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Leptospirosis Pulmonary Hemorrhage Syndrome In Salvador, Brazil From 2003 - 2012

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By

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By enrolling in the Yale MPH program, I am accepting the responsibility to promote and uphold the Code of Academic and Professional Integrity. I agree to be held accountable for maintaining the atmosphere of honesty and professionalism at Yale University and within the greater academic community.

___Annie J. Tsay, May 1, 2014____

ABSTRACT

Objective: Leptospirosis pulmonary hemorrhage syndrome (LPHS) is a severe form of leptospirosis, with a case fatality rate exceeding 50%. Recently, LPHS has become the principal cause of mortality in leptospirosis patients in Salvador, Brazil. This study aims to describe the epidemiology of LPHS since 2003, characterize its clinical presentation, and identify risk factors that distinguish it from non-hemorrhagic pulmonary (NHPL) and non-pulmonary (NPL) forms.

Methods: Patients admitted between January 1, 2003 and December 31, 2012 in the active hospital-based surveillance who met the clinical case definition were included in the study. A standardized questionnaire was used to collect data from patient charts and in-person interviews. Unadjusted logistic regression models identified individual-level risk factors. Maps showing spatial distribution and clustering of leptospirosis cases were created to identify high risk areas.

Results: We identified 1,316 patients meeting our case definition, which included 113 LPHS, 184 NHPL, and 1019 NPL cases. Males were at greater risk for LPHS in all age groups. The LPHS-associated mortality was 65.5% (95% CI: 56.33-73.63), compared to 30.4% and 6.2% for NHPL and NPL cases, respectively. A lower microagglutination test (MAT) titer for LPHS compared to NPL cases in the acute phase suggests the absence of an early robust immune response. A high-risk LPHS cluster (p= 0.01) was identified and rat sightings was associated with NHPL patients (OR: 2.77, 95% CI: 1.23-6.23). Limited unique clinical correlates observed in patients with pulmonary manifestations compared to non-pulmonary forms of leptospirosis.

Conclusion: We describe the clinical features and epidemiology of LPHS in Salvador, Brazil, since its emergence in 2003. Few clinical correlates were identified between LPHS and NHPL patients, suggesting that leptospirosis is a disease on a clinical spectrum. Multidimensional control measures focusing on areas of high risk are necessary to reduce the burden of leptospirosis.

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TABLE OF CONTENTS

LIST OF TABLES

Tables in the body of the Thesis:

Supplementary tables:

LIST OF FIGURES

INTRODUCTION

Leptospirosis pulmonary hemorrhage syndrome (LPHS) is an emerging, fatal form of leptospirosis worldwide [1–8]. LPHS presents differently than the classic form known as Weil's disease, which is characterized by jaundice, acute renal failure, and bleeding. An outbreak of LPHS in Nicaragua in 1995 following a period of heavy rainfall raised awareness for this form of leptospirosis as LPHS patients did not display significant renal dysfunction or jaundice, suggesting that LPHS is a *de novo* form of leptospirosis [3,9]. However, differences in clinical presentation may vary according to geographic locale [10].

Around the world, the incidence of pulmonary involvement ranges from 20 to 70% of cases, with a spectrum of clinical manifestations, including mild or asymptomatic forms to pulmonary hemorrhagic forms [6, 11]. However, the burden of leptospirosis may be grossly underestimated even in endemic areas due to poor diagnostic tools and limited epidemiological studies on the clinical characteristics specific to patients with LPHS. As an example, in Iquitos, Peru, an area endemic to other febrile illnesses, including dengue and malaria, Vinetz et al describe the challenges of identifying leptospirosis-associated pulmonary manifestations due its non-specific febrile presentation [12]. Likewise, in Salvador, Brazil, the co-endemicity of leptospirosis and dengue hampers surveillance efforts. The delay in treatment may explain, in part, the reason behind poor clinical outcomes for patients with severe forms of leptospirosis [13]. The severity of LPHS in Salvador is documented by case fatality rates exceeding 50%, which is among the highest in endemic settings [1].

Prior to May 1, 2003, LPHS was not a recognized form of leptospirosis in Salvador, Brazil [1]. Under-recognition of leptospirosis is a limitation due to early non-specific influenza-like

presentation and the presence of other hemorrhagic fevers, but this was probably not the case in Salvador since other cities in Brazil had previously identified cases of LPHS [1, 14]. Leptospirosis is a reportable disease in Brazil, but estimates of the burden of leptospirosis are poor due to insensitive diagnostic tools, non-differential presentation from other hemorrhagic fevers during acute infection, and limited resources.

This study aims to provide an assessment of the burden of LPHS in Salvador, Brazil since its emergence in 2003. An understanding of the epidemiological burden, risk factors for the acquisition of disease, and clinical characteristics of LPHS is of critical importance as the world's urban population is projected to grow by 2 billion before 2030[15]. A study by Gouveia et al on LPHS patients in Salvador, Brazil, from 2003-2005 identified important characteristics that this study aims to reexamine using additional patient data from 2003-2012. Increased awareness of risk factors for LPHS and an epidemiological assessment of leptospirosis across Salvador are necessary in order to reduce the number of preventable leptospirosis-associated deaths each year.

METHODS

Surveillance site and data collection

All cases of leptospirosis were identified in Salvador, a coastal city with three million inhabitants in northeastern Brazil. Between January 1, 2003 and December 31, 2012, all patients with a clinical suspicion of leptospirosis admitted to Hospital Couto Maia (HCM), the state-run infectious disease referral hospital, were included in the study. All suspected cases must be communicated to the state health secretary and patients in the metropolitan area are referred to HCM.

Informed consent was obtained from patients or caretakers following guidelines instituted by the institutional review boards of the Brazilian Ministry of Health (the Oswaldo Cruz Foundation, FIOCRUZ) and Yale University. A standardized questionnaire was used to collect demographic data, risk factors, including household and occupational exposures through in-person interviews with patients or caretakers for all patients meeting the clinical case definition. Medical chart reviews were performed to collect clinical data, including laboratory or clinical symptoms on admission in addition to data during hospitalization.

Clinical case definition

Patients were enrolled if they met the clinical definition of severe leptospirosis upon admission. The minimum requirement for inclusion was a measured or self-reported fever (>38˚C) and being at least five years of age. In addition, patients must meet at least one of the syndromic criteria on admission to be enrolled. Acute renal insufficiency (oliguria or serum creatinine >1.5 mg/dL), jaundiced appearance noted during physical examination (or self-report), or liver enzyme alteration (total bilirubin >3.0 mg/dL or 75 U/L <TGO <3000 U/L or 75 U/L TGP

<3000 U/L). With respect to bleeding, patients must present with spontaneous hemorrhaging not explained by trauma. Examples of eligible forms of hemorrhage include: pulmonary hemorrhage or hemoptysis, petechiae, or gastrointestinal hemorrhage. Additional clinical presentations for inclusion include: bilateral conjunctival suffusion, enteric fever (malaise, vomiting, diarrhea, constipation, abdominal pain, and headache), undifferentiated febrile syndrome, meningitis or encephalitis with cerebrospinal fluid results consistent with an aseptic inflammatory process (10-2,000 cells/μL; glucose >40 mg/dL; proteins <150 mg/dL), or high clinical suspicion of leptospirosis upon examination.

Patients were excluded from the study if they presented with clinical or laboratory evidence of a diagnosis other than leptospirosis, refused to provide informed consent, readmitted for follow-up care of the same syndrome for the same episode, or experienced more than fifteen days of fever.

Laboratory Confirmation

The microagglutination test (MAT) was performed to confirm the diagnosis of leptospirosis serologically for all cases when possible. Laboratory-confirmed diagnosis of leptospirosis was defined as a four-fold rise in MAT reciprocal titer between paired serum samples, a reciprocal titer greater than 800 in one or more serum samples, or seroconversion was defined as a change in paired serum samples from a negative microagglutination titer to a reciprocal titer greater than 200. We also considered a positive result on the immunoglobulin M (IgM) ELISA (Bio-Manguinhos, Rio de Janeiro, Brazil) from any sample, isolation of leptospires in hemoculture, or detection of leptospires in real-time polymerase chain reaction (PCR) as laboratory-confirmed. Laboratory results interpreted as “probable,” including a single reciprocal MAT titer of 200 or

400 without serconversion; or a borderline positive IgM ELISA without seroconversion, were not considered confirmed for this analysis.

Outcome Case Definition: LPHS, NHPL, and NPL Case Definitions

Three primary outcomes include death, leptospirosis pulmonary hemorrhage syndrome (LPHS), and non-hemorrhagic pulmonary leptospirosis (NHPL). Death was defined as documentation in the patient's chart. LPHS was defined as having clinical suspicion of leptospirosis and massive pulmonary hemorrhage (blood coughed from lungs greater than 250 c.c. or aspiration of fresh blood after endotracheal intubation, which did not clear with suctioning). NHPL was defined as clinical suspicion of leptospirosis and respiratory distress without massive pulmonary hemorrhage. Respiratory distress was defined as having a maximum respiratory rate of at least 35 inhalations per minute or mechanical ventilation. Finally, non-pulmonary leptospirosis (NPL) was defined as having a clinical suspicion of leptospirosis without pulmonary manifestations.

We used the following clinical characteristic definitions for our study: hypotension was defined as a systolic blood pressure less than 90 mmHg; thrombocytopenia as having a platelet count less than 50,000 cells per cubic mm; oliguria as a 24 h urine volume of less than 400 mL at any time during hospitalization; and dialysis as peritoneal or hemodialysis.

Cases included in the study

Interviews were conducted on 1,316, patients who met the case definition for clinical suspicion of leptospirosis. Of the, 1,316 case patients, 1,049 (79.9%) were laboratory-confirmed, 17 (1.3%) were probable cases, and 250 (19.0) were unconfirmed (S3). Serologic confirmation could not be

completed for 21 (8.4%) of the 250 unconfirmed cases because paired serum samples were not obtained. Confirmed, probable and unconfirmed cases differed in demographic and clinical characteristics; however, due to high case fatality ratio among severe cases of leptospirosis, we included all patients who met the clinical case definition for the analysis of individual level exposure analysis and clinical characteristics analyses to prevent selection bias of patients with less severe disease (Tables 3, 4).

Environmental Data

Place of residence for patients enrolled in the hospital-based surveillance at HCM were geo-located during community-home visits following discharge from the hospital. A standardized questionnaire was used to assess individual level risk factors at the household level, including ownership of domesticated pets (dogs, cats, chickens), rat sightings, exposure to contaminated sources at home or at work, contact with floodwaters during the rainy season, and knowledge about incident cases of leptospirosis in the vicinity. All data collected were stored electronically in RedCap. Geographic coordinates of patients' residence were mapped using the GIS software.

Statistical analysis

Demographic analysis

Population estimates for the city of Salvador were obtained from the 2010 population census for Salvador-Bahia, Brazil, collected by the Instituto Brasileiro de Geografia e Estatística (IBGE) (http://www.ibge.gov.br/english/estatistica/populacao/censo2010/). Census data were used to calculate mean annual incidence rates of cases of leptospirosis (LPHS, non-LPHS, all cases) per 100,000 people for the study period using a Poisson model.

The odds ratios and 95% confidence interval (CI) for acquiring LPHS in men compared to women, by age group with the 0-14 age group as the reference category, and the odds of death for LPHS patients compared to non-LPHS patients were calculated using a Chi-square test. CIs for case fatality ratios were calculated using the Wald method in GraphPad (http://graphpad.com/quickcalcs/ConfInterval1.cfm). Confidence intervals that did not include 1.0 were considered statistically significant.

A generalized additive model (GAM) analysis was performed to display the non-linear trend between death and age; the acquisition of the type of leptospirosis (LPHS, NHPL, or NPL) and age. Models displayed show the fitted probability of death by LPHS, NHPL, NPL, or all deaths caused by leptospirosis by age. Age was modeled as a continuous variable.

Spatial cluster detection

SaTScan software [16] was used to identify spatial clustering of LPHS cases and non-LPHS (NHPL and NPL) cases. Kulldorff's scan statistic was calculated by using a moving circular window to test whether cases were distributed randomly over space, and to identify high LPHS clusters. The statistic was set to include a maximum of 50% of the data. A Bernoulli model was used to model the dichotomous outcome variable (LPHS or non-LPHS). The two analyses performed in SaTScan were: purely spatial across the ten-year period, from 2003-2012 and a retrospective spatial-temporal analysis, with time represented in one-year increments to account for seasonal variation. Patient homes were georeferenced to the Universal Transverse Mercator (UTM) zone 24S coordinate system.

Precipitation analysis

Daily rainfall data from January 1, 2003 to December 31, 2012 were obtained from the Instituto Nactional de Meteorologia (INMET) for Salvador-Bahia, Brazil (http://www.inmet.gov.br/portal/). Rainfall was collected each day in millimeters (mm) and summed over the entire month in order to examine the seasonal trend of rainfall (rainy season versus dry season). Cases of leptospirosis were separated by the severity of disease (LPHS, NHPL, and NPL) in order to determine if cases of leptospirosis by severity are associated with rainfall. LPHS cases were plotted beneath the x-axis in order to more closely examine the association between rainfall and the number of LPHS cases compared to non-LPHS cases.

Risk factor and clinical characteristic analyses

We used the Chi-square and Fisher's Exact tests to compare categorical data and the Wilcoxon rank sum test to compare non-parametrically distributed continuous data for two pairwise analyses: LPHS/NPL and NHPL/NPL. A P-value of <0.05 using a two-sided test was considered statistically significant. An unadjusted analysis using unconditional logistic-regression models was used to calculate crude odds ratios and their 95% confidence intervals for individual level risk factors for acquiring leptospirosis. In this study, multivariate analyses were not performed because the primary goal is to provide a descriptive examination of clinical characteristics observed on admission.

Study data were collected and managed using REDCap (v.5.6.4) electronic data capture tools hosted at the Brazilian Ministry of Health (Oswaldo Cruz Foundation, FIOCRUZ).

SAS for Windows (version 9.3) software was used for the statistical analysis of the data, R version 3.0.3 statistical package (R Foundation for Statistical Computing) was used to plot the GAM analysis, GraphPad PRISM (version 6.00) was used to produce figures and associated 95% confidence intervals for the demographic data, and maps were produced in ArcGIS (ArcGIS 10.2 Environmental Systems Resource Institute, Redlands, California).

RESULTS

Descriptive epidemiological findings

Person

Between January 1, 2003 and December 31, 2012, 1,316 hospitalized patients met the surveillance case definition for leptospirosis. Among the cases, 113 (8.6%) had pulmonary hemorrhagic leptospirosis (LPHS), 184 (14.0%) had non-hemorrhagic pulmonary leptospirosis (NHPL), and 1,019 (77.4%) had non-pulmonary leptospirosis (NPL) manifestations. The annual incidence rate of leptospirosis in Salvador, Brazil was 3.73 cases per 100,000 persons, compared to 0.31 and 3.43 cases per 100,000 persons for LPHS and non-LPHS cases (S1).

Young adult males between the ages 15-29 (31.5%) and 30-44 (27.1%) represented the majority of patients enrolled (Figure 1A). The risk of acquiring LPHS was greatest among individuals between the ages 15-29 (OR: 1.06, 95% CI: 0.40-2.80) and 60 years of age or older (OR: 1.93, 95% CI: 0.65-5.67), but were not statistically significant (Table 1). Overall, the odds of males acquiring LPHS was greater than females except for males between 15-29 years of age, where a 58% decrease (OR: 0.42, 95% CI: 0.18-0.98) in acquiring LPHS among men compared to women was observed (Table 1).

The overall case fatality rate was 14.7% (193 deaths); of which 65.6% (74) were caused by LPHS followed by 30.4% (56) and 6.2% (63) for NHPL and NPL forms, respectively (Figure 1B). Patients between the ages 45-59 had the highest case fatality rate (84.0%, 95% CI: 64.7-94.2) (Figure 1B, C, S2). Across all age groups, the odds of LPHS associated death was 17.28 (95% CI: 11.23-26.61) compared to non-LPHS associated death (Table 2). GAM analysis for

the acquisition of leptospirosis by type (i.e. LPHS, NHPL, NPL) and leptospirosis-associated death by age did not display a linear dose-response trend with increasing age (SF1). The fitted probability for death in LPHS and NHPL patients across age increased in a parabolic fashion, with the highest probability of death in individuals between 40 to 60 years of age (Figure 2A, B). Among individuals with non-pulmonary manifestations of leptospirosis, death increased exponentially (Figure 2C). Finally, overall leptospirosis-associated death increased in a semi-linear fashion with age, with a slight elevation in the probability of death for individuals between 35 to 50 years of age (Figure 2D).

Place

During the study period, a total of 999 leptospirosis cases residing in the City of Salvador were enrolled. Of these, 897 (89.8%) patients had associated GIS coordinates, which were obtained during follow-up home visits. Among the cases distributed in Salvador, 75 (8.4%) LPHS, 127 (14.2%) NHPL, and 695 (77.5%) NPL cases were documented (Figure 3A). A significant ($p=0.010$) high-risk cluster for LPHS cases in relation to non-LPHS cases compared to other regions in Salvador was identified in Pirajá by spatial analysis (Figure 3B). Pirajá is a one of the most densely populated districts in Salvador, with a population of 337,667 residents older than 10 years of age, according to the 2010 census[17]. Similarly, a spatial-temporal analysis of high-risk areas for LPHS cases identified a significant ($p=0.014$) geographic cluster in Pirajá. A higher proportion of LPHS cases compared to other locations in the City of Salvador existed from January 1, 2004 to December 31, 2008 (Figure 3C). See Figure 3 for cluster details.

Time

In Salvador, Brazil, the quantity of rainfall was associated with the overall number of cases of leptospirosis, but rainfall did not correspond with the proportion of LPHS cases. The majority of leptospirosis cases were identified during the rainy months, which ranged from April to August (Figure 4). As an example, during the heaviest year of rainfall (2005), 15.1% of all cases of leptospirosis in our study were identified. Out of 199 (15.1%) cases of leptospirosis reported in 2005, 15.1% (30 cases) were LPHS cases. However, the greatest proportion of LPHS cases was identified in 2008, a year with approximately 40% less rain than in 2005. LPHS represented 17.3% out of 104 leptospirosis cases, which accounted for 7.9% of the overall number of leptospirosis cases enrolled in 2008. Furthermore, in the driest year (2012) LPHS cases represented 8.57% of cases in 2012, which is comparable to the proportion of LPHS cases (8.64%) in 2010 (the fifth wettest year in the study and the year with the second largest number of leptospirosis cases, 162).

The number of leptospirosis cases is approximately equally divided between the two five-year segments in the study period. From 2003 to 2007, 55.6% (732) of all cases of leptospirosis cases were identified. Among the 732 cases identified during this time period, 61patients had LPHS, which represents 54% of all LPHS cases from 2003-2012. The number of leptospirosis cases and LPHS cases were marginally less in the second half of the study period (2008-2012).

Individual-level exposures and clinical presentation

Individual level exposures, including occupation, domiciliary, and environmental factors did not differ significantly between LPHS and NPL patients. A high proportion of LPHS (83.7%),

NHPL (81.6%), and NPL (81.4%) patients lived near refuse and consequently reported between two to six rats per day near their homes (Table 3). No significant differences were observed for patients with pulmonary manifestations compared to NPL patients in terms of exposure to contaminated sources potentially harboring *Leptospira* near domiciles including contact with contaminated waters from open sewers or street, house, or backyard flooding during the rainy season. Similarly, no significant differences were identified with respect to contact with contaminated sources at work including mud, trash, flood water, and sewer water. Approximately 20% of patients in each group reported use of protective equipment, such as boots or gloves, when in contact with mud, trash, flood water, or sewer water at work. No significant difference associated with limited use of protective equipment and outcome was detected.

However, we identified two risk exposures related to zoonotic reservoirs that were positively associated with development of NHPL. NHPL patients had an elevated unadjusted odds of observing rats near their homes (OR: 2.93, 95% CI: 1.48-5.81) compared to NPL patients. Additionally, the unadjusted odds of owning a domesticated dog among NHPL patients was 67% higher (95% CI: 1.10-2.55) than NPL patients.

Although individual level exposures did not differ between LPHS and NPL patients, clinical characteristics and laboratory tests based on serology indicate host level differences in the clinical presentation of disease. Among laboratory confirmed cases of leptospirosis, serovar Copenhageni was the primary infecting agent (>90%) for all cases of leptospirosis in Salvador.

LPHS patients had a lower acute phase reciprocal MAT titer (0.0, IQR: 0.0-150.0) compared to NHPL (0.0, IQR: 0.0-400.0) and NPL patients (200.0, IQR: 0.0-1600.0). On the other hand, convalescent titers for LPHS were greater than NPL patients 6400.0 (IQR: 800.0-6400.0) 3200.0 (IQR: 400.0-6400.0), respectively.

LPHS patients admitted to the hospital presented with more severe clinical characteristics on admission and during hospitalization. Among the 95 LPHS patients who responded, 39 (41.1%) had hemoptysis within the first 24 hours of hospital admission. During hospitalization, 107 (99.1%) out of 108 LPHS patients reported having hemoptysis (S4). The proportion of intensive care unit admission was 5.5 times higher for LPHS patients compared to NPL patients, 86.1% and 15.7%, respectively (Table 4). Aggressive therapeutic interventions during hospitalization including dialysis and blood transfusion were performed on a greater proportion of LPHS patients compared to NPL patients. More specifically, dialysis was performed on 55.1% of LPHS patients compared to 18.5% of NPL patients ($p < 0.0001$); 56.1% of LPHS patients received blood transfusion compared to 9.1% of NPL patients (Table 4).

On admission, 66.3% of LPHS patients reported shortness of breath and 16.0% were hypotensive (Table 4). Additionally, 51 (58.6%) of LPHS patients presented with oliguria on admission compared to 263 (30.9%) for NPL patients ($p < 0.0001$). During the course of hospitalization the number of LPHS and NPL patients with oliguria increased to 83 (83.8%) and 474 (53.5%), respectively and remained significant ($p<0.0001$) (Table 4).

Laboratory results detected elevated liver enzyme function of aspartate aminotransferase (TGO) in LPHS patients (126.0 U/L, IQR: 60.0-277.0) compared to NPL patients (86.0 U/L, IQR: 52.0-148.0) (Table 3). The percentage of hematocrit in whole blood was significantly lower ($p<0.0001$) in LPHS patients (30.0, IQR: 26.0-34.0) compared to NPL (35.0, IQR: 31.1-39.1) patients. LPHS patients were more thrombocytopenic (<50,000 platelet cells/mm^3) than NPL patients, 41.9% and 22.7%, respectively (Table 4).

DISCUSSION

Leptospirosis is an important emerging infectious disease as a result of rapid urbanization in Brazil. In Salvador, Brazil, LPHS was not identified prior to May 1, 2003 [1], but cases of pulmonary manifestation had been reported since the mid 1980's in other large urban areas in Brazil (Rio de Janeiro and Sao Paulo) [10, 18,19]. The high case fatality rate observed in LPHS patients calls for heightened awareness and the need to critically evaluate epidemiologic factors associated with this fatal form of leptospirosis. In this study we have continued the work initiated by Gouveia et al by performing a descriptive analysis over ten years (2003-2012) since the first documented case of LPHS in Salvador, Brazil.

Our study attempts to characterize the epidemiology of severe forms of leptospirosis despite diagnostic challenges and high case fatality rates, which hinder surveillance capacity in well-established sites. Laboratory confirmation was obtained for 70% of LPHS cases enrolled, which indicates that LPHS was caused by *Leptospira* infection and not by other endemic hemorrhagic diseases. Although cases of LPHS represent fewer than 10% of all leptospirosis cases in our hospital-based surveillance, it is the leading cause of mortality among hospitalized patients with leptospirosis in Salvador. Despite the attenuation in the overall case fatality rate due to LPHS in Salvador since Gouveia et al's study (74% to 66%), it is important to close the knowledge gap regarding key risk factors associated with LPHS, whether they are individual-level exposures or clinical characteristics.

Specific individual-level exposures unique to the acquisition of LPHS compared to non-pulmonary forms of leptospirosis were not identified in our study and observed by Gouveia et al [1]. Other studies performed in large urban communities in Salvador also did not find an

association between environmental factors and *Leptospira* infection [20]. However, in our study, sighting of rats near the home and the ownership of a domestic dog were significant unadjusted risk factors for the development of NHPL. This suggests that perhaps the development of LPHS does not correspond to an increase in inoculum dose from contact with known reservoirs. Early detection of LPHS cases with respect to individual-level exposures will be difficult since LPHS cases did not have significantly different exposures compared to NPL patients. Therefore, a high clinical index of suspicion for patients with symptoms of respiratory distress is necessary to prevent the progression to LPHS.

Results from our clinical and individual risk factor questionnaire suggest that LPHS does not have a discrete phenotype. Instead, LPHS is a severe form of leptospirosis, which runs on a clinical spectrum and individuals may progress from mild or asymptomatic forms to respiratory distress, and finally massive pulmonary hemorrhagic forms. A greater proportion of LPHS patients present with severe clinical characteristics on admission and progress during hospitalization. Pulmonary hemorrhage was identified in 41.1% of LPHS patients on admission, but increased to 99.1% among LPHS patients during hospitalization (S4). Furthermore, MAT titers of LPHS patients are lower on admission compared to NPL patients, followed by increased titers during hospitalization.

Specific clinical exposures unique to LPHS patients were not identified compared to NHPL patients. This suggests that host specific responses play an important role in the development of severe disease. In Salvador, *L. interrogans* serovar Copenhageni is the causative agent for 90% of cases. A study on rats in Salvador, Brazil positively identified 68.1% of rats (*Rattus*

norvegicus) sampled near the homes of hospitalized patients with severe leptospirosis as carriers of serovar Copenhageni [21]. Genomic studies have not identified differences in isolates from LPHS patients compared to non-LPHS patients suggesting non-differential association between pathogenicity and clinical outcome (unpublished). Additionally, intensity of exposure to rainfall does not result in a greater number of LPHS cases. Although our study supports the established idea that leptospirosis cases are associated with rainfall [3, 9, 22, 23, 24, 25,26], the proportion of LPHS cases does not follow always follow the pattern of rainfall. These observations indicate that host response, such as the role of the immune system, may explain why certain individuals develop LPHS while others do not.

A spatial and temporal analysis identified Pirajá, a densely populated area, as a high risk area for LPHS, particularly from 2004-2008. Although it is difficult to explain why Pirajá is a high risk LPHS area, it is of great public health importance to note that high risk clusters have not been observed since 2008. For public health practitioners, this finding may shed light on future hotspots in Salvador that have similar terrain, exposures, and spatial distribution of residents. Predictive risk mapping for leptospirosis is a novel concept that has only been performed on data from the American Samoa and has the potential to assist with control programs targeting high risk areas[27]. Of note, an ongoing prospective community-based cohort study for leptospirosis, dengue, and non-communicable illnesses is based in an urban slum community situated in Pirajá [13, 20,21, 24, 28]. Reporting bias is not a major concern since our hospital-based surveillance covers the entire city of Salvador. Efforts to reduce the burden of leptospirosis in urban slum communities have led to closure of open sewers, attention to the need for improved sanitation

measures, and awareness of leptospirosis in the community. This may explain the decreasing burden of leptospirosis in the last half of our ten-year study.

Gouveia et al, identified that women were twice as likely as men to acquire LPHS[1]. However, in this follow-up study, the gender effect has been attenuated. Men are at greatest risk for acquiring leptospirosis and LPHS across all age groups, but men between the ages 15-29 have a 58% reduction in the odds of acquiring LPHS (OR: 0.42, 95% CI: 0.18-0.98). Host susceptibility, participation in risk activities, and changes in the social infrastructure may explain the increased risk for LPHS for women in this age group.

The burden of leptospirosis primarily exists among populations residing in resource-poor settings. It is a preventable infectious disease that requires improvements in basic sanitation measures, changes in infrastructure, enhanced awareness of risk factors for disease, and resources for health care [15,29]. Although LPHS constitutes less than 10% of the cases of leptospirosis in Salvador, Brazil, the mortality is exceptionally high. High mortality may result from delayed treatment due to non-specific presentation during acute infection [13, 30]. This study demonstrates that leptospirosis is a disease that manifests on a clinical spectrum, therefore early identification of cases and control programs targeting rat control in densely populated areas may reduce the burden. Studies focusing on host factors and exposure to contaminated sources are needed for the development of effective control programs to reduce the number of leptospirosis-associated deaths.

LIMITATIONS

In this study, information was analyzed from patients who met the surveillance case definition of leptospirosis. These included unconfirmed cases (19.0% of 1,316) and probable cases (1.3% of 1,316) primarily due to incomplete diagnostic testing because of the lack of paired serum samples. Cases without lab confirmation were older, more likely to be female, and had higher mortality (S5). As a result, exclusion of the unconfirmed patients might bias the results by failing to recognize a subgroup of severely ill patients. A potential limitation to this inclusion criterion is the potential for misclassification of patients, which calls for improved diagnostic tests for early detection of leptospirosis. Other limitations of this study are the lack of data for individual-level exposures to contaminated sources, environmental reservoirs, and sanitary conditions. Finally, due to the cross-sectional nature of the data, it is difficult to fully characterize and understand the progression of disease over a complete time course from admission to discharge.

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APPENDIX

Figure 1: Demographics: Clinically suspected cases of leptospirosis, according to age and sex, in Salvador, Brazil, from 2003-2012. A. Demographics of suspected cases of leptospirosis, according to age and sex. Black bars, male cases; gray bars, female cases. **B.** Distribution of 193 leptospirosis-associated deaths among all suspected cases of leptospirosis according to age. Deaths among case-patients with LPHS represented by black bars (▬), death among case-patients with NHPL represented by hatched bars (▨), and death among case-patients with NPL represented by white bars (▭). **C.** Solid circles, LPHS case fatality rate (CFR) (-●-), solid square with dashed line, CFR for NHPL cases (-■·), and solid triangle, CFR for NPL cases (-▲-).

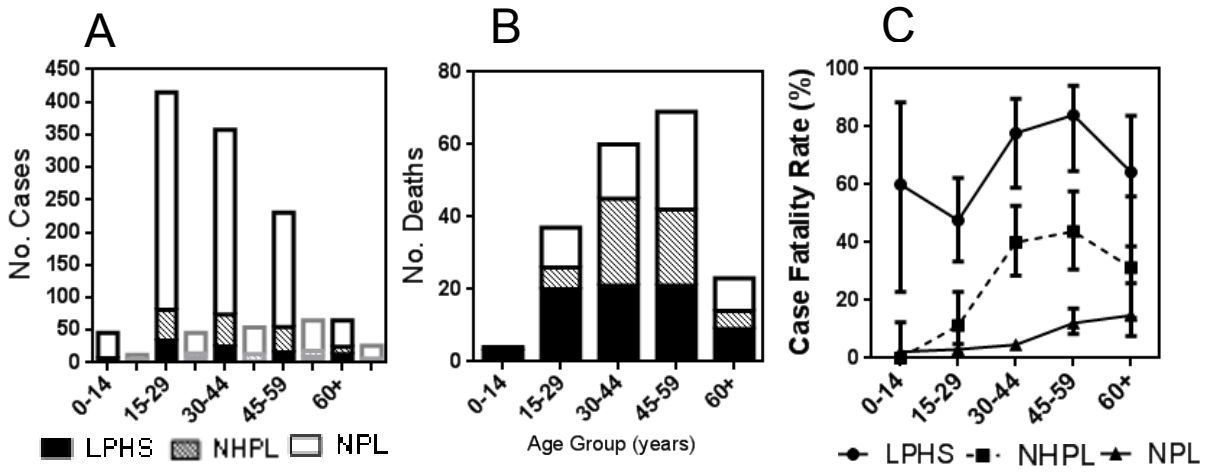


Figure 2: Generalized additive models (GAM) of the association between the probability of leptospirosis-associated death for patients with **A.** LPHS, **B.** NHPL, **C.** NPL, and **D.** all forms of leptospirosis by the continuous variable of age. The coefficient, fitted probability of death, in the GAM model is a measure of the risk of leptospirosis-associated death.

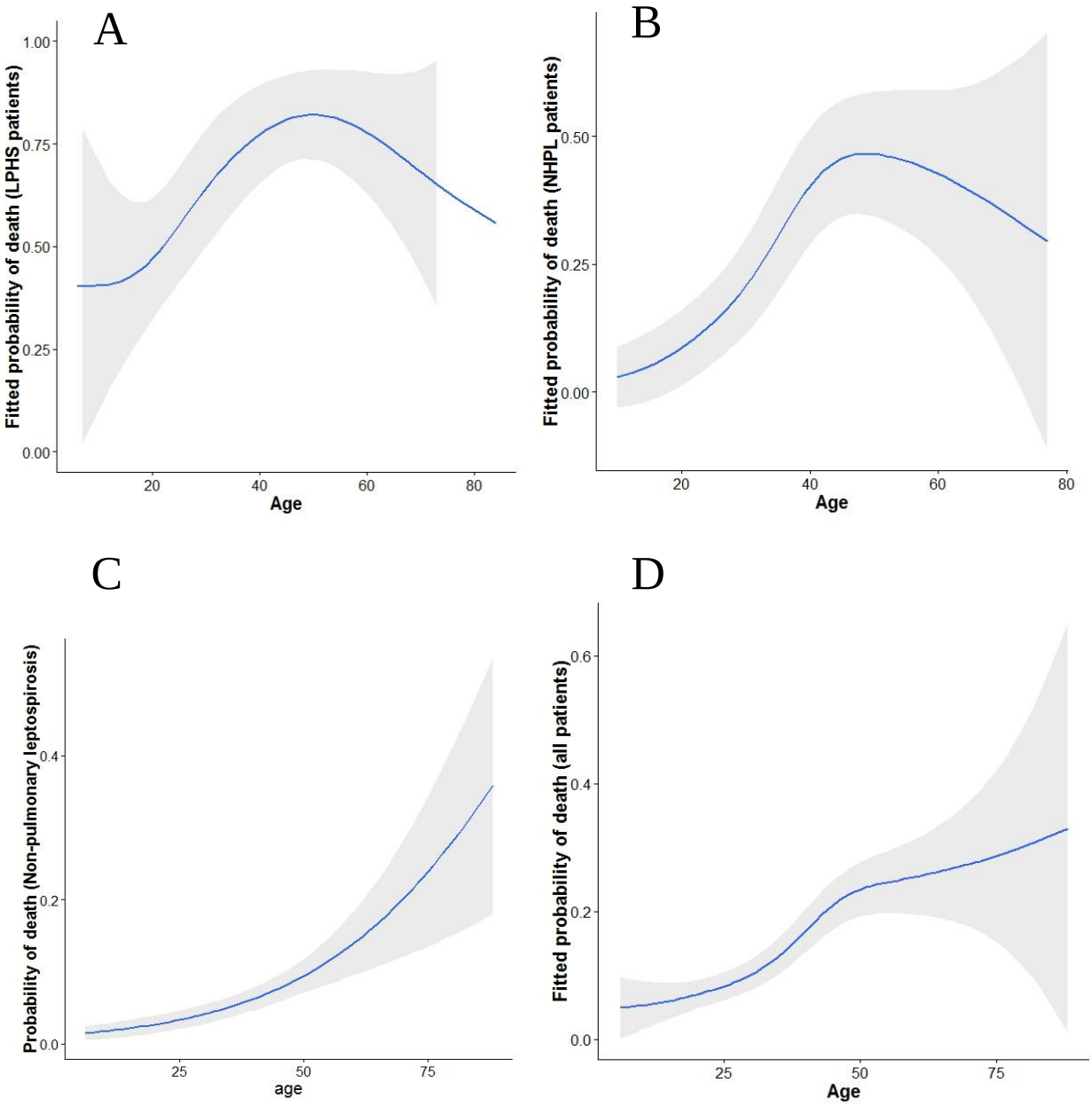
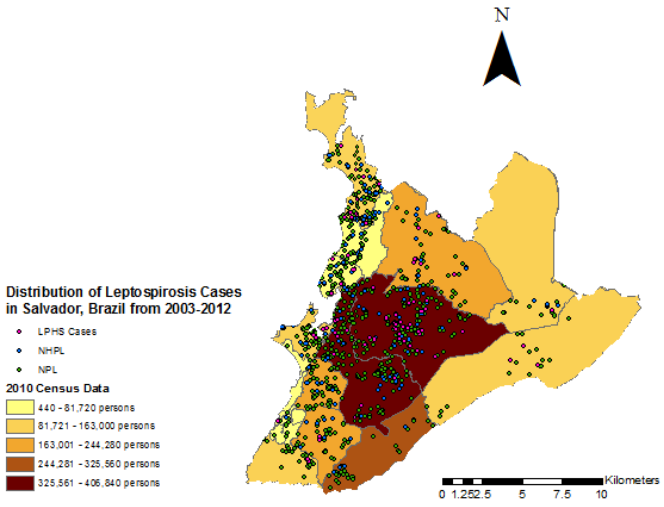


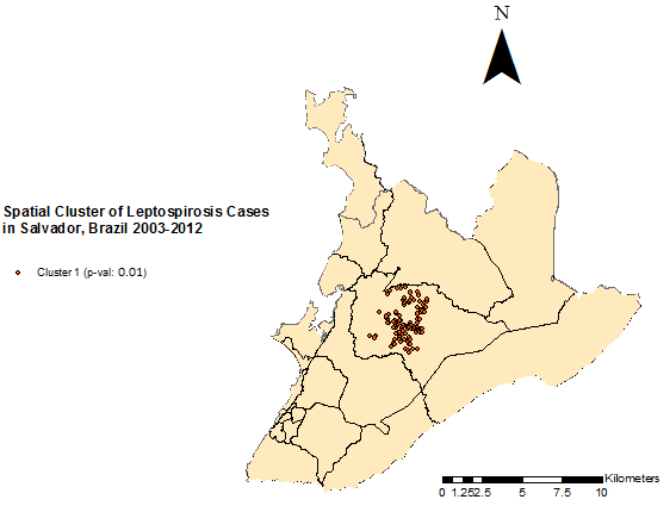
Figure 3: Risk mapping: spatial and temporal distribution of leptospirosis cases in the City of Salvador, Brazil, from 2003-2012. Spatial distribution of 897 suspected cases of leptospirosis in Salvador, Brazil with geocoding from 2003-2012 (N=999). **A.** The distribution of suspected cases of leptospirosis with GIS coordinates residing in the City of Salvador. Pink dots, LPHS cases; blue dots, NHPL cases; green dots, NPL cases. The population (persons older than 10 years of age) by census district obtained from the 2010 Census is shown*. **B.** A high risk area for LPHS compared to non-LPHS. Cluster included 26 LPHS cases out of 121 total leptospirosis cases (RR 3.40, p=0.010). **C.** A high risk area for LPHS compared to non-LPHS using spatial-temporal analysis. Cluster included 7 LPHS cases out of 9 total cases of leptospirosis (RR 10.16, p= 0.014). Cluster significance calculated using Kulldorf's spatial scan statistic [16]. RR= relative risk.

*****2010 Population Census of Salvador-Bahia, Brazil from the Instituto Brasileiro de Geografia e Estatística (IBGE) [17].

A


N
Distribution of Leptospirosis Cases in Salvador, Brazil from 2003-2012
LPHS Cases
NHPL
NPL
2010 Census Data
440 - 81,720 persons
81,721 - 163,000 persons
163,001 - 244,280 persons
244,281 - 325,560 persons
325,561 - 406,840 persons
Kilometers
0 1.252.5 5 7.5 10

B


N
Spatial Cluster of Leptospirosis Cases in Salvador, Brazil 2003-2012
Cluster 1 (p-val: 0.01)
Kilometers
0 1.252.5 5 7.5 10

C

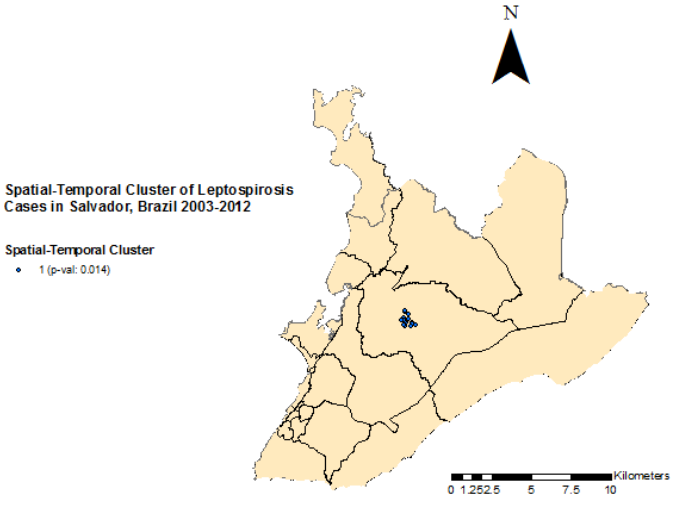

N
Spatial-Temporal Cluster of Leptospirosis Cases in Salvador, Brazil 2003-2012
Spatial-Temporal Cluster
1 (p-val: 0.014)
Kilometers
0 1.252.5 5 7.5 10

Figure 4: Seasonality: leptospirosis cases and monthly rainfall from 2003-2012. Monthly number of clinically suspected cases of leptospirosis (N=1,316) in relation to the monthly pluviometric precipitation (mm) in Salvador, Brazil, from January 1, 2003 to December 31, 2012. The month and year of hospitalization is shown for each case of LPHS, NHPL, and NPL. Red bars, LPHS cases; gray bars, NHPL cases; black bars, NPL cases.

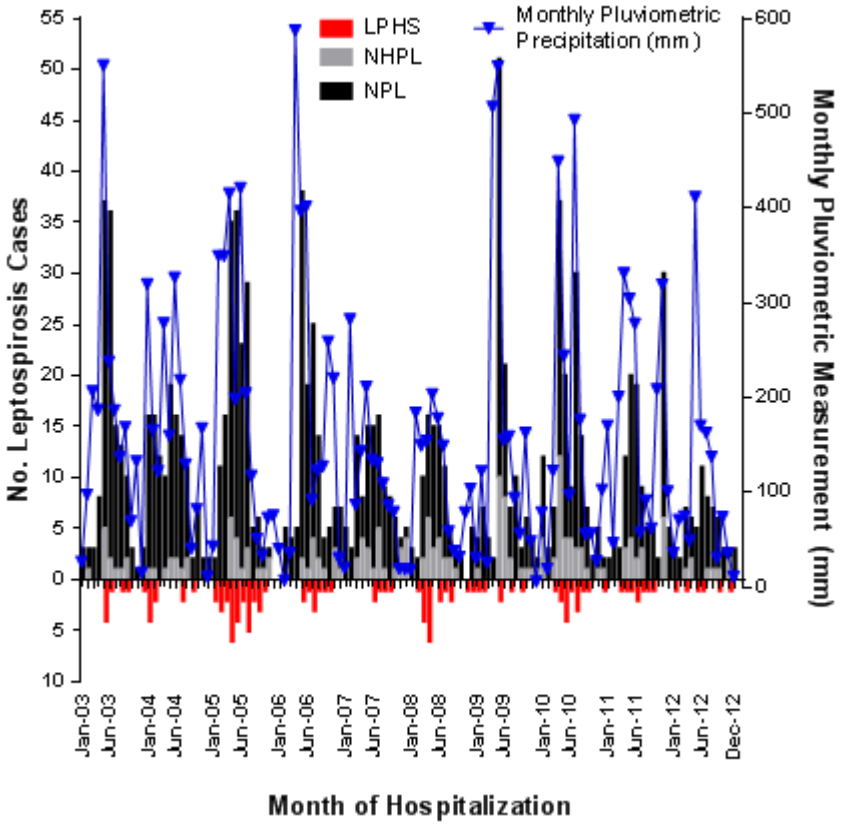


Table 1: Risk of acquiring LPHS among 1,316 suspected cases of leptospirosis, according to age and gender.

	All Cases (N= 1316)			Males (N=1116)			Females (N=200)			
Age Group	No. LPHS Cases	No. Non-LPHS Cases	OR (95% CI)*	No. LPHS Cases	No. Non-LPHS Cases	% LPHS	No. LPHS Cases	No. Non-LPHS Cases	% LPHS	OR (95% CI)**
All ages	113	1203	1.00 (NA)	92	1024	8.24	21	179	10.50	0.77 (0.46-1.26)
0-14	5	53	Ref	4	45	0.36	1	8	0.50	0.71 (0.07-7.21)
15-29	42	419	1.06 (0.40-2.80)	34	381	3.05	8	38	4.00	**0.42 (0.18-0.98)**
30-44	27	384	0.75 (0.28-2.02)	25	332	2.24	2	52	1.00	1.96 (0.45-8.51)
45-59	25	270	0.98 (0.36-2.68)	16	214	1.43	9	56	4.50	0.47 (0.20-1.11)
60+	14	77	1.93 (0.65-5.67)	13	52	1.16	1	25	0.50	6.25 (0.77-50.49)

No., number; OR, odds ratio; CI, confidence interval. Bold values indicate statistical significance. The percentage of LPHS cases among all leptospirosis cases in row.

*OR shown describe the odds of acquiring LPHS in each age group with respect to the 0-14 age group.

**OR shown describe the odds of acquiring LPHS for males compared to females by age group.

Table 2: The odds of death among all suspected cases of LPHS compared to non-LPHS patients by age.

	All Cases				LPHS				Non-LPHS				
Age Group	No. Deaths	Total No. Cases	% CFR	95% CI	No. Deaths	Total No. Cases	% CFR	95% CI	No. Deaths	Total No. Cases	% CFR	95% CI	OR (95% CI)
All ages	193	1316	14.67	12.85-16.68	74	113	65.49	56.33-73.63	119	1203	9.89	8.33-11.71	17.28 (11.23-26.61)
0-14	4	58	6.90	2.24-16.91	3	5	60.00	22.91-88.40	1	53	1.89	0.00-10.88	78.00 (5.41-1123.71)
15-29	37	461	8.03	5.85-10.89	20	42	47.62	33.36-62.28	17	419	4.06	2.50-6.45	21.50 (9.89-46.71)
30-44	60	411	14.60	11.50-18.36	21	27	77.78	58.90-89.74	39	384	10.16	7.49-13.61	30.96 (11.79-81.33)
45-59	69	295	23.39	18.91-28.56	21	25	84.00	64.73-94.21	48	270	17.78	13.66-22.80	24.28 (7.97-73.96)
60+	23	91	25.27	17.42-35.13	9	14	64.29	38.60-83.82	14	77	18.18	11.02-28.36	8.10 (2.35-27.91)

No., number; OR, odds ratio; CI, confidence interval; CFR, case fatality ratio. The OR (95% CI) shown describes the odds of death among LPHS patients compared to Non-LPHS patients.

Table 3: Selected unadjusted individual-level risk factors for the acquisition of leptospirosis among 1,316 suspected cases of leptospirosis enrolled in the active hospital-based surveillance

Characteristics	LPHS (n=113)			NHPL (n=184)			NPL (n=1019)	
	No. Responses	No. (%) or % of group or Median (IQR)	OR (95% CI)	No. Responses	No. (%) or % of group or Median (IQR)	OR (95% CI)	No. Responses	No. (%) or % of group or Median (IQR)
Demographics and Epidemiological Data								
Age (years)	113	38.0 (23.0-49.0)	1.01 (1.00-1.02)	184	39.0 (26.0-48.0)	1.01 (1.00-1.02)	1019	34.0 (23.0-46.0)
Sex								
Male	113	89 (78.8)	0.63 (0.39-1.01)	184	152 (82.6)	0.80 (0.53-1.22)	1019	872 (85.6)
Ethnicity								
Black	113	19 (16.8)	**0.53 (0.32-0.89)**	184	52 (28.3)	1.04 (0.73-1.47)	1019	281 (27.6)
Individual level risk factors								
Smoke	41	20 (48.8)	0.88 (0.47-1.67)	105	54 (51.4)	0.98 (0.65-1.49)	526	273 (51.9)
Diabetes	41	2 (4.9)	1.41 (0.32-6.30)	102	9 (8.8)	2.66 (1.16-6.11)	513	18 (3.5)
Asthma	42	1 (2.4)	0.95 (0.12-7.47)	104	3 (2.9)	1.16 (0.33-4.15)	521	13 (2.5)
Type of Job								
Formal	8	26.7%	1.16 (0.50-2.68)	18	24.7%	1.29 (0.72-2.29)	114	29.6%
Informal	22	73.3%	0.86 (0.37-2.00)	55	75.3%	0.78 (0.44-1.38)	271	70.4%
Occupation								
Sewer worker	30	3 (10.0)	0.93 (0.27-3.20)	73	11 (15.1)	1.48 (0.72-3.04)	384	41 (10.7)
Mechanic	30	0 (0.0)	1.00 (NA)	73	4 (5.5)	0.64 (0.22-1.87)	385	32 (8.3)
Refuse disposal worker	30	0 (0.0)	1.00 (NA)	73	1 (1.4)	0.25 (0.03-1.91)	384	20 (5.2)
Construction worker	30	14 (46.7)	1.97 (0.93-4.17)	74	28 (37.8)	1.37 (0.82-2.30)	384	118 (30.7)
Exposure to contaminated sources*								
Contact with mud at work	27	10 (37.0)	0.90 (0.40-2.01)	64	29 (45.3)	1.26 (0.74-2.15)	381	151 (39.6)
Contact with trash at work	24	5 (20.8)	0.59 (0.22-1.62)	65	17 (26.2)	0.79 (0.44-1.44)	379	117 (30.9)
Contact with flood water at work during rainy season	26	9 (34.6)	0.84 (0.36-1.93)	67	26 (38.8)	1.01 (0.59-1.71)	380	147 (38.7)

Contact with sewer water at work	26	5 (19.2)	0.63 (0.23-1.72)	65	19 (29.2)	1.10 (0.61-1.96)	380	104 (27.4)
Use of boots or gloves during work**	30	6 (20.0)	0.97 (0.38-2.44)	70	14 (20.0)	0.97 (0.51-1.82)	384	79 (20.6)
Environmental Exposures								
Residential sanitary conditions								
Accumulation of trash <10m from home	43	36 (83.7)	1.18 (0.51-2.74)	98	80 (81.6)	1.02 (0.58-1.79)	446	363 (81.4)
Open sewer near home (<10m)	44	15 (34.1)	1.64 (0.85-3.18)	98	26 (26.5)	1.15 (0.70-1.89)	455	109 (24.0)
Street flood during rainy season	42	15 (35.7)	0.68 (0.35-1.31)	101	44 (43.6)	0.95 (0.62-1.46)	519	233 (44.9)
House flood during rainy season	27	5 (18.5)	0.96 (0.35-2.61)	71	17 (23.9)	1.32 (0.72-2.42)	364	70 (19.2)
Backyard flood during rainy season	6	2 (33.3)	0.90 (0.16-5.12)	27	11 (40.7)	1.23 (0.52-2.92)	109	39 (35.8)
Reservoirs								
Rats sighted near home	37	27 (73.0)	0.90 (0.42-1.91)	98	88 (89.8)	**2.93 (1.48-5.81)**	520	390 (75.0)
Number of rats observed near home	27	4.0 (2.0-6.0)	0.99 (0.90-1.09)	87	4.0 (2.0-6.0)	1.02 (0.98-1.07)	385	3.0 (2.0-5.0)
Dog as a domestic pet	44	18 (40.9)	0.94 (0.50-1.75)	105	58 (55.2)	**1.67 (1.10-2.55)**	525	223 (42.5)
Cat at residence	44	5 (11.4)	0.86 (0.33-2.26)	105	18 (17.1)	1.39 (0.79-2.45)	525	68 (13.0)
Chicken near residence	44	6 (13.6)	0.91 (0.37-2.21)	105	13 (12.4)	0.81 (0.43-1.52)	525	78 (14.9)

No., number; %, percentage; IQR, interquartile range; OR, odds ratio; CI, confidence interval. Bold values indicate statistical significance (p-val <0.05).

*Contact with contaminated source three weeks prior to hospitalization

**Use of boots or gloves when in contact with mud or trash or flood water or sewer water.

Table 4: Select characteristics to describe the clinical burden of leptospirosis among 1,316 suspected cases enrolled from the active hospital-based surveillance.

Characteristics	LPHS (n=113)		p-value# (LPHS v. Non-LPHS,NHPL)	NHPL (n=184)		p-value # (NHPL v. Non-LPHS, NHPL)	NPL (n=1019)	
	No. Responses	No. (%) or % of group or Median (IQR)		No. Responses	No. (%) or % of group or Median (IQR)		No. Responses	No. (%) or % of group or Median (IQR)
Clinical Presentation on admission								
Days of symptoms before hospital admission (days)	111	5.0 (4.0-7.0)	0.13	181	5.0 (4.0-7.0)	0.06	999	6.0 (4.0-8.0)
Fever	110	103 (93.6)	0.21	181	174 (96.1)	0.99	1005	966 (96.1)
Dyspnea	95	63 (66.3)	**<0.0001**	164	114 (69.5)	**<0.0001**	833	132 (15.6)
Oliguria (<400ml/24h)§	87	51 (58.6)	**<0.0001**	160	70 (43.8)	**<0.01**	852	263 (30.9)
Jaundice	111	88 (79.3)	0.29	183	147 (80.3)	0.33	1005	837 (83.3)
Hypotension (<90mm Hg)	106	17 (16.0)	**<0.01**	178	31 (17.4)	**<0.0001**	947	74 (7.8)
Laboratory examinations*								
Serum bilirubin (mg/dL)								
Total	70	8.1 (2.6-18.5)	0.76	103	10.0 (3.6-17.5)	0.96	577	10.1 (3.0-18.5)
Blood urea nitrogen (mg/dL)	101	103.0 (61.0-165.0)	0.62	170	114.0 (56.0-170.0)	0.30	867	100.0 (57.0-164.0)
Serum creatinine (mg/dL)	101	3.1 (1.7-5.5)	0.08	171	3.1 (1.6-5.1)	**0.05**	864	2.6 (1.5-4.7)
Serum potassium	91	3.7 (3.5-4.3)	**0.03**	151	3.7 (3.2-4.3)	0.94	771	3.7 (3.3-4.2)
<3.5 mmol/L	21	23.1%		57	37.8%		275	35.7%
3.5-4.9 mmol/L	57	62.6%		82	54.3%		450	58.4%
>4.9 mmol/L	13	14.3%		12	8.0%		46	6.0%
TGO (U/L)	107	126.0 (60.0-277.0)	**<0.001**	173	96.0 (60.0-184.0)	**0.02**	968	86.0 (52.0-148.0)
TGP (U/L)	105	61.0 (44.0-117.0)	0.39	168	68.0 (43.0-108.0)	0.20	953	60.0 (39.0-97.0)
Hematocrit (%)	95	30.0 (26.0-34.0)	**<0.0001**	162	33.5 (28.7-37.0)	**<0.001**	836	35.0 (31.1-39.1)
Platelet Count (cells/mm^3)	93	58000.0 (32000.0-106000.0)	**<0.0001**	159	67000.0 (36000.0-119000.0)	**<0.0001**	816	93000.0 (53000.0-160500.0)

Thrombocytopenia**	93	39 (41.9)	**<0.0001**	159	62 (39.0)	**<0.0001**	816	185 (22.7)
Complications during hospital stay								
Oliguria (<400ml/24h)	99	83 (83.8)	**<0.0001**	171	116 (67.8)	**<0.001**	886	474 (53.5)
Therapeutic Interventions								
Mechanical Ventilation	72	64 (88.9)	**<0.0001**	131	47 (35.9)	**<0.0001**	490	0 (0.0)
Dialysis (Peritoneal or Hemodialysis)	107	59 (55.1)	**<0.0001**	183	80 (43.7)	**<0.0001**	995	184 (18.5)
ICU admission	108	93 (86.1)	**<0.0001**	181	88 (48.6)	**<0.0001**	995	156 (15.7)
Packed erythrocyte transfusion	107	60 (56.1)	**<0.0001**	183	47 (25.7)	**<0.0001**	993	90 (9.1)
Hospital Outcome								
Death	109	74 (67.9)	**<0.001**	184	56 (30.4)	**<0.001**	1001	63 (6.3)
Days of Hospitalization for survivors	33	19.0 (11.0-28.0)	**<0.0001**	128	11.0 (7.0-16.0)	**<0.0001**	936	7.0 (5.0-10.0)
Days of Hospitalization for patients who died	73	3.0 (1.0-5.0)	0.83	54	2.0 (1.0-4.0)	0.60	63	2.0 (1.0-6.0)
Availability of Serum Samples								
All Paired serum samples	113	57 (50.4)	**<0.0001**	184	137 (74.5)	**<0.01**	1019	844 (82.8)
Paired Serum Samples!	113	24 (21.2)	**<0.001**	184	53 (28.8)	**0.02**	1019	388 (38.1)
Single serum sample	113	52 (46.0)	**<0.0001**	184	41 (22.3)	**0.04**	1019	164 (16.1)
No serum sample	113	4 (3.5)	**0.03**	184	6 (3.3)	**0.02**	1019	11 (1.1)
Confirmed Cases$	113	79 (69.9)	**<0.01**	184	134 (72.8)	**<0.01**	1019	836 (82.0)
Serovar Copenhageni as the presumptive infecting agent^	60	56 (93.3)	**0.03**	102	95 (93.1)	**0.03**	650	612 (94.2)
Serological test (MAT) Result								
First Acute sample titer	44	0.0 (0.0-150.0)	**<0.001**	95	0.0 (0.0-400.0)	**<0.001**	406	200.0 (0.0-1600.0)
Second Acute sample titer	23	1600.0 (0.0-6400.0)	0.11	68	3200.0 (800.0-6400.0)	0.98	297	3200.0 (800.0-6400.0)
Convalescent sample titer	19	6400.0 (800.0-6400.0)	0.36	64	6400.0 (1200.0-12800.0)	**0.02**	286	3200.0 (400.0-6400.0)

No., number; %, percentage; IQR, interquartile range. Bold values indicate statistical significance (p-val <0.05). The Chi-square test or Fisher's Exact test was used to evaluate significant differences for categorical data. The Wilcoxon-Rank Sum test was used to compare two groups for non-parametrically distributed continuous data.

#p-value shown is a pairwise comparison between LPHS and Non-LPHS, NHPL cases

##p-value shown is a pairwise comparison between NHPL and Non-LPHS, NHPL cases

§Oliguria within the first 24h upon hospital admission

*Values obtained on hospital admission

**Platelet count <50,000 cells/mm^3

$The MAT, IgM ELISA, real time PCR, and Culture Isolation of Leptospires were used for laboratory confirmation

^Proportions are shown for patients who had an MAT-confirmed diagnosis of leptospirosis and highest agglutination titers against Leptospirosis interrogans serovar Copenhageni

!Paired refers to either paired acute samples or paired first acute sample and convalescent sample

Supplementary Tables

S1. Mean annual incidence rate of leptospirosis in Salvador Brazil, according to age and sex.

LPHS

	All			Male			Female				
Age Group	No. Cases	2010 Census	Incidence	No. Cases	2010 Census	Incidence	No. Cases	2010 Census	Incidence	RR	95% CI
All ages	82	2,675,656	0.31	64	1,248,897	0.51	18	1,426,759	0.13	4.06	4.10-4.11
0-14	0	552,800	0.00	0	301,847	0.00	0	272,707	0.00	1.00	NA
15-29	33	753,900	0.44	25	461,889	0.54	8	392,495	0.20	2.66	2.65-2.66
30-44	18	679,327	0.26	16	271,382	0.59	2	365,530	0.05	10.78	10.73-10.83
45-59	19	441,983	0.43	12	177,211	0.68	7	243,870	0.29	2.36	2.35-2.37
60+	12	247,646	0.48	11	36,568	3.01	1	152,157	0.07	45.77	45.48-46.07

non-LPHS

	All			Male			Female				
Age Group	No. Cases	2010 Census	Incidence	No. Cases	2010 Census	Incidence	No. Cases	2010 Census	Incidence	RR	95% CI
All ages	917	2,675,656	3.43	781	1,248,897	6.25	136	1,426,759	0.95	6.56	6.62-6.63
0-14	40	552,800	0.72	34	301,847	1.13	6	272,707	0.22	5.12	5.11-5.13
15-29	316	753,900	4.19	290	461,889	6.28	26	392,495	0.66	9.48	9.47-9.49
30-44	295	679,327	4.34	253	271,382	9.32	42	365,530	1.15	8.11	8.11-8.12
45-59	206	441,983	4.66	164	177,211	9.25	42	243,870	1.72	5.37	5.37-5.38
60+	60	247,646	2.42	40	36,568	10.94	20	152,157	1.31	8.32	8.31-8.34

All Cases (LPHS and non-LPHS)

	All			Male			Female				
Age Group	No. Cases	2010 Census	Incidence	No. Cases	2010 Census	Incidence	No. Cases	2010 Census	Incidence	RR	95% CI
All ages	999	2,675,656	3.73	845	1,248,897	6.77	154	1,426,759	1.08	6.27	6.33-6.34
0-14	40	552,800	0.72	34	301,847	1.13	6	272,707	0.22	5.12	5.11-5.13
15-29	349	753,900	4.63	315	461,889	6.82	34	392,495	0.87	7.87	7.86-7.88
30-44	313	679,327	4.61	269	271,382	9.91	44	365,530	1.20	8.23	8.23-8.24
45-59	225	441,983	5.09	176	177,211	9.93	49	243,870	2.01	4.94	4.94-4.95
60+	72	247,646	2.91	51	36,568	13.95	21	152,157	1.38	10.11	10.09-10.12

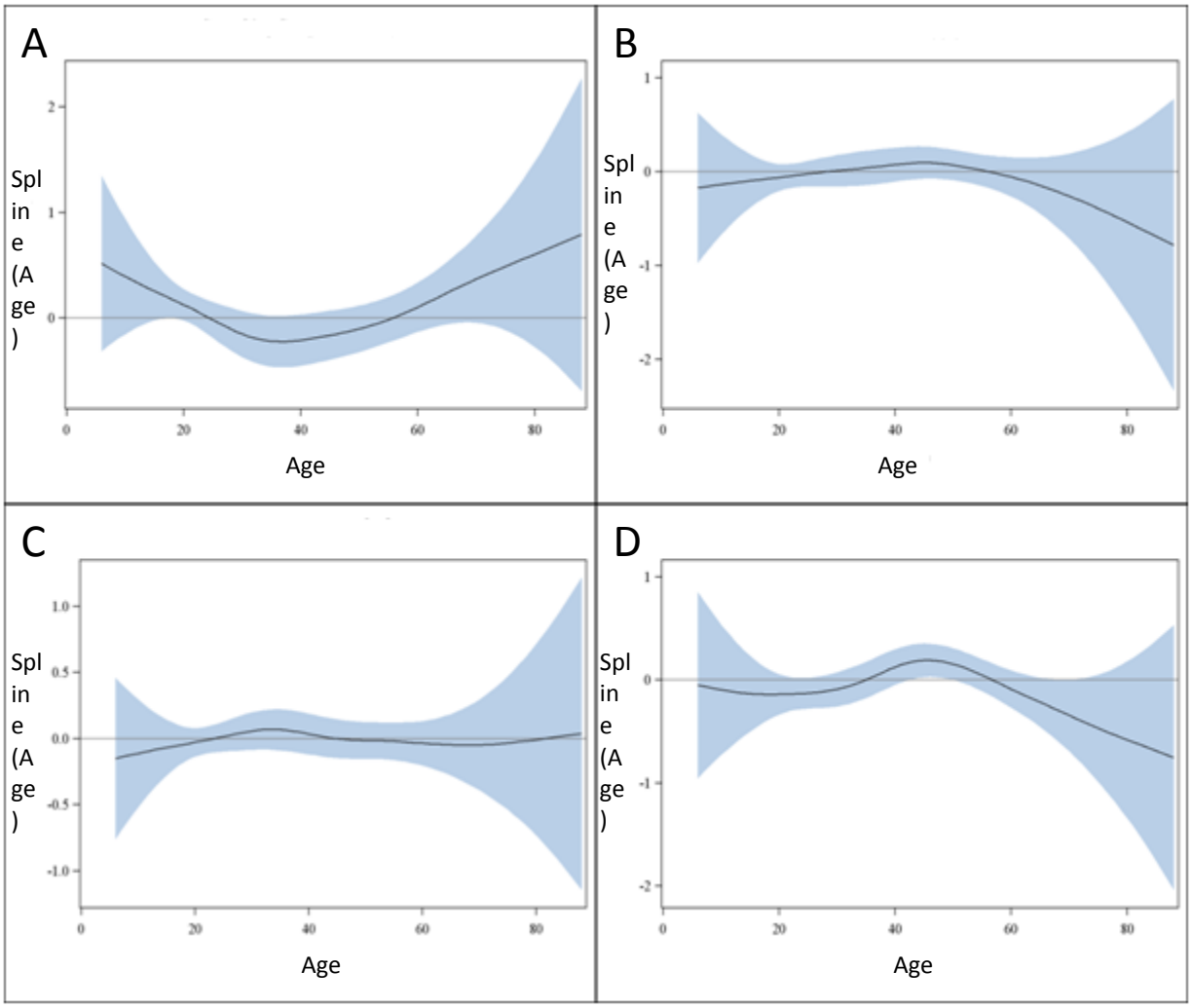
No., number; RR, relative risk; CI, confidence interval. Mean annual incidence is shown as per 100,000 persons. Indirect age standardization performed using the 2010 Population Census of Salvador-Bahia, Brazil from the Instituto Brasileiro de Geografia e Estatística (IBGE). Relative risk and 95% CI values calculated, using Poisson regression modeling for rates of leptospirosis in males compared to females.

S2. Case fatality rate and associated 95% confidence intervals for all suspected cases of LPHS, NHPL, and NPL patients.

Age Group	LPHS Deaths	Total No. LPHS	% CFR	95% CI	NHPL Deaths	Total No. NHPL	% CFR	95% CI	NPL Deaths	Total No. NPL	% CFR	95% CI
0-14	3	5	60.00	22.91-88.40	0	7	0.00	0.00-40.44	1	46	2.17	0.00-12.38
15-29	20	42	47.62	33.36-62.28	6	53	11.32	4.93-22.94	11	366	3.01	1.62-5.36
30-44	21	27	77.78	58.90-89.74	24	60	40.00	28.56-52.65	15	324	4.63	2.77-7.56
45-59	21	25	84.00	64.73-94.21	21	48	43.75	30.69-57.73	27	222	12.16	8.45-17.16
60+	9	14	64.29	38.60-83.82	5	16	31.25	13.91-55.85	9	61	14.75	7.73-25.95
Total	74	113	65.49	56.33-73.63	56	184	30.43	24.23-37.44	63	1019	6.18	4.85-7.84

No., number; CFR, case fatality ratio, CI, confidence interval. Wald method used to calculate the 95% CI for CFRs in GraphPad (http://graphpad.com/quickcalcs/ConfInterval1.cfm).

SF1: Generalized additive models (GAM) of the association between the acquisition of leptospirosis **A.** LPHS, **B.** NHPL, **C.** NPL and the continuous variable of age. **D.** Death and the continuous variable of age. Spline (Age) is the univariate smoothing term used to fit the non-linear relationship between age and outcome (type of leptospirosis, death). Shaded areas represent 95% confidence intervals.



S3. A summary of the serological status of all suspected cases of leptospirosis enrolled in the hospital-based active surveillance. Laboratory confirmation of patients with leptospirosis shown is defined as a positive MAT, IgM ELISA, real-time PCR, or culture isolation of leptospires. Probable cases include individuals who have a borderline positive IgM ELISA without seroconversion or a reciprocal MAT titer of 200 or 400 without seroconversion.

Disease Form (No. Suspected Cases)	Confirmed (n=1049) No. (%)*	Probable (n=17) No. (%)*	Unconfirmed (n=250) No. (%)*
All (1316)	1049 (79.7)	17 (1.3)	250 (19.0)
LPHS (113)	79 (69.9)	5 (4.4)	29 (25.7)
NHPL (184)	134 (72.8)	3 (1.6)	47 (25.5)
NPL (1019)	836 (82.0)	9 (0.9)	174 (17.1)

No., number; MAT, microagglutination test; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction. Among the unconfirmed, 21 out of 1,316 cases did not have a laboratory test performed.

*Row percent shown.

S4. Progression of pulmonary hemorrhage among 113 patients with leptospirosis associated pulmonary hemorrhage syndrome on day of hospital admission compared to developments during hospitalization.

	LPHS (N=113)			
	On Admission*		During Hospitalization**	
Characteristic	No. Responses	No. (%) or group %	No. Responses	No. (%) or group %
Pulmonary hemorrhage in first 24h	91	42 (46.2)	--	--
Any hemoptysis	95	39 (41.1)	108	107 (99.1)
Type of hemoptysis	39		107	
<300 mL blood	23	59.0%	3	2.8%
>300 mL blood	12	30.8%	22	20.6%
Blood in tracheal tube	1	2.6%	60	56.1%
Massive bleeding in endotracheal tube after extubation	3	7.7%	22	20.6%

No., number; LPHS, leptospirosis pulmonary hemorrhage syndrome.

*Presentation on admission or within the first 24hrs post-admission.

**Any point in time between the day of hospital admission and the day of discharge.

S5. A comparison of characteristics among laboratory confirmed versus laboratory unconfirmed patients.

Characteristics	All Suspected Cases (N=1316)		Lab Confirmed (N= 1049)		Not Lab Confirmed (N= 267)		
	No. Responses	**No. (%) or % of group or Median (IQR)**	**No. Responses**	**No. (%) or % of group or Median (IQR)**	**No. Responses**	**No. (%) or % of group or Median (IQR)**	**p-value#**
Demographics and epidemiological data							
Age (years)	1316	34.0 (24.0-47.0)	1049	33.0 (23.0-46.0)	267	38.0 (26.0-49.0)	**<0.01**
05-14	58	4.4%	44	4.2%	14	5.2%	
15-29	461	35.0%	392	37.4%	69	25.8%	
30-44	411	31.2%	321	30.6%	90	33.7%	
45-59	295	22.4%	223	21.3%	72	27.0%	
60+	91	6.9%	69	6.6%	22	8.2%	
Sex	1316		1049		267		**0.04**
Female	203	15.4%	151	14.4%	52	19.5%	
Male	1113	84.6%	898	85.6%	215	80.5%	
Ethnicity	1316		1049		267		
Black	352	26.8%	306	29.2%	46	17.2%	**<0.0001**
Other	964	73.3%	743	70.8%	221	82.8%	
Clinical Presentation on admission							
Days of symptoms before hospital admission (days)	1291	6.0 (4.0-8.0)	1036	6.0 (5.0-8.0)	255	5.0 (3.0-8.0)	**0.001**
Fever	1296	1243 (95.9)	1038	1003 (96.6)	258	240 (93.0)	**0.01**
Jaundice	1299	1072 (82.5)	1044	888 (85.1)	255	184 (72.2)	**<0.0001**
Hypotension (<90mm Hg)	1231	122 (9.9)	988	83 (8.4)	243	39 (16.1)	**<0.001**
Cough	553	190 (34.4)	443	159 (35.9)	110	31 (28.2)	0.13
Dyspnea	1092	309 (28.3)	891	239 (26.8)	201	70 (34.8)	**0.02**
Laboratory examinations*							

Serum bilirubin (mg/dL)							
Total	750	10.0 (3.1-18.1)	614	11.1 (4.4-19.0)	136	3.7 (1.2-12.1)	**<0.0001**
Direct	836	7.8 (2.1-14.2)	678	8.9 (3.2-15.0)	158	2.5 (0.6-10.2)	**<0.0001**
Blood urea nitrogen (mg/dL)	1138	102.5 (57.0-165.0)	927	106.0 (62.0-164.0)	211	76.0 (35.0-172.0)	**<0.001**
Serum creatinine (mg/dL)	1136	2.8 (1.5-4.8)	924	2.9 (1.6-5.0)	212	2.2 (1.2-4.4)	**0.001**
Serum potassium	1013	3.7 (3.3-4.2)	835	3.6 (3.2-4.1)	178	4.0 (3.5-4.6)	**<0.0001**
<3.0 mmol/L	100	2.7 (2.6-2.8)	87	2.7 (2.6-2.8)	13	2.5 (2.2-2.7)	
3.0-3.49 mmol/L	253	3.2 (3.1-3.3)	228	3.2 (3.1-3.3)	25	3.2 (3.1-3.3)	
3.5-5.0 mmol/L	605	3.9 (3.7-4.3)	487	3.9 (3.6-4.3)	118	4.1 (3.8-4.5)	
>5.0 mmol/L	55	5.5 (5.2-5.9)	33	5.4 (5.1-5.9)	22	5.6 (5.2-5.9)	
TGO (U/L)	1248	90.0 (52.0-160.0)	1003	89.0 (54.0-151.0)	245	90.0 (42.0-190.0)	0.80
TGP (U/L)	1226	60.5 (40.0-100.0)	981	60.0 (41.0-93.0)	245	68.0 (38.0-144.0)	**0.04**
Hematocrit (%)	1093	34.5 (30.3-38.8)	893	34.1 (30.3-38.0)	200	36.0 (30.6-42.0)	**<0.001**
Platelet Count (cells/mm^3)	1068	85500.0 (47000.0-151000.0)	881	80000.0 (47000.0-137000.0)	187	149000.0 (55000.0-229000.0)	**<0.0001**
Thrombocytopenia**	1068	286 (26.8)	872	240 (27.5)	196	46 (23.5)	0.25
Platelet Count <50,000 cells/mm^3	286	33000.0 (24000.0-42000.0)	240	33000.0 (25000.0-42000.0)	46	27000.0 (20000.0-39000.0)	**0.02**
Platelet Count <15,000 cells.mm^3	16	11750.0 (11000.0-13500.0)	11	11500.0 (11000.0-14000.0)	5	13000.0 (11400.0-13000.0)	0.65
Complications during hospital stay							
Oliguria (<400ml/24h)	1156	673 (58.2)	938	550 (58.6)	218	123 (56.4)	0.55
Mechanical Ventilation	693	111 (16)	548	73 (13.3)	145	38 (26.2)	**<0.001**
LPHS	1316	113 (8.6)	1049	79 (7.5)	267	34 (12.7)	**0.01**
NHPL without mechanical ventilation	1316	186 (14.1)	1049	140 (13.4)	267	46 (17.2)	0.10
NHPL without LPHS	1316	184 (14)	1049	134 (12.8)	267	50 (18.7)	**0.01**
NPL	1316	1019 (77.4)	1049	836 (79.7)	267	183 (68.5)	**<0.0001**
Hospital Outcome							
Death	1294	193 (14.9)	1036	104 (10.0)	258	89 (34.5)	**<0.0001**
Days of Hospitalization for	1097	8.0 (6.0-11.0)	929	8.0 (6.0-11.0)	168	7.0 (5.0-12.0)	0.11

survivors							
Days of Hospitalization for patients who died	190	2.0 (1.0-5.0)	101	3.0 (1.0-7.0)	89	1.0 (1.0-3.0)	**<0.01**
Availability of Serum Samples							
Paired serum samples	1316	1038 (78.9)	1049	894 (85.2)	267	144 (53.9)	**<0.0001**
Single serum sample	1316	257 (19.5)	1049	155 (14.8)	267	102 (38.2)	**<0.0001**
No serum sample*	1316	21 (1.6)	1049	0 (0.0)	267	21 (7.9)	**<0.0001**

No., number; %, percentage; IQR, interquartile range; OR, odds ratio; CI, confidence interval. Bold values indicate statistical significance (p-val <0.05). The Chi-square test or Fisher's Exact test was used to evaluate significant differences for categorical data.

The Wilcoxon-Rank Sum test was used to compare two groups for non-parametrically distributed continuous data.

#p-value shown is a pairwise comparison between individuals with laboratory confirmation and no laboratory confirmation for leptospirosis.

*Values obtained on hospital admission

**Platelet count <50,000 cells/mm^3