RISK FACTORS FOR LEPTOSPIROSIS DURING A FLOOD DISASTER IN KELANTAN, MALAYSIA.

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ABSTRACT

Leptospirosis is a zoonotic disease caused by the *Leptospira* spirochete. These microorganisms are transmitted through stagnant water or soil via urine of wild and domestic animals which act as reservoir hosts. During flooding incidents, Leptospira spirochetes are dispersed in flood waters and may infect humans who come in contact with the water. The aim of this study is to assess the risk factors for leptospirosis during a major flood disaster in Kelantan at the end of December 2014 until early January 2015. A hospital-based case-control study was conducted from 12 - 30 January 2015 in the state of Kelantan, Malaysia. Leptospirosis cases were confirmed by microscopic agglutination test (MAT) or polymerase chain reaction (PCR). A standardized questionnaire was used to collect demographic and exposure information from both cases and controls. We recruited 54 confirmed leptospirosis cases and 119 controls. Multivariable logistic regression showed staying in flood relief centres (AOR: 4.23; 95% CI: 1.02, 17.53) and history of walking in floodwaters (6.16; 1.40, 27.21) were significantly associated with leptospirosis. Our findings suggest that risk of leptospirosis infection during major floods may be reduced by avoiding walking in floodwaters and ensuring high standards of hygiene and cleanliness in flood relief centers.

KEYWORDS: Leptospirosis, flood, zoonotic disease, Kelantan, Malaysia

INTRODUCTION

Leptospirosis is a systemic infection caused by pathogenic bacteria of the genus *Leptospira* (Johnson 1996) . It is a zoonotic disease, that is, it is transmitted from an animal host to humans. Animal reservoirs of Leptospira spirochetes are comprised of wild and domestic animals such as rodents, cattle, pigs, horses, dogs and cats (Haake and Levett 2015). Spirochetes released when infected animals void into bodies of water or soil, are able to survive the environment for weeks and months.

Humans may be infected through accidental ingestion of water or soil containing spirochetes and through the mucous membrane or broken skin that come in contact with contaminated water or soil. The incubation period for leptospirosis varies from 2 days to 4 weeks. Clinical features are highly variable with no specific defining presentation, and can be broadly categorized into mild, influenza-like illness; Weil's syndrome marked by jaundice, hemorrhage and myocarditis with arrhythmias; pulmonary hemorrhage with respiratory failure; renal failure; meningitis; or meningoencephalitis (World Health Organization 2003). The disease may worsen and become more severe, eventually leading to death if left untreated (Thayaparan et al. 2013).

The number of leptospirosis cases worldwide is estimated to be around 1.03 million each year (Costa et al. 2015). In Malaysia, between January 2004 and December 2012, a total of 12,325 leptospirosis cases were recorded, resulting in 338 deaths and an overall case-fatality rate of 2.74%. The average annual incidence was 4.83 cases per 100,000 population. More cases are observed during the wet season between October and March in Peninsular Malaysia which is, and from October to February in East Malaysia (Benacer et al. 2016).

Floods are associated with an increase in water-borne infectious diseases such as cholera, typhoid, hepatitis A and leptospirosis (Pappas et al. 2008; Naing et al. 2019). During floods, spirochetes are dispersed in the water whereby they may infect the human population (Amilasan et al. 2012). In the Southeast Asian region, Thailand reportedly has the highest incidence of leptospirosis due to frequency of flooding every year (Pappas et al. 2008; Chadsuthi et al. 2021). Leptospirosis outbreaks have been reported after floods in the Philippines (Amilasan et al. 2012), Brazil (Barcellos and Sabroza 2000), Guyana (Dechet et al. 2012), Australia (Smith et al. 2013), Sri Lanka (Agampodi et al. 2014) and Fiji (Togami et al. 2018). Human exposure to leptospira may occur during activities such as cleaning muddy houses and surroundings after the flood without adequate protective clothing like gloves, boots and face shield. Floods may also destroy water treatment facilities and disrupt water supply forcing people to use other unsafe sources of water such as rivers and wells for drinking, bathing, and other activities (Wynwood et al. 2014) which increase their risk of contracting leptospirosis.

Flooding in Malaysia is a regular phenomenon, during the north east monsoon season, especially in the east coast states of Peninsular Malaysia. At the end of December 2014, a particularly massive flood occurred in the state of Kelantan, Malaysia which lasted until the first week of January 2015. It was estimated that more than 200,000 people were evacuated to flood relief centers (Baharuddin et al. 2015). The objective of this study is to assess the risk factors for leptospirosis during this flood disaster.

MATERIALS AND METHODS

This study was conducted as part of the crisis response during the flood, to prevent and control food and water-borne diseases, coordinated by the Crisis Preparedness and Response Centre of the Ministry of Health. The study protocol was approved by the Institute for Medical Research's internal review board and had been exempted from ethical review.

Study design and setting

A hospital-based case control study in eight hospitals was conducted from 12 January 2015 to 30 January 2015. Cases and controls were recruited from Hospital Raja Perempuan Zainab II (Kota Bharu), Hospital Tanah Merah, Hospital Sultan Ismail Petra (Kuala Krai), Hospital Pasir Mas, Hospital Tumpat, Hospital Machang, Hospital Jeli, and Hospital Gua Musang. During the flood, all district hospitals in Kelantan operated as usual and accommodated cases referred for suspected leptospirosis. All patients admitted in hospital with history of exposure to floodwater and presented with clinical signs and symptoms suspected of leptospirosis were tested using a rapid test for leptospirosis. Those found positive for leptospirosis by the rapid test were further confirmed using Microscopic Agglutination Test (MAT) or Polymerase Chain Reaction (PCR).

Definitions of cases and controls

Leptospirosis cases were defined as patients who tested positive by either MAT or PCR. Controls were recruited from the same hospitals and during the same period as the cases, and consisted of patients who were admitted for various reasons, not suspected of leptospirosis and tested negative by MAT or PCR. Patients with history of bleeding tendency and severe dementia were excluded from the study.

Sample size calculation

Prior to the study, the minimum required sample was estimated based on the following assumptions: proportion of controls exposed to floodwater 45%, with odds ratio for leptospiral infection of 3.7[19], equal ratio of cases to controls, 80% power and type I error of 5%, gave a sample size of 30 cases and 60 controls.

Data collection

Written informed consent was taken from cases and controls before data collection. For under-aged subjects (<18 years old), consent was obtained from the parents or guardians. All study subjects who consented were interviewed face-to-face using a structured questionnaire. Interview of under-aged subjects were conducted through their parents/guardian. The questionnaire collected information on socio-demographic variables, comorbidities and exposure to risk factors for leptospirosis. Five milliliters (ml) of venous blood were drawn, collected in plain tubes for MAT and in EDTA tubes for PCR and sent for analysis. MAT was conducted at the Institute or Medical Research's Bacteriology laboratory whereas PCR was carried out by the Kelantan Public Health Laboratory.

Laboratory confirmation of leptospirosis

MAT was performed on a single serum sample, using a standard method outlined by the World Health Organization (World Health Organization 2003). The MAT employed a panel of 20 leptospiral serovars as described in previous studies (Amran, Liow, and Halim 2018; Tan et al. 2014). The cut-off for positivity was agglutination to at least one serovar at titers ≥1:400. Real time PCR was performed using primers targeting the lipL32 gene (Stoddard et al. 2009).

Statistical analyses

Socio-demographic, medical history, environment and activities before and after the flood of cases and controls were described and analysed using Pearson's chi square test. Variables that were significantly associated with leptospirosis at the 0.25 significance level were included in a multivariable logistic regression model. All of the above variables were forced into the multivariable logistic regression model (Enter method) to obtain adjusted odds ratios. The final model was checked for multicollinearity and interaction among the independent variables. All statistical analyses were performed using IBM SPSS version 26.

RESULTS

During the study period, there was a total of 92 patients suspected of leptospirosis, however only 54 met the case definition. We also selected and interviewed 119 eligible patients as controls. Table 1 shows the socio-demographic characteristics, medical history, environment as well as activities before and after the flood, stratified by case/control status. Among the cases, 57.4% were male, 70.3% were adults (age ≥18 years old) and 94.4% were of Malay ethnicity. A majority (76.9%) of the cases were from households with a monthly income of less than RM2000 and 68.4% of adult cases were unemployed.

Results of univariate analysis for associations between each factor with leptospirosis are shown in Table 1. None of the sociodemographic factors were significantly associated with leptospirosis. History of asthma or chronic obstructive pulmonary disease (COPD) was associated with lower odds, while higher odds were linked to multiple factors such as staying in relief centre, distributing aids, accidental swallowing of flood water, flood relief work that involves direct contact with sludge, walking in the floodwaters and coming into contact with left-over stagnant floodwater.

Staying in relief centre during the flood (AOR: 4.23; 95%CI: 1.02, 17.53) and walking in flood waters (AOR: 6.16; 95%CI:1.40, 27.21) were the only independent risk factors which remained significant in multivariable regression (Table 2).

DISCUSSION

A recent systematic review of leptospirosis outbreaks in Latin America and the Caribbean region, Southern Asia, and North America from 1970-2012 found 23% of laboratory-confirmed outbreaks was related to floodwater exposure (Munoz-Zanzi et al. 2020). This phenomenon was coherent with our findings, where the crude analysis showed that staying in a relief centre,

Table 1. Sociodemographic characteristics of cases (n=54) and controls (n=119) by socio-demographic factors

Socio-demographic factors	Cases		Controls		P value[d]
	n	%	n	%	
Gender					
Male	31	57.4	78	65.5	0.304
Female	23	42.6	41	34.5	
Age group (years)					
<7	7	13.0	14	11.8	0.753[f]
7-12	5	9.3	6	5.0	
13-17	4	7.4	7	5.9	
18-39	14	25.9	33	27.7	
40-59	12	22.2	26	21.8	
≥60	12	22.2	33	27.7	
Ethnicity					
Malay	51	94.4	113	95.0	1.000[e]
Non-Malay	3	5.6	6	5.0	
Occupation [a]					
Employed	12	31.6	25	27.2	0.613
Unemployed	26	68.4	67	72.8	
Education level [b]					
Primary school education	9	16.7	24	20.3	0.282
Secondary school education	29	53.7	45	38.1	
Tertiary education	3	5.6	11	9.3	
No formal education	13	24.1	38	32.2	
Monthly household income (RM)					
<2000	40	76.9	94	82.5	0.402
≥2000	12	23.1	20	17.5	
Past medical history [a]					
Diabetes mellitus	7	18.9	17	18.5	0.954
Renal disease	2	5.4	8	8.7	0.723[e]
Asthma /COPD	2	5.4	19	20.7	0.034*
Pneumonia	1	2.7	4	4.3	1.000e
Tuberculosis	1	2.7	2	2.2	1.000e
Hypertension	6	16.2	28	30.4	0.097
Heart disease	3	8.1	11	12.1	0.756e
House and environment					
Stayed in relief centre during flood	23	45.1	29	25.2	0.011*
Stayed in relief centre after the flood	16	32.7	24	21.6	0.137
Presence of rats in flood relief centre or temporary shelter	15	33.3	20	19.2	0.062
Presence of animal carcasses inside the house after the flood	8	15.4	13	11.5	0.487
Outdoor activities					
Distributing aid during the flood [a]	10	29.4	12	13.6	0.042*
Accidentally swallowed flood water	12	27.9	12	12.4	0.024*

Flood relief work with direct contact with sludge during the flood [a]	15	48.4	21	24.7	0.015*
Walking in the floodwaters during the flood [c]	38	82.6	50	48.5	<0.001*
Contact with stagnant floodwater left behind after the flood	12	24.0	9	8.1	0.006*
House cleaning activities					
Cleaning house that is partially covered in mud [a]	21	61.8	39	44.3	0.084
Cleaning house with walls covered in wet sludge [a]	31	68.9	56	51.9	0.053

[a] Excluding subjects age <18 years old
[b] Subjects age <7 years old are included in the 'No formal education' category
[c] Excluding subjects age <5 years old
[d] Pearson's chi squared test
[e] Fisher's exact test
[f] The <7, 7-12, 13-17 years categories were combined into (<18 years) for Pearson's chi square test

Table 2. Crude and adjusted odds ratios for associations between past medical history, environment and outdoor activities with leptospirosis

Socio-demographic factors	Crude		Adjusted [c]	
	OR	95% CI	AOR	95% CI
Past medical history [a]				
Asthma /COPD	0.22	0.05, 1.00*	0.48	0.07, 3.30
Hypertension	0.44	0.17, 1.18	0.57	0.15, 2.18
House and environment				
Stayed in relief centre during flood	2.44	1.22, 4.89*	4.23	1.02, 17.53*
Stayed in relief centre after the flood	1.76	0.83, 3.72	0.63	0.15, 2.65
Presence of rats in flood relief centre or temporary shelter	2.10	0.95, 4.62	1.86	0.55, 6.34
Outdoor activities				
Distributing aid during the flood [a]	2.64	1.01, 6.89*	1.10	0.20, 5.97
Accidentally swallowed flood water	2.74	1.12, 6.74*	1.32	0.24, 7.30
Flood relief work with direct contact with sludge during the flood [a]	2.86	1.21, 6.75*	0.85	0.18, 4.08
Walking in the floodwaters during the flood [b]	5.04	2.14, 11.84*	6.16	1.40, 27.21*
Contact with stagnant floodwater left behind after the flood	3.58	1.40, 9.17*	1.27	0.12, 13.90
House cleaning activities				
Cleaning house that is partially covered in mud [a]	2.03	0.90, 4.56	0.29	0.05, 1.59
Cleaning house with walls covered in wet sludge [a]	2.06	0.99, 4.29	1.37	0.26, 7.19

[a] Excluding subjects age <18 years old
[b] Excluding subjects age <5 years old
[c] Adjusted for all other variables in the model: Hosmer-Lemeshow p-value =0.952, percentage correctly classified =78.4%, AUC=79.3%
OR= Odds Ratio, AOR= Adjusted Odds Ratio
No significant interactions between the independent variables were observed
* P <0.05

distributing aid, swallowing flood water, involvement in flood relief work with direct contact in sludge and walking in the floodwaters during the flood, as well as contact with stagnant floodwater after the flood and history of asthma or COPD, were associated with leptospirosis. All of the above activities (except for asthma/COPD) increase the risk of contact with contaminated floodwater.

In this study, all of the sociodemographic variables were not significantly associated with leptospirosis. However other studies have reported higher proportion of leptospirosis among males, older age, non-Malays (Naing et al. 2019; Munoz-Zanzi et al. 2020; Radi et al. 2018). In the Philippines, incidence of leptospirosis was higher among female workers in lakeshore communities prone to flooding during the monsoon (Arbiol et al. 2016). The reason for gender equality or disparity is probably not biological but rather related to gender differences in occupational exposures (Haake and Levett 2015; Munoz-Zanzi et al. 2020; Garba et al. 2018). Among the self-reported medical history that were assessed, only history of asthma or COPD was found significant in the initial analysis. This could be attributed to asthma/COPD patients being less likely to be involved in strenuous physical activities such as post-flood house cleaning, distributing aid to flood victims and other flood relief work. However, this correlation was eliminated upon control of other factors.

Our finding revealed a significant association between history of walking in floodwaters with risk of leptospirosis infection during flood. This phenomenon was consistent with reported findings from a review of studies in the Asia Pacific region which showed that close contact with flood water, muddy areas, swimming and walking without footwear in contaminated floodwater as well as accidental swallowing of flood water were significant risk factors for leptospirosis in this region (Sakundarno et al. 2014). A study in Southern Thailand among people who were exposed to flood found that having lacerated wounds on the feet and exposure to floodwater for more than three hours a day were significantly associated with risk of leptospiral infection (Chusri et al. 2014).

History of staying in flood relief centers was also independently associated with increased risk of leptospirosis, after adjusting for the other variables. During floods, the evacuees are usually housed in flood relief centers temporarily, which mostly consist of public buildings such as unused public schools. These relief centers are often overcrowded and equipped with inadequate facilities for proper waste disposal, leading to sanitation issues and improper disposal of food waste attracting rats (Zahari and Hashim 2018).

There were several limitations identified in our study. Firstly, we were only able to recruit small number of cases. In addition, we also acknowledge that there could be a possibility of under-reporting of leptospirosis. Under-reporting of leptospirosis in Malaysia is most often due to misdiagnosis owing to its similarity in clinical presentation to dengue. Moreover, lack of availability of rapid tests in the clinics, and requirements for specialized equipment and biosafety level 2 equivalent facility for conducting confirmatory tests also hampers its diagnosis (Garba et al. 2018). Prior to 2010, incidence of leptospirosis in Malaysia was not well documented since it was not a notifiable disease. Even after its inclusion as a notifiable disease on 9 December 2010, (Ministry of Health Malaysia 2011) it is still presumed to be under-reported, primarily due to lack of awareness and low suspicion for leptospirosis among general practitioners. Furthermore, laboratory confirmation for 34 samples from the control group were only based on PCR analysis. However, PCR is one of the most commonly used method for diagnosis of leptospirosis (World Health Organization 2003).

CONCLUSION

We found that staying in flood relief centres and walking in floodwaters were significant risk factors for leptospirosis. During flood events, people in flood affected areas should be advised to avoid walking in floodwaters, and flood evacuees should practice good hygiene and cleanliness during their stay in flood relief centers.

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