



# An outbreak of leptospirosis among reserve military recruits, Hulu Perdik, Malaysia

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## Abstract

Here, we investigated an outbreak of leptospirosis among reserve military recruits that occurred following a survival exercise in the Hulu Perdik forest within the Hulu Langat district, Kuala Lumpur, Malaysia. Blood samples from the 12 patients that presented symptoms for febrile illness on clinical examination were subjected to laboratory investigation, comprising Lepto IgM rapid test, IgM ELISA, and microscopic agglutination test (MAT). All these patients were interviewed for possible risk factors for leptospirosis. Rodent trapping and environmental sampling for possible isolation of leptospires in the outbreak site was performed. The isolated leptospires were genetically characterized and investigated for the potential epidemiological link with human leptospirosis. Among the 12 patients, two (2/12; 16.6%) were confirmed positive for leptospirosis by microscopic agglutination test (MAT with titers 400–800; serovar autumnalis and hardjobovis). Two *Leptospira* species from rodents (*L. interrogans* and *L. borgpetersenii*) and two from the environment (*L. kmetyi* and *L. wolffii*) were identified. The possible epidemiological link between human serovars and animal *Leptospira* species indicates rodents as the potential reservoir while the environment (soil and water) serves as a transmission route. This investigation highlights the robust presence of pathogenic leptospires on Malaysian environment and rodents which may present the risk of infection, especially among high-risk individuals. Hence, occupational risk individuals are cautioned to observe appropriate preventive measures including prophylaxis and seek immediate medical attention for any illness following similar activities.

**Keywords** Outbreak · Leptospirosis · Occupational risk · Malaysia · Recreational exposure · Emerging infectious disease

## Introduction

Leptospirosis is endemic in Malaysia and is documented as a notifiable disease since 2010. Numerous outbreaks have occurred in both Peninsular Malaysia and the Borneo Island regrettably with death [1–4]. Over the 200 serovars detected in Malaysia are mostly from rodents making them the most important source of both human infection and environmental contamination [5–7]. The disease is also associated with occupational exposure where individuals engaged in military services, sewage and garbage collection, and butchery, have shown high prevalence [8–12].

Here, we report a recent outbreak involving reserve military recruits. The outbreak occurred in the state of Selangor near the Perdik River in Hulu Langat district. This river which is surrounded by forest is usually used by the Malaysian military for camping and training exercises. Hulu Langat, in general, is a gem of recreational area with lots of waterfalls and

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also is an awesome location for hiking that is frequently patronized by both local and foreign tourists due to its close proximity to the capital Kuala Lumpur.

## Methods

### Ethics approval

For human samples, the approval for the conduct of this study was obtained from the Medical Research and Ethics Committee, Ministry of Health, Malaysia, with reference number NMRR-15-2148-27536. For animal samples, the study was approved by the Animal Ethics Committee of the Universiti Kebangsaan Malaysia (National University of Malaysia) with reference number (UKMAEC) FST/2016/AR-CAT2.

### Outbreak detection

On 14 October 2016, 12 military personnel were presented to the Tuanku Mizan Armed Forces Hospital in Kuala Lumpur with high-grade fever, chills, headache, myalgia diarrhea, and vomiting, 3 days after participating in a military exercise. All patients were screened for dengue, malaria, and leptospirosis. For leptospirosis, blood samples were subjected to Lepto IgM rapid test (Unimed International Inc., USA) and IgM ELISA (SERION ELISA *classic Leptospira* IgM, SERION® Immunologics, Germany). Samples that were positive for any one of the test was subjected to microscopic agglutination test (MAT). In a month time, all 12 patients were interviewed through phone to ascertain their health status and to establish the presence of any known potential risk factors. The information (data) obtained included nature of the activity, possible exposure prior to the exercise, prophylaxis, or previous antibiotic intake. All 12 patients were invited for the second blood sample (convalescent serum for MAT confirmation); however, only five (including four of the patients whose initial result was positive by screening test) honored the invitation. MAT was performed for the convalescent serum against a panel of 20 live serovars (local and international). The local serovars used included IMR LEP 1, 115, and 175 (saprophytes) and IMR LEP 803/11-Copenhageni, IMR LEP 27-Hardjobovis, IMR LEP 22-Lai (pathogenic), while the international panel ( $n = 14$ ) obtained from WHO Leptospirosis Collaborating Center, Amsterdam, was Australis, Autumnalis, Batavia, Canicola, Celledoni, Grippytyphosa, Hardjoprajtino, Icterohaemorrhagiae, Javanica, Pyrogenes, Tarrasovi, Djasiman, Patoc, and Pomona. For this investigation, a patient is considered having confirmed leptospirosis when positive for MAT (single serum leptospiral antibody titer  $\geq 1:400$  or a fourfold rise in antibody in paired serum; seroconversion) due to the non-availability of the first blood sample for culture or PCR.

## Epidemiological investigation

The location of the exercise was visited in order to have a clear picture of the terrain. Rodents were trapped and kidney extracted for *Leptospira* isolation [13]. On the other hand, a total of 36 environmental samples comprising 18 soil and 18 water samples were collected. The sampling locations were selected based on different activities of the camping group. All samples were brought back to the microbiology laboratory at the Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, and processed for *Leptospira* isolation. The kidney samples were cultured in the EMJH medium supplemented with rabbit serum and 5-fluorouracil [13]. The soil was suspended in sterile PBS and filtered, and 250  $\mu$ l was used to inoculate 7 ml of EMJH media in a 15-ml falcon tube. The cultures were monitored weekly for possible leptospiral growth upon viewing under the dark-field microscope. Subsequently, genomic DNA was extracted (QIAamp DNeasy Blood and Tissue Kit, Qiagen, Valencia, CA) from all the culture-positive media and then subjected to 16S ribosomal RNA [14] and *lipL32* PCR amplification [15].

## Results

### Epidemiological investigation

Overall, 12 patients were interviewed comprising of six males and six females. All the patients were involved in the exercise for the entire period of the drill (2 days) and also admitted receiving prophylactic antibiotic treatment before embarking on the exercise. Among them, 91.6% ( $n = 11$ ) and 58.3% ( $n = 7$ ) of the patients admitted to having accidentally swallowed the river water while swimming and have walked barefoot either during fishing or returning from the river (Table 1). It was intriguing to note that not all of the patients had prior knowledge on the potential risk of contracting the *Leptospira* infection during this kind of exercises. None of them also used any protective clothing like boots or gloves during their over 30 min of submersion in the river and damp soil. Although no mortality was recorded, the majority of the patients presented clinical signs consistent with leptospirosis. These include fever (83%), headache (75%), chills (25%), and myalgia (50%).

### Laboratory investigation

The laboratory examination of samples (first sample from the hospital results) collected from the 12 patients indicated that four have (suspected) *Leptospira* infection. Of these four positive samples, one was positive for Lepto IgM rapid test, another one for IgM ELISA (which also showed an elevated

**Table 1** Risk factors associated with leptospirosis in suspected patients who participated in the survival exercise

Risk exposed	No. of subjects (n = 12)	Percentage (%)
Swallowing river water	11	91.6
Walking barefoot	7	58.3
Hunting and fishing	11	92
Duration of submersion ( $\geq 30$ min)	12	100
Cuts and bruises	2	16.6
No prior knowledge of potential risk	12	100
Prophylactic treatment prior to the drill	12	100

anti-leptospiral antibody by MAT), while the other two were equivocal for both ELISA and MAT (with a titer of 1:200) (Table 2).

Routine hematological and biochemical analysis of blood collected during admission showed that the blood parameters were within the normal range except for a slight elevation in leukocyte counts. The patient that was positive for Lepto IgM rapid test was treated in the hospital with ceftriaxone at 2 g daily for 3 days, after which the fever subsided at day 3 and was discharged on day 4.

For the result of five out of the 12 patients who provided the convalescent serum samples, two showed diagnostic titers for leptospirosis (Table 2).

### Environmental and rodent sample analysis

From the 5 days of trapping, 12 rodents were caught. Of the 12 rodent kidneys and the 36 environmental samples cultured, 6 rodents, 6 water, and 8 soil samples (amounting to 20) were positive (spirochetes) after viewing under the dark-field

**Table 2** Laboratory diagnostic profile of leptospirosis-suspected patients

Patient	IgM rapid test	ELISA	MAT (1st sampling)	MAT (2nd sampling)
1	+	+	200	800 (Autumnalis)
2	–	–		
3	–	Equivocal	200	100 (Patoc)
4	–	+	400	400 (hardjobovis)
5	–	–		None detected
6	–	–		
7	–	–		
8	–	–		
9	–	–		None detected
10	–	Equivocal	200	
11	–	–		
12	–	–		

microscope. A total of 14 environmental and six animal culture showed positive amplification for *lipL32* and 16S rRNA PCR. Sequence analysis of the 16S rRNA-amplified fragment identified *L. kmetyi* (n = 8) and *L. wolffii* (n = 6) among the environmental samples while *L. interrogans* (n = 5) and *L. borgpetersenii* (n = 1) among the rodent kidney. The phylogenetic tree constructed using the neighbor-joining method with the help of MEGA software version 7.0 produced four clusters and clustered all isolates according to the species as shown in Fig. 1.

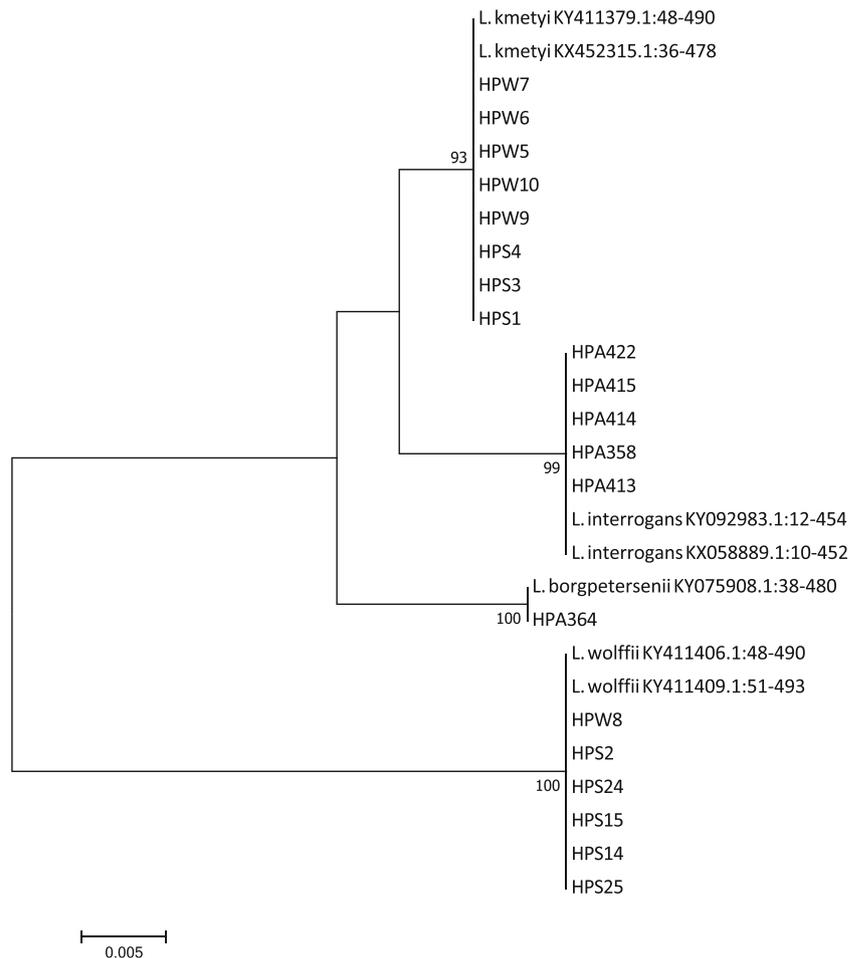
### Discussion

The persistence, associated mortality, and economic implication of leptospirosis have prompted the Malaysian government through the Ministry of Health to integrate leptospirosis in the surveillance network as well as declaring it a notifiable disease. Unfortunately, due to the non-specific nature of the clinical manifestations and lack of highly effective rapid or early diagnostic assays, the disease continues to be misdiagnosed and under-reported [16, 17]. Hence, it has become imperative to document any outbreak case, including conducting a thorough epidemiological assessment and microbiological investigations in order to ascertain the possible role of the environment and reservoir animals in the persistence and changing epidemiology of the disease in Malaysia.

The risk factors investigated in the present study show that 92 to 100% of the subjects were involved in water activities such as fishing and submersion for more than 30 min and admit swallowing water. Importantly, among the 56 participants who received pre-exposure prophylaxis with doxycycline (administered at 200 mg daily for 3 days prior to the commencement of the activity), it resulted in a protective efficacy of 96.4% (54/56 subjects). This observation is consistent with the results obtained in Thailand, where a single dose of doxycycline (200 mg) was administered to residents exposed to flooding and the protective efficacy was found to be up to 95.6% [18].

In order to track the source of the leptospirosis outbreak in the campsite, we performed an epidemiological investigation of the carrier status of rodents and environmental contamination. Four species of *Leptospira* were isolated, two from the animal (*L. interrogans* and *L. borgpetersenii*) and two from environmental samples (*L. kmetyi* and *L. wolffii*). *L. kmetyi* is a pathogenic species, native to Malaysia, and was first described in 2009 isolated from soil in Johor, a southern state of Malaysia, followed by Kelantan, northern state, and now in central Malaysia [19, 20]. From the above-mentioned studies, it is clear that the pathogenic strains of *L. kmetyi* is ubiquitous present in Malaysian soil and water and may play a significant role in human infection. Thus, it has become imperative

**Fig. 1** Phylogenetic analysis of the 443-nucleotide region of *rrs* for the *Leptospira* species isolated from rodent and environmental samples collected from the outbreak site. HPS, isolate from soil; HPW, isolate from water; HPA, isolate from the animal. Isolates with *Leptospira* species names are obtained from GenBank (NCBI-NIH) for reference



to investigate the possibility of human infection due to the intermediate *L. wolffii* and *L. kmetyi* which have both been isolated from environmental samples [21, 22].

It is interesting to confirm again that *L. interrogans* and *L. borgpetersenii* as the species colonizing rodent kidneys as in agreement with earlier reports in Malaysia [23]. The available MAT data from the patients identified two serovars, autumnalis and hardjobovis. A dominant clone of *L. interrogans* Autumnalis ST34 associated with human leptospirosis outbreak in the neighboring country Thailand cautions the circulation of similar clones in Malaysia [24].

In conclusion, two *Leptospira* species from rodents (*L. interrogans* and *L. borgpetersenii*) and two from the environment (*L. kmetyi* and *L. wolffii*) as well as their corresponding serovars (autumnalis and hardjobovis) from humans were successfully identified. The possible epidemiological link between human serovars and animal *Leptospira* species indicates rodents as the potential reservoir while the environment (soil and water) serves as the transmission route. To date, there is no available vaccine covering all serogroups of pathogenic *Leptospira* for both human and animal use [25]. Nonetheless, the identification of leptospirosis as the cause of this outbreak

among military recruits in Perdik should remind us of the epidemic potential of this disease, and its correspondence with certain established epidemiologic scenarios. The main recommendations from the study include wearing proper clothing including shoes or boots, using gloves while working with mud and water, avoid swallowing water while swimming, reduce the contact time with water, ensure no wounds or cut in the body surface during the activities, and practice proper prophylaxis prior to the activity. Most importantly, occupation risk population should have awareness about leptospirosis and promptly report to the consultant when any sign of illness observed within 2 to 30 days of such activities as febrile illness often have diagnostic challenges in the tropics.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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