

RESEARCH ARTICLE

Pulmonary haemorrhage as a frequent cause of death among patients with severe complicated Leptospirosis in Southern Sri Lanka

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Abstract

Background

Leptospirosis is a tropical disease associated with life threatening complications. Identifying clinical and investigation-based parameters that predict mortality and morbidity is vital to provide optimal supportive care

Methods

We conducted an observational study in an endemic setting, in the southern Sri Lanka. Consecutive patients having complicated leptospirosis were recruited over 18 months. Clinical, investigational and treatment data were collected and the predictors of mortality were analysed.

Results

Out of 88 patients having complicated leptospirosis, 89% were male. Mean age was 47yrs (± 16.0). Among the total major complications 94.3% had acute kidney injury, 38.6% pulmonary haemorrhages, 12.5% fulminant hepatic failure, 60.2% hemodynamic instability and 33% myocarditis. An acute significant reduction of haemoglobin (Hb) was observed in 79.4% of patients with pulmonary haemorrhage. The mean of the highest haemoglobin reduction in patients with pulmonary haemorrhage was 3.1g/dL. The presence of pulmonary haemorrhage (PH) and hemodynamic instability within first 48 hours of admission significantly predicted mortality ($p < 0.05$) in severe leptospirosis. Additionally, within first 48 hours of admission, elevated SGOT (AST), presence of atrial fibrillation, presence of significant haemoglobin reduction, higher number of inotropes used, prolonged shock, invasive ventilation and admission to ICU significantly predicted mortality. Out of major complications during

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the first week after admission, pulmonary haemorrhage and fulminant hepatic failure (FHF) combination had significant adjusted odds of mortality (OR = 6.5 and 4.8, $p < 0.05$). Six patients with severe respiratory failure due to PH underwent ECMO and four survived. The overall mortality in complicated leptospirosis was 17%. In PH and FHF, the mortality rate was higher reaching 35.4% and 54.5%, respectively.

Conclusions

Within first 48 hours of admission, major complications such as pulmonary haemorrhage and haemodynamic instability and other parameters such as atrial fibrillation, acute haemoglobin reduction, elevated SGOT level could be used as early parameters predictive of mortality in severe leptospirosis. PH and FHF during the first week of admission in leptospirosis are associated with high morbidity and mortality requiring prolonged ICU care and hospitalisation. Above parameters could be used as parameters indicating severity for triaging and intensifying treatment. Using ECMO is a plausible treatment option in patients with severe pulmonary haemorrhage.

Author summary

Leptospirosis is a tropical infectious disease predominantly affecting the lower socioeconomic groups in Sri Lanka. It is associated with significant morbidity and mortality, especially in an endemic setting. Hence, it is vital to identify clinical and biochemical parameters that can predict mortality for triaging and for escalation of care. We observed that pulmonary haemorrhage, myocarditis, hemodynamic instability and hepatic dysfunction are frequent complications of leptospirosis in southern Sri Lanka. Additionally, we identified that mortality was associated with the presence of two major complications of leptospirosis: pulmonary haemorrhage and haemodynamic instability. Therefore, early detection of these two complications along with other parameters that predict mortality such as elevated SGOT levels on admission, acute haemoglobin reduction, atrial fibrillation, prolonged shock and need for invasive ventilation would assist to recognise critically ill patients. Within the first week of admission, PH and development of FHF predicted mortality. One-third our population had acute kidney injury in isolation and they had lesser mortality. Other major complications were almost always detected in combination and were associated with a higher mortality. Pulmonary haemorrhage was detected in one-third of patients and the majority warranted intensive care. Other than usual treatment modalities, ECMO (Extracorporeal Membrane Oxygenation) was used in six patients with critical respiratory failure due to pulmonary haemorrhage, where four survived. Out of the total group of complicated leptospirosis, more than one-third required intensive care treatment and 17% succumbed. Additionally, we mapped the case rates of complicated leptospirosis in Galle district, and observed that severe cases are detected in specific localities. These features are helpful in early detection of severe disease and proactive management for those who are having predictors of mortality.

Introduction

Leptospirosis has emerged as a major health threat in tropical and even in sub-tropical settings, estimated to infect more than a million people with approximately 60,000 deaths annually

[1, 2]. Natural disasters and extreme weather events including floods are now recognized to precipitate leptospirosis related epidemics [3] and its emergence in tropical settings is observed even in Sri Lanka [4]. Severe leptospirosis is characterized by dysfunction of multiple organs including the livers, kidneys, lungs, and brain. The combination of jaundice and renal failure was commonly known as Weil's disease [5].

The major burden attributed to leptospirosis is due to its severe life-threatening manifestations such as acute renal failure, myocarditis, pulmonary haemorrhage, and liver involvement. Out of the life-threatening manifestations, pulmonary haemorrhage (PH) is recognized as the most fatal. This life-threatening complication was observed to be prevalent in several leptospirosis endemic countries such as Nicaragua, Brazil, Sri Lanka and Greece [6–10]. The case fatality for pulmonary haemorrhage syndrome was reported to be more than 50% [11], but in an endemic setting it could be as high as 100% [12, 13]. A recent review noted 1.4–45.4% of lung involvement among hospitalized patients in Sri Lanka [14], and a recent study done in southern Sri Lanka mentioned an incidence just above 60% [10].

Different studies have mentioned parameters or manifestations that can be associated with mortality from leptospirosis. Few studies have mentioned that altered mental status, oliguria, and respiratory insufficiency, hypotension, arrhythmias were independently associated with death [15–17]. Out of major complications, acute kidney injury (AKI) is found to be the most common manifestation with a median series mortality around 10% [18]. Myocarditis was found in 7–15% of confirmed cases in Sri Lanka [19, 20]. Hepatic involvement is common and can commonly present as slight disturbances in serum liver function tests. However, fulminant hepatic failure is occasionally reported as a recognised complication in a few instances of severe leptospirosis [21]. It was evident that frequency of the complication could vary depending on the infecting serovar and geographic location.

In severe cases of pulmonary haemorrhage, some preferred using advanced therapeutic modalities such as plasmapheresis and ECMO. Although these techniques were previously reserved for developed countries, currently there are attempts to use these in developing countries where leptospirosis is endemic [22]. Currently, there is a paucity of knowledge on usage of ECMO in patients with pulmonary haemorrhage in an endemic tropical setting, only reported several case reports [22].

In our study, we describe morbidity, mortality and their predictors in a potentially severe group of leptospirosis who had major complications, excluding relatively benign mild cases of leptospirosis without significant complications. Additionally, we describe details from a large case series of critically ill patients with pulmonary involvement who required ECMO.

Methods

Ethics statement

Ethical approval for the study was obtained from the Ethical Review Committee, Faculty of Medicine (Approval No. 15.08.2018:3.6), University of Ruhuna. Approval for conducting the research at Teaching Hospital, Karapitiya (THK) was obtained from the Director of the hospital. Informed written consent was taken from all study participants.

Study sample

An observational study was conducted at the Teaching Hospital Karapitiya (THK), Galle, the largest tertiary care centre in southern Sri Lanka, for 18 months, from March 2017 to September 2018. THK is the main referral center for complicated leptospirosis and for ECMO, due to its geographic location surrounding paddy farming communities. All consenting consecutive patients meeting the criteria for complicated leptospirosis (with at least single organ

involvement/complication) as defined below were recruited from all medical units, emergency care unit and intensive care units (ICU) of THK. The criteria included patients with documented fever or a history of fever within 7 days of admission with or without myalgia with one or more of the following: evidence of acute kidney injury without prerenal aetiology: creatinine >1.5 mg/dL ($130\mu\text{mol/L}$) and /or urine output less than $500\text{mL}/24\text{h}$ with active urinary sediments (at least two of WBC, RBC and albumin on dipstick or >5 RBC/WBC per HPF), evidence of respiratory failure: Respiratory rate >28 cycles per min, evidence of hypoxia requiring oxygen, or requiring non-invasive or mechanical ventilation, evidence of spontaneous haemorrhage, pulmonary haemorrhage on chest radiograph or haemoptysis, conjunctival haemorrhage, epistaxis, haematuria, haematemesis, haematochezia or melena, unexplained vaginal bleeding, or petechiae), evidence of cardiac arrhythmias or ECG changes (new atrial fibrillation, new frequent ventricular ectopic beats, other arrhythmias) or new echocardiographic abnormalities indicative of myocarditis, evidence of hepatic dysfunction—clinical jaundice or elevated bilirubin (total bilirubin $>2\text{mg/dL}$ or $51.3\mu\text{mol/L}$) or laboratory evidence of hepatitis (transaminases elevated above 2 times the upper limit of normal (ULN), for male and female: AST ULN—35, ALT ULN—45).

The above clinical criterion was used to screen patients to detect patients who are clinically suspected of leptospirosis and to conduct confirmatory testing. Individuals who had positive confirmatory testing (mentioned under laboratory confirmation) were included in the study (S1 Table). Individuals who met the clinical criteria, however had negative tests of confirmation were excluded from the study.

Patients whose age less than 18 years, patients who are unable or unwilling to give consent for participation, leukopenia (white cell count below $4.0 \times 10^9/\text{L}$) within 48 hours prior or after hospital admission with positive virology or serology for Dengue or infections other than leptospirosis, evidence of focal bacterial infection as an aetiology of fever (i.e., urinary tract infection/ pyelonephritis, cellulitis, sinusitis, lobar pneumonia), evidence of end organ failure (renal, cardiac, respiratory) due to other etiologies unrelated to the current illness were excluded from the study.

Definitions of complications

An acute rise in serum creatinine above 1.5 mg/dL ($130\mu\text{mol/L}$) with a rise ≥ 0.3 mg/dL ($26.5\mu\text{mol/L}$) within 48 hours with or without oliguria was considered as acute kidney injury. Oliguria was defined as urine output <0.5 mL/kg/hr at least for 6 hours and urine output >0.5 mL/kg/hr was considered normal [23]. Pulmonary haemorrhage (PH) was defined from the presence of hypoxia ($\text{SpO}_2 < 92\%$, $\text{paO}_2 < 80$ mmHg/ 10.7 kPa) / requirement of oxygen by non-invasive or invasive methods with the presence of bilateral diffused alveolar shadows and/or sudden reduction in haemoglobin. Acute liver failure or fulminant hepatic failure was defined as the presence of PT/INR >1.5 and/or presence of hepatic encephalopathy. Cardiac involvement was defined by the presence of elevated troponin levels, changes in electrocardiogram or presence of arrhythmias or echocardiographic changes. Significant acute haemoglobin reduction due to bleeding was defined as $\geq 2\text{g/dL}$ reduction of haemoglobin within 48 hours. ‘Major complications’ in leptospirosis were considered acute kidney injury, pulmonary haemorrhages, fulminant hepatic failure, hemodynamic instability, myocarditis.

Data collection

Clinical and epidemiological data were collected using an interviewer administered questionnaire by a trained research assistant by conducting direct interviews of patients, and from

hospital records after obtaining written informed consent from study participants. Symptoms on admission to the hospital were recorded. Other clinical details and investigation records were assessed every other day to extract relevant details. The interviewers gathered details from patient and patient records every other day excluding the weekends. Laboratory and clinical parameters on first 48 hours of admission were used to analyse predictors of mortality. When the patient was in critical condition, the consent was obtained from the next-of-kin. The patients were visited every other day by the investigators to record clinical data and laboratory data. Follow-up was done by a brief assessment at the hospital performed one month after discharge from the hospital or by a telephone conversation.

Laboratory confirmation

Laboratory confirmation was based on the WHO LERG report, by symptoms consistent with leptospirosis and a single Microscopic Agglutination Test (MAT) titre $\geq 1:400$ and/or by detection of *Leptospira* DNA by PCR and/or by the presence of IgM antibodies [24]. Leptospirosis PCR and MAT (15 pathogenic serovar panel) was performed at Medical Research Institute, Colombo, Sri Lanka.

Spatial distribution of leptospirosis occurrence

Location of probable exposure to an environmental source of leptospirosis or home was recorded during the study. The spatial distribution of the patients was overlaid with the local Divisional secretariat (DS) division map of the Galle district. The base layer of Galle, Sri Lanka was extracted from Global administrative areas (GADM data-version 4.1) (https://gadm.org/download_country.html). The direct link to the base layer (shapefile) of Sri Lanka can be accessed via the following link (<http://www.medi.ruh.ac.lk/medicine/research-data-repository/>). The link to the license agreement details can be accessed at <https://gadm.org/license.html>. The number of leptospirosis cases per hundred thousand people in each DS division was illustrated using ArcGIS 10.3.1 (ESRI, New York, USA).

Statistical testing

All data were analysed with SPSS version 26.0. Results were expressed by mean \pm standard deviation (SD) or percentages. Comparison between the two groups was performed using Pearson's Chi-square test and Student's T test. Mann-Whitney test was used for parameters with a non-normal distribution. A logistic regression model was used for quantitative variables for binary outcome of mortality. Unadjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated. A multivariate logistic regression (stepwise forward and backward analysis) was performed to analyse the possible risk factors associated with mortality. Significance level was set at 5% (p value ≤ 0.05).

Results

One hundred and twenty-two patients with complicated leptospirosis were recruited to conduct confirmatory testing. Eighty-eight were confirmed by PCR and/or MAT testing. Among them, mean age was 47 (± 16.0) years, and 89% were male (Male: Female= 9:1). Almost all patients had fever (97.7%) on presentation, and myalgia (81.8%), arthralgia (78.4%), vomiting (46.6%) and headache (62.5%) were other common symptoms. Eighty-three percent had a history showing exposure to a probable environmental source of leptospirosis.

Complications associated with Leptospirosis (Table 1)

Acute kidney injury. Among the study group, acute kidney injury (AKI) was the most common complication. Out of 88 patients, 83(94.3%) developed AKI. Among the group who had AKI, 73.9% had oliguria and 15.9% had non-oliguric AKI. Out of the total, 30.7% (n = 27) had AKI without other major complications.

Pulmonary haemorrhage

Pulmonary haemorrhage (PH) was detected in 34(38.6%) patients. All of them had significant hypoxia and bilateral diffuse alveolar opacities. Out of them, 26.1% had cough and shortness of breath. Seven (8%) complained of haemoptysis on admission to the hospital. Twenty-four (70.6%) had bilateral diffused involvement in the chest radiograph and the rest (29.4%) had patchy bilateral alveolar shadows. Among patient who developed PH, all required

Table 1. Frequency of demographic and clinical features or complications in severe leptospirosis among the study group.

	Number (n = 88)	%
Demographic details		
Age, yrs	47(± 16.0)	
Male	78	88.6
Symptoms on admission		
Fever on presentation	86	97.7%
Myalgia	72	81.8%
Arthralgia	69	78.4%
Headache	55	62.5%
Vomiting	41	46.6%
Fatigue	31	35.2%
Calf pain	35	39.8%
Cough	23	26.1%
Hemoptysis	7	8.0%
Shortness of breath	23	26.1%
Abdominal pain	21	23.9%
Yellowing discolouration of eyes	20	22.7%
Diarrhea	27	30.7%
Reduced urine output	39	44.3%
Leptospirosis exposure in history	73	83%
Disease related complications		
Acute kidney injury	83	94.3%
Oliguric	65	73.9%
Non-oliguric	14	15.9%
Pulmonary haemorrhage	34	37.4%
Myocarditis	29	33.0%
Atrial fibrillation	11	12.5%
Hepatic dysfunction	43	48.9%
Fulminant hepatic failure	11	12.5%
Haemodynamic instability	53	60.2%
Intensive care unit admission	32	36.4%
Overall mortality	15	17%

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supplementary oxygen, 15 (44.1%) were on non-invasive ventilation, 29 (79.4%) required intubation and ventilation.

Hemodynamic instability

Fifty-three (60.2%) had hemodynamic instability (hypotension with blood pressure $\leq 90/60$) and 47 (53.4%) patients required usage of at least one inotrope during the course of treatment. Out of them, in 57.4%, 25.5%, 17% patients required one, two, three or more types of inotropes, respectively, to gain hemodynamic stability.

Cardiac manifestations

Out of total, 33.0% (29/88) had at least a single indicator of cardiac involvement. Elevated Troponin, abnormal ECG or an abnormality in echocardiography was detected in 18.2%, 14.8%, 11.4%, respectively. Eleven participants (12.5%) had atrial fibrillation during the illness. One had ST elevation myocardial infarction detected on admission.

Hepatic dysfunction

Out of total, 43 (48.9%) patients had hepatic dysfunction evidenced by deranged transaminases, alkaline phosphatase, total bilirubin and/or PT/INR. Thirty (34.1%) had elevated ALT and/or AST and 8 had elevated alkaline phosphate levels at the first 48 hours of the admission. Eleven (12.5%) patients had evidence of fulminant hepatic failure late after 48 hours of admission. Deranged PT/INR was observed at a later stage 48 hours after admission. All of these individuals who fulfilled the definition of acute hepatic failure had prolonged hypotension and were supported on multiple inotropes.

Haematological parameters—acute haemoglobin reduction and platelet count

A significant reduction in haemoglobin (Hb) of $\geq 2\text{g/dL}$ within 48 hours was observed in 27 out of 34 (79.4%) having PH with a mean highest haemoglobin(Hb) level reduction of 3.1g/dL (± 1.2 , range = 1.3–5.9g/dL) over 48 hours. Median day of detection of a significant haemoglobin reduction was on day 5 after onset of fever (Q1 = 4, Q3 = 6 days). In the non-pulmonary haemorrhage group, a minority (16.7%) had significant haemoglobin reduction. The mean significant haemoglobin reduction in this group was 1.15g/dL (± 1.09 , range = 0–4.5). Median day of detection of a significant haemoglobin reduction in the non-PH group was on day 6 after onset of fever (Q1 = 4.75, Q3 = 8.75 days). The maximum mean haemoglobin reduction was significantly higher in the PH group than non-PH group ($p = < 0.001$). Furthermore, significant hemoglobin reduction in non-PH group has occurred later than the PH group ($p = 0.046$).

Among the whole group, 44 (50.0%) and 70 (79.5%) had platelet counts less than $50,000\text{cm}^3$ and less than $100,000\text{cm}^3$, respectively.

Overall morbidity and mortality in patients with Leptospirosis

Overall, the median duration of hospitalisation was 6 (IQR–5,10) days. Thirty-two (36.4%) patients had received ICU care and the median duration of ICU care was 6 (IQR–3.5,8) days. Out of them, 17 received ICU care for < 7 days, 4 for 7–14 days and 6 for more than 2 weeks. The patients were intubated and ventilated for mean days of 5.97 (SD ± 4.8) days. The case fatality rate of the study sample was 17% with 15 deaths, including one patient who succumbed on

admission to the hospital. Patients who developed PH and FHF had a mortality rate of 35.2% and 54.5%, respectively.

The fatality rate of patient with pulmonary haemorrhage who has undergone plasmapheresis is 32.3%. Direct comparison for efficacy was not made as the majority (91.2%) of patients with pulmonary haemorrhage have undergone plasmapheresis and only 3 in PH group did not receive plasmapheresis.

Association of the number of major complications with mortality in severe Leptospirosis

Then, we analysed the association of major complications in patients with severe leptospirosis within the first week after admission. Acute kidney injury, myocarditis, pulmonary hemorrhage, hepatic dysfunction and hemodynamic instability were considered as major complications.

Table 2 reveals the association of major complications among confirmed leptospirosis patients. Twenty-nine (33.0%) patients had a single complication associated with leptospirosis. Out of them, nearly all (27 patients, 93.1%) had isolated acute kidney injury, one had isolated pulmonary haemorrhages and another had isolated hemodynamic instability mimicking septic shock. In the group of patients who had only two major complications, majority (n = 13, 68.4%) had a combination of acute kidney injury with hemodynamic instability. In patients who had two or more complications, haemodynamic instability was almost always associated with acute kidney injury. Increasing number of complications significantly increased the admission to ICU, duration of hospital stays and mortality (p = <0.05).

Early predictors of mortality in patients with severe Leptospirosis

Clinical and investigation parameters within first 48 hours of admission were considered for analysis. Symptoms present on admission were not predictive of mortality, which included symptoms such as haemoptysis, shortness of breath, abdominal pain etc. However, the presence of atrial fibrillation, presence of a significant acute haemoglobin reduction, increase number of inotropes used, prolonged shock, intubation and ventilation, and admission to ICU significantly predicated mortality in the severe leptospirosis group. Although, platelet, white cell count, serum bilirubin during admission were not predictive of mortality, high SGOT (AST) level on admission (but not SGPT/ALT) level significantly predicted mortality (**Table 3**).

Table 2. Morbidity and Mortality in patients having major complications of severe Leptospirosis.

Number of Complications	1 (n = 29)	2 (n = 19)	3 (n = 20)	4 (n = 17)	5 (n = 3)	p-value
Complications						
AKI	27 (93.1%)	17 (89.5%)	19 (95.0%)	17 (100%)	3 (100%)	0.711
Pulmonary Haemorrhage	1 (3.4%)	1 (5.3%)	13 (65.0%)	16 (94.1%)	3 (100%)	<0.01**
Myocarditis	0	4 (21.1%)	10 (50.0%)	12 (70.6%)	3 (100%)	<0.01**
Fulminant Hepatic Failure	0	1 (5.3%)	0	7 (41.2%)	3 (100%)	<0.01**
Hemodynamic instability	1 (3.4%)	15 (78.9%)	18 (90.0%)	16 (94.1%)	3 (100%)	<0.01**
Morbidity & Mortality						
Admission to ICU	1 (3.4%)	2 (10.5%)	12 (60.0%)	14 (82.4%)	3 (100%)	<0.01**
Duration of ICU care (Days)	2	6.5 (±2.1)	6.1 (±4.4)	8 (±6.1)	5.3 (±3.2)	0.7
Duration of hospital stay	5.5 (±1.8)	7.7 (±4.1)	8.6 (±4.9)	13.2 (±9.6)	6.7 (±2.9)	<0.01**
Mortality	1(3.4%)	1 (5.3%)	4 (20.0%)	6 (35.3%)	3 (100%)	<0.01**

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Table 3. Clinical indices and major complications predictive of mortality in patients with severe leptospirosis. (Logistic regression analysis of predictors of mortality).

	Unadjusted Odds ratio	p Value	Adjusted Odds ratio	p Value
Clinical and investigation indices				
Presence of high SGOT	5.3 (CI = 1.6–17.1)	0.006*		
Atrial fibrillation	4.7 (CI = 1.21–17.8)	0.22		
Presence of a significant reduction of Hb ($\geq 2\text{g/dL}$ over 48 hours)	5.3 (CI = 1.5–18.9)	0.009*		
Number of inotropes used in shock	10.7 (CI = 2.5–45.4)	0.001*		
Longer duration of shock / inotrope requirement (more than 48 hours)	2.5 (CI = 1.07–5.9)	0.034*		
Intubation and ventilation	18.5 (3.8–89.5)	<0.001**		
Admission to ICU	10.6 (CI = 2.7–41.5)	0.001*		
Type of major complication				
Pulmonary Hemorrhage	9.3 (CI = 2.4–36.1)	<0.001**	6.5 (CI = 1.6–27.0)	0.01*
Fulminant Hepatic Failure	9.1 (CI = 2.3–35.9)	0.002*	4.8 (CI = 1.1–21.0)	0.04*
Hemodynamic instability	12.2 (CI = 1.5–97.7)	0.018*	-	
Myocarditis	-	0.07	-	
AKI	-	0.9	-	

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Association of major complications with mortality in severe Leptospirosis

We analysed whether these five major complications were predictive of mortality in patients with severe leptospirosis (Table 3). Due to the long hospital stay in certain individuals the complications within the first week of stay in hospital was considered for this analysis. The presence of pulmonary haemorrhage (PH), fulminant hepatic failure (FHF) and hemodynamic instability significantly predicts mortality ($p = <0.05$). Additional analysis with stepwise logistic regression showed that, out of the five complications, PH and FHF combination were most predictive of mortality.

Comparison of mortality and morbidity in patients having Pulmonary Haemorrhage (PH)

Table 4 depicts a comparison of clinical characteristics and morbidity and mortality among patients who had pulmonary haemorrhage compared with those who did not.

Table 4. Characteristics of complications and morbidity among patients having pulmonary haemorrhage (PH) in severe Leptospirosis.

	No PH	PH	p-value
Number of patients	54 (61.4%)	34 (38.6%)	-
Age	45.2±17.8	45.7±13.4	0.96
Gender	Male	27 (30.7%)	<0.05*
	Female	7 (7.8%)	
Highest serum creatinine	410.0±210.6	397.9±199.3	0.79
Oxygen support	15 (31.3%)	33 (68.8%)	<0.01*
Patients with significant acute haemoglobin reduction	9 (16.7%)	27 (79.4%)	<0.01*
Highest acute haemoglobin reduction prior to transfusion	1.14±1.1	2.8±1.4	<0.01*
Number of patients in ICU	5 (9.3%)	27 (79.4%)	<0.01*
Days in ICU	4± 1.8	7.96±5.4	0.01*
Number of patients with 3 or more major complications	8 (14.8%)	32 (94.1%)	<0.01**
Total hospitalization days	5.57±2.1	11.3±8.2	<0.01**
Mortality	3/54 (5.6%)	12/34 (35.3%)	<0.01*

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This shows that females had a significantly higher risk of developing PH than males. The PH group required significantly higher non-invasive ventilation, intubation and ICU care and had a higher number of days in ICU and in hospitalization. Out of the patients who had PH, all most all had two or more major complications and had a significantly higher mortality rate compared to the group who had no PH (35.3% vs 5.6%). Nearly 80% patients with PH required management in an ICU. Among the haematological parameters, PH group ($71,400/\mu\text{l} \pm 46,600$) had significantly lower mean platelets counts on admission ($104,000/\mu\text{l} \pm 69,300$) ($p = 0.03$). However, white blood cell count on admission was not significantly between the two groups.

Non-invasive and invasive treatment modalities used in patients with severe Leptospirosis

Next, we analysed the treatment modalities used in patients with severe leptospirosis. All patients received antibiotics. Penicillin, ceftriaxone and doxycycline were the most frequently used antibiotics either alone or in combination: Penicillin or Ceftriaxone monotherapy for 27.3%, penicillin and doxycycline for 13.6%, ceftriaxone and doxycycline for 38.6%. Methylprednisolone was administered to 56(63.6%) and intravenous immunoglobulin (IV Ig) was given to 22(25.0%) patients. Tranexamic acid was given to 49(55.7%) patients.

Out of total, sixteen (18.2%) patients required dialysis. Two patients required continuous renal replacement therapy (CRRT) concurrently during ECMO. Supplementary oxygen was administered to 55.7%, non-invasive ventilation to 21.6% and intubation and ventilation to 36.4%. Plasmapheresis was performed in 39(44.3%) and 6 (6.6%) required ECMO.

ECMO in patients with Leptospirosis

ECMO was required in 6 (6.6%) patients with severe pulmonary haemorrhage. Eligibility of ECMO was decided on the severity of respiratory failure calculated by the 'Murray score' which consist of $\text{PaO}_2/\text{FiO}_2$ (P/F) ratio, extent of alveolar involvement on chest radiograph and peak end-expiratory pressure (PEEP) in the ventilator setting [25], physician's discretion and the presence of other co-morbidities. All patients had evidence of severe respiratory failure with Murray scores of 3.25 or above and poor oxygenation despite high ventilator settings. They had a mean P/F ($\text{PaO}_2/\text{FiO}_2$) ratio of 93 before ECMO. All had dense bilateral diffused opacities in the chest radiograph (S1A and S1B Fig). All underwent veno-venous ECMO and were supported by ECMO for a mean duration of 151.4 hours (± 55.83). They were intubated and ventilated for a mean of 12 days (± 5.70) and were in ICU for a mean 14.8 ± 5.44 days (total of 118 days). All required renal replacement therapy (RRT). Serial chest radiographs of these patients typically showed resolution of alveolar shadows within 4 to 5 days after initiation of ECMO (S1C, S1D and S1E Fig). Four got completely cured and were well at 1 and 3 month follow-up. Two patients succumbed despite ECMO therapy (S2 Table).

Spatial distribution of the abundance of Leptospirosis cases

Total leptospirosis cases, leptospirosis cases with pulmonary haemorrhage and the number of deaths due to leptospirosis were mapped for Galle district of Sri Lanka. Fig 1 illustrates that Elpitiya and Yakkalamulla DS divisions are the hot spots for overall leptospirosis cases and leptospirosis cases with pulmonary haemorrhage (Fig 1(A) and 1(B)). On the contrary, Neluwa DS division had the highest death rate due to leptospirosis among all divisions (Fig 1 (C)).

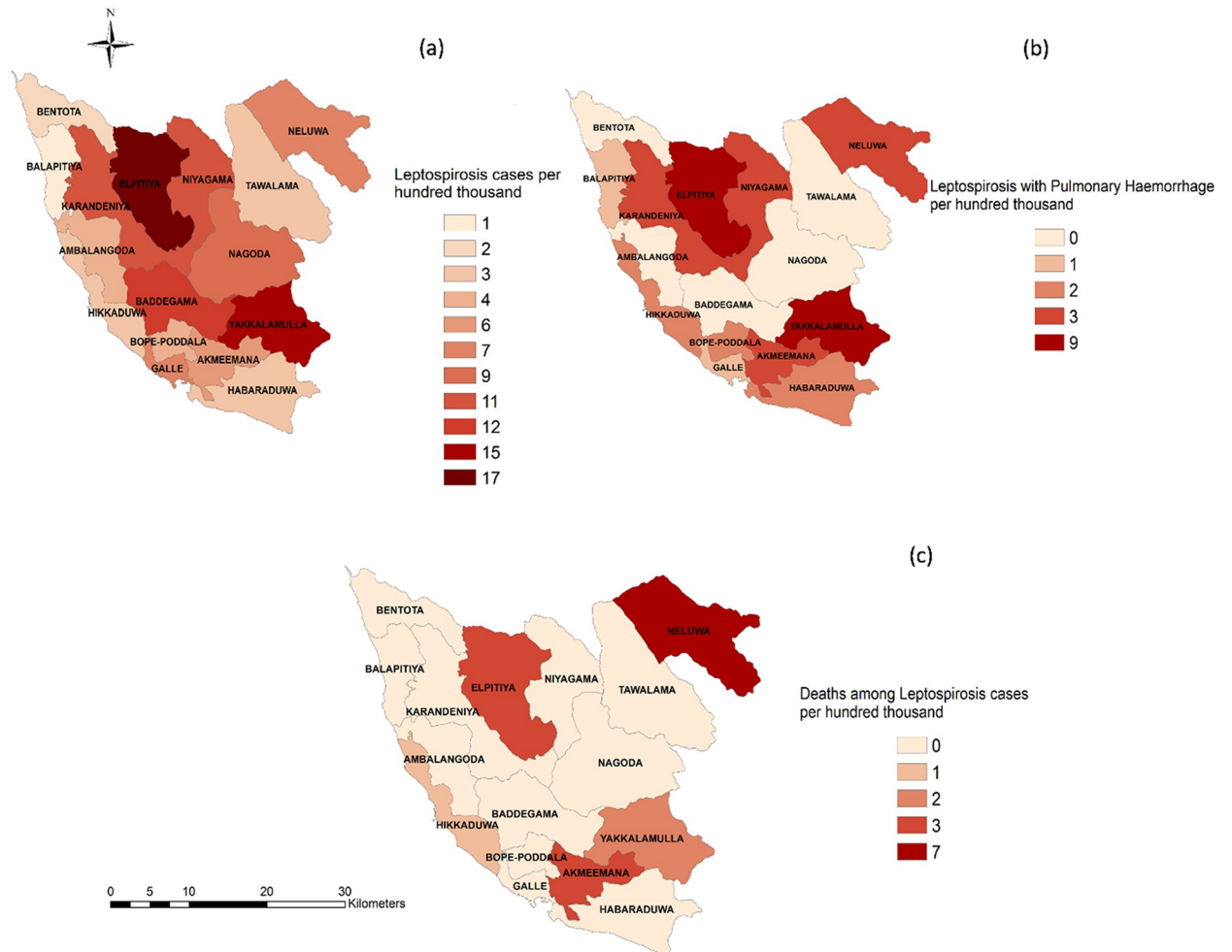


Fig 1. The spatial distribution of the cases and deaths of leptospirosis in Galle district, Sri Lanka. The base layer file of Galle, Sri Lanka was extracted from Global administrative areas (GADM data–version 4.1) (https://gadm.org/download_country.html). The direct link to the base layer (shapefile) of Sri Lanka can be accessed in the following link (<http://www.medi.ruh.ac.lk/medicine/research-data-repository/>).

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Discussion

In this study, we observed that pulmonary hemorrhage (PH) and hemodynamic instability early in the presentation were significantly associated with mortality. Galle is an area prevalent in complicated leptospirosis where PH were present in one-third and FHF was present in 12.5% of patients with severe leptospirosis. We observed that 80% of patients requiring ICU admission had pulmonary hemorrhage, warning us of the significant morbidity in this group. Hepatic involvement with FHF is considered as an unusual clinical manifestation of severe leptospirosis [26, 27], and has only been reported in several instances [21]. Features of fulminant hepatitis were not present early in the illness and developed in patient who require inotropic support which indicate that the pathogenesis could be related to ischaemic liver injury as opposed to direct hepatic involvement. However, the presence indicated risk of morbidity and mortality. Therefore, other than the routinely performed such as hematological tests and renal function tests, we suggest that focused examination and investigations should be performed for early detection of PH and FHF. Therefore, in suspected or confirmed cases of leptospirosis we suggest to perform examination for tachypnoea, work of breathing, and investigations such

as arterial blood gas to detect arterial hypoxia and perform investigations to detect hemoglobin reduction, chest radiograph, prothrombin time (INR), transaminases in view of identifying these complications early. AKI presented as the only manifestation in the majority and other major complications usually associated with AKI. The presence of a single major complication is associated with lesser mortality and with addition of each major complication an increase in mortality is observed. Additionally, we have shown the 'disease hot spot' areas of leptospirosis cases and deaths in Galle district. This would give us more understanding on how to triage and escalate care in patients that require strict monitoring and advanced supportive care.

Septic shock is observed in severe cases of leptospirosis associated with high morbidity and mortality [28, 29]. In our study, hemodynamic instability was observed in two-thirds of patients with severe leptospirosis. Most of them required inotropic support, where 50% required 2 or more inotropes. Majority had haemodynamic instability in combination with AKI. Severe leptospirosis behaves similar to septic shock caused by Gram positive or negative bacteria [30]. However, it was observed that the septic shock in leptospirosis is much difficult to manage than septic shock due to other conventional bacterial organism. Further comparative studies on clinical and cytokine correlated will be of value to understand the pathogenesis. It was shown that persistence of *leptospirosis spp.* antigen was present in the spleen in a case of lethal leptospirosis who had septic shock-like features. This may indicate that leptospirosis or associated mediators may be directly responsible for septic shock in leptospirosis [30].

Fluid management in severe leptospirosis is more challenging than bacterial sepsis due to the co-occurrence of AKI, myocarditis or multiple organ dysfunction syndrome. Additionally, we should be aware that myocardial dysfunction and severe bleeding can mimic septic shock. Therefore, it has been suggested that a more conservative fluid management approach may be more appropriate in complicated leptospirosis than managing according to standard sepsis guidelines, given the high mortality associated with pulmonary involvement [31]. Yilmaz et al. examined ICU patients and found that the clinical and laboratory findings of leptospirosis are similar to those of sepsis. They recommended to think about leptospirosis while examining a patient with SIRS/sepsis etiology in an area endemic for leptospirosis [32]. In a study done in a tropical Australian setting, they have suggested that prompt ICU support, early antibiotics, conservative fluid resuscitation, protective ventilation strategies, traditional thresholds for RRT initiation, and corticosteroid therapy, associated with a very low case-fatality rate [33]. Given the high incidence of hemodynamic instability in our group, we should be more vigilant that septic shock, bleeding and myocarditis should be identified promptly and managed accordingly.

Although, we could not find any symptoms present on admission or hematological parameters other than SGOT, that can predict mortality; atrial fibrillation, significant acute hemoglobin reduction, longer duration of shock (more than 48 hours duration), requirement of intubation and ICU care were important clinical indicators predicting mortality. It has been reported that disproportionate exaggerated rise of SGOT associated with grave prognosis in the late phase of leptospirosis in a limited number of patients [34]. However, we observed that SGOT (AST) within first 48 hours of admission can predict mortality at an early phase of leptospirosis.

There is a dearth of evidence regarding the extent of hemoglobin reduction detectable in patients with leptospirosis associated pulmonary hemorrhage. We observed that in the PH group, the majority had a significant hemoglobin reduction on 4–6 days after symptom onset. We also observed a mean highest hemoglobin reduction of 3.1g/dL in patients with PH. This hemoglobin reduction was observed despite efforts to immediately resuscitate and transfuse patients having acute bleeding. Due to the fact that acute hemoglobin reduction predicted mortality, we suggest that it is advisable to cross match 4–5 units of blood upon suspicion of PH. Moreover, in a leptospirosis endemic area of Brazil, it has been noted that 13% had

gastrointestinal bleeding while a similar percentage developed pulmonary hemorrhages[35]. Therefore, it is plausible that other than pulmonary hemorrhages, leptospirosis could lead to concealed bleeding elsewhere, judging from the fact that significant hemoglobin reduction was observed in nearly 15% of patients who did not have features to suggest pulmonary hemorrhages.

The spatial distribution data indicated that pulmonary hemorrhages are detected in similar areas where increased case numbers of leptospirosis are detected. Detection of increased case numbers are important in identifying 'hot spots' of complicated leptospirosis. Identifying disease 'hot spots' and areas of increased leptospirosis morbidity and mortality will be beneficial in triaging patient in the process of transferring patients early for advanced modalities of care in tertiary care hospitals. We suggest that real-time surveillance of leptospirosis cases may be beneficial to identify new or emerging 'hot spots' of the disease and initiating focus preventive strategies in such areas.

As we have only included patients with one or more complication related to leptospirosis, we consider that these patients represent a potentially severe group that needs hospital care and advanced treatment modalities. Moreover, our study includes a large case series of leptospirosis who underwent ECMO in the world. Altogether, there were 11 reported individual cases and two retrospective studies mentioning 13 patients with ARDS due to leptospirosis [22, 36–38] who underwent ECMO. Patients in our study were selected for ECMO when the Murray score was 3–4. ECMO improved oxygenation in these patients who had inadequate oxygenation despite being on maximum ventilatory support. We described 6 patients who underwent ECMO and four survived. Respiratory failure occurring due to pulmonary hemorrhages are transient and reversible, whereas the chances of survival may be improved if they are supported adequately during this critical period. Initiation of ECMO enabled to maintain adequate oxygenation because the lungs could be maintained on 'rest settings' at low ventilatory pressures until haemorrhagic lungs recover. Therefore, 'rest settings' potentially could reduce inotrope requirement due to restoration of venous return and tissue perfusion due to lesser ventilatory pressures. Due to prevalent septic shock clinical phenotype requiring multiple inotropes, theoretically ECMO would assist to stabilise haemodynamic parameters initially. Traditionally, severe bleeding has been a relative contraindication to extra-corporeal membrane oxygenation (ECMO), which requires systemic anticoagulation to maintain circuit patency. However, cases of diffuse alveolar haemorrhage have been reported to be successfully managed using ECMO [39]. In our case series, standards levels of activated clotting time were used and any aggravation of bleeding was not observed. This should be used cautiously in patients with PH and anticoagulation should be withheld immediately with exacerbation of bleeding. In a tropical setting where funding and resources are sparse, we consider this a major step forward to improve mortality in patients with severe pulmonary haemorrhage due to leptospirosis.

Ideally, further observational studies would have been beneficial to decide on surrogate respiratory parameters (Murray score, P/F ratio, oxygenation index) where ECMO can be initiated. This would invite the need for further research or trials to observe a benefit of ECMO and plasma exchange in patients with pulmonary haemorrhage due to leptospirosis.

Having a significant morbidity and mortality with leptospirosis in our setting despite advanced treatment modalities calls out for strengthening preventive measures of the diseases through measures such as chemoprophylaxis. The uptake of chemoprophylaxis in a risk group of farmers was shown be less than 30% [40]. Therefore, education of risk groups performed through public health sector would be an important measure to potentially reduce the disease burden. In addition, further studies to understand pathogenesis would shed light for therapeutic options to reduce complications of the disease.

Conclusions

Pulmonary haemorrhage and haemodynamic instability can be used as early predictors of mortality in severe leptospirosis. Other than the above atrial fibrillation, acute haemoglobin reduction, prolonged shock, elevated SGOT level at admission can be used as warning signs of mortality. These findings will have a significant effect on the management of patients in taking decisions on triaging and escalation of care. ECMO can be used as a plausible option to support patients with leptospirosis associated severe pulmonary haemorrhage. Identifying disease hot spots of leptospirosis cases and deaths will help in decisions to improve regional hospital facilities and organised transfer for advanced care.

Supporting information

S1 Fig. Serial chest radiographs showing gradual resolution of pulmonary shadows in a patient who underwent ECMO.

(DOCX)

S1 Table. Leptospirosis testing data for Rapid IgM, PCR and Microscopic Agglutination Test (MAT).

(DOCX)

S2 Table. Summary table depicting data of patients who underwent ECMO having pulmonary haemorrhage due to Leptospirosis.

(DOCX)

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