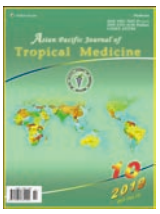

Original Article
Asian Pacific Journal of Tropical Medicine
journal homepage: www.apjtm.org

doi: 10.4103/1995-7645.269904 Impact factor: 1.77

Manifestations and outcomes of leptospirosis during local outbreaks in high endemic districts of Sri Lanka: A retrospective multi-center study

Thilini Nisansala[1], Kanchana Bandara[2], Manjula Weerasekera[1], Chinthika Gunasekara[1], Chamil Marasinghe[3], Chandika D. Gamage[4], Neluka Fernando[1], Nilantha Ranasinghe[5]✉

[1]*Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka*

[2]*Department of Basic Sciences, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Sri Lanka*

[3]*Department of Medicine, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka*

[4]*Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka*

[5]*Base Hospital, Panadura, Sri Lanka*

ARTICLE INFO

Article history:
Received 14 December 2018
Revised 15 October 2019
Accepted 18 October 2019
Available online 30 October 2019

Keywords:
Case definitions
Diagnosis
Leptospirosis
Sri Lanka

ABSTRACT

Objective: To determine the clinical presentations and disease outcomes of suspected and confirmed cases of leptospirosis from 3 high endemic districts of Sri Lanka, during outbreaks reported between 2013 and 2017.

Methods: The retrospective multi-center study was carried out during 2013-2017 in 5 selected hospitals representing 3 high endemic districts in Sri Lanka. Clinically suspected leptospirosis patients were recruited according to the Communicable Disease Epidemiology Profile Sri Lanka, WHO. Leptospirosis was confirmed by either single microscopic agglutination test titre 1: 400 or by positive polymerase chain reaction (PCR) test result.

Results: Out of 372 clinically suspected cases, 29.00% were confirmed as leptospirosis cases by either microscopic agglutination test (50.00%) or positive polymerase chain reaction (52.77%) and 12.90% were presumptively identified as leptospirosis. Clinical symptoms (headache, vomiting, jaundice and dyspnoea) and variations in haematological parameters (haemoglobin, platelet count) and biochemical parameters (serum creatinine, serum urea, serum bilirubin and C-reactive protein) were associated with confirmed leptospirosis ($P<0.05$). Acute kidney injury, meningitis, myocarditis, pulmonary haemorrhage and acute liver failure was seen among 21.30%, 12.04%, 6.48%, 6.48%, 5.56%, respectively with 4.63% fatality among the leptospirosis confirmed patients. The sensitivity, specificity, positive predictive value and negative predictive value of the case definition of Ministry of Health, Sri Lanka were 96.29%, 9.09%, 31.13%and 85.71%, respectively, when benchmarked against either positive polymerase chain reaction or microscopic agglutination test as the gold standard.

Conclusions: Acute kidney injury is the predominant complication observed among the leptospirosis confirmed patients. However, pulmonary haemorrhage is predominantly associated with mortality. The case definition of Ministry of Health, Sri Lanka is found to have higher sensitivity and enabled the screening of all probable cases of leptospirosis.

✉Corresponding author: Nilantha Ranasinghe, Consultant Physician, Base hospital, Panadura, Sri Lanka.
Tel.: 0094-071-8267184
E-mail: nilranasinghe@gmail.com

Foundation project: This study was funded by grants awarded by University of Sri Jayewardenepura, Sri Lanka (No. ASP/01/RE/MED/2015/37, ASP/01/RE/MED/2016/48 and ASP/01/RE/MED/2017/29).

How to cite this article: Nisansala T, Bandara K, Weerasekera M, Gunasekara C, Marasinghe C, Gamage CD, et al. Manifestations and outcomes of leptospirosis during local outbreaks in high endemic districts of Sri Lanka: A retrospective multi-center study. Asian Pac J Trop Med 2019; 12(10): 442-449.

1. Introduction

Leptospirosis has emerged as an endemic zoonotic infection of public health importance, occurring throughout the year with two peaks generally observed coinciding with the rice cultivation season in Sri Lanka[1]. *Leptospira* (*L.*) *interrogans* has been reported to be a major pathogen in the country, while several other pathogenic species including *L. borgpetersenii*, *L. kirschneri* and *L. weilli* have also been identified from patients[1,2]. The annual disease incidence vary from 31 to 164 per 100 000 population in Sri Lanka[3]. The climatic conditions such as high humidity, heavy rainfall and periodical flooding favour the disease outbreaks in Sri Lanka[4].

The clinical presentations of leptospirosis have a wide spectrum ranging from mild illness to severe forms leading to life threatening complications with multi organ failure (Weil's disease) and death[5]. Early treatment using antibiotics may prevent the complications, however, the outcome of the disease, despite early antibiotic treatment, may also be determined by several other factors including the virulence of the infecting serotype, level of leptospiraemia and cytokine involvement[6]. Leptospirosis can result in a spectrum of complications including pulmonary haemorrhage, acute respiratory distress syndrome, hepatitis, myocarditis, acute kidney injury, neuroleptospirosis and multi-organ involvement[2,3]. This study presents a comprehensive analysis of clinical outcomes of suspected and confirmed cases of leptospirosis reported from 5 selected hospitals in 3 districts of Sri Lanka.

2. Materials and methods

2.1. Study design

This was a retrospective multicenter study carried out in 5 different hospitals from 3 districts in Sri Lanka (Figure 1), including one tertiary care hospital (Colombo District) and 4 base hospitals (Colombo, Kaluthara and Hamanthota districts). Colombo and Kalutara districts in the Western province belong to the wet zone that receives high mean annual rainfall over 2 500 mm without noticeable dry periods. Hambantota District of Southern province is in the intermediate zone, located between wet and dry zones, receiving a mean annual rainfall of 1 750 to 2 500 mm. This region has also reported increasing incidence of leptospirosis cases over the years[7]. The study was conducted between 2013 and 2017, which experienced several leptospirosis outbreaks during this period.

2.2. Patients

A total of 372 clinically suspected leptospirosis patients admitted to the medical wards were included during each reported outbreak as per the suspected case definition described in Communicable Disease Epidemiology Profile Sri Lanka, WHO[8]. Socio-demographic data and exposure history was obtained using a pretested interviewer administered questionnaire. Clinical and laboratory findings were recorded serially from admission until discharge.

2.3. Specimen

A 5 mL venous blood sample was collected on admission in a plain tube for serum separation and in an ethylene diamine tetra acetic acid tube for deoxyribonucleic acid (DNA) extraction following standard procedures. All samples were transported at 4 ℃ to the Department of Microbiology, University of Sri Jayewardenepura, Sri Lanka.

2.4. Serological identification

Leptospirosis was presumptively diagnosed by detecting *Leptospira* specific IgM using a rapid immune-chromatographic assay kit (Leptocheck WB, Zephyr Biomedicals, India). Microscopic agglutination test (MAT) was performed at Medical Research Institute, the reference laboratory for leptospirosis diagnosis in Sri Lanka. A single MAT titre of ⩾1: 400 against the genus specific *Leptospira* biflexa serovar Patoc strain was considered positive[9,10].

2.5. Leptospira sec Y gene segment amplification using PCR and sequencing

PCR assay was used to amplify a 203 bp *sec Y* gene segment

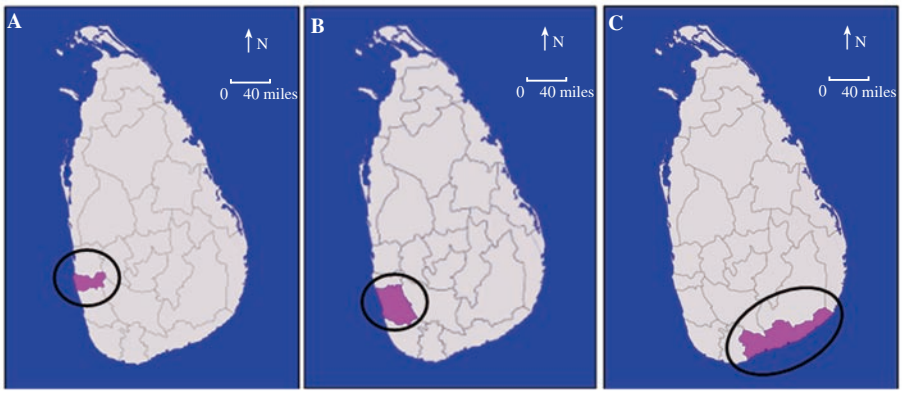

Figure 1. Map of Sri Lanka with the localization of habitats of the confirmed leptospirosis patients described. A: Colombo District; B: Kaluthara District; C: Hambanthota District.

encoding a preprotein translocase present in pathogenic *Leptospira* species using sec Y ⅠⅤF (5'- GCG ATT CAG TTT AAT CCT GC-3') and sec Y ⅠⅤ (5'- GAG TTA GAG CTC AAA TCT AAG-3') as described by Ahmed *et al*[11]. DNA was extracted from 200 μL whole blood using QIAamp DNA blood mini kit (Qiagen GmbH, Germany) according to the manufacturer's instructions. Amplification of isolated DNA was carried out in 25 μL volume with 5 μL template DNA, 5 μL of 5X green GoTaq® Flexi buffer (pH 8.5), 3.0 mM $MgCl_2$, 0.16 μM of each primer, 0.25 mM deoxy nucleotide triphosphate mix and 0.25 unit of Taq DNA polymerase (Promega, USA). *L. interrogans* DNA was used as a positive control and a negative control without the template DNA were included in each PCR assay. PCR amplification was initiated at 95 ℃ for 10 min followed by 45 cycles of 94 ℃ for 1 min, 54 ℃ for 30 s, 72 ℃ for 1 min and a final elongation step at 72 ℃ for 8 min with final hold at 4 ℃ in a thermal cycler (Flexigene Techne, UK). Each PCR product was mixed with 1/5 volume of the gel loading buffer (Promega, USA) and loaded into the agarose gel and electrophoresis was carried out.

PCR products were purified and sequenced bidirectionally at Macrogen Inc (South Korea). DNA sequences were obtained using v.3.1 BigDye chemistry (Thermo Fisher Scientific, US). Individual gene sequences were aligned using Bio Edit v.7.0.9.0 (Ibis Biosciences Inc, CA). Consensus sequences were generated using Chromas v.5.0 (Technelysium Pty Ltd, Australia) and species were identified by National Center for Biotechnology Information BLAST search.

2.6. Evaluation of case definition for diagnosis of leptospirosis

The case definition for suspected case of leptospirosis were evaluated based on the recommendations of WHO, Centers for Disease Control and Prevention and from the National Guidelines on Management of Leptospirosis, Epidemiology Unit, Ministry of Health, Sri Lanka[8,12,13]. In this study, WHO LERG criteria was used to define the confirmed and presumptive cases of leptospirosis[9]. A patient was considered as a confirmed case if positive by either PCR or MAT, while others were considered as leptospirosis cases without a confirmed diagnosis. A patient who was negative for either MAT or PCR but positive for *Leptospira* specific IgM was considered as a presumptive leptospirosis case.

2.7. Statistical analysis

Data were analyzed using Statistical Package for Social Science (SPSS version 15). The strength of the association between confirmed leptospirosis patients and all patients positive by either PCR, MAT (confirmed) or *Leptospira* specific IgM (presumptive) and exposure factors was measured by odds ratio (*OR*), with 95% confidence intervals (*CI*). Comparison of clinical parameters and laboratory investigations between confirmed and unconfirmed patients were performed using two sample proportion test and Mann-Whitney *U* test respectively. The sensitivity, specificity, positive predictive value and negative predictive value of three case definitions were calculated considering either MAT or PCR as the gold standard.

2.8. Ethics approval

Ethical clearance for the study was obtained from the Ethics Review Committee of the University of Sri Jayewardenepura, Sri Lanka (ERC application No.702/12, 02/17) and from the regional director of health services and the respective hospitals.

3. Results

3.1. Patients

Three hundred and seventy two (372) clinically suspected leptospirosis patients who were admitted to general medical units at selected hospitals were recruited for the study following informed written consent. The distribution of patients from each district was 192 patients from Colombo District and 107 patients were from Kalutara District of Western province, while 73 patients were from Hambantota District of the Southern province. The mean age was (44.0±15.8) years and 91.94% (*n*=342) were male. Average days of fever after hospital admission were (6.0±3.9) days.

3.2. Diagnosis of leptospirosis

Of the 372 leptospirosis suspected patients, 156 (41.94%) were positive for either *Leptospira* IgM, MAT or PCR. Of them, 108 were positive by either MAT or PCR and were considered as confirmed patients. Out of the 108 confirmed patients, 51 were positive only by PCR while 54 were positive only by MAT and 3 were positive for both PCR and MAT. All MAT positive patients were also *Leptospira* IgM positive and majority (87.71%) had a MAT titre of 1: 800 or above. Forty eight (48) patients were identified as presumptive cases.

Table 1. Association between exposure factor and confirmed/presumptive leptospirosis.

Exposure factor	Confirmed leptospirosis[a] (*n*=108)		Confirmed[a]+presumptive[b] leptospirosis (*n*=156)	
	OR	95% *CI*	*OR*	95% *CI*
Paddy and agricultural water sources	2.12	1.35-3.35	3.46	2.24-5.35
Domestic sewage, canal water or ditch water	1.14	0.65-2.03	1.15	0.68-1.96
Flood water	1.68	0.67-4.23	1.14	0.46-2.82
River, lake or pond water while swimming	0.98	0.37-2.59	1.04	0.43-2.53

[a]Confirmed diagnosis of leptospirosis included patients having symptoms consistent with leptospirosis and a single MAT titre≥1:400; or *Leptospira* DNA detected by a method based on the PCR; [b]Patients positive only for *Leptospira* specific IgM.

Table 2. Reported clinical parameters of clinically suspected leptospirosis patients included in the study (%).

Clinical features	Confirmed diagnosis of leptospirosis[a] (*n*=108)	Without a confirmed diagnosis of leptospirosis[b] (*n*=264)	*P* value*
Fever	98.14	98.48	0.78
Headache	84.25	64.77	<0.01
Myalgia	74.07	78.40	0.36
Vomiting	54.62	40.90	0.02
Conjunctival haemorrhage	20.30	15.53	0.26
Oliguria/Anuria	25.92	19.31	0.15
Jaundice	24.07	11.36	<0.01
Diarrhoea	20.37	21.59	0.80
Haematuria and mucosal haemorrhage	16.66	16.66	-
Dyspnoea	15.74	6.81	<0.01

[a]Confirmed diagnosis of leptospirosis included patients having symptoms consistent with leptospirosis and a single MAT titre≥1:400; or *Leptospira* DNA detected by a method based on the PCR; [b]Patients negative for either MAT or PCR. *Two sample proportion test.

3.3. Exposure history of the patients

Out of the 372 clinically suspected patients, 23.66% (*n*=88) were farmers and 32.53% (*n*=121) were outdoor workers. Exposure to paddy and agricultural water sources was reported in 39.78% (*n*=148) patients while 18.01% (*n*=67) had a history of exposure to domestic sewage, canal water or ditch water. Twenty (20) patients (5.38%) reported exposure to flood water and 5.65% (*n*=21) patients reported exposure to river, lake or pond water while swimming.

Considering the history of exposure among the presumptive leptospirosis cases (*n*=48), 66.67% (*n*=32) reported exposure to paddy and agricultural water sources, 18.75% (*n*=9) reported contact with domestic sewage, canal or ditch water, 6.25% (*n*=3) were exposed to river, lake or pond water while swimming and one patient was exposed to flood water. Among the confirmed cases (*n*=108), 57, 21, 6 and 8 patients were reported exposure to the above sources respectively. Sixteen (14.81%) confirmed and 3 (6.25%) presumptive cases had no known exposure. Majority (91.66%) of confirmed were males and 74.80% of the patients were employed as outdoor workers and farmers. Table 1 shows the results of the analysis to identify exposure factors for infection in this study population. Exposure to paddy and agricultural water sources was a major risk factor related to *Leptospira* infection.

3.4. Distribution of the patients

Three peaks of high incidence were observed in the selected setting, with the highest incidence occurring in October and November which may be attributed to exposure to agricultural (34.23%) and construction activities (5.56%) and an intermediate peak was observed in the months of June and July. Majority of the patients were from 5 divisional secretariats in Colombo District (Figure 1).

3.5. Clinical presentation and laboratory parameters of the patients

Out of the 372 clinically suspected patients, 98.39% (366) had fever. Interestingly, 4 out of the 6 patients who did not have fever had leptospirosis (2 were confirmed cases, while 2 with presumptive diagnosis). All 6 claimed to have an exposure history. Other common

Table 3. Biochemical and haematological parameters of clinically suspected leptospirosis patients included in the study.

Haematological/ biochemical parameter	Median (IQR) of patients with confirmed diagnosis of leptospirosis[a] (*n*=108)	Median (IQR) of patients without a confirmed diagnosis of leptospirosis[b] (*n*=264)	*P* value*
Haemoglobin	12.00 (10.80-13.40)	12.60 (11.30-13.90)	0.03
WBC	10.55 (6.68-13.57)	8.87 (6.52-12.73)	0.21
Neutrophils (%)	82.45 (71.75-88.93)	79.44 (68.19- 87.45)	0.10
Lymphocytes (%)	11.95 (6.20-20.00)	14.25 (7.84-23.35)	0.07
Platelet count	97.50 (47.25-147.75)	124.00 (74.50-176.00)	0.04
Serum creatinine	129.55 (97.24-297.96)	100.58 (81.32-132.70)	<0.01
Serum Urea	14.06 (7.84-28.55)	7.75 (5.23-15.65)	<0.01
Serum potassium	4.10 (3.60-4.50)	4.00 (3.60-4.60)	0.74
Serum sodium	136.50 (131.22-140.00)	136.50 (133.00-140.00)	0.69
AST	58.65 (41.32-92.14)	52.60 (31.75-95.95)	0.16
ALT	64.15 (38.53-99.47)	56.63 (35.45-101.35)	0.67
Serum bilirubin	33.42 (11.82-67.79)	15.16 (9.69-35.72)	<0.01
CRP	155.45 (88.45-201.75)	96.00 (44.00-186.75)	0.01

[a]Confirmed diagnosis of leptospirosis included patients having symptoms consistent with leptospirosis and a single MAT titre≥1:400; or Leptospira DNA detected by a method based on the PCR. [b]Patients negative for either MAT or PCR; *P<0.05 is considered as statistically significant. Data are expressed using Mann-Whitney *U* test. CRP: C-reactive protein. IQR: Interquartile range.

Table 4. Performance characteristics of case definitions [*n* (%)].

Performance characters	Suspected case (WHO)	Case definition (CDC)	Suspected case (MOH)
Sensitivity	70 (64.81)	99 (91.66)	106 (96.29)
Specificity	114 (43.18)	29 (10.98)	24 (9.09)
PPV	70 (31.81)	99 (29.64)	104 (31.13)
NPV	114 (75.00)	29 (76.31)	24 (85.71)

PPV: Positive predictive value; NPV: Negative predictive value; CDC: Centers for Disease Control and Prevention; MOH: Ministry of Health, Sri Lanka.

symptoms included myalgia (87.09%), headache (67.47%), oliguria/anuria (21.23%) and conjunctival haemorrhage (18.54%)..

Median duration of fever among confirmed leptospirosis patients on admission was 6 d (interquartile range: 5-7). Headache (P<0.01), vomiting (P<0.05), jaundice (P<0.01) and dyspnoea (P<0.01) had a significant association with patients having confirmed leptospirosis compared to patients without confirmed diagnosis of leptospirosis (Table 2).

Table 3 describes the haematological and biochemical profiles in the patients with and without a confirmed diagnosis of leptospirosis. A significant difference was observed with haemoglobin (P<0.05), platelet (P<0.05), serum creatinine (P<0.01), serum urea (P<0.01), serum bilirubin (P<0.01) and C-reactive protein (P<0.05) between the two groups.

3.6. Complications reported in patients

Among the confirmed leptospirosis patients the main organ involvements were found to be haematological followed by hepatic and renal (Figure 2). Acute kidney injury (AKI) was seen among 23 (21.30%) patients and 3 of them had undergone haemodialysis. Myocarditis was observed among 7 (6.48%) patients and there were 13 (12.04%) meningitis patients. Seven (7) patients (6.48%) had pulmonary haemorrhage, with 6 (85.71%) patients requiring mechanical ventilation. There were 6 (5.56%) patients who developed acute liver failure, and 19 patients (17.59%) were admitted for intensive care unit treatment.

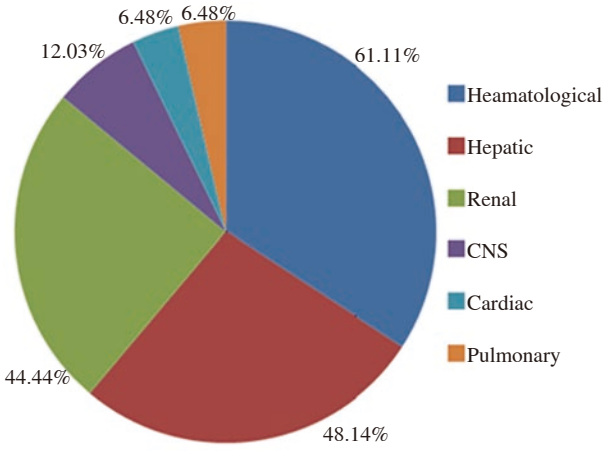

Figure 2. Organ involvement in confirmed leptospirosis patients (n=108).

Features of Weil's disease includes jaundice (n=40, 37.04%), haemorrhage with mucosal bleeding and disseminated intravascular bleeding (n=43, 39.81%) and renal impairment (n=42, 38.89%), which were observed in less than 50% of the patients. These 3 clinical features of typical Weil's disease were only observed in 16 (14.81%) leptospirosis confirmed patients. Of these 16 patients, 87.50% (n=14) developed organ failure and 92.85% (n=13) of them had AKI. However, out of 30 leptospirosis confirmed patients who developed organ failure, only 13 (43.33%) had all three clinical features of Weil's disease.

Five deaths (3 males and 2 females) were recorded among the 108 confirmed leptospirosis patients, resulting in a case fatality ratio of 4.63%. All these 5 patients were treated in the intensive care unit. All 5 patients were late presenters to the hospital (more than 5 d) and 2 were admitted after 10 d of fever onset. Four (4) were confirmed by PCR and the infecting species were identified as *L. interrogans* based on the results of sec y sequencing. Among confirmed patients with organ involvement, mortality due to pulmonary, cardiac, renal, hepatic and haematological involvement was reported in as 42.86% (3/7), 14.29% (1/7), 8.33% (4/48), 7.69% (4/52) and 4.55% (3/66), respectively. Out of the 5 deaths, only 2 had developed multi-organ failure.

3.7. Case definitions of leptospirosis

The performance characteristics of the case definitions used in the study to identify clinically suspected leptospirosis patients are tabulated in Table 4. The suspected case definition proposed by Ministry of Health had a sensitivity of 96.29% and a specificity of 9.09%. This was comparable with the sensitivity and specificity of leptospirosis case definition by the Centers for Disease Control and Prevention. The suspected case definition proposed by Ministry of Health had a higher sensitivity compared to the suspected case definition of the WHO.

3.8. Prophylaxis and treatment of patients

It is important to note that out of 148 farmers, only 4 had taken prophylactic antibiotics and among them only 2 had completed the antibiotic course. Majority of leptospirosis patients were treated with

third generation cephalosporins (25.00%), Ⅳ penicillin (17.32%) and doxycycline (8.33%) during first two days of hospitalization. The most commonly used antibiotic combination was doxycycline and third generation cephalosporins (12.00%) followed by Ⅳ Penicillin and third generation cephalosporins (8.33%).

4. Discussion

Being a tropical country, Sri Lanka has reported increasing incidence of leptospirosis from almost all the districts in the recent years, emphasizing its public health importance. The Colombo and Kalutara districts of Western and Hambantota District of Southern provinces are among the districts with high incidence of the disease[3,14].

In this study, farming or exposure to agricultural water sources were found as major risk factors while construction site was also an importance source. Other occupations with exposure to domestic sewage, canal water or ditch water were also identified as an emerging risk in the highly populated Colombo District of Western province in Sri Lanka. All 5 divisional secretariats in Colombo district of which cases have been reported can be considered as urban, out of which 3 are undergoing great urbanization and a boom of construction possibly contributing to the high incidence. In 2 out of the 5 divisions, paddy cultivation is wide spread and thus is suggested to be the possible source of infection. Rapid urbanization with mushrooming construction projects in the district, loss of vegetation, slum dwellings, open sewers, increasing burden of waste and improper waste management, poor rainwater drainage systems and associated flooding contribute to the transmission of the infection with the expansion of zoonotic reservoirs[15-17]. A study done in urban settings in Brazil has reported household clustering of leptospirosis due to increased population density and poor housing facilities, indicating the increased risk of transmission of leptospirosis among the household members[15]. Sri Lanka being a developing country with rapid urbanization and a booming construction industry may face a similar challenge in the near future. The outbreaks of leptospirosis coincided with the two main paddy cultivation seasons, 'Yala' and 'Maha' season, which is determined by the monsoon pattern in Sri Lanka. In this study, a higher incidence of patients were reported during October and November, which may be attributed to exposure to agricultural land preparation for paddy cultivation and rice harvesting of the Maha season. The paddy harvesting season of the Yala cultivation takes place during months of June and July which the intermediate peak has been observed. These agricultural seasons are based on the southwestern monsoon rain which occurs during the months of May to September. The disease transmission in Sri Lanka is mainly considered to occur due to the rodent population, cattle and domestic mammals such as dogs[1]. In Sri Lanka, *L. interrogans* has been reported as the main species associated with human infection and a recent review suggests that at least 10 serogroups including Icterohaemorrhagiae, Autumnalis, Sejroe, Grippotyphosa, Javanica, Louisiana, Canicola, Hebdomadis, Pomona and Pyrogenes are circulating in the country[2,18].

In this study, fever, myalgia and headache were the prominent clinical presentations among the leptospirosis confirmed cases. However, these clinical symptoms are nonspecific and are common for most of the other tropical diseases. Dyspnoea was significantly associated with confirmed leptospirosis cases, suggesting pulmonary involvement. Pulmonary involvement was seen among 3 of the 5 deaths in this study, which is an important finding in the clinical context. All these 3 patients were infected with *L. interrogans*. Several serovars have been associated with pulmonary haemorrhage in other studies including *L. interrogans* serovar, Copenhageni, Icterohaemorrhagiae, Canicola and Lai, which have also been reported in several districts in Sri Lanka[19,20].

As leptospirosis mimics most of the other common tropical infections including dengue, Hanta and typhus fever, it is important to consider the laboratory parameters for the identification of these infections. Six laboratory parameters including haemoglobin, platelet count, serum creatinine, serum urea, serum bilirubin and C-reactive protein had a significant association with leptospirosis in this study population[21]. Previous studies also suggest an association of low hemoglobin and platelet counts with severe leptospirosis[22,23]. Serum creatinine, serum urea and serum bilirubin were elevated in this study, indicating renal impairment and liver involvement respectively. Daher *et al.* also reported elevation of serum urea, serum creatinine and bilirubin levels among thrombocytopenic patients with leptospirosis[21].

In this study, out of the 108 confirmed leptospirosis patients, only 14.81% presented with all 3 typical Weil's clinical features: jaundice, haemorrhage and renal impairment. Similarly in the 2011 outbreak in Sri Lanka these typical Weil's features were seen in less than 50% of the patients[24]. Of the 5 deaths reported in the current study, only 2 patients had typical Weil's features while the other 3 had one or two of those features.

A study conducted in coastal south India during 2011 to 2014 has revealed higher incidence of AKI as the most common complication of leptospirosis, which is similar to the findings of this study[25]. Pulmonary haemorrhage was the main cause of death in this study and findings of a retrospective study of leptospirosis during 2001 to 2012 in Thailand also reported pulmonary haemorrhage as the major cause of death[26].

The higher sensitivity of case definition of Ministry of Health,

Sri Lanka enables the detection of a higher proportion of clinically suspected leptospirosis patients. The fact that case definition of Ministry of Health, Sri Lanka considered febrile illness with at least any one of the other symptoms as opposed to the recommendations of WHO, which included febrile illness with headache, myalgia and prostration strongly diminishes the chance of missing any possible cases. However, the low specificity observed in this case definition can be due to the broad coverage of clinical symptoms, which may mimic other important tropical diseases, including dengue fever. Further the guideline was benchmarked against either MAT titre >1:400 using the Patoc strain for single specimen or PCR. This may lead to include higher false negatives. The MAT in this study was carried out using *L.* biflexa Patoc strain, which was offered by the *Leptospira* reference center in Sri Lanka at the time of the study. The Patoc MAT has inherent drawbacks, therefore, it is not as ideal as the serovar specific MAT for leptospirosis confirmation. This may lead to include higher false negatives, which is one of the limitations of the present study. The administration of antibiotics prior to admission may also be a reason for negative PCR results, which can ultimately affect the sensitivity of the guideline. Based on the recommendations of WHO, the history of exposure was limited to exposure to infected animals or an environment contaminated with infected animal urine. However, in the case definition of Ministry of Health, the history of exposure has been defined to include more possible contacts to improve the sensitivity.

In the present study, either MAT or PCR was used to confirm the infection. However, it is important to note that these tests need specialized laboratories, therefore, they are not available to cater for the demand during outbreaks. And the case definition of Ministry of Health, Sri Lanka can be useful in identification of suspected leptospirosis cases in resource poor settings.

Sri Lankan government has implemented a system whereby antibiotic prophylaxis is available to those engaging in farming activities through the office of the medical officer of health. It forms an important component of the public health system, offering services to the public with the contribution of public health inspectors and other public health care staff. Although such a system is available, it is of significant note that only 4 of the suspected patients involved in farming utilized this system and only 2 completed the prophylactic antibiotic course. This is of grave concern as majority of the farming community, although aware of the availability of prophylaxis, did not consider the importance to take necessary preventive measures. In a national household survey conducted in Sri Lanka, 64% of farmers were aware of the availability of chemoprophylaxis for leptospirosis, which is in contrast with their actual practices reported in the current study where only 4 farmers (2.70%) were reported to have taken doxycycline as prophylactic treatment[27]. Further, this study identified the increased risk of those associated with domestic sewage and construction sites. Therefore, it is important to consider this target group for prophylactic antibiotic treatment. The findings of this study also emphasizes the importance of renewed attention of policy makers to address the gaps in communication and distribution of prophylaxis among the high risk groups. It is recommended that prophylaxis is closely monitored and easily accessible to the farmers and other high risk groups that have not so far been considered.

This study highlights the deviating clinical outcomes of leptospirosis in Sri Lanka with emergence of pulmonary haemorrhage as an important complication in mortality among leptospirosis patients. The proposed suspected case definition of Ministry of Health, Sri Lanka was found to be highly sensitive to screen probable cases of leptospirosis.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We wish to acknowledge all patients, staff of relevant hospitals, institute and the members of the Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka.

Foundation project

This study was funded by grants awarded by University of Sri Jayewardenepura, Sri Lanka. [ASP/01/RE/MED/2015/37, ASP/01/RE/MED/2016/48 and ASP/01/RE/MED/2017/29].'

Authors' Contributions

TN and KB involved in sample and data collection, laboratory work, data analysis and interpretation and writing the manuscript. MW designed the study, involved in proposal writing, data analysis and writing the manuscript. CG involved in proposal writing, research question, data analysis, writing the manuscript. NR and CM selected and recruited the patients for the study and involved in drafting the article. CDG involved in laboratory diagnosis and writing the manuscript. NF involved in proposal writing, research question, data analysis and writing the manuscript. All authors read and approved the final manuscript.

References

[1] Nisansala T, Muthusinghe D, Gunasekara C, Weerasekera M, Fernando N, Ranasinghe KNP, et al. Isolation and characterization of *Leptospira* interrogans from two patients with leptospirosis in Western Province, Sri Lanka. *J Med Microbiol* 2018; doi: 10.1099/jmm.0.000800

[2] Bandara KK, Weerasekera M, Gunasekara CP, Ranasinghe N, Marasinghe C, Fernando N. Molecular characterisation and disease severity of leptospirosis in Sri Lanka. *Mem Inst Oswaldo Cruz* 2015; **110**(4): 485-491.

[3] Bandara K, Weerasekera MM, Gunasekara C, Ranasinghe N, Marasinghe C, Fernando N. Utility of modified Faine's criteria in diagnosis of leptospirosis. *BMC Infect Dis* 2016; **16**(1): 446.

[4] Ehelepola N, Ariyaratne K, Dissanayake WP. The correlation between local weather and leptospirosis incidence in Kandy district, Sri Lanka from 2006 to 2015. *Glob Health Action* 2019; **12**(1): 1553283.

[5] Haake DA, Levett PN. Leptospirosis in humans. *Leptospira and leptospirosis*. Berlin: Springer; 2015, p. 65-97.

[6] Bandara K, Gunasekara C, Weerasekera M, Marasinghe C, Ranasinghe N, Fernando N. Do the Th17 cells play a role in the pathogenesis of leptospirosis? *Can J Infect Dis Med Microbiol* 2018; doi: https://doi.org/10.1155/2018/9704532.

[7] Epidemiology Unit, Ministry of Health, Nutrition & Indigenous Medicine. *Weekly epidemiology report*; 2013. [Online] available at: http://www.epid.gov.lk/web/ [Accessed on 10 December 2018].

[8] World Health Organization. *Communicable disease epidemiological profile Sri Lanka*. 2010. World Health Organization; 2010. [Online] available at: https://apps.who.int/iris/bitstream/handle/10665/70514/WHO_HSE_GAR_DCE_2010.7_eng.pdf;sequence=1[Accessed on 15 December 2018].

[9] World Health Organization. *Report of the second meeting of the leptospirosis burden epidemiology reference group*; [Online] available at: https://apps.who.int/iris/bitstream/handle/10665/44588/9789241501521_eng.pdf;sequence=1[Accessed on 18 January 2019].

[10]Rajapakse S, Weeratunga P, Niloofa R, Fernando N, de Silva NL, Rodrigo C, et al. A Diagnostic scoring model for leptospirosis in resource limited settings. *PLoS Negl Trop Dis* 2016; **10**(6): e0004513. doi: 10.1371/journal.pntd.0004513.

[11]Ahmed A, Engelberts MF, Boer KR, Ahmed N, Hartskeerl RA. Development and validation of a real-time PCR for detection of pathogenic *Leptospira* species in clinical materials. *PLoS One* 2009; **4**(9): e7093. doi: 10.1371/journal.pone.0007093.

[12]National Notifiable Diseases Surveillance System, Centers for Disease Control and Prevention. *Leptospirosis (Leptospira interrogans) case definition 2013*. [Online]Available at: https://wwwn.cdc.gov/nndss/conditions/leptospirosis/case-definition/2013/[Accessed on 20 March 2018].

[13]Epidemiology unit, Ministry of Health, Nutrition and Indigenous Medicine. *National guidelines on management of leptospirosis; 2016*. [Online]Available at: http://www.epid.gov.lk/web/images/pdf/Publication/leptospirosis/lepto_national_guidelines.pdf [Accessed on 25 March 2018].

[14]Sanjeewani R. Temporal & spatial trends of leptospirosis casesin Sri Lanka. *Int J Innov Res Dev* 2017; **6**(6). doi:10.24940/ijird/2017/v6/i6/116041-268418-1-SM.

[15]Ko AI, Reis MG, Dourado CMR, Johnson Jr WD, Riley LD. Urban epidemic of severe leptospirosis in Brazil. *Lancet* 1999; **354**(9181): 820-825.

[16]Barcellos C, Sabroza PC. Socio-environmental determinants of the leptospirosis outbreak of 1996 in western Rio de Janeiro: A geographical approach. *Int J Environ Heal R* 2000; **10**(4): 301-313.

[17]Sarkar U, Nascimento SF, Barbosa R, Martins R, Nuevo H, Kalofonos I, et al. Population-based case-control investigation of risk factors for leptospirosis during an urban epidemic. *Am J Trop Med Hyg* 2002; **66**(5): 605-610.

[18]Naotunna C, Agampodi SB, Agampodi TC. Etiological agents causing leptospirosis in Sri Lanka: A review. *Asian Pac J Trop Med* 2016; **9**(4): 390-394.

[19]Ludwig B, Zotzmann V, Bode C, Staudacher DL, Zschiedrich S. Lethal pulmonary hemorrhage syndrome due to *Leptospira* infection transmitted by pet rat. *ID Cases* 2017; **8**: 84-86.

[20]Segura ER, Ganoza CA, Campos K, Ricaldi JR, Torres S, Silva H, et al. Clinical spectrum of pulmonary involvement in leptospirosis in a region of endemicity, with quantification of leptospiral burden. *Clin Infect Dis* 2005; **40**(3): 343-351.

[21]Daher EF, Silva GB, Silveira CO, Falcão FS, Alves MP, Mota JAAA, et al. Factors associated with thrombocytopenia in severe leptospirosis (Weil's disease). *Clinics (Sao Paulo)* 2014; **69**(2): 106-110.

[22]De Silva NL, Niloofa M, Fernando N, Karunanayake L, Rodrigo C, De Silva HJ, et al. Changes in full blood count parameters in leptospirosis: A prospective study. *Int Arch Med* 2014; **7**(1): 31.

[23]Daher EF, Lima RS, Silva Júnior GB, Silva EC, Karbage NNN, Kataoka RS, et al. Clinical presentation of leptospirosis: A retrospective study of 201 patients in a metropolitan city of Brazil. *Braz J Infect Dis* 2010; **14**(1): 3-10.

[24]Agampodi SB, Dahanayaka NJ, Nöckler K, Anne MS, Vinetz JM. Redefining gold standard testing for diagnosing leptospirosis: Further evidence from a well-characterized, flood-related outbreak in Sri Lanka. *Am J Trop Med Hyg* 2016; **95**(3): 531-536.

[25]Holla R, Darshan B, Pandey L, Unnikrishnan B, Kumar N, Thapar R, et al. Leptospirosis in coastal south India: A facility based study. *BioMed Res Int* 2018; doi: https://doi.org/10.1155/2018/1759125.

[26]Thipmontree W, Suputtamongkol Y, Tantibhedhyangkul W, Unnikrishnan B, Kumar N, Thapar R, et al. Human leptospirosis trends: Northeast Thailand, 2001-2012. *Int J Environ Res Public Health* 2014; **11**(8): 8542-8551.

[27]Agampodi SB, Agampodi TC, Thalagala E, et al. Do people know adequately about leptospirosis? A knowledge assessment survey in post-outbreak situation in Sri Lanka. *Int J Prev Med* 2010; **1**(3): 158.