

Leptospirosis on a Rafting Tour in Thailand

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Summary

Tourists who are exposed to possibly contaminated water are likely to contact leptospirosis, especially in activities such as rafting.

Abstract

There have been reported cases of leptospirosis among foreign travellers in Thailand infected while on eco-tourism programs such as rafting tours. The current study investigated the leptospirosis cases in 2007 returning from a rafting trip along a flooded river in a southern Thai province. This study aimed to assess the risk factors amongst tourists on a rafting trip and tour guides at risk in the Sohl district of Thailand. Microscopic agglutination test (MAT) among tour guides and animals found along the river were also conducted. Risk for illness among the rafting participants showed no true association with open wounds during rafting (RR 1.96, 95% CI 0.37-10.43) and being submerged in water upon falling into the river (RR 3.69, 95% CI 0.78-17.53). The sero-prevalences were 59.8% among the guides, 76.2 % for the cattle, 68.2% among the dogs, and 100 % for the frogs. Of the infected guides, most were infected with *Leptospira interrogans* serovar Shermani and Bratislava, which were also present in the sera of cattle, dogs and frogs. The PCR assay for pathogenic strains was positive in kidneys of 1/13 frogs (7.7%) and 5/33 bats (15.2%). *Leptospirae* could not be purely isolated from all of the specimens.

Introduction

Leptospirosis is a zoonotic disease that can be acquired from infected animal reservoirs. In Thailand, the primary reservoir animals are rodents *Leptospira* can survive best in soil/mud and water with temperatures between 28-30°C for 2-6 weeks but can be eliminated in dry soil and sunlight within 3 hours. Essentially, humans usually become infected by con-tact of the skin, especially if abraded, or of mucous membrane with water, moist soil or vegetation contaminated with urine of infected animals.¹

The incubation period is approximately 10 days from 4 to 19 days.² In some areas where leptospirosis is common, most of the infected population may have no symptoms at all or few symptoms, which are hard to diagnose.^{3,4} About 41% of those who have contact with a contaminated environment will be infected and 40% of the infected persons show symptoms.^{5,6} Therefore, only 16% of those who have contact with a contaminated environment manifest symptoms.

The transmission of disease was found among water-sport enthusiasts (e.g., rafting). Fresh water swimmers (waterfall, lake or river), specifically triathlon participants, have also been found to be infected continuously. In a 2007 medical report in Hong Kong,⁷ a tourist was found infected in Pa Ton Parivart Wildlife Sanctuary of Pungnga. In 2006, a German patient in the northern part of Thailand was found to be infected,⁸ and in 2001 a Dutch traveler was diagnosed with leptospirosis.⁹

Materials and Methods

Retrospective cohort study of rafting participants 44 officers and lecturers of Prince of Songklanagarind University who traveled in La Ghu, Stul were investigated. The program tour included a waterfall tour, cave exploring and rafting. Blood samples were collected from 38 of these travellers to detect anti-bodies by indirect immunofluorescence assay (IFA). Leptospirosis was identified as follows:

- Suspected leptospirosis patients who had fever, headache or muscle pain that started from December 2007 to January 2008, and returned from recreation in La Ngu, Stul in December 2006.
- Confirmed leptospirosis patients refer to suspected cases whose antibody titers, evaluated by the IFA technique, was > 1:200 for single samples or a 4-fold raise on the second sample (paired sera). Some cases without any symptom were found to have titer > 1:400.
- Epidemiological data were collected and analyzed in order to recognize the risk factors of infection in patients who returned from travel in December 2006. Relative Risk (RR) and 95% confidence interval was calculated.

In order to determine the possible sources of leptospirosis, the environment and animals within the vicinity were also investigated. This was done by tracing the routes of the tour and by collecting samples from soil in 8 locations, freshwater from 6 sources, 13 frog kidneys, 5 mice, 33 bats and 6 of bat urine samples. Polymerase chain reaction (PCR) using 16S rRNA gene was performed on the environmental and animal samples. These samples were also cultured to detect for the presence or absence of *Leptospira*.

Cohort Study on the prevalence of Leptospira infection amongst rafting and river guides

225 participants amongst rafting and cave guides in the Stul area volunteered to participate in this study. Of these 87 guides were selected by purposive sampling method. The guides consisted of 59 rafting guides, 7 guides working in Phupapetch Cave and the rest worked in both activities. The sera of these subjects were tested for the presence or absence of anti-bodies against 24 *Leptospira* serovars by microscopic agglutination test (MAT). A positive result was set at a titre of 1:100 or greater.

Further blood samples were collected from 21 cat-tle, 22 dogs, 3 frogs and 1 cat in suspected areas of infection in Stul. The sera taken were tested using MAT.

Results

Study on rafting participants

Of the 44 travellers 2 who returned became sick, had fever, chill, severe headache and were diagnosed as being infected with leptospirosis. Thirty-eight officers consisted of 23 females (60.5%) and 15 males (39.5%). The median age was 41 (age range was 13-59 years old). Investigation reported that 7 officers (2 males, 5 females) were suspected of having *Leptospira* infection and their symptoms also fit in the definition of suspected leptospirosis patients. Median the age of the suspected cases was 49 (13-53 years old). The median incubation period was 18 days (4-20 days), as shown on Figure 1.

After the rafting tour, the patients presented with fever, and at least another symptom. Most of the 7 patients had severe headache (71%), muscle pains (57%), vomiting (57%), and other symptoms as shown in Figure 2.

Based on the result of antibody analysis by IFA technique, 6 patients were found to have a titer \geq 1:200. Interestingly, 2 of the 6 had 4-fold raise in anti-body analysis and 1 did not have any symptom but *Leptospira* infection was indicated by 1:3,200 titre. Risk factor analysis indicated that the travelers wore non-protective clothes, such as shorts and T-shirt, that made them prone to injuries. Furthermore, the tourists did not take any prophylactic medicines. Most of the travelers got wounds on knees and legs during the rafting and there was one who got wounded on the face. Those who got wounds did not clean their wounds, and showered with freshwater in resort. (Freshwater was taken from a canal without any treatment). Some were immersed (choked) and during 2 hours of the rafting because of a boat sinking. Based on the results of this study, having skin wounds and immersion in water are potential risks of infection (Table 1). There were no statistically significant differences however observed between these risks and infection.

One risk factor that may have caused leptospirosis among the tourists was that all of them were wearing shorts. Also, they had to walk bare foot through 5-10 meters of sharp rocks. Furthermore, 3 dogs and 1 cow were found along the canal. Additionally, there were bats in the humid cave, where the travellers had to pass through bat urine and excrement. The guides often suggested the tourists to gather under the water dripping point, to wipe their bodies and faces with flowing water. It is unlikely however to acquire wounds from the cave trekking on the grounds of having safe paths. Moreover, tourists hardly got any wounds or accident from swimming in the waterfall as the waterfall is small and not many people spend much time in it.

Samples were collected from bats, rats, soil, and water from Phupapetch Cave and the white water rafting canal. These samples were examined by using a dark field microscope. Then, the PCR method was conducted and the results are shown in Table 2. *Leptospira* could not be purified from any of these cultures.

Study on the Guides

Fifty-two of the 87 guides (59.8%) were positive by MAT. These MAT-positive cases consisted of 42 men and 10 women. The median age of the cases was 20.5 years old (age range was 13-57 years old). Table 3 shows the distribution of the guides according to their workplace. All the guides did not experience any fever, shivering or muscle pains. The outcome of *Leptospira* antibodies test in the guides, who worked in the outbreak location related to the dogs and cattle within that zone. The highest serovars of *Leptospira* spp were found Shermani and Bratislava as shown in

Table 5.

Results from animal blood samples

Results of antibody detection among animals in Ma Nang and La Ngu districts showed that 15/22 dogs (68.2%), and 16/21 cattle (76.2%) were positive MAT-positive (Table 4). All 3 frog samples had *Leptospira* antibodies.

Discussion

Tourists who are exposed to possibly contaminated water are likely to contact leptospirosis, especially in activities such as rafting by that they could cause wounds. Nevertheless, reports from the Ministry of Health still have flaws in the exact numbers of tourists who are infected since most of the travellers are not local people. It is quite hard therefore, to follow up Thai travellers. On the other hand, most of the reports regularly come from overseas, stating that foreign tourists are infected from travelling in Thailand.⁷⁻⁹

The most risky activity that tourists can engage in and may make them prone to leptospirosis, is rafting. In this activity, tourists are likely to have cuts or wounds and they may be submerged in water for a long time. Aside from rafting, a cave is also a risky location for its humidity, darkness, and presence of many reservoirs such as bats.

It appears that bats are contagions, showing by the evidence of PCR test in the guides that work in the caves are more likely to be infected by *Leptospira* (Table 3) comparing with guides in other locations apart from rafting activities.

Additionally, rafting is considered to accidentally cause people wounds to drink water from the infection source, though this issue has not been proven statistically owing to limited numbers of samples. There are some noticeable probabilities and trends which have to be researched further including locations and risk of the infection.

By conducting a MAT test in seeking antigen in 24 serovars of *Leptospira* the 52 guides have contracted the same pathogen *Leptospira interrogans* serovar Shermani and Bratislava. By using descriptive paradigm, this leptospirosis outbreak has the same location in which dogs, cattle and frogs are found to have the same antibody. Nonetheless, the antibody to serovar was not done by MAT in the tourists.

Interestingly, individuals who lived in endemic areas and exposed to *Leptospira* in daily lives had high prevalence of seropositivity but low prevalence of severe leptospirosis.^{10,11} It is possible that naturally acquired antibodies provide protection in these people. In this study, we have demonstrated that 55.9% of guides in flooded areas were seropositive for *Leptospira* antibody. However, they did not demonstrate leptospirosis manifestations. As suggested by several reports,¹²⁻¹⁶ the pre-exposure to *Leptospira* in their daily lives could induce antibodies to the organism which provide protection during later *Leptospira* exposure.

Nevertheless, many *Leptospira* serovars found in dogs, cattle, frogs or guides will not get us to the assumption that it is the main reason of the outbreak. It only tells us about how many these animals are infected or the number of serovars that are infected and might also be involved in the infection in people. Even though, this research may not tell the species of serovars in *Leptospira*, which found in bats rats and frogs in consequence of the limited sample of bat and frog blood but the solution to this issue is to use PCR testing in the bat, rat and frog kidney sample. The bat kidney sample could tell us the fact that bats can release the disease to the atmosphere.¹⁷ Besides, the frequency in discovering *Leptospira* in this case is dramatically high with 15.2% comparing to the *Leptospira* in the Amazon location, which is only 3.4%.¹⁸ This might be the reason why leptospirosis is found mostly in guides who work in the cave, comparing to guides who work in other venues. However, it is still to be debated whether bats are the main cause of outbreak due to the lack of capability in the disease testing process. Serovars infecting reptiles and amphibians (frogs) have not been shown to infect humans but have been suspected in Barbados and Trinidad. In carrier animals, an asymptomatic infection occurs in the renal tubules, with leptospiuria persisting for a long period or, especially in reservoir species, for life.¹

Conclusion

There was an outbreak of leptospirosis in the traveller attractions in La Ngu and Manang district in Stul province. Proved by the infected historical data finding in the guides and animals around the location. Guides are infected by the same type of *Leptospira* serovar Shermani and Bratislava as well as in cattle and dogs. We may not assume that these animals are the main contagion, because bats and frogs are also contagion. It is yet to be proven that the bat was the main reason of this outbreak.

Recommendations and Implementations

Since leptospirosis is a threat to humans and especially, in tourist attractions, makes this incidence much more harmful. Hence, there should be solutions and preventive measures to protect people from the disease. Fundamental solutions are:

- 1 Sanitation of resorts and communities around the canal should be improved. Maintaining sanitation of restrooms and refuse areas will keep away contagious animals.
- 2 Guides and tourists should be educated about rafting activity risks, appropriated outfits and emergency first aid in order to lessen the chance of being infected with leptospirosis.
- 3 Guides should be educated about misunderstood beliefs, which lead to insanitary behaviours. For example, the guides should not tell the travellers to gather the water dripping from caves to wash faces or bodies.
- 4 The registration system of sickness awareness should be improved by registering guests at the hotels and follow up if any guest is sick.

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Table 1
Risk factor analysis of the officers of Prince of Songklanagarind in travelling path

Risk Factor	Risk Factor		Non- Risk Factor		Crude RR (95%CI)
	Sick	Not Sick	Sick	Not Sick	
Waterfall*	5	20	1	8	1.80 (0.24-13.40)
Cave*	5	22	1	6	1.30 (0.17-9.38)
Rafting	6	27	0(1)**	5	Undefined 1.09 (0.16-7.52)**
Got wounded before or while doing activities***	3	13	2	19	1.97 (0.37-10.43)
Immersion or drinking water rafting ***	4	9	2	22	3.69 (0.78-17.53)

Note: *4 people lacked information **substitute 0 with 1***1 person lacked information

Table 2
The prevalence of Leptospire found by conducting a PCR test in bats, rats, soil, and water in travellers on rafting in La Ngu and Ma-Nang district, Stul 2008

Species	Specimen	Samples	Samples positive <i>Leptospira</i> spp (%)	Pathogenic leptospire (%)	Non-Pathogenic leptospire (%)
Bat	Urine	6	2 (33.3)	0/8 (0.0)	2/6 (33.3)
	Kidney	33	26 (78.8)	5/33 (15.2)	21/33 (63.6)
Frog	Kidney	13	3 (23.1)	1/13 (7.7)	2/13(15.4)
Rat	Kidney	5	3 (60.0)	0/5 (0.0)	3/5(60.0)
Environment	Soil	8	7 (87.5)	0/8 (0.0)	7/8 (87.5)
Environment	Water	6	6 (100.0)	0/6 (0.0)	6/6 (100.0)

Table 3
The result of *Leptospira* antibodies by MAT in guides at La Ngu and Ma Nang, Stul province, 2008 divided by workplace

Guides' Workplace	Infection Rate (%)
Phupapetch cave	5/7 (77.4%)
Float raft downstream	11/59 (18.6%)
Phupapetch cave + Rafting	7/21 (33.3%)

Table 4
Results of MAT-positive (%) from serum samples among species examined in Stul province, 2008.

Species	No. of serum samples examined	No. of MAT-positive (%)
Cattle	21	16 (76.2)
Dog	22	15 (68.2)
Cat	1	0 (0.00)
Frog	3	3 (100.0)
Human	87	52 (59.8)

Table 5
Numbers (percentage) of each species examined, based on infecting serovar as determined by microscopic agglutination test (MAT), Stul province.

Serovars	Animals				
	Cattle	Dog	Cat	Frog	Human
Bratislava	0 (0.0)	5 (22.7)	0 (0.0)	3 (100.0)	40 (46.0)
Autumnalis	0 (0.0)	3 (13.6)	0 (0.0)	0 (0.0)	12 (13.8)
Ballum	1 (4.8)	1 (4.6)	0 (0.0)	0 (0.0)	1 (2.4)
Bataviae	1 (4.8)	1 (4.6)	0 (0.0)	0 (0.0)	0 (0.0)
Canicola	0 (0.0)	1 (4.6)	0 (0.0)	0 (0.0)	0 (0.0)
Cellidoni	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)
Cynopteri	0 (0.0)	1 (4.6)	0 (0.0)	0 (0.0)	0 (0.0)
Djasiman	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)
Grippotyphosa	1 (4.8)	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.4)
Hebdomadis	1 (4.8)	3 (13.6)	0 (0.0)	0 (0.0)	6 (6.9)
Icterohaemorrhagiae	0 (0.0)	3 (13.6)	0 (0.0)	0 (0.0)	8 (9.2)
Javanica	0 (0.0)	2 (9.1)	0 (0.0)	0 (0.0)	1 (1.2)
Louisiana	0 (0.0)	1 (4.6)	0 (0.0)	0 (0.0)	1 (1.2)
Manhao	0 (0.0)	1 (4.6)	0 (0.0)	0 (0.0)	0 (0.0)
Mini	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.4)
Panama	0 (0.0)	2 (9.1)	0 (0.0)	0 (0.0)	2 (2.3)
Pomona	0 (0.0)	1 (4.6)	0 (0.0)	0 (0.0)	1 (1.2)
Pyrogenes	1 (4.8)	3 (13.6)	0 (0.0)	0 (0.0)	4 (4.6)
Ranarum	16 (76.2)	6 (27.3)	0 (0.0)	0 (0.0)	0 (0.0)
Sarmin	0 (0.0)	1 (4.6)	0 (0.0)	0 (0.0)	1 (1.2)
Sejroe	0 (0.0)	2 (9.1)	0 (0.0)	0 (0.0)	2 (2.3)
Shermani	16 (76.2)	15 (68.2)	0 (0.0)	3 (100.0)	39 (44.8)
Tarassovi	0 (0.0)	1 (4.6)	0 (0.0)	0 (0.0)	3 (3.4)
Patoc	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Figure 1
Number of patients as to the first day of illness.

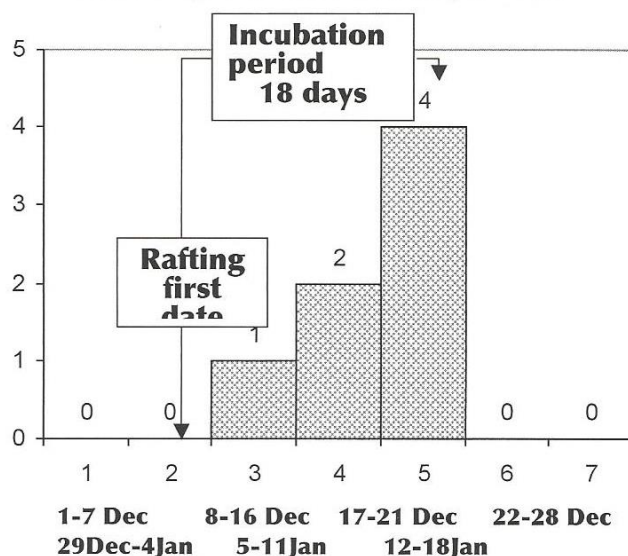


Figure 2
Signs and symptoms of seven suspected leptospirosis patients who returned from the rafting tour in December 2006, La Ngu, Stul province.

