the *Leptospira* species were *L. interrogans* (Fig. 1). The demographic and epidemiological data for the two confirmed patients are summarized in Table 2. qPCR was only successful in patient SLUSJ_183, whose bacterial load was 134 353 *Leptospira*/ml. The serum samples from the two patients reacted with the serogroups Sejroe and Canicola at a maximum titre of 1 : 200.

Positive culture isolation provides strong confirmatory evidence for diagnosis. As observed in this study, a qPCR-negative patient was found to be positive by culture, probably due to low bacterial load in the sample, which emphasizes the usefulness of culture over other techniques. MAT requires acute and convalescent specimens for definitive diagnosis, which is a daunting task in resource-poor settings with no proper follow-up. Although culture isolation also has limitations and is affected by prior antibiotic usage, time of collection and the fastidious nature of the organisms, it has a definitive role, not only in diagnosis but also in the epidemiology of leptospirosis [14].

Although ideally serogroups are identified by using the MAT on isolates using reference antiserum against various serogroups, these are unavailable in Sri Lanka and the transportation of this zoonotic pathogen to other countries is difficult and expensive. Therefore, as an alternative, the MAT was performed using patient serum samples to deduce the infecting *Leptospira* serogroup in this study. The serum samples reacted with a certain serovar strain, but their titres were relatively low, probably due to sample collection in the early stage of disease (5 and 6 days after the onset of fever).

Serogroups Canicola and Sejroe are considered to be highly adapted to dogs [15] and cattle [16], respectively, while rodents are known to harbour both strains [17, 18]. These serogroups have been isolated from leptospirosis-suspected

patients in India and Uruguay, supporting its role as an important zoonotic pathogen [19, 20]. Although there was no evidence of direct exposure to potential reservoir animals in culture-positive patients, occupational or daily exposure to contaminated environments, such as domestic sewage and a gutter, suggest a zoonotic health issue in this study setting. This study emphasizes the importance of the contribution of zoonotic reservoir hosts to the public health risks of urban human leptospirosis in Western Province, Sri Lanka.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

Ethical clearance for the study was obtained from the Ethics Review Committee of the University of Sri Jayewardenepura, Sri Lanka (ERC application no. 02/17).

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Table 2. Demographic and epidemiological data for leptospirosis-confirmed patients

	SLUSJ_182	SLUSJ_183
Age	94 years	42 years
Gender	Female	Male
Occupation	–	Outdoor labourer
Exposure	Domestic sewage	Gutter filled with muddy water
Days of fever on admission	05	06

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