**Building prognostic models for adverse outcomes in a prospective cohort of hospitalised patients with acute leptospirosis infection in the Philippines**

Running title: Leptospirosis in the Philippines

Nathaniel Leea\*, Emi Kitashojib, Nobuo Koizumic, Talitha Lea V. Lacuestad, Maricel R. Ribod, Efren M. Dimaanod, Nobuo Saitob, Motoi Suzukib, Koya Ariyoshib,e, Christopher M. Parrye,f

a London School of Hygiene and Tropical Medicine, London, UK

b Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

c National Institute of Infectious Diseases, Toyama, Shinjuku-ku, Tokyo, Japan

d San Lazaro Hospital, Manila, Philippines

e School of Tropical Medicine and Global Health, Nagasaki University, Japan

f Liverpool School of Tropical Medicine, Liverpool, UK

Corresponding author: Nathaniel Lee; Tel +447771888958; E-mail Nathaniel.lee@doctors.org.uk

Word Count

Abstract: 204

Text: 3,325

**Abstract**

Leptospirosis is endemic to the Philippines. 10% of cases will develop severe or fatal disease. Predicting progression to severity is difficult. Risk factors have been suggested, but few attempts have been made to create predictive models to guide clinical decisions. We present two models to predict the risk of mortality and progression to severe disease.

Data was used from a prospective cohort study conducted between 2011-2013 in San Lazaro Hospital, Manila. Predictive factors were identified from a literature review.  A strategy utilizing backwards stepwise-elimination and multivariate fractional polynomials identified key predictive factors.

203 patients met the inclusion criteria. The overall mortality rate was 6.84%. Multivariable logistic regression revealed that neutrophil counts [OR 1.38, 95% CI 1.15 -1.67] and platelet counts [OR 0.99, 95% CI 0.97 – 0.99] were predictive for risk of mortality. Multivariable logistic regression revealed that male sex (OR 3.29, 95% CI 1.22 – 12.57) and number of days between symptom onset and antibiotic use (OR 1.28, 95% CI 1.08 - 1.53) were predictive for risk of progression to severe disease.

The multivariable prognostic models for the risks of mortality and progression to severe disease developed could be useful in guiding clinical management by the early identification of patients at risk of adverse outcomes.

**Keywords**

Leptospirosis, risk factors, prognostic models, severity score

**Introduction**

Human leptospirosis is a zoonotic infection caused by the obligate aerobic spirochete bacteria of the genus *Leptospira*. 1 The disease was first characterised by Adolf Weil in 1886. Leptospirosis has a global distribution, and is thought to be the most widespread zoonosis in the world.2 The principle routes of transmission are through pre-existing abrasions on the skin, by contact with mucous membranes and following prolonged submersion in contaminated water.3 Rodent species are the most widely-reported maintenance hosts and reservoirs, but domestic and agricultural animals have also been implicated.4 The interrelationships between environmental factors, human behaviour, and animal reservoirs defines the pattern of human leptospirosis disease.5 Human infections can be acquired through occupational, recreational and avocational exposure.3,4

The Philippines is a lower-middle-income country with a mixed private-public health system, where leptospirosis is endemic. Case Fatality Rates (CFR) between 6% and 43% have been reported and vary depending on presentation, season, outbreak status and hospital location. 6 At San Lazaro Hospital (SLH), the National Infectious Diseases tertiary referral centre based in Manila, a 2009 outbreak following two successive typhoons resulted 471 patients hospitalized and a CFR of 10.8% in patients with confirmed leptospirosis.7

Clinical presentations of leptospirosis range from mild flu-like symptoms to life-threatening illness requiring intensive treatment unit (ITU) admission with mechanical ventilation and haemodialysis.3 The initial non-specific presentation is similar to other acute febrile syndromes, making clinical diagnosis difficult and possibly delaying appropriate treatment. More than 90% of infections will exhibit a mild anicteric disease, with the remaining developing severe icteric disease.4 In the absence of a reliable reference diagnostic test, predictive models may aid in management of leptospirosis and could be of particular value in low-resource settings.

The aim of this study was to establish simple models, using an evidence-based predictor selection, that would predict the risk of progression to severe disease or mortality in patients presenting to health care providers with leptospirosis. Such models could guide treatment choice, reduce mortality rates, and improve the effective allocation of scarce resources.

**Materials and Methods**

Patient Selection

The study used data previously collected in a prospective cohort study designed to examine the diagnostic accuracy of a recombinant immunoglobulin-like protein A-based IgM (LigA) ELISA for the early diagnosis of leptospirosis.8 The cohort case definition included patients with an acute admission to SLH between 2011-2013 who were clinically suspected to have leptospirosis based on 1) presence of fever plus at least two other signs and symptoms of leptospirosis (headache, myalgia, conjunctival suffusion, jaundice, tea-coloured urine, oliguria, anuria, or unusual bleeding) and 2) history of exposure to floodwaters or animals. 6–8 Only patients with laboratory confirmed leptospirosis were considered in this analysis. The laboratory confirmation criteria is a modified criteria presented in Kitashoji *et al*8 and includes 1) If Leptospira cultures were positive, or 2) If specific antibodies were detected with seroconversion or at least a 4-folds increase in reciprocal MAT titer between paired samples or with a reciprocal MAT titer of > = 400 in at least 1 plasma sample, or 3) A positive Patoc or LigA ELISA tests. Primary data collection was performed and provided by investigators based at SLH in conjunction with Nagasaki University.8 All patients gave written informed consent before participation in the study. Sample size calculations were linked to Kitashoji *et al*’s study, which were calculated to meet adequate significance and power calculations for a case-control study based on 120 laboratory confirmed cases and 100 health controls.8 The STROBE checklist was used to ensure adequate and meaningful reporting.

Selection of Risk Factors

A literature search for prognostic factors for leptospirosis infection was conducted. The following strategy was employed: *human leptospir$ or Explode Mh leptospirosis*, *risk$ factor$ or clinical outcome$ or prognos$*, *mortality or death*, and *sever$ or sever$ disease or sever$ clinic$ outcome$*. Detail of the search strategy is given in **Supplemental 1**. Criteria for inclusion in the summary estimates were: laboratory-confirmed or strong clinical suspicion (defined by study) of leptospirosis, and primary outcomes of mortality or severe disease (defined by study). 17 studies were identified and analysed. The following is a summary of recognized predictive risk factors.

* **Associated with mortality** - Elderly age, oliguria, thrombocytopaenia, elevated creatinine, pulmonary infiltrates on x-ray, altered mental status, dyspnoea, delay in antibiotic initiation, AST/ALT ratio, elevated white blood cell count, electrocardiogram abnormalities, haemodynamic compromise, and hyperkalaemia.
* **Associated with severe disease** - cigarette smoking, delay in antibiotic initiation, infecting serovar, thrombocytopaenia, elevated creatinine, elevated lactate, elevated amylase, elevated AST, leptospiraemia, haemodynamic compromise, reduced consciousness, dyspnoea, hypokalaemia, jaundice, and oliguria.

Data Collection

Demographic data collected on patients reflected previously reported risk factors. 8–12Patients were followed up in-hospital as far as discharge. Data on primary outcomes collected included mortality during admission, and the development of severe disease. Severe disease was modified from Kitashoji *et al* and defined as Acute Kidney Injury according to RIFLE (Risk, Injury, Failure, Loss of kidney function, End-stage kidney disease) criteria or the need for dialysis 6, or evidence of clinically-recognized pulmonary haemorrhage, or liver dysfunction (2.5x the upper limit of AST and ALT in IU/L, or presenting with jaundice).

Patient demographics were collected and divided into those prior and subsequent to admission. Risk factors prior to admission included age, sex, geographic location, occupation, body mass index (BMI, kg/m2), smoking history, the number of days between onset of symptoms and first antibiotic treatment, referral from primary health post to current hospital admission, the use of any antibiotics in community prior to admission, and the presence of skin abrasion.

Risk factors related to the period of hospital admission included symptoms and signs on admission (Pyrexia [>38 °C], headache, cough, dyspnoea, haemoptysis, jaundice, calf pain, conjunctival suffusion [defined as eye redness without exudates6]), oliguria (<500 mL per day) or anuria (<100 mL per day), blood pressure (mmHg), heart rate (beats/min), respiratory rate (breath/min), complete blood counts, chest X-ray findings, initial antibiotic choice, corticosteroid use, blood transfusions, intravenous fluid support, and catecholamine use.

*Missing Data*

A strategy of multiple imputation was employed to manage missing data.13,14 Variables chosen for imputation were *Missing at Random* or *Missing Completely At Random*, and were those to be included in the final multivariable model along with auxiliary variables which were either predictive of the pattern of missingness or correlated with variables used in the analysis. Multiple imputations by chained equations and predictive mean matching was performed as appropriate.14 A total of 4,060 iterations were generated over 20 imputation sets. Twenty imputation cycles were chosen based on the size of the dataset, on the maximum proportion of missingness seen, and because increasing the number of imputed data sets improves power.

Statistical Analysis

The data from the prospective study was recorded on a case report form and then entered into a password-protected Excel spreadsheet (Microsoft Corporation). This was imported to and analysed in Stata 13 (StataCorp LP). Risk factors and outcomes were described using summary statistics and reported to two decimal places. All primary outcome variables were categorical.

In the univariable analysis categorical dependent variables were analysed by univariable logistic regression, or a χ2/Fisher’s exact test where appropriate. All tests were reported with respective summary statistics and 95% confidence interval. P-values were reported to three decimal places in data tables, and were assumed to be at most p≤0.05 when described in the manuscript as significant.

In the multivariable analysis, candidate variables considered for inclusion in the multivariable analysis were selected for their predictive performance.15,16 To satisfy temporal requirements in building predictive models, candidate variables representing only risk factors prior to hospital admission were chosen to model the probability of severe disease. To model mortality, the same risk factors were included, as well as all variables measured after hospital admission. Candidate predictors were not barred from inclusion in multivariable analysis based on non-significance in univariable analysis, and our study employed an evidence-based predictor selection strategy.15,16 Predictor variables identified from our literature search were included, provided similar variables were available in our dataset. The predictor-selection strategy was modified to employ multivariate fractional polynomial (MFP) analysis to avoid dichotomizing continuous variables.17 Inclusion and exclusion criteria for stepwise elimination were tightly set.15 The P-value-to-include was p=0.05, and the P-value-to-exclude was p=0.055.

Multivariable logistic regression for both primary outcome variables was chosen as the post-MFP regressive method. Coefficients were reported as calculated to improve model accuracy. Estimates were rounded to two decimal places, with significance levels rounded to three decimal places. Post-regression analysis was conducted for model calibration and discrimination.15 Multiple Receiver Operating Characteristics (ROC) curves based on each imputed dataset were generated using a modified code incorporating Rubin’s rules, and the averaged area under the ROC (AUROC) and pseudo-R2 were calculated from these. An additional analysis of calibration was to bootstrap the original data set by 100 samples and analyse the resulting AUROCs for agreement with the first method, as well as establish variable selection patterns and overall model stability. An optimal threshold marker (Youden’s J statistic) was calculated in order to determine the optimal cutoff point for probability. A method for performing this on imputed data has not been described, and therefore analysis was manually performed on the original dataset and superimposed on the final model.

Ethics statement.

The study received ethical approval from the London School of Hygiene and Tropical Medicine (Reference 9316), and under the ethical approval granted for the Kitashoji et al study from the Institute of Tropical Medicine Nagasaki University and, locally, by the Ethics Committee of San Lazaro Hospital.

## Results

Data on 349 patients with suspected leptospirosis was collected between October to December 2011, September to October 2012, and August to September 2013. There were 203 patients (**Figure 1**) included in the study (mean age 30.64, 95%CI 29.78 – 32.49, range 7 – 64 years), with 13 inpatient deaths (CFR 6.84%). There were 14 women (7%) and 189 men (93%). The majority of participants were unemployed (33%) followed by occupations involving exterior manual labour (22%), interior manual labour (11%), and non-manual labour (9%). 25% of participants had an unknown employment status.

There were 145 (71%) patients with a severe complication reported. 91 (45%) had a prolonged hospital stay, with a mean of 6.33 (95% CI 5.97 – 6.70) days. The mean total durations of illness was 10.91 days (95%CI 10.41 – 11.43). The mean total number of days before presenting to hospital was 4.92 (95%CI 4.25 – 4.90). **Tables 1 and 2** summarize the univariable analysis for factors assessed in the final predictive models.

Five binary (male sex, dyspnoea, and anuria, haemoptysis, and jaundice) and eleven continuous (creatinine, ALT, AST, age, days between onset of symptoms and antibiotic use, WBC count, neutrophil count, platelet count, serum potassium, and systolic/diastolic blood pressure) variables were included in the multivariable analysis for risk of mortality. Two variables were selected as significant in the model (**Table 3**): platelet and neutrophil counts. Given that: ***Pi = 1/(1+e^-(β + α1X1p1 +…αmXm pm)*** Where P is the probability of outcome ***I***, ***e*** is the natural log, ***β*** is the estimated constant regression coefficient, ***α*** is the estimated regression coefficient of explanatory variable ***m***, ***p*** is the fractional power, ***X*** is the value of explanatory variable ***m***. The formula for the probability of mortality is as follows: **Pr (Mortality) = 1/(1+e^-(-4.427654 + (0.3243045 *× (Neutrophil – 8.8269576)1) + (-0.0130227 × (Platelet -155.9719212)1))))*** where “Neutrophil” and “Platelet” are given as 109 cells/L. A further breakdown is given on **Table 3**, with predictive curves illustrated in **Figure 2**. The AUROC was 0.85 (**Figure 3A**), with a pseudo-R2 of 0.22. Analysis by bootstrapping showed average repeat selection in agreement with AUROC and model stability, with a variable selection rates of 82% for Platelet and 97% for Neutrophil. **Figure 3B** showed the sensitivity/specificity curve from which the Youden’s J-statistic was derived, calculated as 0.09 using the original dataset (n=189).

Three binary (male sex, occupation involving exterior work, and use of antibiotics in the community prior to admission) and three continuous (age, days between onset of symptoms and antibiotic use, and BMI) variables were included in the analysis of risk to progression to severe disease. Two predictive variables were selected as significant in the multivariable model : male sex and days between antibiotics and admission. The formula structure for probability of progression to severe disease was as for the mortality model (see above), and the model is as follows - ***Pr(Severe leptospirosis) = 1/(1+e^-(-0.2745557 + (1.366016 × Male sex) + (0.2505996 × (Days between onset and antibiotics – 4.555665025)1)))*** where “Days between onset and antibiotics” is given as number of whole days, and “Male sex” is given the value 1 for patient who are male and 0 for patients who are not. A further breakdown is given on **Table 4** with a predictive curve illustrated in **Figure 4**. The AUROC was 0.67 (**Figure 5A**), with a pseudo-R2 of 0.06. Analysis by bootstrapping showed agreement with AUROC and model stability. Sensitivity/specificity plots (**Figure 5B**) allowed for calculation of a J-statistic of 0.75 (original dataset, n=189).

**Discussion**

Several prognostic clinical factors were identified that could be used to predict mortality and development of severe disease in human leptospirosis. The cohort showed a sex distribution typical of human leptospirosis, with men being more affected than women.10,12,18 There was a higher proportion of men between the ages of 20-40 years primarily affected compared to other age groups: 53% compared to 22% (0-19), 21% (40-59) and 8% (60+). There was also a similar pattern amongst women who were young adults.

Risk of Mortality

*Neutrophilia*

A predictor of mortality for leptospirosis infection in our model was changes in neutrophil counts. Biomarkers for inflammation such as neutrophils are useful not only for documenting presence of leptospires infection, but differentiating between severe and uncomplicated disease. Dupont *et al* report a 2.5 OR for death (95%CI 2.8-48.5) for WBCs over 12,900/mm3. 9 Amilasan *et al* report 2.1 RR (95% CI 1.05 – 4.17) for death in neutrophil counts over 12 x 109 cells/L.7 One aspect of the trend of neutrophilia relates to its association with the diseases’ biphasic nature and severe manifestations. Neutrophil counts spike and then decline in the first week of leptospirosis infection, before climbing in the second week.19 This is consistent with a second phase of illness, when complications are known to occur.3 In patients with severe disease, neutrophil counts are significantly higher in the first phase of illness.19 These differences disappear during the second phase of illness, when neutrophil counts in mild disease match those of severe disease.

Host immune response to pathogen factors may be associated with mortality.1,4 *Craig* et al reported significant differences between neutrophil counts in patients presenting with 11 different leptospires serovars.20 This supports findings of differences in pathogenicity between the various serovars.21,22

#### Platelet Count

Low platelet counts have been linked with leptospirosis.4 The mechanism leading to an increased mortality risk is thought to be the exacerbation by a thrombocytopaenia of an existing haemorrhagic state often present in disease. Spichler *et al* report an 2.2 OR (95% CI 1.2 - 4.7) against mortality for platelet counts <70,000.23 At SLH in the Philippines, Amilasan *et al* reports in a univariable analysis the significant association of thrombocytopaenia of <50 x 103 cells/L with death.7 Conversely, Daher *et al* reported no difference in mortality amongst patients admitted with leptospirosis and a thrombocytopaenia (defined as <100,000/mm3).24 It is likely that variable dichotomization contributed to the varied results, causing a loss in power to detect changes.

The mechanism linking thrombocytopaenia and mortality is unclear. Thrombocytopaenic presentations are common, but usually not associated with spontaneous haemorrhage.3,4 Platelet counts may exhibit indirect effects on the severity of complications such as AKI 24 and leptospirosis-associated severe pulmonary haemorrhage syndrome, thereby increasing mortality risk.25

### Progression to Severe Disease

#### Time Between Onset and Antibiotic Use

A prognostic indicator previously reported was the timing of patients receiving definitive antibiotic therapy.7,22 Tubiana *et al* reports severe disease to be associated with a delay >2 days between onset of symptoms and initiating antibiotic therapy [OR 2.78, 85% CI 1.31 – 5.91].26 Another study describes severe disease to be associated with > 10 days of illness before antibiotic therapy [OR 4.8, 95% CI 1.1-20.2].22 A similar association with mortality has been previously noted.6 A recent Cochrane Review showed that the choice of antibiotic, including placebo, did not significantly affect leptospirosis mortality. 27 The Review included trials that had attempted to risk-stratify by disease severity. Overall there was insufficient evidence to recommend an optimal timing for antibiotic delivery.27

#### Male Sex

The association between male sex and leptospirosis infection has been documented previously.4,18 Interpretation of demographic factors such as sex and gender must be seen through a cultural and sociological lens. Such an analysis is outside the scope of this paper. Relevant to our study are the following considerations: gender-associated norms (high risk behaviour and occupational exposure), biological differences between sexes, and differences in health seeking behaviour.28

Gender-associated norms play a significant role. In the Philippines, a sero-epidemiological study between 1998 and 2001 revealed 87% of suspected seropositive cases were male, of whom 72% were outdoor workers (stall keepers, farmers, construction, etc).29 This reflects the occupational breakdown of our own dataset.

Biological factors may contribute to the association between males and severity. Jansen *et al* report that males were more likely to be hospitalized (OR 2.6, p<0.01) and exhibit symptoms consistent with icteric disease such as AKI (ORMH 3.4, 95% CI 1.7 – 6.5) and haemorrhage (ORMH 7.8, 95% CI 1.03 – 60.0) even after controlling for exposure risks, infecting serovar, and health-seeking behaviour. 30 Whether force of infection or duration of exposure are factors remains unknown.

Health-seeking behaviour by sex in our sample does not appear to be different. The mean number of days between onset of symptoms and admission to hospital are not significantly different between men and women (data not reported). In the context of Filipino society, health-seeking behaviour is a complex field that our dataset cannot address.

Limitations and Bias

A limitation in our dataset is the lack of chronological follow-up, which is addressed by separating predictive factors into “before” and “after” hospital admission. Additionally, the accuracy of our prognostic model is limited to the accuracy of current diagnostic tests.

The risk of inter-personnel sampling bias and measurement error was minimized by allocating one person to perform the patient selection and data collection. The population from which our sample is drawn is reflective of those typically seen at SLH who are at risk of leptospirosis infection. As a tertiary infectious diseases referral centre, SLH receives cases from its immediate surroundings as well as other regions nationally. Our population sample included those in at-risk occupations typical of leptospirosis endemic regions (**Table 1**).4,10,18 The number of cases in our sample who were unemployed reflected the avocational exposure risks commonly seen in these groups.4 Recall bias may have affected responses with respect to duration of illness, but this was addressed by the prospective design for the collection of the primary data set.

Patients who were lost to follow up were transferred for haemodialysis, which is not available at SLH. The fact that they would have been classified as “Severe”, or possibly have died, represents a potential bias due to their exclusion. However, as these exclusions were small (n=2, **Figure 1**), attrition bias was minimized.

When building our prognostic models, all potential confounders were inserted into the variable selection procedure. Adjusting for confounding is not as vital as when building aetiological models.16 The strength of association of predictive factors selected by the model was not based on cause of disease, but the risk of the outcome.

**Conclusions**

This study generated easy and intuitive prognostic models that can be used to calculate risk probability of mortality and progression to severe disease. The equations formulated can be integrated into a risk calculator, perhaps using online or mobile application platforms, to facilitate computation. The resulting probability can be used as an adjunct to guide clinical decision-making. In order of descending significance, the predictive factors for mortality were neutrophil and platelet counts, and the predictive factors for progression to severe disease were male sex and number of days between symptom onset and antibiotic use.

**References**

1 Evangelista, KV and Coburn, Jenifer (2010) ‘Leptospira as an emerging pathogen: a review of its biology, pathogenesis and host immune responses’. *Future microbiology*, 5(9), pp. 1413–1425. [online] Available from: http://www.futuremedicine.com/doi/abs/10.2217/fmb.10.102 (Accessed 21 December 2014)

2 Haake, David A. and Levett, Paul N (2015) ‘Leptospirosis in Humans’ Adler, B. (ed.). *Current topics in microbiology and immunology*, 387, pp. 65–97. [online] Available from: http://link.springer.com/10.1007/978-3-662-45059-8

3 Haake, David A and Levett, Paul N (2015) ‘Leptospirosis in Humans’. *Current topics in microbiology and immunology*, 387, pp. 65–97. [online] Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4442676/

4 Levett, P N (2001) ‘Leptospirosis.’ *Clinical microbiology reviews*, 14(2), pp. 296–326. [online] Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=88975&tool=pmcentrez&rendertype=abstract

5 de Vries, Sophia G, Visser, Benjamin J, Nagel, Ingeborg M, Goris, Marga G a, et al. (2014) ‘Leptospirosis in Sub-Saharan Africa: a systematic review.’ *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases*, 28C, pp. 47–64. [online] Available from: http://www.ncbi.nlm.nih.gov/pubmed/25197035 (Accessed 15 October 2014)

6 Mendoza, Myrna T, Roxas, Evalyn A, Ginete, Joanne Kathleene, Alejandria, Marrisa M, et al. (2013) ‘Clinical profile of patients diagnosed with leptospirosis after a typhoon: A multicenter study’. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 44(6), pp. 1021–1035. [online] Available from: http://www.tm.mahidol.ac.th/seameo/2013-44-6-full/10-5469-3.pdf (Accessed 27 December 2014)

7 Amilasan, Al-shere T., Ujiie, Mugen, Suzuki, Motoi, Salva, Eumelia, et al. (2012) ‘The Outbreak of Leptospirosis after Flood’. *Emerging Infectious Diseases*, 18(1), pp. 91–94.

8 Kitashoji, Emi, Koizumi, Nobuo, Lacuesta, Talitha Lea V., Usuda, Daisuke, et al. (2015) ‘Diagnostic Accuracy of Recombinant Immunoglobulin-like Protein A-Based IgM ELISA for the Early Diagnosis of Leptospirosis in the Philippines’. *PLOS Neglected Tropical Diseases*, 9(6), p. e0003879. [online] Available from: http://dx.plos.org/10.1371/journal.pntd.0003879

9 Dupont, H, Dupont-Perdrizet, D, Perie, J L, Zehner-Hansen, S, et al. (1997) ‘Leptospirosis: prognostic factors associated with mortality.’ *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 25, pp. 720–724.

10 WHO (2003) ‘Human leptospirosis: guidance for diagnosis, surveillance and control’. [online] Available from: http://apps.who.int/iris/handle/10665/42667 (Accessed 21 December 2014)

11 Segura, Eddy R, Ganoza, Christian a, Campos, Kalina, Ricaldi, Jessica N, et al. (2005) ‘Clinical spectrum of pulmonary involvement in leptospirosis in a region of endemicity, with quantification of leptospiral burden.’ *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 40, pp. 343–351.

12 McBride, Alan Ja, Athanazio, Daniel a, Reis, Mitermayer G and Ko, Albert I (2005) ‘Leptospirosis’. *Current Opinion in Infectious Diseases*, 18(5), pp. 376–386. [online] Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00001432-200510000-00003

13 Donders, a. Rogier T, van der Heijden, Geert J M G, Stijnen, Theo and Moons, Karel G M (2006) ‘Review: A gentle introduction to imputation of missing values’. *Journal of Clinical Epidemiology*, 59(10), pp. 1087–1091.

14 Azur, Melissa J, Stuart, Elizabeth a, Frangakis, Constantine and Leaf, Philip J (2012) ‘Multiple Imputation by Chained Equations: What is it and how does it work?’ *International Journal of Methods in Psychiatric Research*, 20(1), pp. 40–49.

15 Moons, K. G. M., Kengne, a. P., Woodward, M., Royston, P., et al. (2012) ‘Risk prediction models: I. Development, internal validation, and assessing the incremental value of a new (bio)marker’. *Heart*, 98(9), pp. 683–690.

16 Shmueli, Galit (2011) ‘To Explain or to Predict?’ , 25(3), pp. 289–310. [online] Available from: http://arxiv.org/abs/1101.0891

17 Sauerbrei, W., Meier-Hirmer, C., Benner, a. and Royston, P. (2006) ‘Multivariable regression model building by using fractional polynomials: Description of SAS, STATA and R programs’. *Computational Statistics and Data Analysis*, 50(12), pp. 3464–3485.

18 Galloway, R, Guerra, M and Shadomy, S (2015) ‘Control of Communicable Diseases Manual’, in Heymann, D. L. (ed.), Washington D.C., American Public Health Association, pp. 348–353.

19 De Silva, Nipun, Niloofa, Mjr, Fernando, Narmada, Karunanayake, Lilani, et al. (2014) ‘Changes in full blood count parameters in leptospirosis: a prospective study’. *International Archives of Medicine*, 7(1), p. 31. [online] Available from: http://www.intarchmed.com/content/7/1/31

20 Craig, S B, Graham, G C, Burns, M.-A., Dohnt, M F, et al. (2009) ‘Haematological and clinical-chemistry markers in patients presenting with leptospirosis: a comparison of the findings from uncomplicated cases with those seen in the severe disease’. *Annals of Tropical Medicine & Parasitology*, 103(4), pp. 333–341. [online] Available from: http://dx.doi.org/10.1179/136485909X435058

21 Taylor, Andrew J., Paris, Daniel H. and Newton, Paul N. (2015) ‘A Systematic Review of the Mortality from Untreated Leptospirosis’. *PLOS Neglected Tropical Diseases*, 9(6), p. e0003866. [online] Available from: http://dx.plos.org/10.1371/journal.pntd.0003866

22 Herrmann-Storck, Cécile, Saint Louis, Magalie, Foucand, Tania, Lamaury, Isabelle, et al. (2010) ‘Severe leptospirosis in hospitalized patients, Guadeloupe’. *Emerging Infectious Diseases*, 16(2), pp. 331–334.

23 Spichler, Anne S., Vilaça, Pedro J., Athanazio, Daniel a., Albuquerque, J. O M, et al. (2008) ‘Predictors of lethality in severe leptospirosis in urban Brazil’. *American Journal of Tropical Medicine and Hygiene*, 79(6), pp. 911–914.

24 Daher, E F, Silva, G B, Silveira, C O, Falcao, F S, et al. (2014) ‘Factors associated with thrombocytopenia in severe leptospirosis (Weil’s disease)’. *Clinics (Sao Paulo)*, 69, pp. 106–110. [online] Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list\_uids=24519201

25 Wagenaar, J. F P, Goris, M. G a, Partiningrum, D. L., Isbandrio, B., et al. (2010) ‘Coagulation disorders in patients with severe leptospirosis are associated with severe bleeding and mortality’. *Tropical Medicine and International Health*, 15(2), pp. 152–159.

26 Tubiana, Sarah, Mikulski, Marc, Becam, Jérôme, Lacassin, Flore, et al. (2013) ‘Risk Factors and Predictors of Severe Leptospirosis in New Caledonia’. *PLoS Neglected Tropical Diseases*, 7(1).

27 Brett-Major, David M and Coldren, Rodney (2012) ‘Antibiotics for leptospirosis.’ *The Cochrane database of systematic reviews*, 2(2), p. CD008264. [online] Available from: http://www.ncbi.nlm.nih.gov/pubmed/22336839

28 Skufca, Jozica and Arima, Yuzo (2012) ‘Sex, gender and emerging infectious disease surveillance: a leptospirosis case study.’ *Western Pacific surveillance and response journal : WPSAR*, 3(3), pp. 37–9. [online] Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3731007&tool=pmcentrez&rendertype=abstract

29 Yanagihara, Yasutake, Villanueva, Sharon Y a M, Yoshida, Shin-Ichi, Okamoto, Yoshihiro and Masuzawa, Toshiyuki (2007) ‘Current status of leptospirosis in Japan and Philippines.’ *Comparative immunology, microbiology and infectious diseases*, 30(5–6), pp. 399–413. [online] Available from: http://www.ncbi.nlm.nih.gov/pubmed/17614131 (Accessed 24 December 2014)

30 Jansen, Andreas, Stark, Klaus, Schneider, Thomas and Schöneberg, Irene (2007) ‘Sex differences in clinical leptospirosis in Germany: 1997-2005.’ *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 44(9), pp. e69–e72.

### Authors’ statements

**Authors’ contributions**   
  
CMP and NL conceived the study; CMP, EK, NK, NS, TLVL, MRR, EMD, and KA completed the previous study for which the data was made available for the current study; CMP, MS, KA, NL designed the study protocol. NL performed the data analysis and initial manuscript drafting and edits; CMP, EK, NK, NS, TVLV, MRR, EMD, and KA contributed to the drafts of the manuscript. All authors read and approved the final manuscript. CMP and NL are the guarantors of the paper.

**Acknowledgements**

We would like to thank Dr Winston S Go and Dr Jose B Villarama for their support, the medical and nursing staff of San Lazaro Hospital and all the participants of the study.

**Funding**   
This work was supported by funds provided Chadwick Travelling Fellowship award.

**Competing interests**   
None declared.

**Ethical approval**   
As provided in the manuscript