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Douadi Benacer
University of Malaya

Kwai Lin Thong
University of Malaya

Ng Choung Min
University of Malaya

Khebir Bin Verasahib
Disease Control Division, Ministry of Health, Malaysia

Renee L. Galloway
Bacterial Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA

See next page for additional authors

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Epidemiology of human leptospirosis in Malaysia, 2004–2012

Douadi Benacer[a], Kwai Lin Thong[a], Ng Choung Min[b], Khebir Bin Verasahib[c], Renee L. Galloway[d], Rudy A. Hartskeerl[e], Marc Souris[f], Siti Nursheena Mohd Zain[a,*]

[a] Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia
[b] Institute of Mathematical Sciences, Faculty of Science, University of Malaya, Malaysia
[c] Disease Control Division, Ministry of Health, 62590 Putrajaya, Malaysia
[d] Bacterial Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA
[e] Royal Tropical Institute (KIT), Amsterdam, The Netherlands
[f] Institut de Recherche pour le Développement, UR 178 Marseille, France

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ABSTRACT

Leptospirosis is an emerging disease, especially in countries with a tropical climate such as Malaysia. A dramatic increase in the number of cases has been reported over the last decade; however, information on the epidemiological trends of this disease is lacking. The objective of this study is to provide an epidemiological description of human leptospirosis cases over a 9-year period (2004–2012) and disease relationship with meteorological, geographical, and demographical information.

A retrospective study was undertaken to describe the patterns of human leptospirosis cases and their association with intrinsic (sex, age, and ethnicity) and extrinsic (location, rainfall, and temperature) factors. Data was grouped according to age, sex, ethnicity, seasonality and geographical distribution, and analyzed using statistical tools to understand the influence of all the different factors on disease incidence.

A total of 12,325 cases of leptospirosis were reported between 2004 and 2012 with an upward trend in disease incidence, with the highest in 2012. Three hundred thirty-eight deaths were reported with an overall case fatality rate of 2.74%, with higher incidence in males (9696; 78.7%) compared with female patients (2629; 21.3%), and overall male to female ratio of 3.69:1. Patients aged cohorts between 30–39 years old (16.22 per 100,000 population) had the highest disease incidence while the lowest incidence occurred between <1 to 9 years old (3.44 per 100,000 population). The average incidence was highest amongst Malays (10.97 per 100,000 population), followed by Indians (7.95 per 100,000 population). Stratification according to geographical distribution showed that the state of Malacca had the highest average disease incidence (11.12 per 100,000 population) followed by Pahang (10.08 per 100,000 population). The states of Terengganu, Kelantan, and Perak recorded similar rates of incidence (≈8.00 per 100,000 population), while Johor with the least number of reported cases (1.80 per 100,000 population). Positive relationships were recorded between the number of reported cases with the number of raining days per month and monthly average temperature (p-value < 0.05). However, no significant association was noted between rainfall volume and number of reported Leptospirosis cases.

This collaborative efforts between medical, academic and governmental institutions has enabled the construction of this comprehensive database that is essential to understand the disease trends in Malaysia and add insights into the prevention and control of this disease.

1. Introduction

Leptospirosis is an important emerging global zoonotic disease, which is caused by the pathogenic spirochete belonging to the genus *Leptospira*. Twenty-two different species have been classified according to DNA–DNA hybridization and phylogenetic analysis (Bourhy et al., 2014; Saito et al., 2013), and over 300 serovars are based on agglutinating lipopolysaccharide antigens

* Corresponding author at: Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
E-mail address: nsheena@um.edu.my (S.N.M. Zain).

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(Victoriano et al., 2009). Domestic and wild mammals, including rodents, have been implicated as reservoirs for these organisms (Levett, 2001). Human infection is transmitted via direct contact with the pathogenic species through contaminated urine, blood, or infected tissue from animal reservoirs or indirectly from contaminated water and soil (Bharti et al., 2003). The clinical manifestations of leptospirosis vary from asymptomatic to a severe potentially fatal form (Levett, 2001). Leptospirosis is often poorly recognized and may be confused with a variety of other febrile diseases or systemic illness (Lau et al., 2010a) because symptoms are nonspecific and often lead to misdiagnosis.

Leptospirosis is recognized as a reemerging infectious disease with an estimate of more than one million cases per year globally (Cosson et al., 2014). Human infection is centered mainly in the tropical and subtropical regions, with marked incidence in Southeast Asia (Amilasan et al., 2012; Bahaman and Ibrahim, 1988; Hinjoy, 2014; Kawaguchi et al., 2008) as well as the Central and South America regions (Costa et al., 2012; Schneider et al., 2012). The warm and humid weather in these regions allow leptospires to thrive longer in the environment and increases the risk of exposure.

Previous studies have highlighted the influence of ethnicity, gender, season and age on the distribution of leptospirosis in Malaysia (El Jalii et al., 2000; Tan, 1979). Weather conditions, especially in tropical countries, have a significant effect on the occurrence of the disease (Levett, 2001), particularly during the rainy season. The monsoon season, normally accompanied by heavy rainfall and floods, increases the risk of leptospirosis. Numerous outbreaks of leptospirosis have been reported following these extreme weather events in many countries (Amilasan et al., 2012; Kawaguchi et al., 2008; Pappachan et al., 2004).

Leptospirosis is endemic in Malaysia. The first human study was reported in 1925, when Fletcher diagnosed 32 cases from rubber plantation workers and rural inhabitants (Fletcher, 1928). Historically, the annual incidence of leptospirosis in the Malaysian population ranged between 1 to 10 per 100,000 population (Lim, 2011). However, in the previous decade, there has been a dramatic increase in reported cases, with several disease outbreaks primarily related to recreational activities and natural disasters (Badrul Hisham et al., 2009; Sapian et al., 2012).

The emergence of leptospirosis in Malaysia has become a significant public health concern and highlights the putative roles of increased surveillance, including the assessment of local socioeconomic and environmental factors, on the transmission of leptospirosis. In this study, we discuss the epidemiology of leptospirosis over a 9-year period (2004–2012) to obtain a better understanding of the disease trends using a combination of statistical tools to correlate the influence of the demographical, geographical, and socio-environmental factors on the incidence of leptospirosis locally.

2. Methods

2.1. Study area

Malaysia consists of Peninsular Malaysia, which covers the southern most point of Eurasia, and Malaysian Borneo (East Malaysia), which is on the island of Borneo located in the South China Sea. Malaysia borders Thailand in the north of the peninsula, and Indonesia and Brunei on the island of Borneo with both parts separated by the South China Sea. Malaysia is composed of 14 states and covers an area of 329,847 square kilometers (land, 328,657 km^2; water, 1190 km^2) close to the equator. The average temperature is 27 °C and the climate is humid throughout the year, with an annual rainfall exceeding 2000 mm.

The climate on the east coast of Peninsular Malaysia and the coastal parts of Sabah and Sarawak is very much influenced by the monsoon season, particularly from November until March. During this season, the heavy monsoon rain sweeps across these areas. Meanwhile, the western coast of Peninsular Malaysia is less affected by the monsoon because of the protection afforded by the mountain ranges in the central part of the peninsula.

2.2. Data collection

Reported cases of leptospirosis during 2004–2012 from hospitals and private health care facilities from 14 states were provided by the Disease Control Division, Ministry of Health Malaysia. The clinical cases were confirmed based on serological tests (MAT $\geq$ 400) and PCR. Data were stratified according to age, sex, ethnicity, time of onset (time of year) and geographic location (states).

2.3. Meteorological data

Meteorological data was provided by the Malaysian Meteorological Department (Malaysian Meteorological Department, 2014) and corresponded to the main meteorological station of each state. The information given included the monthly accumulated rainfall, monthly number of raining days, monthly average temperature, monthly average of daily minimum temperature and monthly average of daily maximum temperature for the 9 years of the study (2004–2012).

2.4. Demographical data

Population statistics based on sex and ethnic groups by state for the 9-year period (2004–2012) were obtained from the Malaysian Department of Statistics (Malaysian Department of Statistics, 2014). Data based on ethnicity was available only for a 3-year period (2010–2012). Detailed population data by age was obtained for 2012 and was analyzed thoroughly to determine the incidence of disease according to 7 age groups: <1–9, 10–19, 20–29, 30–39, 40–49, 50–59, and $\geq$60 years.

2.5. Statistical analysis

Data were analyzed using R software version 2.15.0 (http://www.r-project.org) and Microsoft Excel. Incidences were calculated based on the number of reported cases in the population per 100,000 population. Case fatality rates (CFRs) were obtained by dividing the number of deaths by the number of reported cases of leptospirosis. Analysis of variance (ANOVA) is used to infer about the equality of averages for incidence as well as case fatality rates between the states. Pearson's chi-square (χ^2) test was used to test the difference in counts between groups. The negative binomial regression (Cameron and Trivedi, 2013) was used to estimate the effect of factors (state, gender, ethnicity, and season) with the number of reported leptospirosis cases from 2004 to 2012, where the factors are linked through a log link function to the means of the data. For count data, this approach is commonly used instead of the Poisson regression for situations where overdispersion (variance is greater than the mean) is observed. A p-value < 0.05 was used to indicate statistical significance of the results obtained.

3. Results

3.1. General

Between January 2004 and December 2012, there were 12,325 leptospirosis cases recorded resulting 338 deaths. This gives the

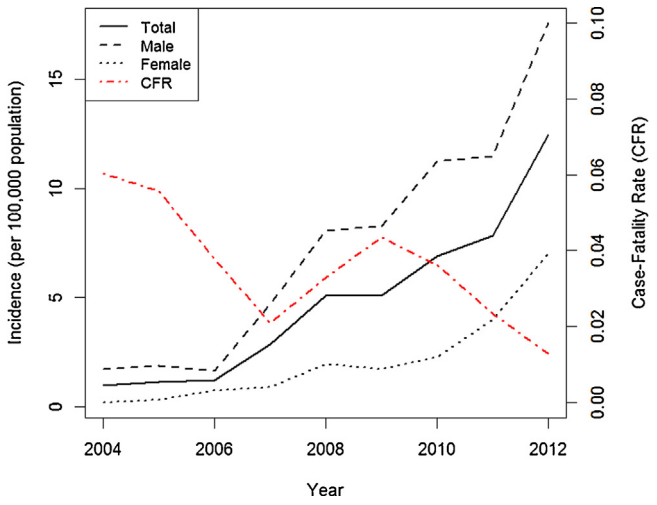

Fig. 1. Trends of incidence and case fatality rate (CFR) of reported cases of leptospirosis for all states in Malaysia during the years 2004–2012.

overall CFR of 2.74%. The annual incidence rate ranged from 0.97 to 12.47 cases per 100,000 population over the 9-year period with an average annual incidence of 4.83 cases per 100,000 population. The CFR and incidence are plotted against time (in reported years) to examine the trends across years (Fig. 1). The incidence markedly increased from 2004 to 2012; however, the CFR declined between those periods apart from a peak from 2007 to 2009.

3.2. Infection according to sex and age groups

Significantly more males were infected than females, with male subjects accounting for 9696 (78.67%) cases compared with 2629 (21.33%) female cases (χ^2 test, p-value = $2.2 \times 10^{-16} < 0.01$). This corresponded to an overall male to female ratio of 3.69:1, and an average annual incidence of 7.40 and 2.11 per 100,000 population for males and females, respectively. According to state, Sabah (15.62) recorded the highest male-to-female disease incidence ratio in 2010 (Table 1).

Based on the detailed patient demographic description in 2012 (Table 2), analyses showed the mean (±standard deviation) age of the infected patients was 33.79 (±17.55) years with a median of 31 years. The highest incidence occurred in the 30–39-year-old age group (16.21 per 100,000 population) while the lowest incidence occurred in the <1–9 year old (3.4) and 10–19 year old (11.5) age groups. Malacca recorded an unusually high incidence of leptospirosis across all age groups compared with the other states, while Johor recorded the lowest incidence across all age groups compared with the rest (Table 2). Fig. 1 illustrates the incidence trends and CFR for all states from the period of 2004–2012.

3.3. Ethnicity

Malays, the predominant ethnic group in Malaysia, were found to be the highest infected compared with other ethnic groups. The average incidence over the period of 2010–2012 for Malays was 10.97 per 100,000 Malays population, rising from 9.12 in 2010 to 14.18 in 2012. Malays were followed by Indians, the third largest ethnic group, with an increasing incidence from 4.78 in 2010 to 11.94 in 2012, giving an average incidence of 7.95 per 100,000 Indians population. Chinese showed the lowest average incidence of 2.22 per 100,000Chinese population compared with the other two ethnic groups. Over the 3 year (2010–2012) period for which ethnicity data was available, Malacca, showed a dramatic increase in the disease rate in the Malay community (Tables 3 and 4).

3.4. Geographical distribution

Between January 2004 and December 2012, all the states in Malaysia showed an increase in recorded cases. Malacca recorded the highest confirmed leptospirosis cases with an average incidence rate of 11.12 per 100,000 population (Table 5), followed by Pahang (10.90 per 100,000 population). Terengganu, Kelantan, and Perak recorded similar incidence rates of confirmed leptospirosis cases over the 9-year period (about 8.00 per 100,000 population). Johor recorded the lowest average disease incidence (1.79 per 100,000 population) in 2005, however accounted the highest number of fatal cases with a CFR of 0.20. Overall, the CFR declined throughout the 9-year period. The geographic distribution of the reported human cases is summarized in Table 5 (ANOVA, p-values = 0.03 (incidence) and 0.28 (CFR)).

3.5. Season

It is observed that more cases in the peninsular were reported during the wet season than the dry, particularly between the months of October to March and between October to February in east Malaysia. From the negative binomial regression analyses, the number of rain days in a month and the average monthly temperature were significant factors associated with the number of reported leptospirosis cases (Table 6). However, no significant association was observed between the average rainfall with number of reported cases (Figs. 2 and 3).

4. Discussion

Leptospirosis is a long-standing disease in Malaysia. To date more than 37 *Leptospira* serovars from 13 different serogroups have been identified in Malaysia with more than half carried by rodents (Bahaman and Ibrahim, 1988). Since the first human case reported in the early 1920s, the number of cases increased subsequently, particularly over the 9-year study period showing a clear increase in annual incidence rising from 0.97 in 2004 to 12.47 per 100,000 population in 2012, with an average incidence of 4.83 cases per 100,000 population.

The disease trend showed that the number of the cases were linked to frequency of outbreaks (Badrul Hisham et al., 2009; Sapian et al., 2012) especially in the last four years of the study particularly after a major flood during the monsoon seasons (Thayaparan et al., 2013) and in combination of other such as socio-demographic, occupation types, recreational activities, environment and climatic changes. However, the overall disease incidence was still much lower compared our country neighbor, Thailand (6.6 per 100,000 population) (Hinjoy, 2014).

Similarly, the average case fatality rate over the 9-year period (0.03) in this country was also lower compared to Thailand and the Philippines (Hinjoy, 2014; Yanagihara et al., 2007). Mortality rates declined in the last 3 years of this study and despite lower fatality, disease incidence continued to rise, indicating improvements in monitoring and surveillance by the authorities, increased awareness among the population, as well as an improvement in early diagnosis and initiation of supportive therapy and antimicrobial drugs.

Higher number of cases in neighboring countries such as Thailand and Philippines were as a result of natural disasters such as flooding. In Thailand, cases were mainly in the underdeveloped northern-east region at the Thai-Myanmar and mostly occurring amongst agricultural workers primarily to those working in the rice fields.

Similarly, the Philippines is situated in a cyclone zone and infection outbreaks primarily affects urban slum inhabitants of the city

Table 1
The male to female disease incidence ratio, according to all states in Malaysia from 2004 to 2012.

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012
State									
Johor	12.81	12.30	3.81	6.26	9.31	4.40	5.67	3.58	1.17
Kedah	13.96	13.91	–	8.56	5.78	4.79	6.14	4.19	3.37
Kelantan	13.97	8.72	1.82	8.94	3.28	9.73	5.45	2.26	4.55
Malacca	5.92	–	1.01	3.52	11.33	1.83	2.30	1.87	2.13
Negeri Sembilan	7.89	–	–	6.26	4.09	3.08	2.90	2.78	2.03
Pahang	6.28	8.51	3.67	5.92	5.17	5.38	4.09	3.30	2.61
Perak	6.15	9.66	2.29	2.55	3.89	4.84	5.24	2.60	2.53
Perlis	–	–	–	4.13	6.20	–	2.59	3.83	5.81
Penang	8.01	6.00	5.99	10.63	7.30	4.31	6.95	3.11	3.26
Sabah	11.12	2.77	2.77	11.68	7.14	5.52	15.62	3.88	2.11
Sarawak	9.18	4.14	6.12	13.46	1.42	10.43	4.00	4.69	2.96
Selangor	4.71	4.23	3.51	5.14	5.60	4.80	6.20	2.87	2.63
Terengganu	–	3.12	4.08	2.81	2.99	3.72	5.71	3.89	3.66
W.P. Kuala Lumpur	8.98	3.84	2.28	3.99	3.83	2.49	3.48	3.32	1.92
Mean Ratio	8.89	6.45	2.28	5.29	4.12	4.79	4.99	2.89	2.51

Table 2
Incidence per 100,000 population according to age groups and states in the year 2012.

Age Group	Age Groups (Years)							All Age Groups
	<1–9	19–20	20–29	30–39	40–49	50–59	≥60	
State								
Johor	0.34	1.76	2.55	1.88	2.82	3.15	2.31	2.00
Kedah	2.56	17.07	18.32	14.96	13.20	15.13	13.07	13.39
Kelantan	0.86	8.93	16.00	16.74	15.39	11.62	9.62	10.19
Malacca	25.84	44.25	61.59	76.29	39.84	69.60	60.82	52.34
Negeri Sembilan	1.12	10.34	17.21	15.48	17.26	26.78	20.77	14.28
Pahang	4.12	9.51	15.16	16.84	12.59	9.35	5.24	10.51
Perak	5.92	14.70	15.91	13.88	11.78	11.10	5.63	11.54
Perlis	4.95	12.24	15.49	11.95	21.90	0.00	10.99	11.30
Penang	0.87	6.46	5.63	5.43	7.93	13.24	21.89	7.87
Sabah	1.45	7.44	11.14	16.53	18.29	22.32	34.72	12.00
Sarawak	1.52	6.58	16.08	19.95	16.04	12.05	11.74	11.37
Selangor	5.82	16.59	19.11	17.08	14.82	14.11	15.83	14.97
Terengganu	2.98	9.80	15.74	20.54	12.00	11.85	14.29	11.42
W.P. Kuala Lumpur	1.99	16.54	22.76	22.53	13.53	22.74	10.04	16.49
Mean Incidence	3.44	11.48	15.74	16.22	13.63	15.21	14.33	12.47

Table 3
Incidence per 100,000 population based on ethnicity for years 2010–2012.

Year	2010			2011			2012		
Ethnicity	Malay	Chinese	Indian	Malay	Chinese	Indian	Malay	Chinese	Indian
State									
Johor	2.66	0.38	0.92	1.79	0.48	0.91	2.09	1.23	1.35
Kedah	7.31	0.39	5.85	6.35	0.39	2.19	15.13	2.34	7.98
Kelantan	10.16	5.73	22.22	14.00	15.15	0.00	9.61	7.53	0.00
Malacca	3.09	0.00	2.02	28.77	6.17	25.10	64.61	15.57	60.74
Negeri Sembilan	9.56	0.44	3.37	16.43	3.97	11.26	17.32	3.51	10.43
Pahang	19.96	4.76	14.26	5.71	2.15	4.69	7.47	1.28	4.64
Perak	22.77	2.86	11.94	11.47	2.57	5.60	11.46	1.85	9.08
Perlis	3.47	0.00	0.00	8.39	5.29	33.33	9.81	0.00	225.81
Penang	4.24	0.60	3.83	2.62	0.74	0.63	11.33	4.56	7.45
Sabah	0.89	2.37	0.00	0.43	1.01	0.00	4.56	9.66	0.00
Sarawak	0.70	1.20	0.00	3.11	1.19	0.00	11.05	2.52	42.86
Selangor	5.93	1.24	2.50	9.51	1.37	8.89	18.88	3.87	14.62
Terengganu	15.80	0.00	0.00	11.08	0.00	0.00	10.47	7.41	0.00
Kuala Lumpur	9.33	0.30	5.70	23.25	2.12	13.81	24.14	4.67	14.34
Mean Incidence	9.12	1.22	4.78	9.60	1.69	7.12	14.18	3.77	11.94

NB: The value 0.00 indicates there is no reported case of leptospirosis for that particular State during that period.

(Metro-Manila) which is characterized by the high population density and poor sanitation.

In contrast, Singapore is a small developed country and reports annually of 26–70 cases only. It has developed an electronic death or infectious disease notification system (Communicable Diseases Live & Enhanced Surveillance System or CDLENS), that facilitates good communication among healthcare professionals and authorities to communicate updates.

Gender played a big role in the disease distribution with incidence in males was three and a half times higher than in women. Similarly, several published works have shown that leptospirosis was gender-biased with more males infected (Chou et al., 2008; Goris et al., 2013) and is thought to be influenced by occupa-

Table 4
The average incidence rate for each ethnic group per 100,000 population for all 14 states over the years 2010–2012.

Year	Average over Years 2010–2012		
Ethnicity	Malay	Chinese	Indian
State			
Johor	2.18	0.70	1.06
Kedah	9.60	1.04	5.34
Kelantan	11.26	9.47	7.41
Malacca	32.16	7.25	29.29
Negeri Sembilan	14.44	2.64	8.35
Pahang	11.05	2.73	7.86
Perak	15.23	2.42	8.87
Perlis	7.22	1.76	86.38
Penang	6.06	1.97	3.97
Sabah	1.96	4.34	0.00
Sarawak	4.96	1.64	14.29
Selangor	11.44	2.16	8.67
Terengganu	12.45	2.47	0.00
Kuala Lumpur	18.91	2.37	11.28
Average Incidence	10.97	2.22	7.95

Table 5
The average incidence (per 100,000 population) and CFRs for all states from 2004 to 2012.

Average over years 2004–2012		
State	Incidence	CFR
Johor	1.79	0.05
Kedah	4.17	0.03
Kelantan	8.01	0.03
Malacca	11.12	0.04
Negeri Sembilan	6.57	0.01[a]
Pahang	10.09	0.02
Perak	7.99	0.06
Perlis	3.21	0.09[a]
Penang	2.28	0.01
Sabah	2.33	0.04
Sarawak	3.61	0.05
Selangor	3.90	0.02
Terengganu	8.07	0.06
Kuala Lumpur	5.57	0.01
Overall Average	4.83	0.03

[a] Average over all years except years 2005 and 2006, where no cases were reported.

Table 6
The negative binomial regression analyses between number of rain days and temperature with the number of reported leptospirosis cases from 2004 to 2012.

Weather	Coefficient	95% Confidence Interval
Rain days		
(Constant)	7.28*	(5.79, 9.19)
Number of Rain Days	1.02*	(1.00, 1.03)
Temperature		
(Constant)	0.04*	(0.004, 0.33)
Average Temperature	1.23*	(1.13, 1.34)

* Estimate significantly different from 1.0 (p-value < 0.05).

tional exposure and activities that put men at a higher risk (Hinjoy, 2014; Mohan et al., 2009). Previous investigations in Malaysia have shown high leptospirosis incidence especially among male-dominated occupations such as paddy planters, rubber tappers, and palm oil workers, laborers dealing with sewage, draining, town cleaning, forestry, those involved in antimalarial programs, and military personnel (El Jalii et al., 2000; Shafei et al., 2012; Tan, 1973).

Unfortunately, it was not possible to analyze the impact of occupation on disease incidence as this information was grossly lacking. In essence, gender may have a compounding effect on occupation.

Males were also known to be more involved in outdoor activities such as water sports and recreational activities compared with

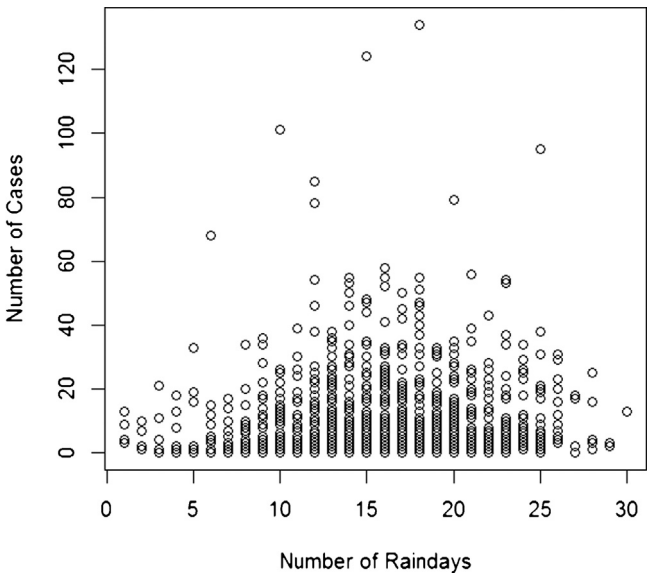

Fig. 2. Number of leptospirosis cases according to the monthly number of raining days from 2004 to 2012.

females. These activities are known risk factors for leptospirosis (Lau et al., 2010a) and have been linked to several outbreaks in this country (Koay et al., 2004; Sapian et al., 2012).

Leptospirosis affects a vast range of age groups, with a higher number of cases occurring amongst the working class ranging from 20 to 49 years old, with the highest amongst the 30–39 years old. This was in agreement with previous studies (Goris et al., 2013; Jansen et al., 2007) as the middle age groups are generally more mobile thus at higher risk of exposure compared to the young and school-going children. Despite known association between leptospirosis with occupation and recreational activities (Bharti et al., 2003; Jansen et al., 2007) as previously reported, we were unable to conclude this information, as data on occupation was not available.

Based on the patient's ethnicity, infections were highest among Malays (10.97 per 100,000 Malays population), followed by Indians (7.95 per 100,000 Indians population). This is not surprising because Malays make up 50% of the total population (Malaysian Department of Statistics, 2014) although, infections in the Indian community exceeded Malays in the two states of Perlis and Sarawak in 2012. The low Indian population in Sabah and Terengganu also corresponded with the low number of cases among Indians in both states.

In a previous serological study, Tan (1979) correlated the ethnic distribution among 18 occupations though serological testing of 4646 febrile patients with leptospirosis. Indians recorded the highest infection (44.1%), followed by Malays (33.9%) and Chinese (22.0%). Indians employed as oil palm plantation workers, rubber tappers, miners, and farmers were at higher risk for infection (Tan, 1979) compared with the rest. Chinese recorded the lowest infection (2.22 per 100,000 population) even in population densities where the Chinese were higher or equal to the Malay population as observed in Pulau Pinang, Kuala Lumpur and Perak.

In relation to geographical distribution, Malacca noted a record high of 50 times more cases in the 9-year study. Based on the national statistics, more than 85% of the population resides in the urban districts of Jasin, Alor Gajah, and central Malacca. The impact of rural-to-urban demographic migration has often led to rapid urbanization and creation of urban slums and this factor has been associated with the emergence of leptospirosis in many countries (Lau et al., 2010b). Urban slums are typically associated with bad sanitation infrastructure, poor waste management and

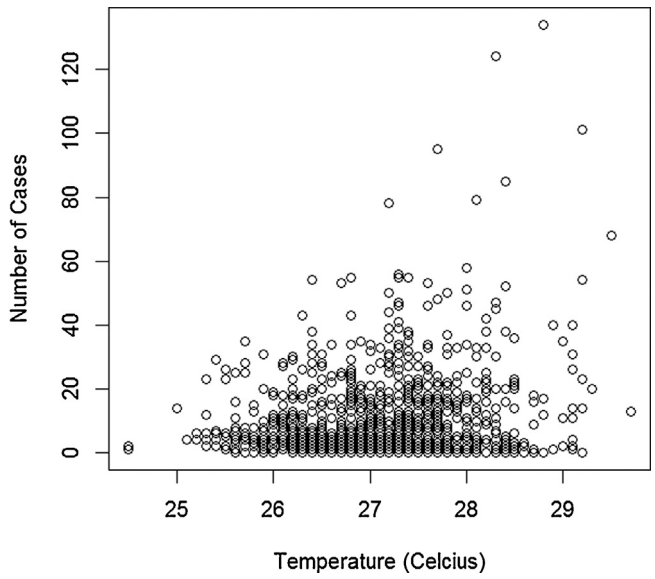

Fig. 3. Number of cases by average monthly temperature from 2004 to 2012.

where humans typically inhabit in close proximity with animal and environmental reservoirs of infectious agents. It is also reported the emergence of *Leptospira* transmission are associated with proximity of residence to open sewers and accumulated refuse, flood-risk areas and areas with high rat infestation (Barcellos, 2000; Felzemburgh et al., 2014). In addition to environmental features, low socioeconomic status among slum residents was also reported to contribute to the risk of leptospirosis (Barcellos, 2000; Felzemburgh et al., 2014; Oliveira et al., 2009). The impact of season can be excluded as, the state of Malacca is on the west coast of Peninsular Malaysia and protected by the mountain ranges and least prone to the effects of flooding by the northeast monsoon.

It is estimated that urban population growth in Malaysia was expected to rise from 54.7% in 1995 to approximately 73% by 2020 (Osman et al., 2004). This rapid growth if not managed with proper would indirectly create the growth of slums with poor irrigation and flash-flood management, all of which create favorable conditions for the survival of the host reservoir and subsequently the contamination of environmental waters and soils via their excreta and urine (Benacer et al., 2013a).

In Malaysia, the majority of leptospirosis cases are caused by exposure to an environment contaminated by *Leptospira* spp. (Benacer et al., 2013b). To date, more than 29 pathogenic *Leptospira* serovars have been isolated from natural water and soil locally (Alexander et al., 1975; Benacer et al., 2013b). The climatic conditions such as high humidity (70–90%) and warm weather provided an appropriate niche for this organism to survive longer in the environment. Thus, the association between the disease frequency and meteorological parameters were explored. The monthly number of reported cases of leptospirosis all year round showed a seasonality pattern. The reported cases were significantly higher during the wet season than the dry season, especially between October to March in the peninsula and between October to February in east Malaysia. This result agrees with other investigations in the region such as Indonesia, Laos, and Thailand (Chadsuthi et al., 2012; Kawaguchi et al., 2008; Victoriano et al., 2009). A significant association was also shown between the number of rain days and infection incidence as observed in Malacca, Pahang, Perak, Terengganu, and Kelantan.

Continuous rain over several days or weeks keeps soil moist and forms small lakes, muddy ponds, and streams that allow leptospires to survive longer in the environment. This ultimately leads to an increase in human and animal exposure to the bacteria (Ullmann and Langoni, 2011), especially near forests and farms. Heavy rain can also unclog drains, flush out urine or infected dead animals, which contaminates water bodies and becomes a source of contagion. Flooding often destroys properties and damages the water and sanitation networks, and contaminates drinking water with pathogens. Interestingly, the association between the number of cases in relation to rainfall volume was not significant in the current study despite being recognized as a risk factor associated with leptospirosis (Bharti et al., 2003; Levett, 2001). When compared with neighboring countries such as Thailand and the Philippines, only a handful of flooding incidents in Malaysia were known to be associated with a leptospirosis outbreak. During the period of this study, only one incident occurred between 2006 and 2007, in Johor, after a major flood caused more than 20 confirmed leptospirosis cases with two deaths among 2000 cases of waterborne diseases reported (Badrul Hisham et al., 2009).

This study found significant association between environmental temperature and the number of leptospirosis cases, with most cases being recorded between 27 to 28 °C (27.5 °C). This finding was in agreement with previously published studies (Desvars et al., 2011; Ullmann and Langoni, 2011) that explained the warm climate relationship to leptospirosis, where the pathogen survives and thrives at an average temperature of about 23.5 °C (Chadsuthi et al., 2012).

The high temperatures in this region encourage water-based activities such as swimming, bathing, and drinking, which promotes contact between humans, livestock, pets, and wildlife through more intense sharing of shrinking surface-water sources (Lau et al., 2010b). Furthermore, prolonged immersion can make the skin and mucous membranes more penetrable and allow the entrance of leptospires (Ullmann and Langoni, 2011). In addition, recreational water activities such as fishing, kayaking, and water skiing in contaminated water have been reported as risk factors for infection, especially when ingestion of water occurred (WHO, 2003). In Perlis, a family of 8 men developed symptoms and were hospitalized after fishing at a nearby swamp. Investigation revealed that the swamp was the source of the infection (Baharudin et al., 2012).

Leptospirosis is an endemic disease in Malaysia. A better understanding of the epidemiology of leptospirosis in this country is necessary to link factors that contribute to the emergence of this disease, such as climatic events, environmental factors, animal reservoirs, human demographic and social trends, as well as cultural and anthropological factors.

The involvement of more health workers is necessary to educate the public about the dangers of the disease, its mode of transmission among the population and those most at risk, so that the disease can be recognized and treated immediately. In addition, implementation of a robust surveillance system to monitor temporal and spatial changes in incidence and prevalence is indispensable to identify risk areas and risk behavior and, in turn, design adequate control and prevention measures.

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References

Alexander, A.D., Evans, L.B., Baker, M.F., Baker, H.J., Ellison, D., Marriapan, M., 1975. Pathogenic leptospires isolated from Malaysian surface waters. Appl. Environ. Microbiol. 29, 30–33.

Amilasan, A.S., Ujiie, M., Suzuki, M., Salva, E., Belo, M.C., Koizumi, N., Yoshimatsu, K., Schmidt, W.P., Marte, S., Dimaano, E.M., Villarama, J.B., Ariyoshi, K., 2012. Outbreak of leptospirosis after flood: the Philippines 2009. Emerg. Infect. Dis. 18, 91–94.

Badrul Hisham, A.S., Nor Azian Shaharom, C.M.D., Marzukhi, M.I., Norli, R., Fatimah, O., Kee, K.F., Arbaiah, O., Mohd Yusof Adam, A.M., 2009. Spectrum of flood-related diseases encountered during flood disaster in Johore, Malaysia. Malays J. Community Health 15, 15–23.

Bahaman, A.R., Ibrahim, A.L., 1988. A review of leptospirosis in Malaysia. Vet. Res. Commun. 12, 179–189.

Baharudin, B., Rohayati, M.N., Mohd Sade, Y., Amizah, O., Zulhizzam, A., 2012. Outbreak of Leptospirosis in Pauh, Arau, Perlis. Abstract of the 11th International Annual Symposium on Sustainability Science and Management.

Barcellos, C., 2000. Socio-environmental determinants of the leptospirosis outbreak of 1996 in western Rio de Janeiro: a geographical approach. Int. J. Environ. Health Res. 10, 301–313.

Benacer, D., Mohd Zain, S.N., Amran, F., Galloway, R.L., Thong, K.L., 2013a. Isolation and molecular characterization of *Leptospira interrogans* and *Leptospira borgpetersenii* isolates from the urban rat populations of Kuala Lumpur, Malaysia. Am. J. Trop. Med. Hyg. 88, 704–709.

Benacer, D., Who, P.Y., Mohd Zain, S.N., Amran, F., Thong, K.L., 2013b. Pathogenic and saprophytic *Leptospira* species in water and soils from selected urban sites in Peninsular Malaysia. Microbes Environ. 28, 135–140.

Bharti, A.R., Nally, J.E., Ricaldi, J.N., Matthias, M.A., Diaz, M.M., Lovett, M.A., Levett, P.N., Gilman, R.H., Willig, M.R., Gotuzzo, E., Vinetz, J.M., 2003. Peru-United States Leptospirosis Consortium. Leptospirosis: a zoonotic disease of global importance. Lancet Infect. Dis. 3, 757–771.

Bourhy, P., Collet, L., Brisse, S., Picardeau, M., 2014. *Leptospira mayottensis* sp. nov.: a pathogenic species of the genus *Leptospira* isolated from humans. Int. J. Syst. Evol. Microbiol. 64, 4061–4067.

Cameron, A.C., Trivedi, P.K., 2013. Regression analysis of count data. In: Econometric Society Monograph No.53, 2nd edition. Cambridge University Press, New York.

Chadsuthi, S., Modchang, C., Lenbury, Y., Iamsirithaworn, S., Triampo, W., 2012. Modeling seasonal leptospirosis transmission and its association with rainfall and temperature in Thailand using time–series and ARIMAX analyses. Asian Pac. J. Trop. Med. 5, 539–546.

Chou, Y.-L., Chen, C.-S., Liu, C.-C., 2008. Leptospirosis in Taiwan, 2001–2006. Emerg. Infect. Dis. 14, 856–857.

Cosson, J.F., Picardeau, M., Mielcarek, M., Tatard, C., Chaval, Y., Suputtamongkol, Y., Buchy, P., Jittapalapong, S., Herbreteau, V., Morand, S., 2014. Epidemiology of *Leptospira* transmitted by rodents in Southeast Asia. PLoS Negl. Trop. Dis. 8, e2902, http://dx.doi.org/10.1371/journal.pntd.0002902.

Costa, F., Martinez-Silveira, M.S., Hagan, J.E., Hartskeerl, R.A., dos Reis, M.G., Ko, A.I., 2012. Surveillance for leptospirosis in the Americas, 1996–2005: a review of data from ministries of health. Rev. Panam Salud. Publica. 32, 169–177.

Desvars, A., Jégo, S., Chiroleu, F., Bourhy, P., Cardinale, E., Michault, A., 2011. Seasonality of human leptospirosis in Reunion island (Indian ocean) and its association with meteorological data. PLoS One 6, e20377, http://dx.doi.org/10.1371/journal.pone.0020377.

El Jalii, I.M., Bahaman, A.R., Mohd-Azmi, M.L., Mutalib, A.R., 2000. Occurrence of human leptospirosis in Malaysia: a retrospective study. J. Trop. Biomed. 16, 1–5.

Felzemburgh, R.D.M., Ribeiro, G.S., Costa, F., Reis, R.B., Hagan, J.E., Melendez, A.X.T.O., Fraga, D., Santana, F.S., Mohr, S., dos Santos, B.L., Silva, A.Q., Santos, A.C., Ravines, R.R., Tassinari, W.S., Carvalho, M.S., Reis, M.G., Ko, A.I., 2014. Prospective study of leptospirosis transmission in an urban slum community: role of poor environment in repeated exposures to the leptospira agent. PLoS Negl. Trop. Dis., e2927, http://dx.doi.org/10.1371/journal.pntd.0002927.

Fletcher, W., 1928. Recent work on leptospirosis: tustsugamushi disease and tropical typhus in the Federal Malay States. Trans. R. Soc. Trop. Med. Hyg. 21, 265–288.

Goris, M.G.A., Boer, K.R., Duarte, T.A.T.E., Kliffen, S.J., Hartskeerl, R.A., 2013. Human leptospirosis trends the Netherlands, 1925–2008. Emerg. Infect. Dis. 19, 371–378.

Hinjoy, S., 2014. Epidemiology of leptospirosis from Thai National Disease Surveillance System 2003–2012. Outbreak Surveill. Investig. Rep. 7, 1–5.

Jansen, A., Stark, K., Schneider, T., Schoeneberg, I., 2007. Sex differences in clinical leptospirosis in Germany 1997–2005. Clin. Infect. Dis. 44, 69–72.

Kawaguchi, L., Sengkeopraseuth, B., Tsuyuoka, R., Koizumi, N., Akashi, H., Vongphrachanh, P., Watanabe, H., Aoyama, A., 2008. Seroprevalence of leptospirosis and risk factor analysis in flood-prone rural areas in Lao PDR. Am. J. Trop. Med. Hyg. 78, 957–961.

Koay, T.K., Nirmal, S., Noitie, L., Tan, E., 2004. An epidemiological investigation of an outbreak of leptospirosis associated with swimmin, Beaufort, Sabah. Med. J. Malaysia 59, 455–459.

Lau, C., Smythe, L., Weinstein, P., 2010a. Leptospirosis: an emerging disease in travellers. Travel. Med. Infect. Dis. 8, 33–39.

Lau, C.L., Smythe, L.D., Craig, S.B., Weinstein, P., 2010b. Climate change, flooding, urbanisation and leptospirosis: fuelling the fire? Trans. R. Soc. Trop. Med. Hyg. 104, 631–638.

Levett, P.N., 2001. Leptospirosis. Clin. Microbiol. Rev. 14, 296–326.

Lim, V.K., 2011. Leptospirosis: a re-emerging infection. Malaysia J. Pathol. 33, 1–5.

Malaysian Department of Statistics, http://www.statistics.gov.my. (accessed 15.08.14.).

Malaysian Meteorological Department, http://www.met.gov.my. (accessed 05.08.14.).

Mohan, A.R., Cumberbatch, A., Adesiyun, A.A., Chadee, D.D., 2009. Epidemiology of human leptospirosis in Trinidad and Tobago, 1996–2007: a retrospective study. Acta Trop. 112, 260–265.

Oliveira, D.S., Guimaraes, M.J., Portugal, J.L., Medeiros, Z., 2009. The socio-demographic: environmental and reservoir factors associated with leptospirosis in an urban area of north-eastern Brazil. Ann. Trop. Med. Parasitol. 103, 149–157.

Osman, A., Zaleha, M.I., Mohd Rizam, A.R., 2004. The effect of urbanisation on the health of urban residents. Akademika 65, 111–124.

Pappachan, M.J., Sheela, M., Aravindan, K.P., 2004. Relation of rainfall pattern and epidemic leptospirosis in the Indian state of Kerala. J. Epidemiol. Community Health 58, 1054.

Saito, M., Villanueva, S.Y., Kawamura, Y., Iida, K., Tomida, J., Kanemaru, T., Kohno, E., Miyahara, S., Umeda, A., Amako, K., Gloriani, N.G., Yoshida, S., 2013. *Leptospira idonii* sp. nov.: isolated from environmental water. Int. J. Syst. Evol. Microbiol. 63, 2457–2462.

Sapian, M., Khairi, M.T., How, S.H., Rajalingam, R., Sahhir, K., Norazah, A., Khebir, V., Jamalludin, A.R., 2012. Outbreak of melioidosis and leptospirosis co-infection following a rescue operation. Med. J. Malaysia 67, 293–297.

Schneider, M.C., Nájera, P., Aldighieri, S., Bacallao, J., Soto, A., Marquino, W., Altamirano, L., Saenz, C., Marin, J., Jimenez, E., Moynihan, M., Espinal, M., 2012. Leptospirosis outbreaks in Nicaragua: identifying critical areas and exploring drivers for evidence-based planning. Int. J. Environ. Res. Public Health 9, 3883–3910.

Shafei, M.N., Sulong, M.R., Yaacob, N.A., Hassan, H., Wan Mohammad, W.M.Z., Daud, A., Ismail, Z., Abdullah, M.R., 2012. Seroprevalence of leptospirosis among town service workers on northeastern State of Malaysia. IJCRIMPH 4, 395–403.

Tan, D.S.K., 1973. Occupational distribution of leptospiral (SEL) antibodies in West Malaysia. Med. J. Malaysia 27, 253–257.

Tan, D.S.K., 1979. Leptospirosis in West Malaysia-epidemiology and laboratory diagnosis. Malaysia J. Pathol. 2, 1–6.

Thayaparan, S., Robertson, I., Fairuz, A., Suut, L., Abdullah, M., 2013. Leptospirosis: an emerging zoonotic disease in Malaysia. Malaysia J. Pathol. 35, 123–132.

Ullmann, L.S., Langoni, H., 2011. Interactions between environment, wild animals and human leptospirosis. J. Venom. Anim. Toxins Incl. Trop. Dis. 17, 119–129.

Victoriano, A.F., Smythe, L.D., Gloriani-Barzaga, N., Cavinta, L.L., Kasai, T., Limpakarnjanarat, K., Ong, B.L., Gongal, G., Hall, J., Coulombe, C.A., Yanagihara, Y., Yoshida, S., Adler, B., 2009. Leptospirosis in the Asia Pacific region. BMC Infect. Dis. 9, 147–156.

World Health Organization and the International Leptospirosis Society, 2003. Human Leptospirosis: Guidance for Diagnosis, Surveillance and Control. The Organization, Geneva.

Yanagihara, Y., Villanueva, S.Y.A.M., Yoshida, S., Okamoto, Y., Masuzawa, T., 2007. Current status of leptospirosis in Japan and Philippines. Comp. Immunol. Microbiol. Infect. Dis. 30, 399–413.