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Leptospirosis Outbreak in Sri Lanka in 2008: Lessons for Assessing the Global Burden of Disease

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Abstract. Global leptospirosis disease burden estimates are hampered by the lack of scientifically sound data from countries with probable high endemicity and limited diagnostic capacities. We describe the seroepidemiologic and clinical characteristics of the leptospirosis outbreak in 2008 in Sri Lanka. Definitive/presumptive case definitions proposed by the World Health Organization Leptospirosis Epidemiology Reference Group were used for case confirmation. Of the 404 possible cases, 155 were confirmed to have leptospirosis. Highest titers of patient seum samples reacted with serovars Pyrogenes (28.7%), Hardjo (18.8%), Javanica (11.5%), and Hebdomadis (11.5%). Sequencing of the 16S ribosomal DNA gene identified six infections: five with *Leptospira interrogans* and one with *L. weilli*. In this patient population, acute renal failure was the main complication (14.8%), followed by myocarditis (7.1%) and heart failure (3.9%). The case-fatality rate was 1.3%. This report strengthens the urgent need for increasing laboratory diagnostic capabilities to determine the causes of epidemic and endemic infectious diseases in Sri Lanka, a finding relevant to other tropical regions.

INTRODUCTION

Leptospirosis is a globally widespread, neglected, and emerging zoonotic disease,[1] posing important public health threats in the developing and developed world alike. Millions of persons are estimated to be affected annually, and increasing number of outbreaks have been reported recently from several countries.[2] The disease is endemic in humid, tropical, and subtropical areas of the world where most of the developing countries are located.[3] Emerging leptospirosis mostly affects vulnerable communities living in resource poor settings. Often the disease is either not suspected under or misdiagnosed in marginalized populations because of the need for laboratory resources to confirm leptospirosis; typically, such resources are neither accessible nor affordable. From the clinical perspective, better diagnostics are needed to prevent severe complications and death. From the public health perspective, the lack of reliable and efficient diagnostics tests makes assessing the burden of disease, whether regionally or globally, impossible.

With the recent emerging global threat of leptospirosis worldwide, the first meeting of Leptospirosis Epidemiology Reference Group (LERG) was held in December 2009 with guidance of the World Health Organization (WHO). LERG conducted a systematic review of literature on existing evidence to obtain accurate estimates of global leptospirosis incidence and prevalence. Of 12,033 reports reviewed, only 64 studies fulfilled the inclusion criteria and had low or moderate risk of bias. One major concern of the LERG group was the geographic bias of the selected data that might cause regional gaps in global estimates. Some of the countries such as Sri Lanka in which leptospirosis has been declared as highly endemic were not included in the systematic review because of lack of scientifically sound data.[4]

Despite lack of scientific publications, routinely reported data published by the epidemiology unit of Sri Lanka suggests that Sri Lanka has experienced one of the largest global leptospirosis outbreak reported in recent years.[5] In 2008, the total number of clinically suspected cases reported to the surveillance system was 7,406 and 204 deaths.[6] The reported incidence rate based on notification data was 35.7 per 100,000 population. In 2009, 4,980 cases and 145 deaths were reported[7] and the outbreak persisted in 2010 with 4,553 cases and 121 deaths.[8] The probable case incidence remains more than 22.5 per 100,000 population, making it the second highest reported incidence of leptospirosis worldwide; the highest has been reported from Seychelles with 43.2 per 100,000 population.[9] However, these cases are reported on the basis of clinical suspicion and fewer than 10% were laboratory confirmed because of lack of diagnostic capacity. These data available in the national surveillance program did not conform with definitive or presumptive case definitions proposed by LERG and were not published in scientific literature, making the utility of such data questionable for disease burden estimates.

Sri Lanka is a tropical island with a population of approximately 20 million persons. The economic activities, environment, hygienic conditions, recent ecological changes, and climate in this country combine to provide an ideal environment for leptospirosis transmission. A large part of the economy in Sri Lanka is based on agriculture, especially rice farming. Farming activities depend on two monsoons where the average rainfall is approximately 200 cm per year. Water buffalo and cattle are commonly used in rice-farming activities. A small holder dairy industry is a major component of the rural economy. Rodents and small mammals that could harbor *Leptospira* are abundant in and around households and rice fields. Rapid unplanned urbanization during the past two

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decades has brought wild and domesticated animals into close contact. Rapid urbanization has also led to the proliferation of rodent populations in human population centers.

Despite having the second highest reported incidence worldwide on a per capita basis and a significant number of deaths, little has been published about leptospirosis in Sri Lanka. Laboratory confirmation of the disease is not routinely made because of lack of diagnostic capabilities. The Medical Research Institute of Sri Lanka in Colombo provides diagnostic capacity for the entire country and uses an abbreviated form of the microscopic agglutination test (MAT) with only genus-specific *L. biflexa* serovar Patoc strain for diagnosis, but does not use a broad panel of serovars. Only one published report is available on disease confirmation during the 2008 outbreak, which confirmed 26 cases in 107 suspected cases using a single-sample MAT. In the same report, molecular diagnosis and species identification was reported for three cases and the deduced leptospirosis species were *L. interrogans* (2 samples) and *L. kirschneri* (1 sample).[10] In the same sample of suspected leptospirosis patients, the authors confirmed eight cases of hantavirus infection, which clinically mimics leptospirosis.[11]

We report a study conducted in three leptospirosis-endemic districts of Sri Lanka during the 2008 outbreak of leptospirosis using WHO LERG case definitions and a disease model. The objective of this study was to obtain scientifically sound data on leptospirosis in Sri Lanka within the ascertainment limits of a resource-poor setting and to place these limitations within the scope of emerging issues in assessing the global burden of this disease. The lessons drawn from this analysis are applicable to tropical regions where the burden of leptospirosis is likely to be substantial but remains unknown because of limited diagnostics capacity.

METHODS

Study settings, study samples, and patient selection. The present study was conducted in the districts of Kegalle, Kandy, and Matale in Sri Lanka. All three districts have been identified as leptospirosis-endemic by the Epidemiology Unit of Sri Lanka. One hospital in each of the three districts was selected as a study site, namely, District General Hospital-Kegalle, Teaching Hospital - Kandy, and District General Hospital - Matale.

The study population consisted of consecutively identified male and female patients with acute febrile illness admitted to medical wards in the three hospitals during August 20, 2008–January 6, 2009. All fever patients admitted to selected wards were screened. Patients conforming to the study inclusion criteria (Table 1) were invited to participate in the study. Inclusion criteria for possible cases were designed to increase the sensitivity of screening procedure. Thus, the stringent case definition proposed by the WHO and adopted by the Epidemiology Unit of Sri Lanka for surveillance activities was not used at this initial screening stage. Less stringent criteria to include undifferentiated fever, which reduced specificity, were used to detect even mild-to-moderate cases. A dedicated interviewer administering a clinical checklist carried out initial screening and obtained standardized clinical data. Socio-demographic profiles, exposure histories, and environmental data were obtained by using a pre-tested, interviewer-administered, questionnaire.

TABLE 1
Inclusion criteria for selecting fever patients during leptospirosis outbreak in Kegalle, Kandy and Matale, Sri Lanka, 2008

Inclusion criteria
Criterion 1
1. Patients admitted to medical wards in the selected hospitals
2. Presenting complaint is acute febrile illness (fever less than 15 days and temperature > 37.8°C)
3. With any one of the following major symptoms
Headache
Myalgia
Prostration
4. Associated with any of the following signs (at least one)
Conjunctival suffusion/conjunctival hemorrhage
Meningeal irritation
Anuria or oliguria/proteinuria/hematuria
Jaundice
Hemorrhages
Purpuric rash
Cardiac arrhythmia or failure
Criterion 2
Any other patient suspected and treated as a leptospirosis patient by a treating physician

Sample collection and transport. On admission, 5 mL of whole blood was obtained in a plain tube to obtain serum for enzyme-linked immunosorbent assay (ELISA) and MAT; an additional 5 mL was collected into a tube containing EDTA for polymerase chain reaction (PCR) on whole blood. Another 5-mL blood sample was obtained at least 7–10 days later as the convalescent-phase sample for serologic testing, which was conducted at least 10 days after the onset of symptoms. All samples were stored at 4–8°C[12] and transported in cooled boxes to the study laboratory within 24 hours. In the laboratory, serum samples were separated by centrifugation and IgM ELISA testing was carried out by using a commercially available kit (Panbio, Sinnamon Park, Queensland, Australia)[13] for all cases. Remaining samples were stored at –60°C until send in dry ice to Thailand and Australia for further testing.

Case definitions for leptospirosis. Two case definitions were used in diagnosing leptospirosis on the basis of the WHO LERG report. A definitive case was classified by any one of the following: 1) seroconversion (negative first sample and a titer ≥ 1:100 in the second sample), or a 4-fold increase in MAT titer between acute-phase and convalescent-phase samples; 2) *Leptospira* DNA detected by PCR. A presumptive case definition was defined as a patient with symptoms consistent with leptospirosis and presence of IgM against *Leptospira* shown by ELISA. Presumptive case definition was necessary because MAT was carried out only for patients with paired serum samples, and PCR was applied only to a sample of patients because of logistic issues. For remaining patients with only acute-phase serum samples, the ELISA-based presumptive case definition was used to confirm the diagnosis. For the presumptive diagnosis, a patient with symptoms consistent with leptospirosis was defined according to WHO recommended surveillance case definition of leptospirosis; "A suspected case involves a person presenting with acute febrile illness; headache, myalgia and prostration; associated with any of the following symptoms: conjunctival suffusion; meningeal irritation; anuria, oliguria, or proteinuria; jaundice; hemorrhage (from intestines; lung bleeding often notorious); cardiac arrhythmia or failure; skin rash; a history of exposure to infected animals or an environment contaminated with infected animal urine."[14]

Sample processing and analysis. The MAT was performed by the WHO/Food and Agricultural Organization/World Organization for Animal Health Collaborating Center for Reference and Research on Leptospirosis, Western Pacific Region (Archerfield, Queensland, Australia) on 167 paired serum samples. The reference panel represented 5 species and 16 serogroups of *Leptospira* spp. and included four isolates from Sri Lanka (Table 2).

Molecular diagnosis was carried out at the Oxford-Mahidol Welcome Trust Research Unit (Bangkok, Thailand). DNA was extracted from the 400 μL of EDTA blood sample by using the QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany) and eluted in 200 μL of Tris-EDTA buffer. DNA samples were stored at –20°C prior to use. A single-tube nested PCR was used to amplify a region of the 16S ribosomal DNA gene specific for pathogenic and intermediate *Leptospira* spp. The PCR primers were rrs-outer-F (5-CTCAGAACTAACGCTGGCGGCGCG-3′), rrs-outer-R(5′-GGTTCGTTACTGAGGGTTAAAACCCCC-3′), rrs-inner-F (5′-CTGGCGGCGCGTCTTA-3′), and rrs-inner-R (5′-GTTTTCACACCTGACTTACA-3′).

The resulting amplicon was predicted to be approximately 547 basepairs. A 25-μL PCR reaction contained 4.5 mM $MgCl_2$, 200 μM dNTP, 1.25 units of Tag DNA polymerase (Roche, Indianapolis, IN), 0.150 pmol of each outer primer, 1.25 pmol of rrs-inner-F, 5 pmol of rrs-inner-R, 1 M betaine (Sigma-Aldrich, St. Louis, MO) and either 1 μL of DNA extracted from laboratory cultures or 5 μL of DNA extracted from EDTA blood samples taken from febrile patients. The PCR was performed in duplicate for each sample by using a PTC-200 Peltier Thermal Cycler (MJ Research, Boston, MA) and the following conditions: one cycle at 95°C for 2 minutes; 40 cycles at 95°C for 10 seconds, 67°C for 15 seconds, and 72°C for 30 seconds; 40 cycles at 95°C for 10 seconds, 55°C for 15 seconds, and 72°C for 30 seconds; and one cycle at 72°C for 7 minutes. Positive and negative controls were included in each test. The positive control was genomic DNA extracted from *L. interrogans* serovar Lai strain Lai spiked with human DNA. Human DNA was extracted from a 5-mL blood sample obtained from one person and extracted as described above for clinical samples. The negative control was reaction mixture minus DNA template. Amplicons were visualized by using 1.5% gel electrophoresis and staining with ethidium bromide. A positive PCR result was defined as the visualization of a band of the predicted size for one or both samples. Purified PCR products were sequenced by Macrogen Inc. (Seoul, South Korea) by using the rrs-inner-F and rrs-inner-R primers. Sequences were aligned using SeqManII software (DNASTAR Inc., Madison, WI), and reduced to a 443-basepair region of the ribosomal DNA gene (positions 89–531 of the ribosomal DNA gene of *L. interrogans* serovar Lai strain 56601 (GenBank accession no. NC_004342).

Sequelae. All case-patients were followed-up until the end of hospitalization to obtain data on complications and sequelae.

Data analysis. Data were managed and analyzed by using Epi-Info version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA) and Statistical Package for Social Sciences version 13.0 (SPSS Inc., Chicago, IL), respectively. The proportion of definitive and presumptive cases among suspected cases and the 95% confidence intervals (CIs) for proportions were calculated. Descriptive analysis of the socio-demographic profile of patients and common clinical symptoms and signs was conducted. Results are presented as percentages with 95% CIs.

Ethical approval. The protocol was reviewed and approved by the Post Graduate Institute of Medicine and the Ethical Review Board of the Faculty of Medicine, University of Peradeniya, Sri Lanka.

RESULTS

Of 746 fever patients screened, 401 patients were enrolled in the study (Figure 1). Of these patients, 370 fulfilled the inclusion criteria and were clinical treated as cases of leptospirosis

TABLE 2

Panel of *Leptospira* serovars used for MAT analysis and the rationale of including these serovars in the MAT panel to diagnose leptospirosis cases during the leptospirosis outbreak in Kegalle, Kandy and Matale, Sri Lanka, 2008*

Serogroup	Serovar	Strain	Species	Reasons
Icterohaemorrhagiae	Copenhageni	M20	*L. interrogans*	Classic Weil's disease organism, common in *Rattus norvegicus*
Hebdomadis	Hebdomadis	Hebdomadis	*L. interrogans*	Common in cattle throughout Asia
Autumnalis	Autumnalis	Akiyami A	*L. interrogans*	Common in rodents in rice-growing areas
Autumnalis	Alice	Alice	*L. santarosai*	Isolated from human source in Sri Lanka in 1968
Autumnalis	Weerasinghe	Weerasinghe	*L. interrogans*	Isolated from human source in Sri Lanka in 1965
Pyrogenes	Pyrogenes	Salinem	*L. interrogans*	Common in various *Rattus* spp. throughout Asia
Bataviae	Bataviae	Swart	*L. interrogans*	Common in various *Rattus* spp. throughout Asia
Grippotyphosa	Ratnapura	Wumalasena	*L. kirschneri*	Isolated from human source in Sri Lanka in 1966 and has since been isolated from cattle/buffalo
Canicola	Canicola	Hond Utrecht IV	*L. interrogans*	Often associated with disease in dogs worldwide
Australis	Australis	Ballico	*L. interrogans*	Common in rodents near rice-growing areas in Asia and can have severe pulmonary symptoms
Pomona	Pomona	Pomona	*L. interrogans*	Found in domestic pigs
Javanica	Ceylonica	Piyasena	*L. borgpetersenii*	Isolated from human source in Sri Lanka in 1964
Sejroe	Geyaweera	Geyaweera	*L. borgpetersenii*	Isolated from human source in Sri Lanka in 1965
Sejroe	Hardjo	Hardjoprajitno	*L. interrogans*	Found in domestic cattle worldwide
Tarassovi	Tarassovi	Perepelitsin	*L. borgpetersenii*	Found in domestic pigs
Ballum	Ballum	Mus127	*L. borgpeterseni*	Common in mice and some rat species
Loisiana	Lanka	R740	*L. interrogans*	Isolated from a human source in Sri Lanka in 1967
Celledoni	Celledoni	Celledoni	*L. weilii*	Representing species *L. weilii*
Sarmin	Sarmin	Sarmin	*L. weilii*	Representing species *L. weilii*
Semaranga	Patoc	Patoc 1	*L. biflexa*	Used in MRI MAT panel

*MAT = microscopic agglutination test; MRI = magnetic resonance imaging.

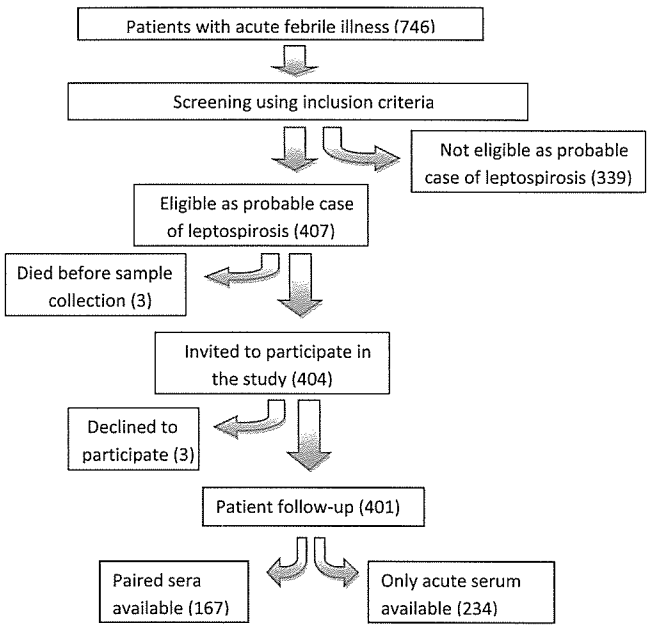

FIGURE 1. Selection of probable cases of leptospirosis, during the 2008 outbreak of leptospirosis in Kegalle, Kandy and Matale, Sri Lanka.

by the primary physicians. The remaining 31 patients were recruited on the basis of inclusion criteria alone and were treated by the primary physicians for a range of other conditions. Only 167 (41.6%) patients returned for follow-up and provided a second serum sample.

Disease confirmation. Of the 401 probable cases, 155 (38.7%) were identified as having leptospirosis (Table 3) either by definitive (n = 92) or presumptive (n = 63) criteria according to LERG criteria (outlined above). Of the 167 patients for whom paired serum samples were available, 154 patients were managed clinically as having leptospirosis by treating physicians, of whom only 81 (52.6%) showed the required 4-fold increase/seroconversion in titer. Of the 13 patients treated as having other conditions, but recruited based on inclusion criteria, six (46.2%) had a positive MAT result.

Serologic data. Of the battery of 22 serovars used in the MAT panel, 17 were reactive for one or more serum samples (Table 4). The most frequent antibody response (based on the highest titer for a given patient) was against *L. interrogans* serogroup Pyrogenes serovar Pyrogenes, which accounted for 28.7% of positive serum samples. Three isolates in the test panel (representing *L. interrogans* [n = 2] and *L. borgpetersenii* [n = 1]) each had the highest titer for 10 patients.

TABLE 3

Summary of case confirmation for 401 probable cases of leptospirosis during the leptospirosis outbreak in Kegalle, Kandy and Matale, Sri Lanka, 2008*

Category	Diagnosis criteria	No.	%
Definitive cases (n = 92, 23.0%)	MAT, PCR positive	3	0.8
	MAT positive	84	21
	PCR positive	5	1.2
Presumptive cases	ELISA, surveillance case definition positive	63	15.7
Unconfirmed		246	61.3
Total		401	100.0

*MAT = microscopic agglutination test; PCR = polymerase chain reacrion; ELISA = enzyme-linked immunosorbent assay. Total number tested with MAT = 167; PCR = 100; ELISA = 368.

Species identification. Of the 8 PCR-positive samples, 16S rDNA sequencing was carried out on 6 samples. The deduced *Leptospira* species were *L. interrogans* (5) and *L. weilli* (1) (Figure 2).

Clinical profile. Median duration of fever before admission among confirmed cases was 5 days (interquartile range = 4–7), and the median duration of hospital stay was 4 days (interquartile range = range 3–6). The classical clinical picture of leptospirosis was not observed in most of the cases (Table 5).

Validity of presumptive case definition. Diagnostic validity of proposed presumptive case definition (clinical leptospirosis and ELISA) was evaluated among 167 patients with paired serum samples, assuming paired sample MAT as the gold standard. Of these patients, definitive ELISA results (omitting the equivocal results) was available for 147 patients. The diagnostic sensitivity of the presumptive case definition was 66.2% and the specificity was 89.0% (Table 6).

Sequelae. Acute renal failure was classified based on the report of the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group.[15] Serum creatinine levels were greater than 1.5 μmol/L in 23 (14.8%) of 155 patients, and 11 of these patients had acute bilateral renal paranchymal disease detected by ultrasonography. For 76 (49%) patients, serum creatinine levels were not available. On the basis of the final diagnosis provided by the treating physicians, 11 (7.1%) patients had myocarditis and 6 (3.9%) patients had heart failure. Of the patients with myocarditis, 7 patients had T wave inversions, 3 had ST wave abnormalities, and 3 had tachyarrhythmia. We did not observe hyperbilirubinemia or pulmonary hemorrhage in this patient population. Three deaths were recorded among the 155 patients, giving a case-fatality rate of 1.9%. The three patients who died were hospitalized on the seventh day of illness and died within 24 hours of admission because of fulminant myocarditis. The mean ± SD duration of fever on admission among patients who had complications (n = 33) was 7.7 ± 3.8 days compared with 5.25 ± 2.2 days among other patients ($t = 4.714$, degrees of freedom = 153, $P < 0.001$).

DISCUSSION

Leptospirosis is a globally important disease with a broad range of clinical manifestations that mimic a wide variety of acute infectious diseases. Because of the variable clinical manifestations, laboratory methods are essential for disease confirmation. The purpose of this analysis was to obtain scientifically sound information on leptospirosis in Sri Lanka and to discuss the implications of diagnosing leptospirosis in resource-poor settings in assessing the global burden of this disease. A central issue in leptospirosis is the difficulty in readily establishing the diagnosis. In the present study, of patients treated as if they did have leptospirosis, 52.6% (95% CI = 43.8–59.3%) were laboratory confirmed to have leptospirosis. Paired serum samples required for optimal serologic diagnosis (MAT) were available for only 167 of 404 patients. Serologic diagnosis ultimately only retrospectively confirms diagnosis and does not contribute actively to patient care. Because of this limitation of MAT, patients may not be efficiently referred for leptospirosis diagnosis outside a research setting. These data yet again argue that the lack of point of care diagnostics for

TABLE 4

Frequency of MAT antibody titers to the test panel of *Leptospira* spp. among 87 MAT-positive cases during the leptospirosis outbreak, Kegalle, Kandy, and Matale, Sri Lanka, 2008*

Species	Serogroup	Serovar	No.	%	Range of antibody titers in MAT†
L. interrogans	Pyrogenes	Pyrogenes	25	28.7	200–6,400
L. borgpetersenii	Javanica	Ceylonica	10	11.5	100–3,200
L. interrogans	Sejroe	Hardjo	10	11.5	100–6,400
L. interrogans	Hebdomadis	Hebdomadis	10	11.5	100–6,400
L. borgpetersenii	Sejroe	Geyaweera	6	6.9	400–3,200
L. interrogans	Loisiana	Lanka	5	5.8	100–3,200
L. interrogans	Autumnalis	Weerasinghe	4	4.6	400–6,400
L. santarosai	Autumnalis	Alice	2	2.3	400–3,200
L. interrogans	Australis	Australis	2	2.3	200–400
L. interrogans	Autumnalis	Autumnalis	2	2.3	100–800
L. interrogans	Icterohaemorrhagiae	Copenhageni	2	2.3	1,600–3,200
L. interrogans	Pomona	Pomona	2	2.3	100–400
L. kirschneri	Grippotyphosa	Ratnapura	2	2.3	200–1,600
L. interrogans	Canicola	Canicola	2	2.3	100–200
L. interrogans	Bataviae	Bataviae	1	1.2	800
L. weilii	Celledoni	Celledoni	1	1.2	200
L. weilii	Sarmin	Sarmin	1	1.2	400

* MAT = microscopic agglutination test.
† Results represent highest titer for each patient.

leptospirosis not only impairs clinical care but also significantly limits regional and global public health assessments of the burden of this disease. This lesson is supported by the findings in leptospirosis outbreaks in Sri Lanka, a region where the disease is highly endemic and diagnosis remains a challenge, even with close cooperation with governmental health ministry laboratory resources.

Specifically with regard to the leptospirosis outbreak in Sri Lanka in 2008, the differential diagnosis of acute febrile illness is problematic. Even if one considers the 167 patients with paired serum samples, the confirmed diagnosis of only approximately 50% of suspected leptospirosis cases during this epidemic shows the limitations of clinical diagnosis of the syndrome of acute undifferentiated fever. One probable explanation is that there may have been a concurrent outbreak of another infectious disease with similar clinical characteristics, which may have been masked by the leptospirosis outbreak. A number of tropical diseases such as dengue,[16–18] hantavirus infection,[19,20] rickettsial infection[18,21] and malaria[22,23] may mimic the clinical presentation of leptospirosis. In Sri Lanka, dengue is highly endemic but not efficiently diagnosed. Rickettsial diseases are also common in small pockets in all three districts but difficult to diagnosis. Hantavirus infection is usually not considered in the differential diagnosis of leptospirosis patients in Sri Lanka, the incidence is not known, and diagnostic testing is not routinely available. Nevertheless, in 1995–1996, Sunil Chandra and others showed that 34.5% of patients with leptospirosis-like illness had hantavirus infections.[24] During the 2008 outbreak, Gamage and others also described eight cases of hantavirus infection among 103 suspected cases of leptospirosis in Peradeniya.[11] These data suggest that hantavirus infection among patients with leptospirosis-like illness in Sri Lanka is a possibility.[24] The major drawback of the lack of definitive diagnosis is that a large number of patients developed a leptospirosis-like illness, 10–20% of whom had complications and occasional deaths. If confirmatory diagnostic testing was available and the correct diagnosis was made, some of the deaths caused by other conditions and complications may have been prevented.

Serologic data showed that multiple serovars likely circulate in Sri Lanka. However, proper interpretation of these data is difficult because of probable cross-reactivity among different serogroups. Smythe and others have shown that correct detection of serovars by using a panel of standard serovars in MAT was as low as 33% when compared with titers against the homologous isolate obtained from a patient.[25] Levett reported similar findings but a slightly higher sensitivity (46.4% detection rate for 151 isolates) and that high MAT titer did not necessarily predict the serovar isolated from clinical samples.[26] Thus, the results of the present study should not be used as definitive evidence of the serovars circulating in Sri Lanka. Nevertheless, it seems evident that a range of serovars are causing human leptospirosis in Sri Lanka. Prevalence of serovars Hardjo, Hebdomadis, and Sejroe in the present study might be an indication of the important role of cattle/buffaloes in human leptospirosis in Sri Lanka.

This study expands our knowledge of circulating *Leptospira* in Sri Lanka. The first report of species identification was published by Koizumi and others, which reported two cases of infection with *L. interrogans* and one case of infection with *L. krischneri*.[10] The present study is consistent with a diversity of *Leptospira* infection in Sri Lanka. However, the molecular epidemiology of *Leptospira* species in Sri Lanka needs further investigation.

The proportion of renal complications among the confirmed cases of leptospirosis in this sample was 14.8% (95% CI = 9.8–21.1%). In some studies, rates of renal complications are as high as 44–67%[27] whereas most studies report a range of renal complications ranging from 10% to 40%.[17,28,29] In previously reported studies in Sri Lanka, renal complication rates ranged from 25%[30] to 70%.[31] The most probable explanation for the low complication rates is that the present study involved active case finding, which resulted in detecting mild-to-moderate cases, which could have missed in routine clinical practice. It is also possible that the set of infecting *Leptospira* in the region of study had different virulence capacities, which led to varying complications. Interestingly, pulmonary hemorrhage and other pulmonary complications were not observed among these confirmed cases. Previous studies in the National

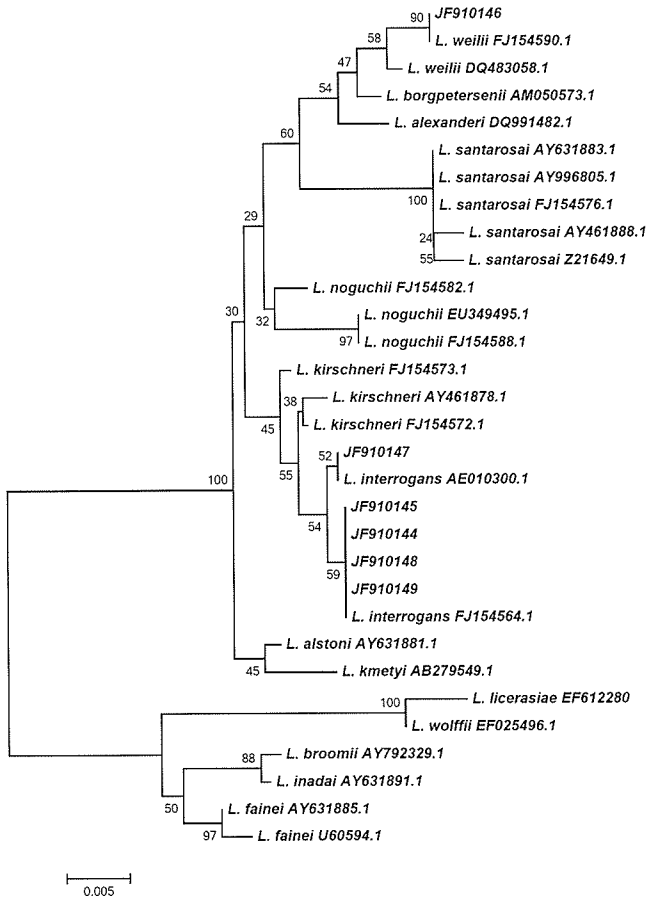

FIGURE 2. Phylogenetic tree based on the *Leptospira* 16S ribosomal DNA gene. Sequences obtained in this study are indicated in sample numbers 334, 68, 109, 229, 75, and 82. Sequences were aligned in MEGA4 using CLUSTALW, and phylogenetic distances were calculated in MEGA4 using maximum-likelihood. Numbers at nodes are bootstrap support after 500 replicates.

Hospital of Sri Lanka (Colombo) reported acute lung injury among 31% of cases,[30] and at Peradeniya Hospital (Central Province) at least 6–8 deaths because of pulmonary hemorrhage during this study period were attributed to leptospirosis.[32] This finding could be caused by varying virulence of infecting serovars in different studies. Different clinical manifestations caused by specific serovars are well documented in the literature. *Leptospira interrogans* serovars Icterohaemorrhagiae, Copenhageni, Javanica, and Bataviae are reported as causing severe icteric, typical Weil's disease, and most of the other serovars are associated with mild disease.[33]

The frequency of leptospirosis with undifferentiated fever and the absence of the classical clinical picture of leptospirosis in this study complicates the accurate disease burden estimate in countries where proper diagnostic facilities are not available. Use of presumptive diagnosis was having a diagnostic sensitivity of only 60% in this study, if the proposed case definition for surveillance activities is used. Use of this proposed presumptive case definition carries a risk of systematically underestimating the disease burden in countries with no access to diagnostic facilities and also missing considerable number of patients who will end up with severe complications. Clinicians need more efficient, precise, and specific point of care diagnostic tools, which would enable prompt individual

TABLE 5

Prevalence of clinical symptoms and signs among 155 confirmed case-patients with leptospirosis during the leptospirosis outbreak, Kegalle, Kandy, and Matale, Sri Lanka, 2008

	Definitive cases (n = 92)		Presumptive cases (n = 63)	
Clinical symptoms/signs	No.	%	No.	%
Headache	76	82.6	55	87.3
Myalgia	71	77.2	47	74.6
Arthralgia	67	72.8	45	71.4
Prostration	55	59.8	52	82.5
Nausea/vomiting	57	62.0	45	71.4
Anorexia	46	50.5	35	55.6
Conjunctival suffusion	42	45.7	33	52.4
Chills	35	38.0	26	41.3
Jaundice	32	34.8	25	39.7
Oliguria	33	35.9	25	39.7
Abdominal pain	25	27.2	22	34.9
Muscle tenderness	28	30.4	18	28.6
Diarrhea	20	21.7	10	15.9
Hepatomegaly	16	17.4	8	12.7
Shortness of breathing	11	12.0	4	6.3
Neck stiffness	6	6.5	7	11.1
Mental confusion	5	5.4	2	3.2

treatment. Contrary to popular belief, accurate and more sensitive diagnostic tests are equally important to epidemiologists and public health professionals for rapid outbreak response and better disease surveillance. We proposed widening of clinical criteria for surveillance purposes to improve the sensitivity of presumptive case definition and urge scientific community and funding agencies to develop rapid and accurate diagnostic tools.

The present study was carried out in only three districts in Sri Lanka and was based in only three large hospitals. A large network of other primary and secondary care hospitals exists within these three districts. Patients with mild-to-moderate leptospirosis could be visiting these other hospitals. Furthermore, because of the protean manifestations of leptospirosis, a large number of patients could be having only mild symptoms for which they visit general practitioners or outpatient departments and are not treated as in-ward patients. Thus, this study is not representative of all patients with leptospirosis in these three districts. The clinical manifestations discussed are only for persons with moderate-to-severe cases who required hospitalization. The percentage of patients having specified clinical features is an overestimation because of this selection bias.

TABLE 6

Validity of the presumptive case definition of leptospirosis among 147 possible cases for whom paired serum samples were obtained during the leptospirosis outbreak in Kegalle, Kandy and Matale, Sri Lanka, 2008*

Parameter	Estimate	95% CI
Sensitivity	66.2%	54.9–76.0
Specificity	89.0%	79.8–94.3
Positive predictive value	86.0%	74.7–92.7
Negative predictive value	72.2%	62.2–80.4
Diagnostic accuracy	77.5%	70.1–83.5
Likelihood ratio of a positive test result	6.04	4.63–7.88
Likelihood ratio of a negative test result	0.38	0.35–0.41
Diagnostic odds	15.93	6.62–38.3

*Eighteen patients with equivocal results in the enzyme-linked immunosorbent assay (ELISA) and two patients in whom ELISA results were available were not included in analysis. CI = confidence interval.

Diagnosis of patients with a single serum sample was achieved mainly by ELISA, for which the sensitivity is not optimal. The probability of missing several cases with leptospirosis because of this diagnostic procedure could not be excluded. Selecting hospital controls makes selection bias likely. Furthermore, hospitalized patients are a population that are sicker than ambulatory patients, and thus represents a biased sampling. Therefore, any generalization of the case-control study results should be made cautiously.

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