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Epidemiological Investigation of Visceral Leishmaniasis Caused by *Leishmania martiniquensis* in a Non-endemic Area of Thailand

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Abstract

On 5 Sep 2013, a patient suspected to have leishmaniasis from Lamphun, a northern province of Thailand, was reported to the Bureau of Epidemiology. An investigation was carried out to identify reservoirs and vectors. Active case finding was carried out among those who lived within 200-meter radius from the patient's residence. Blood samples were collected from humans, domestic mammals and rodents, and adult sandfly trapping was conducted by light traps. The patient was a 38-year-old man infected with human immunodeficiency virus (HIV) who had worked as a lumberjack and a woodcraftsman. In addition to many kinetoplasts in the bone marrow and amastigote form of *Leishmania*, *Leishmania martiniquensis* was identified by polymerase chain reaction (PCR). A total of 12 suspected cases out of 123 surveyed villagers were identified. However, all human specimens were tested negative by direct agglutination test and PCR. Female *Sergentomyia* sandflies were found. Deltamethrin fogging was done to control the vectors. Visceral leishmaniasis was confirmed in an HIV positive male from northern Thailand. Awareness of leishmaniasis among immunocompromised people should be raised for timely appropriate medical attention and specific vector control for sandflies in the area should be implemented.

Keywords: leishmaniasis, kala azar, *L. martiniquensis*, northern Thailand

Introduction

Leishmaniasis is a vector-borne zoonosis which is caused by intracellular protozoa, *Leishmania* species. There are two main forms of leishmaniasis, namely cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL or kala azar). CL often involves only skin with painless papules. Common symptoms of VL are prolonged fever, weight loss, signs of bone marrow invasion (anemia, thrombocytopenia and leucopenia), abdominal distension with hepatosplenomegaly, and lymphadenopathy.[1] In Thailand, VL was first reported in 1960 among south Asians who were probably infected from abroad. During 1998-2010, there were 43 CL cases and 13 VL cases reported in Thailand, including autochthonous and imported cases. At least six of the 13 VL cases were autochthonous and most were reported from the southern Thailand.[2]

Both humans and animals could be reservoirs, depending on type of *Leishmania* species. The parasite is transmitted by sandflies. Among over 1,000 known species of sandflies, about 70 female

sandfly species can transmit *Leishmania* spp.[3], especially *Plebotomus* and *Lutzomyia* genera. Sandflies tend to bite humans and animals in the evenings within 200 meters from its breeding site.[4]

On 5 Sep 2013, the Bureau of Epidemiology, Ministry of Public Health, Thailand, received a notification from a local authority that a suspected leishmaniasis patient was admitted to a university hospital in Chiang Mai, a northern province of Thailand. We conducted an investigation in collaboration with the local authorities. The objectives of the investigation were to confirm the diagnosis of the reported patient; identify other possible cases through active case finding, reservoirs and vectors; institute control measures; and provide recommendations for prevention of future infection.

Methods

Epidemiological Investigation

We reviewed the medical records, and conducted interview and physical examination of the patient. Active case finding was performed among neighbors who lived within 200-meter radius from the patient's residences in two villages (Villages A and B) since onset of the illness. Information on gender, age, nationality, occupation, signs and symptoms of leishmaniasis, travel history, and behaviors related to insect bites was obtained by face-to-face interview. They were also physically examined and blood samples were collected for direct agglutination test (DAT) and polymerase chain reaction (PCR).

A suspected case was defined as a person who had at least one of the followings: wound or ulcer lasting more than one month, fever or feverish for more than six weeks, anemia, hepatomegaly or splenomegaly, or weight loss. A probable case was a suspected case with positive DAT for *Leishmania* species. A confirmed case was a suspected or probable case with positive result for *Leishmania* spp. by PCR, Giemsa stain of wound or ulcer biopsy, or amastigote form in bone marrow.

Reservoirs and Potential Vectors

An environmental study was performed by surveying the patient's residences in Villages A and B, including the presence of foreign workers in those villages. In addition, blood samples were collected from villagers and domestic mammals such as cow, dog, cat, rabbit living within 200-meter radius from the patient's residence. Serum and blood samples were tested by DAT and PCR respectively, while DAT of more than 1:100 for *Leishmania* spp. was considered as positive.

Furthermore, we set up 60 live rodent traps in Village A and 30 live rodent traps in Village B for two consecutive nights from 6 pm to 7 am. Traps were placed around 20 meters apart, starting from the patient's residences. Banana or fish was used as bait.

We also laid light traps at six locations in each village from 6 pm to 5 am, once during the rainy season and once in the cold season. Female sandflies caught were sent for PCR testing for *Leishmania* species. The density of trapped sandflies was calculated by the following formula.

$$\text{Trapping rate (TR)} = \frac{\text{Number of trapped sandfly}}{\text{Number of light trap} \times \text{Night}}$$

Results

Case Investigation

A 38-year-old Thai male with human immunodeficiency virus (HIV) infection had been under medication for antiretroviral therapy with zidovudine 250 mg, lamivudine 150 mg and nevirapine 200 mg. He developed itchy papules on both hands for the first time in 2010, and later slowly spread to elbows, feet, legs, knees, body and ears bilaterally. He had no fatigue or fever at that time. During 2011-2012, he developed sclerodactyly (Figure 1) and visited a community hospital where leprosy was ruled out by slit skin smear and he received symptomatic treatment.

Figure 1. Skin manifestations (a) hands with sclerodactyly, (b) elbow and (c) legs of a visceral leishmaniasis case in Mae Tha District, Lamphun Province, Thailand, 2013

On 2 Jul 2013, he was referred to a university hospital and his skin biopsy was tested positive for fungus, possibly histoplasmosis. Although he received 10% urea in triamcinolone acetonide cream,

clobetasol cream and salicylic acid 40% ointment, his lesions showed no improvement. He was admitted to the same university hospital on 23 Aug 2013 as the dermatologist suspected that his chronic skin lesions were caused by leishmaniasis. Physical examination on admission to the hospital revealed multiple non-tender hyperpigmented nodules on legs, forearms and hands, with deformity of fingers (sclerodactyly). In addition, bone marrow biopsy on 27 Aug 2013 showed many kinetoplasts and amastigote form of *Leishmania* spp. (Figure 2), which were later identified as *Leishmania martiniquensis* by PCR.[5] After receiving intravenous amphotericin B 60 mg daily for 15 days, follow-up bone marrow biopsy found no *Leishmania* species.

Regarding the patient's exposure history, he lived in Village A and worked as a lumberjack in a nearby forest for 10 years until 2010 and later worked as a woodcraftsman for two years. While working in the forest which was about three kilometers far from his house, he used to wear long sleeves and long pants. Then, he moved to Village B where he worked as a woodcraftsman for a year. Village B is located about 30 kilometers from Village A. In March 2013, he moved back to Village A where he was currently living with his 82-year-old mother and a 10-year-old daughter. Village A is situated in Pong Mae Lob Sub-district while Village B is in Tha Tung Luang Sub-district of Mae Tha District, Lamphun Province.

He had no history of injection drug use (IDU) or blood transfusion. He and his family had never traveled to the southern Thailand or abroad.

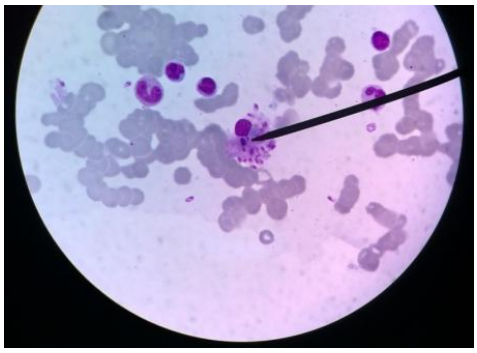


Figure 2. Amastigote form of *Leishmania* spp. in white blood cell from bone marrow biopsy of a visceral leishmaniasis case in Mae Tha District, Lamphun Province, Thailand, 2013

Active Case Finding and Behavior Risk Assessment

In Village A with the patient's current residence, there were 106 villagers living within 200-meter radius. Among them, 88 (83.0%) from 36 households were interviewed and examined, including 47 (53.4%) males and 41 (46.6%) females. The median age was 49 years, with the range of 10-91 years. All of them were Thai, with the main occupation as farming (51.1%). Of 11 participants who met the suspected case definition, all were tested negative for *Leishmania* by both DAT and PCR. Behavior risk assessment showed that 51 (58.0%) villagers always protected themselves from insect bites using mosquito nets while sleeping, 44 (50.0%) villagers sometimes used insect repellant when going outside, and 50 (56.8%) villagers used indoor insecticide spraying.

In Village B where the patient lived previously, 46 villagers lived within 200-meter radius from the patient's house and 35 (76.1%) from 17 households were enrolled in the investigation. There were 21 (60.0%) females and 14 (40.0%) males. The median age was 53 years (range 12-78 years). They were Thai who were mainly working as woodcrafts (48.6%). Only one suspected case was identified and *Leishmania* spp. was not found in her blood samples by both DAT and PCR. Moreover, 17 (48.6%) participants never used mosquito nets while sleeping, 18 (51.4%) never used insect repellant when going outside, and 21 (60.0%) used indoor insecticide spraying (Table 1).

In both villages, none of them had traveled to the southern Thailand or abroad in the past two years. There was no history of IDU or blood transfusion in any participants.

Environmental Study

The patient's house in Village A was a one-storey wooden house which was built eight years ago, located one meter above the ground and surrounded by fig trees, with plenty of longan plant sprouts in plastic bag pots underneath the house. There was also a cottage situated nearby to store sculptures. There was a neighbor's cattle pen 30 meters away from his house as well. Inside his bedroom, there was a long-lasting insecticide impregnated net that he received two years ago. His sister's house, a one-storey cement house with a grocery store, was approximately 500 meters away. Before he developed symptoms, the patient visited his sister's shop every evening after logging trees in the forest and stayed for 1-1.5 hours.

In Village B, the house that he had stayed was a renewed one-storey cement house with two bedrooms, which was adapted from a wooden house. The mosquito-screened windows in the house were torn and three windows were always left opened.

No foreign worker or people from the southern Thailand had visited, lived or worked in these two villages during the past two years.

Table 1. Characteristics of villagers who lived within 200-meter radius from a visceral leishmaniasis case in Mae Tha District, Lamphun Province, Thailand, 2013

Variable	Village A (n = 88)		Village B (n = 35)	
	Number	Percent	Number	Percent
Gender				
Male	47	53.4	14	40.0
Median age in year (range)	49 (10-91)		53 (12-78)	
Thai nationality	88	100.0	35	100.0
Occupation				
Farming	45	51.1	5	14.3
Housekeeper	11	12.5	5	14.3
Woodcraftsman	1	1.1	17	48.6
General laborer	12	13.6	1	2.9
Student	7	8.0	4	11.4
Others	12	13.6	3	8.6
Protection behavior				
Using mosquito net				
Never	28	31.8	17	48.6
Sometimes	7	8.0	3	8.6
Always	51	58.0	15	42.9
Unknown	2	2.3	0	0
Using insect repellent				
Never	24	27.3	18	51.4
Sometimes	44	50.0	11	31.4
Always	18	20.5	6	17.1
Missing	2	2.3	0	0
Insecticide spraying				
No	36	40.9	14	40.0
Yes	50	56.8	21	60.0
Unknown	2	2.3	0	0

Table 2. Potential reservoirs and laboratory results of villagers who lived within 200-meter radius from a visceral leishmaniasis case in Mae Tha District, Lamphun Province, Thailand, 2013

Pontential reservoir	Village A				Village B			
	Total	Sample collected	DAT*	PCR†	Total	Sample collected	DAT*	PCR†
Human	106	77	0	0	46	34	0	0
Domestic animal								
Dog	20	18	18	0	0	0	-	-
Cat	3	0	-	-	0	0	-	-
Cow	2	1	1	0	0	0	-	-
Rabbit	2	1	1	0	0	0	-	-
Rodent	NA	2	-	0	NA	0	-	-

*DAT: Positive result by direct agglutination test

†PCR: Positive result by polymerase chain reaction

Reservoirs and Potential Vectors

Total 77 (87.5%) people in Village A and 34 (97.1%) people in Village B without symptoms were all tested negative by DAT and PCR.

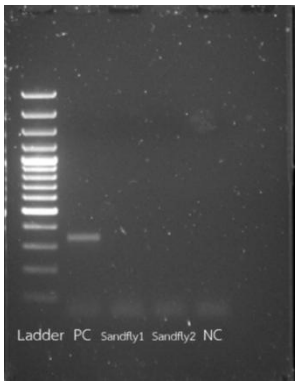
In Village A, all 20 blood samples of 18 dogs, one cow and one rabbit revealed positive DAT (Antibody titer >1:100). However, all PCR results from 20 samples were negative for *Leishmania* species. PCR results of two rodents trapped showed negative as well (Table 2). No domestic animal, rodents or sandflies were trapped in Village B.

To identify the potential vectors, the first trapping was performed just after the case investigation which was during the rainy season. In Village A, sandflies were captured in three traps, including one male sandfly (TR = 0.2 per trap-night) from a trap placed at the patient's workplace and two female sandflies (TR = 0.3 per trap-night) from a trap under the

(a) (b) (c)

Figure 3. Locations of the light traps (a) under patient's house, (b) patient's workplace, (c) cattle pen of neighbor's house that captured sandflies in the village of a visceral leishmaniasis case in Mae Tha District, Lamphun Province, Thailand, 2013

patient's house and a trap in neighbor's cattle pen (Figure 3). All sandflies were identified as *Sergentomyia* species. Thoraxes of female sandflies were tested by PCR and negative for *Leishmania* (Figure 4). Trapping was repeated for Village A during the cold season and two more male *Sergentomyia punjabensis* (TR = 0.5 per trap-night) were identified.



Remark:
PC = positive control
Sandfly1 = sandfly captured under the patient's house
Sandfly2 = captured from cattle pen
NC = negative control

Figure 4. Result of polymerase chain reaction amplification of a visceral leishmaniasis case in Mae Tha District, Lamphun Province, Thailand, 2013

Public Health Actions

Health education on clinical symptoms, reservoirs and prevention measures for leishmaniasis were provided to headmen, health volunteers and villagers in the villages to reduce panic and stigmatization to the patient and his family. We encouraged the communities to improve environmental sanitation in the area and cooperate with the local authorities to control adult vector by deltamethrin fogging.

Discussion

Visceral leishmanisis was confirmed in a man with HIV from Lamphun, a northern province of Thailand. No additional cases were found through active case finding. There were no human or animal reservoirs found in this study. Five *Sergentomyia* sandflies were identified.

This was an unusual event since leishmaniasis was usually found in the southern part of Thailand[2]. Only three cases were reported from the northern part during 1996-2012, with the latest case reported in Chiang Rai Province during 2012[2,6].

The case identified in this study was similar to the second leishmaniasis case reported from Chiang Rai Province in 2012 who also had underlying HIV infection and history of working in the forest and bamboo jungle for 4-5 times per month[6]. Immunocompromised hosts with HIV/AIDS are at higher risk of developing leishmaniasis when they contract the infection.[3,7] Source of the outbreak might not be in the community because we found no evidence of leishmaniasis from reservoirs and vectors. However, the evidence of sandflies in the village should be known as potential risk area. Though history of lumberjack working was likely to be associated with exposure to the parasite in the forest, other risk factors should be considered as well since the incubation period of leishmaniasis ranges from weeks to months[8]. The case might be using the mosquito net at home. However, as sandflies are 1.3-3.5 mm in size which is about one third of mosquitoes[2,9] (Figure 5), pores of the mosquito net cannot protect from sandflies.[4,8]

Figure 5. Photo of *Culex* mosquito (top) compared with a sandfly (bottom)

The diagnosis of VL was obtained by amastigote form of *Leishmania* spp. in bone marrow. Despite the fact that the result of skin biopsy revealed negative, CL could not be ruled out as *Leishmania* spp. could escape from necrotic tissue.[10]

We found female sandflies of genus *Sergentomyia* which can suck blood from humans and animals. However, there was no strong evidence that it could act as a vector for this disease while other genera such as *Phlebotomus* and *Lutzomyia* are known effective vectors in transmitting *Leishmania* parasites.[1] In Thailand, *Leishmania martiniquensis* was recently found.[11] Nevertheless, there was no evidence on the relationship between *Leishmania martiniquensis* and potential vector, which was *Sergentomyia* spp. in our study. Thus, we could not conclude that *Sergentomyia* spp. had a role in transmitting leishmaniasis.

Only few sandflies could be trapped as there was heavy rain during the first trapping and the thin wings of sandfly cannot tolerate flying through the rain. Despite the second attempt to trap in late cold season, the climate was drier than last year and this might lead to less number of sandflies than we expected. The second trapping was carried out only in Village A due to limited light traps available.

The antibody titer of all animal samples was more than 1:100 by DAT although PCR testing revealed negative. This might be caused by cross-reaction to other parasites such as *Trypanosome* spp. which belongs to the same *Trypanosomatidae* family as *Leishmania* species.[7]

In this investigation, a total of 90 live rodent traps were used. Despite that, only two rodents could be trapped in two villages after two nights. According to a study by Weihong published in 1999, rodents capture rate was the highest on the third day for grid trapping method.[12] Blood samples from rodent were sent only for PCR testing because of insufficient volume.

Our control measure by deltamethrin fogging could be appropriate to reduce number of sandflies.[13] Some studies have found that only 0.1% female phlebotomine sandflies are resistance to deltamethrin.[14,15]

The study was conducted on the weekday and it might be difficult for some villagers to participate in the study. In addition, due to limited field workers, survey on types of mosquito net that could protect from sandfly was not performed in these villages.

Conclusion

The results revealed that VL was confirmed in a HIV-positive patient by identifying *Leishmania* spp. in bone marrow biopsy in Lamphun, a northern province of Thailand. DAT and PCR testing of all suspected cases were negative. False positive of DAT was found in domestic animals. Only *Sergentomyia* sandflies were captured from the trapping. We recommended that people with symptoms such as chronic wound or ulcer, chronic fever, fatigue, abdominal discomfort and continuous weight loss should be made aware of leishmaniasis to receive appropriate investigation and medical attention, especially in immunocompromized people. Specific and effective vector control for sandflies in the area should be considered and implemented.

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We would like to thank all field workers and staff from Lamphun Provincial Health Office, Office of Diseases Prevention and Control (ODPC) for Region 10, and Center of Vector Borne Disease Control 10.5 in Phrae Province for their contribution. We also appreciate all the people in the studied communities for their collaboration.

Suggested Citation

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