

SOCIO-DEMOGRAPHIC, LABORATORY AND CLINICAL FEATURES OF PATIENTS ADMITTED FOR LEPTOSPIROSIS TO TWO MAJOR HOSPITALS IN EAST MALAYSIA (2011-2014)

Mohan Rao, Fairuz Amran, Nurul Atiqah Noor Halim and Rashidah Mohammad

Bacteriology Unit, Institute for Medical Research, Kuala Lumpur, Malaysia

Abstract. Leptospirosis is a widespread zoonotic disease caused by pathogenic *Leptospira* mostly affecting people in tropical and subtropical countries where environmental and socio-cultural conditions favor disease transmissions. Borneo, Malaysia is endemic for leptospirosis and a survey was conducted to analyze profiles of leptospirosis patients in Borneo obtained from records of confirmed cases admitted to Queen Elizabeth Hospital and Sibu Hospital from 2011-2014. Among 268 patients, 76% were males, age ranging from <1 to 89 years with 19% in 30-39 age bracket and 79% detected in the indigenous population. Fever with chills and rigor, nausea and vomiting were predominant symptoms, with abnormal liver and renal profiles. Mortality (5%) was due to complications from the disease. As leptospirosis continues to be an important public health concern, more work is needed to evaluate risk factors and improve awareness to improve strategies for control and prevention.

Keywords: *Leptospira*, leptospirosis, zoonosis, Borneo, Malaysia

INTRODUCTION

Leptospirosis is an acute febrile illness caused by pathogenic members of the genus *Leptospira*, which can infect both humans and animals (WHO, 2009). It is transmitted through urine of infected mammals or consumption of contaminated water (Andre-Fontaine *et al*, 1992). Entry into human system occurs by direct penetration through skin lesion, mucosal membrane or conjunctiva. Mammals such as rat is an essential reservoir host for several pathogenic *Leptospira* (Levett, 2001).

Correspondence: Mohan Rao, Bacteriology Unit, Institute for Medical Research, Jalan Pahang, Kuala Lumpur 50588, Malaysia.
Tel: +603 26162762; Fax: +603 2693 9335
E-mail: rao.mohan@imr.gov.my

Clinical features of the illness range from subclinical to severe fatal disease (Haake and Levett, 2015). Leptospirosis is known for its wide spectrum of symptoms, which share similar clinical picture to those of other tropical infections, such as dengue, malaria and scrub typhus, thereby, can often be overlooked and misdiagnosed (Suttinont *et al*, 2006; Kingston *et al*, 2015).

Leptospirosis is endemic worldwide but sporadic, and outbreaks are more common in tropical and subtropical regions including countries in East Asia and South America (Vinetz, 2001). The island of Borneo, Malaysia is endemic for leptospirosis and several outbreaks have occurred during the past decades (Mohammed *et al*, 2015; Benacer *et al*, 2016b). Prevalence of leptospirosis among the

rural population is significantly related to agricultural and animal husbandry activities, and a close association exists between outbreaks and the above mentioned occupations, recreational activities, adventure tourism, and rainy seasons (Sejvar *et al*, 2003). Gaps in knowledge related to epidemiology of leptospiral infection have delayed treatment and prevention measures (Garba *et al*, 2017).

Hence, socio-demographic, clinical features and laboratory findings of leptospirosis among patients in Borneo, Malaysia were determined to identify significant parameters for predicting possible occurrence of leptospirosis in this region of the country.

MATERIALS AND METHODS

Data collection

A retrospective descriptive survey was conducted in two major hospitals, Queen Elizabeth Hospital (QEH), Sabah and Sibuh Hospital (SH), Sarawak, Malaysia. Demographic, clinical and laboratory data of laboratory confirmed leptospirosis cases during 2011-2014 were collected from admission records.

The research protocol was approved by the National Health Institute Human Research Ethics Committee (JKKM/NIHSEC/P14-926). It did not involve any types of intervention in diagnosis and treatment, and thus prior written informed consent was not considered necessary. The study involved use of anonymous patients' medical records.

Serological diagnostic test

Microscopic agglutination test (MAT) (World Health Organization and International Leptospirosis Society, 2003) was performed using a panel of 20 different *Leptospira* serovar cultures (*L. Australis*,

Autumnalis, *Bataviae*, *Ballum*, *Canicola*, *Copenhageni*, *Hardjobovis*, *Grippotyphosa*, *Icterohaemorrhagiae*, *Javanica*, *Pomona*, *Pyrogenes*, *Tarrasovi*, *Sejroe* and *Patoc*) from WHO Collaborative Centre for Leptospirosis, Amsterdam, The Netherlands and five local isolates (*L. Lep 1*, *Lep115*, *Lep 175*, *Lep 22*, and *HOSHAS*). Agglutination with human serum was examined under a dark-field microscope (10x magnification) and >50% of test L. serovars were agglutinated in comparison to respective negative control is considered positive (World Health Organization and International Leptospirosis Society 2003). Cut-off MAT titer for serum sample was 400 and titer ≥ 400 is considered as positive for acute leptospirosis. When paired samples were available, a 4-fold rise in titer of the second sample from the same patient is considered serologically positive for leptospirosis (Ministry of Health, 2011).

Qualitative (q)PCR assay

DNA was extracted from 200 μ l of whole blood, plasma or serum sample (obtained within seven days of illness) using DNeasy Blood & Tissue kit (QIAGEN, Valencia, CA) and stored in 50 μ l of elution buffer at -20°C until used. PCR mixture consisted of 12.5 μ l of Sso Advance Universal probe Supermix (BioRad Lab, Berkeley, CA), 10 nM forward (5'-AAGCATTACCGCTTGTGGTG-3') and reverse (5'-GAACTCCCATTTCAGC-GATT-3') primers targeting for *Leptospira* LipL32 gene, 10 nM TaqMan probe (5'-FAM-AAAGCCAGGACAAGCGCCG-3LABkFQ-3') (Integrated DNA Technologies, Coralville, IA), 7 μ l of DNA extract and ultrapure water to a final volume of 20 μ l. TaqMan real-time PCR assay was carried out in a Lightcycler 480, thermocycler (Roche Diagnostics, Indianapolis, IN) as follows: 95°C for 8 minutes; followed by 45 cycles of 95°C for 30 seconds

and 60°C for 60 seconds (Stoddard *et al*, 2009). Threshold cycle between 25 and 40 is considered positive for detection of *Leptospira* in sample.

Statistical analysis

Statistical analysis was performed using a Statistical Package for the Social Sciences (SPSS), version 16 (SPSS, Chicago, IL). Categorical data are reported as percent and continuous data as mean \pm standard deviation (SD).

RESULTS

Socio-demographic profile

Patients ($n = 268$; male:female ratio = 3:1) recruited from QEH (55%) and SH (45%) had a mean age of 37 years (range = 1-85 years), the majority (19%) in age group of 30-39 years (Table 1). Thirty-eight (14%) patients were Ibans from Sarawak, 39 (15%) from Kota Kinabalu District, 40 (15%) were Bajaus from Sabah, and 60 (22%) from Sibu District. One hundred and nineteen (41%) patients were agricultural workers (in paddy fields, on estates,

as farmers, and in slaughter houses), and 84 (31%) had a recent history of visits to recreational sites.

Clinical profile

All patients had fever, with chills and rigor, nausea and vomiting, jaundice, myalgia, abdominal pain and coughing the prominent symptoms. The most common clinical feature was hepatomegaly (27%), followed by hemoptysis (5%) and splenomegaly (1%) (Table 2).

Laboratory findings

Blood culture was negative for all patients, qPCR assay positive for 61 (23%) samples and MAT titer of ≥ 400 for 151 (56%) and 4-fold rise in second sample from the same patient in 56 (21%) samples (Table 3). Patients ($n = 15$, 21% and 7, 3%) were seropositive for dengue and hepatitis A IgM, respectively. Only one patient had a positive blood film for plasmodium parasites. Elevated liver function test above normal value, abnormal renal function, leukocytosis, thrombocytopenia, anemia, and hypo-albuminemia was pres-

Table 1
Age and gender of leptospirosis patients admitted to Queen Elizabeth Hospital, Sabah and Sibu Hospital, Sarawak, Malaysia (2011-2014).

Age group (years)	Female Number (%) ($n = 65$)	Male Number (%) ($n=203$)	Total Number (%) ($n = 268$)
<1-9	3 (1)	4 (2)	7 (3)
10-19	9 (3)	39 (15)	48 (18)
20-29	8 (3)	41 (15)	49 (18)
30-39	14 (5)	37 (14)	51 (19)
40-49	10 (4)	35 (13)	45 (17)
50-59	8 (3)	20 (7)	28 (10)
60-69	10 (4)	17 (6)	27 (10)
70-79	3 (1)	9 (3)	12 (4)
80-89	0 (0)	1 (0.5)	1 (0.5)

Table 2
Clinical profile of leptospirosis patients ($n = 268$) admitted to Queen Elizabeth Hospital, Sabah and Sibiu Hospital, Sarawak, Malaysia (2011-2014).

Clinical feature	Number (%) of patients
Fever	268 (100)
Chill and rigor	167 (62)
Nausea and vomiting	161 (60)
Jaundice	159 (59)
Myalgia	117 (44)
Abdominal pain	115 (43)
Coughing	110 (41)
Headache	105 (39)
Arthralgia	96 (36)
Diarrhea	89 (33)
Hepatomegaly	73 (27)
Anorexia	48 (18)
Calf pain	38 (14)
Conjunctival redness	26 (10)
Rash	20 (7)
Retro-orbital pain	18 (7)
Hemoptysis	13 (5)
Splenomegaly	4 (1)

ent in 107 (40%), 85 (32%), 141 (54%), 94 (35%), 115 (43%), and 161 (60%) patients, respectively (Table 4).

Treatment

Patients ($n = 29$, 11% and 56, 27%) required ventilator support and intensive care, respectively. No patient needed dialysis. Of the 13 (5%) mortalities, nine (69%) were local indigenous people.

DISCUSSION

Among the 268 leptospirosis patients admitted to two hospitals in Borneo, 56% were in the economically productive age group (20-49 years) mainly involved in labor and farming. Male predominance of infected patients was likely to be due to differences in occupations rather than to a gender-linked susceptibility (Baker and Baker 1970; Sejvar *et al*, 2003; Garba *et al*, 2017). In our study, 28% of the patients worked in water-logged areas, which pose a high risk to leptospira infection, as reported in earlier studies (Vinetz, 2001;

Table 3
Diagnostic tests of leptospirosis patients admitted to Queen Elizabeth Hospital, Sabah and Sibiu Hospital, Sarawak, Malaysia (2011-2014).

Diagnostic test	Number of patients tested	Number (%) of positives
Leptospira IgM	106	99 (93)
Leptospira qPCR assay	61	61 (100)
Leptospira blood culture	76	0 (0)
Microscopic agglutination test, MAT	207	207 (100)
Dengue IgM	72	15 (21)
Hepatitis A IgM	11	7 (64)
HBc ^a IgM	21	3 (14)
Hepatitis C antibody	18	2 (11)
Blood film for Plasmodium parasites	109	1 (1)

^aHepatitis B core antigen.

Table 4

Laboratory features of leptospirosis patients admitted to Queen Elizabeth Hospital, Sabah and Sibuh Hospital, Sarawak, Malaysia (2011-2014).

Laboratory parameter	Number / number of patients tested (%)	Mean \pm SD
Anemia (Hb <120 g/l) ^a	115/268 (43)	12 \pm 2
Leucocytosis (>11x10 ⁹ /l) ^a	141/263 (54)	12 \pm 6
Thrombocytopenia (<120x10 ⁹ /l) ^a	94/264 (35)	199 \pm 124
Hyperkalemia (K >5 mM) ^a	5/265 (2)	3.7 \pm 0.6
Hypokalemia (K <3.5 mM) ^a	88/265 (33)	3.7 \pm 0.6
Urea (>8 mM) ^a	111/264 (42)	12 \pm 13
Creatinine (>120 μ M) ^a	107/264 (40)	210 \pm 231
Albumin (>35 g/l) ^a	161/259 (60)	32 \pm 8
Alanine transaminase (>40 IU/l) ^a	166/266 (62)	175 \pm 354
Aspartate transaminase (>40 IU/l) ^a	197/266 (74)	160 \pm 299
Alkaline phosphatase (>100 IU/l) ^a	170/266 (43)	162 \pm 117
Total bilirubin (<21 μ M) ^a	115/268 (43)	55 \pm 104
Prothrombin time (>13 s) ^a	85/103 (82)	17 \pm 9
Activated protromboplastin time (>38 s) ^a	32/52 (61)	42 \pm 12

^aNormal value at the hospitals of the study.

Lau *et al*, 2010).

Local ethnic people were predisposed to illness from leptospirosis. Dense population with inadequate local infra-structure and poor flash-flood management could be contributing factors for infection (Benacer *et al*, 2016a). In addition, climatic conditions may assist in disease transmission: high rainfall, high humidity and warmer environment increase incidence of leptospirosis (Reis *et al*, 2008; Storck *et al*, 2008; WHO, 2010).

Leptospirosis is associated with a broad range of clinical presentations (Haake and Levett, 2015). Over half of infected patients present with anicteric febrile illness (Forbes *et al*, 2012). However, other signs and symptoms observed are insufficient to distinguish leptospirosis

from other acute febrile illnesses (Kishimoto *et al*, 2004; Pappas *et al*, 2008; Benacer *et al*, 2016a). In the present study, leucocytosis was the most frequently noted laboratory finding, followed by anemia, elevated liver function tests, azotemia, and thrombocytopenia, similar to a previous report (Pamar *et al*, 2013).

Leptospira is difficult to culture and diagnosis usually relies on serology test. However, serology is neither rapid nor specific (Korver, 1992). At times, seroconversion or 4-fold rise in MAT titer is required for evidence of current leptospirosis infection (Budihal and Perwez, 2014). In the present study, qPCR assay was used in 23% of patients for accurate, specific and rapid diagnosis (Riediger *et al*, 2017), thereby allowing more rapid appropriate

antibiotic administration and reducing possible complications stemming from delayed treatment.

The study suffers from a number of weaknesses. Firstly, the data of hospitalized patients used in this study were retrospective. Secondly, any conclusion could not be extrapolated to the spectrum of the disease that is not present in the hospital records. Thirdly, potentially useful clinical findings could not be included in the analysis. Fourthly, due to the selection procedures of leptospirosis cases, an inherent bias might have affected the analysis.

In summary, the investigation highlights the importance of assessing patient's history and risk factors, such as social background, in conjunction with clinical presentation and laboratory findings in arriving at a definitive diagnosis of leptospirosis. Notable clinical symptoms were fever with chills and rigor, nausea and vomiting, jaundice, hepatomegaly, and myalgia; and leukocytosis, abnormal liver function profile, elevated creatinine level and hypoalbuminemia were prominent laboratory findings. The majority of patients were males, age ranging from 10-69 years, and from local indigenous people. Clinician should initiate antibacterial therapy in early stage of the illness. Mortality was low (5%). Thus, a subject presented with fever, chill and rigor, nausea and vomiting, with a predisposing history and exposure to leptospira, should be suspected for leptospirosis and referred to a tertiary care center for diagnosis and treatment. Medical professionals and the general public need to be educated regarding leptospirosis and the latter should be urged to seek early medical intervention if suspected of having contracted this debilitating and potentially fatal disease.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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