Sandfly Distribution and Risks of Leishmaniasis Transmission in Kinmen

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From Chinese version, pp,269-278

Abstract:

A suspected case of kala-azar was reported by First Hospital in Xiamen City, Fujian Province, China. Kinmen County is an important harbor counterpart to Xiamen City, and has climatic and geographic similarities with Xiamen City. In order to evaluate the risk of leishmaniasis transmission in Kinmen, we studied sandfly distribution during May to July, 2007. The results showed that sandflies do exist in Kinmen County; they are *Sergentemyia barraudi*, which do not consume human blood. In addition, because there have been no reported cases of leishmaniasis in Kinmen, our initial evaluation showed that there is no immediate threat of *Leishmania* transmission. The environment in Kinmen is suited for sandfly to grow because there are lots of combat trenches and rodent caves. We recommend long term sandfly surveillance in collaboration with mosquito

Received: Feb 1,2008 ; Accepted: April 3, 2008

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investigation to provide the basis for future disease control policies.

Introduction:

Leishmaniasis is one of the infectious diseases requiring immediate attention identified by the World Health Organization (WHO). There are an estimated 2 million new cases in the world annually (1.5 million with cutaneous disease and 0.5 million with visceral disease), distributed in 88 countries (including North America, Central and South America, Europe, Asia, and Africa); 350 million people live in areas with disease transmission. Ninety percent of the cutaneous diseases occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia, and Syria; 90% of the mucocutaneous diseases occur in Bolivia, Brazil, and Peru; 90% of the visceral diseases (as known as kala-azar) occur in Bangladesh, Brazil, India, Nepal and Sudan [1].

Records of leishmaniasis in Taiwan began after World War II. A total of 22 cases of kala-azar were reported among Japanese soldiers who arrived in Taiwan from Shandong. There was no related research after that time until the Nationalist Government moved to Taiwan in 1949. At that time, around 2 million people, including soldiers, came to Taiwan, and cases began to appear in the medical journals. There were approximately 100 cases of kala-azar and post-kala-azar cutaneous leishmaniasis reported, occurring mainly in new immigrants from China (mostly soldiers). In 1970, there were two cases of leishmaniasis in aboriginals in Jianshi, Hsinchu County. Neither of them had travel history and the two lived only 100 meters apart. Dr. Zi-Chin Lian conducted research on sandfly distribution in that area and found that the sandflies there did not transmit *Leishmania*. There were also no animals with suspect symptoms in the area. Continued surveillance showed no additional

cases [3]. In 2005, a new case was reported in Fuhsing, Taoyuan County. In 2008, the parasite was identified as *Leishmania tropic* .

Leishmaniasis in China usually occur in the northwest. According to published data in 2005, new cases were found in Sinkiang, Gansu, Szechwan, Shaanxi and Inner Mongolia (prevalence rate = 0.59%). Kala-azar was rarely reported in the southeast. No cases had been reported for many years in Fujian Province, which is close to Taiwan. In 1989, Chinese researcher L.M. Chang and Y.G. Lung conducted research in 11 provinces in southern China and found sandflies in Fujian [5]. Therefore, if *Leishmania* were present, an epidemic might occur. In March, 2007, First Hospital in Xiamen City, Fujian Province, China, reported one kala-azar case. The patient did not have any travel history to affected areas (northwest China), and was suspected to have acquired the infection locally.

The geography and climate are similar between Kinmen County and Xiamen City. Kinmen County is the most important harbor counterpart to Xiamen City. There has been little investigation of vectors which may transmit *Leishmania* in Kinmen. To protect the health of people, Taiwan Centers of Disease Control conducted research on sandfly distribution in Kinmen County, to provide the basis to evaluate the possible transmission of *Leishmania.*

Literature Reviews:

Leishmaniasis is caused by *Leishmania* infection. *Leishmania* is transmitted by sandflies. Because *Leishmania* is difficult to categorize based on morphology, the disease, leishmaniasis, is categorized into cutaneous, mucocutaneous, and visceral types. The life cycle of *Leishmania* can be divided into two stages, promastigote and amastigote. After development in the female

sandfly gut, promastigotes migrate to the proboscis. When the sandfly takes a blood meal, promastigotes are injected into the skin. The amastigote will develop inside the host's macrophages and reproduce by asexual reproduction. As the host cell swells then bursts, amastigotes are released, in order to infect other cell. Some protozoa will remain in the skin, resulting in ulcers. Other protozoa may invade macrophages of internal organs, such as lymph nodes, bone marrow, spleen and liver, resulting in visceral leishmaniasis, also called kala-azar [6].

Diagnosis of leishmaniasis is based on clinical presentation and laboratory results. In the past, most laboratories relied on microscopy of bone marrow, spleen, or lymph nodes obtained through invasive procedures. Recently, diagnosis using antibody in the blood had been developed .Traditionally, treatment of leishmaniasis consisted of pentavalent antimonials or the more expensive amphotericin B; both needed to be administered in the hospital, which means higher cost. New treatment using mitefosine, an oral drug, is safe and cheap. It had become the new trend of treatment in affected areas.

The risk factors of *Leishmania* infection include the presence of vector and infected cases. Vectors may be sandflies that feed on human blood, animal blood, or both. In order to understand these, we needed to investigate sandfly distribution. Sandflies belong to the family *Phlebotomidae*, of which, there are around 500 species in the world. Approximately 30 of these can transmit *Leishmania*, and most of them are distributed in the tropical and sub-tropical area.

Sandflies are grayish-brown in color, covered by fine hair, and approximately 1.5 to 5.0 mm in length. They have a pair of wings on the notum; the wings are small and slender. When resting, the wings are raised at

45-degree angle, like hatchback roof. Sandfly metamorphosis is complete; other than its adult stage, sandfly egg, larva, and pupa are all developed in the soil. Sandflies like dark, moist soil, rich in organic material; soil in the house and wild rodent caves are ideal breeding places for sandflies. Different sandfly species in different regions and environments have different feeding patterns. For example, Chinese sandflies in the plains rarely feed on dog blood, but those in the mountain do feed on dog blood. The choice of host also differ: *Sergenteyia* feed on cold blooded animals mainly; *Phlebotomus* feed on blood of human and other higher class animals*; Idiophlebotomus* mainly feed on bat blood

The earliest sandfly report was written by Japanese Tokunaga. He found an uncategorized male sandfly in Tatung Township of I-lan. In 1966, Dr. Zi-Chin Lian used lights and animals as lures to collect sandflies in I-lan, Taipei, Kao-hsiung, Pin-tung, and Hua-lian. Dr. Lian found four species of sandflies, including *Phlebotomus barraudi, Phlebotomus kiangsuensis*, *Phlebotomus iyengari taiwanensis*, and *Phlebotomus squamipleuris.* Between 1995 and 1996, Taiwan National Institute of Preventive Medicine conducted field investigations in 16 townships of nine counties in Taiwan using the Malaise trap and light traps (including black light bulb light trap [BLB light trap], and ultra-violet light traps). BLB light trap was found to be most effective. Specimens collected were similar to what was found by Dr. Lian in 1966, that no *Leishmania*-transmitting sandflies were found [13]. Sandfly identification in the past was mainly based on microscopy. Currently, laboratories are using biotechnology methods for more precise identification. Recently, large-scale screening of sandflies for the presence of *Leishmania* had also been developed to estimate the risk of infection[14].

Materials and Methods:

(A) Investigation time and location: peak period for sandfly activity is from May to July. Investigation was conducted one day of each month in areas of Kinmen where the ground was moist, soft, had lush forest, or where rodent caves were abundant.

(B) Methods: Based on research by Ting-Hsiang Lin conducted in 1997, BLB light traps were hung 1.5 meters above ground, from the afternoon until 7:00 am the next day. Collected specimens were frozen to immobilize the insects. Larger insects, such as moths, were manually removed. Finally, sandflies and mosquitoes were selected from the sample of small insects.

(C) Specimen preservation:

1. Adult insect preserved in 70% alcohol.
2. Followed by soaking in 10% KOH solution for 12-16 hours.
3. Use distilled water to rinse off KOH four times, 20 minutes each.
4. Pour in 20% carbol fuchsin for staining; after 16 hours, pour in pure alcohol and allow specimen to set for 30 minutes.
5. Place specimen in 70%, 80%, 90%, then 100% alcohol sequentially for dehydration. Allow 30 minutes interval between each alcohol concentration.
6. Place in phenol.
7. Tease apart the head, thorax, abdomen, wings and genitals using a needle, then mount the specimen using Canada balsam; extract the mouthparts through the neck, place on the right side of the slide, and mount separately.
8. Allow specimen to air dry at room temperature before microscopy.

Results:

Kinmen County had a total area of 150,456 square kilometers, including 12 islands (Big Kinmen, Small Kinmen, Da-Dan, Erdan, East Peten, Peten, etc.) Most of the population lived on Big and Small Kinmen islands. Kinmen's port, Shuitou, is the counterpart to the port in China after the implementation of mini-three links between Kinmen and China in 2001. The investigation focused on Big Kinmen, an area of frequent access since direct link with China. Between May and July 2007, a total of 13 locations were investigated (Figure 1).

We captured a total of three sandflies. All three were *Phlebotomus barraud*, and were found in sampling location 7. This location was a park under the management of the Forestry Bureau, open for visitors. The 34 hectares park had dense forest and a pond. The study collected specimens once during each month of May, June, and July. In order to provide the power needed for the ovitrap lights, we put the ovitrap lights close to the park's front, central, and rear toilets (each approximately 300 meters apart). In May, the investigation found no sandflies. One female sandfly was found in the rear toilet trap of the park in June; the toilet was situated next to a large forest, and the soil near the toilet was soft. In July, in addition of the ovitrap lights, we also set up manual traps at night. We found one female sandfly near the central toilet and a male sandfly near the rear toilet. The central toilet was close to a large pond.

In addition, 1574 mosquitoes of 16 different species were collected (Table 1). It was interesting to had captured 70 *Anopheles sinensis* at locations 3 and 4. There were 537 *Culex tritaeniorhynchus* captured mainly from locations 3, 4, and 5. Locations 3 and 4 were both located in the shores of Shuitou, an important port for the Mini-three links. Location 5 was at the Kuningtou World

War II History Museum Scenic Area.

Discussion:

This investigation showed that there are *Sergentemyia barraudi* sandflies in Kinmen County. According to the literature, this sandfly does not feed on human blood. Because, there are also no cases of leishmaniasis in Kinmen County, our preliminary assessment is that there is no immediate threat of *Leishmania* transmission in Kinmen County. In November after the conclusion of this investigation, information from China's 24th National Pesticides and Plants Science and Technology Exchanges and Exhibition, the suspected case reported Xiamen City was diagnosed with and treated successfully for histoplasmosis by the Infectious Diseases Section of Huashan Hospital, a subsidiary of Shanghai Fudan University [15].

This investigation was subject to time and manpower constraints, therefore, we choose BLB light traps, the most effective method based on past experiences. We were unable to compare the effectiveness of BLB light traps to other methods of specimen collection. In addition, because of the short collection period and the limited number of sandflies collected, we were unable to test for protozoa. Long term surveillance is needed in the future for a comprehensive assessment.

Although sandflies were the focus of this investigation, we were also able to gather information on the distribution of mosquitoes in this area. *Culex tritaeniorhynchus* were found in large numbers, concentrated in locations 3, 4, and 5. This information could be used by the local government to prevent Japanese encephalitis.

In conclusion, the many trenches and rodents caves in Kinmen were

suitable breading ground for sandflies. We recommend long term sandfly surveillance in collaboration with mosquito investigation to provide the basis for future disease control policies.

Acknowledgment:

Many thanks to Dr. Ting-Hsiang Lin and Dr. Hwa-Jen Teng from the Vector Laboratories at the Centers for Disease Control, and Dr. Dar-Der Ji from the Parasitology Laboratory, who provided valuable information during the study. In addition, many thanks to colleagues at Kinmen Office, CDC First Branch, who provided much assistance, actively participated in negotiating with different agencies to set up ovitraps. Setting up ovitraps in appropriate locations was the key to the successful completion of this project.

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Figure 1

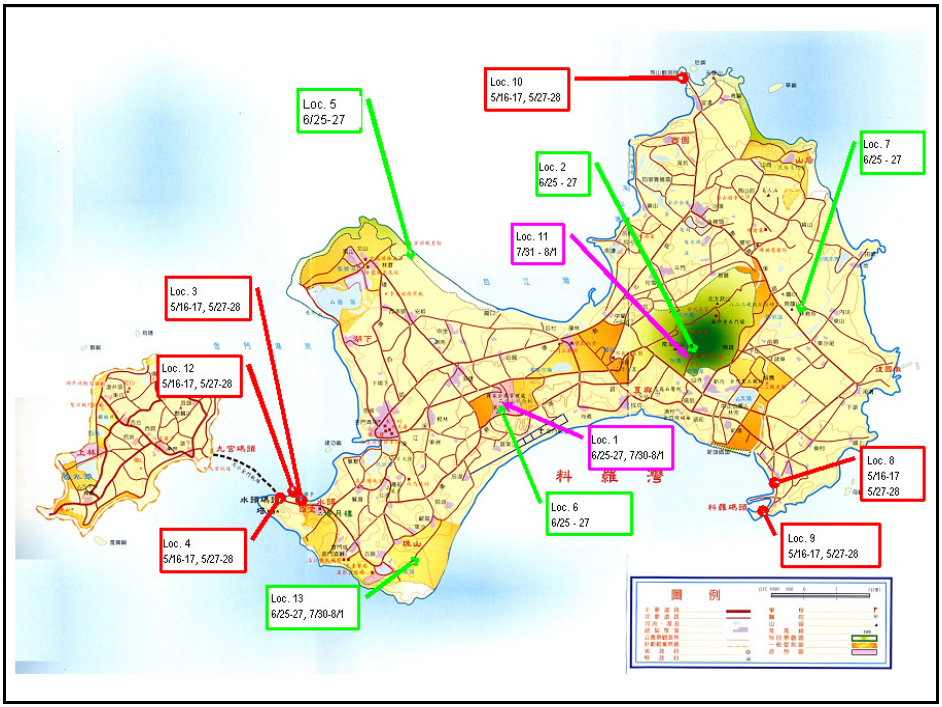
Loc. 10
5/16-17, 5/27-28
Loc. 5
6/25-27
Loc. 2
6/25 - 27
Loc. 7
6/25 - 27
Loc. 11
7/31 - 8/1
Loc. 3
5/16-17, 5/27-28
Loc. 12
5/16-17, 5/27-28
Loc. 1
6/25-27, 7/30-8/1
Loc. 8
5/16-17
5/27-28
Loc. 6
6/25 - 27
Loc. 9
5/16-17, 5/27-28
Loc. 4
5/16-17, 5/27-28
Loc. 13
6/25-27, 7/30-8/1
圖 例

Table 1

Sampling Location	*Phlebotomus barraudi*	*An. sinensis*	*Ar.* subalbatas	*Ae. albopicus*	*Fin. togoi*	*Cx. tritaeniorhynchus*	*Cx. annulus*	*Cx. quinquefasciatus*	*Cx. bitaeniorhynchus*	*Cx. sitiens*	*Lut. fuscanus*	*Lut. halifaxii*	*Cx. pallidothorax*	*Lop. rubithoracis*	*Coq. crassipes*	*Mon. uniformis*	*Mi.luzonensis*
1	0	2	1	5	0	2	0	47	0	0	0	0	0	0	0	0	0
2	0	0	18	7	0	3	0	17	0	0	1	0	1	0	0	0	0
3	0	31	1	2	0	85	33	42	0	11	0	0	0	0	0	0	0
4	0	13	1	7	7	64	12	12	0	2	1	0	0	0	0	0	0
5	0	0	3	3	0	187	43	16	0	12	6	2	5	2	2	0	0
6	0	0	1	2	0	1	0	17	0	1	0	0	2	0	0	0	0
7	3	1	14	10	0	78	0	26	0	0	0	0	1	2	8	3	3
8	0	11	36	8	17	42	14	85	0	15	2	0	1	0	0	0	0
9	0	2	2	2	135	16	0	26	0	7	3	0	1	0	0	0	0
10	0	2	0	3	24	10	2	51	0	1	0	0	0	1	0	0	0
11	0	0	0	0	0	4	0	0	1	0	0	0	1	0	2	0	3
12	0	8	0	6	2	37	5	52	0	12	0	0	0	0	0	0	0
13	0	0	0	7	0	8	4	7	2	8	0	0	4	0	3	0	0
Total	3	70	77	62	185	537	113	398	3	69	13	2	16	5	15	3	6

Sampling Location	*Phlebotomus barraudi*	*An. sinensis*	*Ar.* subalbatas	*Ae. albopictus*	*Fin. togoi*	*Cx. tritaeniorhynchus*	*Cx. annulus*	*Cx. quinquefasciatus*	*Cx. bitaeniorhynchus*	*Cx. sitiens*	*Lut. fuscanus*	*Lut. halifaxii*	*Cx. pallidothorax*	*Lop. rubithoracis*	*Coq. crassipes*	*Mon. uniformis*	*Mi.luzonensis*
1	0	2	1	5	0	2	0	47	0	0	0	0	0	0	0	0	0
2	0	0	18	7	0	3	0	17	0	0	1	0	1	0	0	0	0
3	0	31	1	2	0	85	33	42	0	11	0	0	0	0	0	0	0
4	0	13	1	7	7	64	12	12	0	2	1	0	0	0	0	0	0
5	0	0	3	3	0	187	43	16	0	12	6	2	5	2	2	0	0
6	0	0	1	2	0	1	0	17	0	1	0	0	2	0	0	0	0
7	3	1	14	10	0	78	0	26	0	0	0	0	1	2	8	3	3
8	0	11	36	8	17	42	14	85	0	15	2	0	1	0	0	0	0
9	0	2	2	2	135	16	0	26	0	7	3	0	1	0	0	0	0
10	0	2	0	3	24	10	2	51	0	1	0	0	0	1	0	0	0
11	0	0	0	0	0	4	0	0	1	0	0	0	1	0	2	0	3
12	0	8	0	6	2	37	5	52	0	12	0	0	0	0	0	0	0
13	0	0	0	7	0	8	4	7	2	8	0	0	4	0	3	0	0
Total	3	70	77	62	185	537	113	398	3	69	13	2	16	5	15	3	6