

Overseas Practice on (Field Epidemiology • Collaborative Research)

2015/11/30 (Year/Month/Day)

report form (For Student)

Name	Wallaya Phongphaew
Laboratory	Division of Molecular Pathobiology, Research Center for Zoonosis Control
Year (Grade)	D2
Place of practice	School of Veterinary Medicine, University of Zambia Chipata District, Eastern Province, Republic of Zambia
Period of practice	November 4 th -17 th , 2015
Purpose	Mosquito sample collection for field epidemiological study of mosquito-borne viral diseases and bat and tick sampling for field epidemiological study of viral pathogens in ticks and bats in Zambia

Summary of activities (about 800 words, provide photos, tables and figures that clearly show the activities during the period)

Background and objectives

Republic of Zambia is a country in southern Africa. Zambia is landlocked country (Figure 1). Zambia has two main seasons, rainy (November to April) and dry season (May to October/November). Dry season is subdivided into cool dry (May to August) and hot dry season (September to October/November). We performed three activities during Zambia visiting. The first one is mosquito trapping and other activities are tick sampling and collection of bat tissues and feces.



Figure 1: Map of Republic of Zambia

The main activity is mosquito trapping for epidemiological study of mosquito-borne viral diseases in Zambia. Zambia has a potential for mosquitoes-borne viral diseases, because Zambia has suitable climate and ecology for mosquito survival. Recently, there were reports of sero-prevalence of several mosquito-borne diseases in Zambia, including Dengue, West Nile fever and Yellow fever (Liwewe *et.al*, 2014; Ndumba *et.al*, 2015; Masaninga *et.al*, 2015). However, these viruses have not been isolated in Zambia. In addition, the situation of other mosquito-borne viruses have not been elucidated yet. For this reason, continuous investigation of presence of the arboviruses in mosquitoes in Zambia is necessary. Our research group aims to reveal the current distribution of mosquito-borne viruses in Zambia.

Our group also conducted tick sampling to analyze the tick-borne diseases in Zambia. Another research activity is collection of feces of bats and sampling of tissues of Egyptian fruit bats (*Rousettus aegyptiacus*). It has been reported that Egyptian fruit bats might be reservoir of Ebola and Marburg virus in Africa. Marburg virus has been isolated from Egyptian fruit bats collected from Kitaka cave in Uganda in 2009 (Towner *et.al*, 2009). Our aim is investigation of viral pathogens in Egyptian fruit bats in the Republic of Zambia.

Mosquito trapping activity

- Sampling method

The mosquito trapping was performed under permission from the Zambia Wildlife Authority. Mosquitoes were trapped with two types of mosquito trap, the CDC miniature light trap and the BG-sentinel. The CDC miniature light trap is composed of trap assembly with light, motor and fan, lid, collection cup, power cable and with CO₂ production apparatus (Figure 2A). The mosquitoes were attracted by light and CO₂. The other trap is the BG-sentinel trap (Figure 2B) in combination with a lure which releases artificial human skin emanations. Both types of mosquito trap were set up in the late morning and picked up in the morning of next day. The trapped mosquitoes were kept in -20°C until species identification using morphological method. Only female mosquitoes are used for further examination.

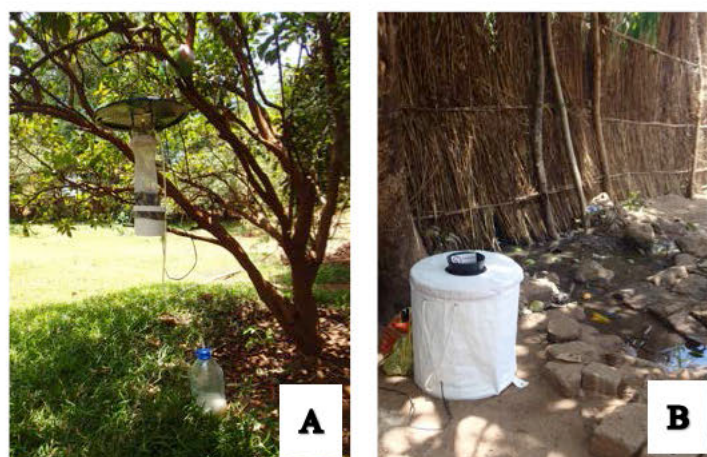


Figure 2: the mosquito traps, CDC miniature light mosquito trap (A) and BG-sentinel trap (B).

- Sampling activity

The sampling locations at Chipata district, Eastern province, Zambia were decided by Prof. Hirofumi Sawa and Dr. Yasuko Orba from Division of Molecular Pathobiology, CZC, Dr. Bernard M. Hang'ombe from the Department of the Veterinary Medicine, University of Zambia.

Chipata district which is the biggest district in Eastern province, is located at the border with Malawi and 568 km from Lusaka in distance (Figure 1).

The mosquito traps were set up in different locations, including urban and suburban areas (Figure 3) with the cooperation of a local officer, Mr. Anthony Chupa from the Provincial Veterinary office, Ministry of Agriculture and Livestock. We put the mosquito traps in many places (Figure 4) as below table (Table 1).

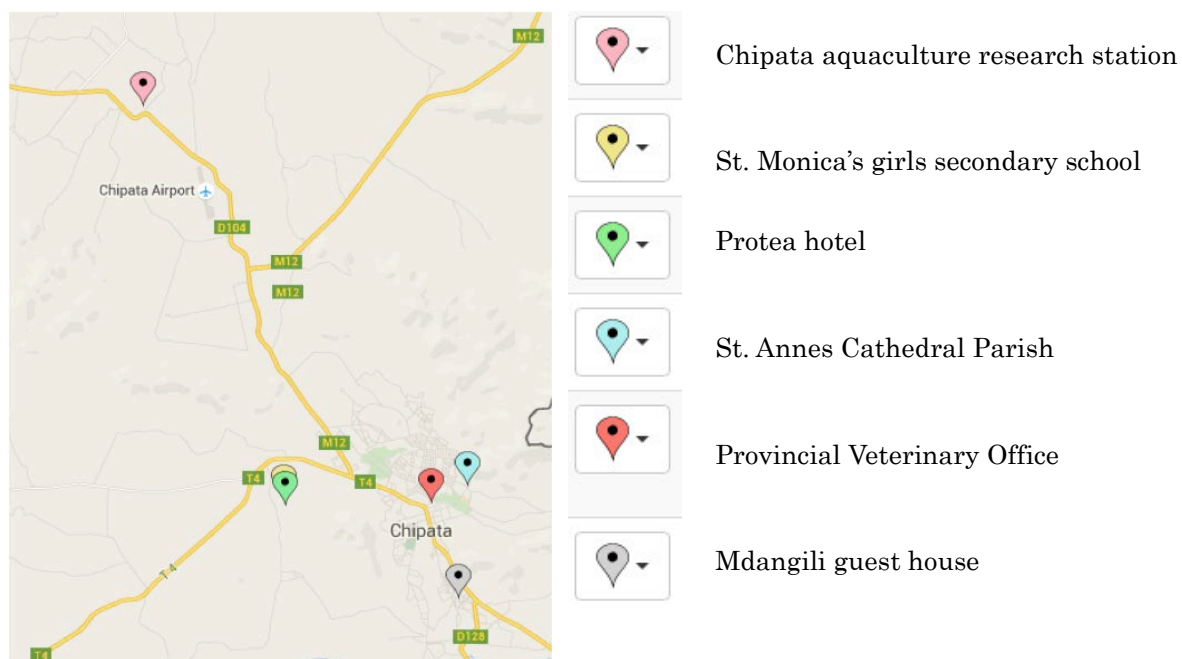


Figure 3: Locations of mosquito trap installation

In summary, 292 female mosquitoes were collected with two genus, including *Culex* and *Aedes*. Almost of samples were *Culex* species (Figure 5), especially *Culex quiquefasciatus* (Table 2). However, the number of collected mosquito was lower than our expectation because of marked dry and windy weather which was unusual in this period in Zambia.



Figure 4: Mosquito traps were set up in several locations around the Chipata district.

Table 1. List of locations and mosquito traps during the activity

Date	Location	Type of trap	Number of traps	Number of mosquitoes
Nov 8 th	Provincial Veterinary office	CDC	3	68
		BG-sentinel	3	16
Nov 9 th	St. Annes Cathedral Parish	CDC	3	47
		BG-sentinel	3	10
Nov 10 th	Saint Monica's girls secondary school	CDC	3	13
		BG-sentinel	3	6
Nov 11 th	Chipata aquaculture research station	CDC	4	1
		BG-sentinel	2	
	Protea hotel	BG-sentinel	1	38
Nov 12 th	Mdangili guest house	Hand		14
		CDC	4	31
		BG-sentinel	2	48

Table 2. Mosquitoes collected in the activity

Mosquito species	Number of mosquitoes
<i>Culex quiquefasciatus</i>	271
<i>Culex univittatus</i>	19
<i>Culex tigripes</i>	1
<i>Aedes aegypti</i>	1
Total	292

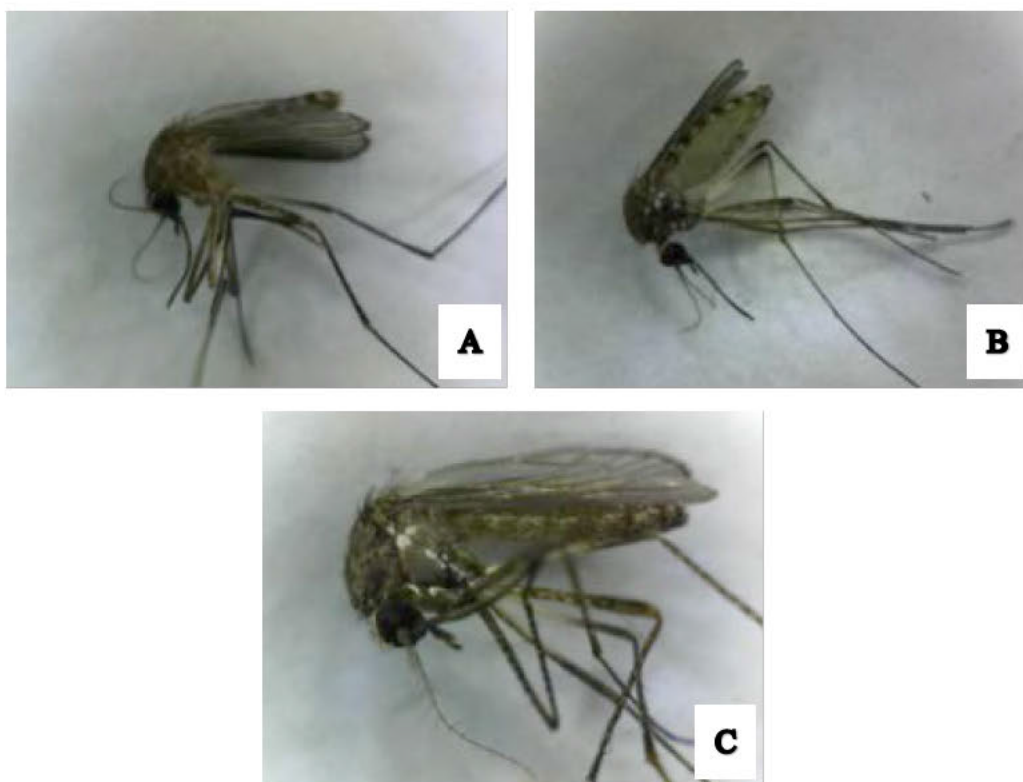


Figure 5: *Culex quiquefasciatus* (A), *Culex univittatus* (B) and *Culex trigrupes* (C)

Other activities of sampling of tick and collection of Egyptian fruit bats and their feces

- Sampling methods

The sampling activities were conducted under permission from the Zambia Wildlife Authority. Ticks were collected by hands from the ground of the cave and also harvested using the dragging method with white cloth. Bat feces were collected using plastic sheet on the ground with aseptic technique. In addition, Egyptian fruit bats were caught by portable harp traps which were set up at the entrance of cave in the evening (Figure 6). The collected bats were euthanized by inhalation of ether, then blood samples were collected (Figure 6). Thereafter, the body weight, length of head to tail and wingspan was measured (Figure 7). The bat samples were dissected in the Biosafety Level-2.5 experimental room at the University of Zambia. The visceral organs, including liver, lung, spleen, kidney, salivary gland, colon and testes were collected for further examination. Some of tissue samples were fixed with 10% formalin.

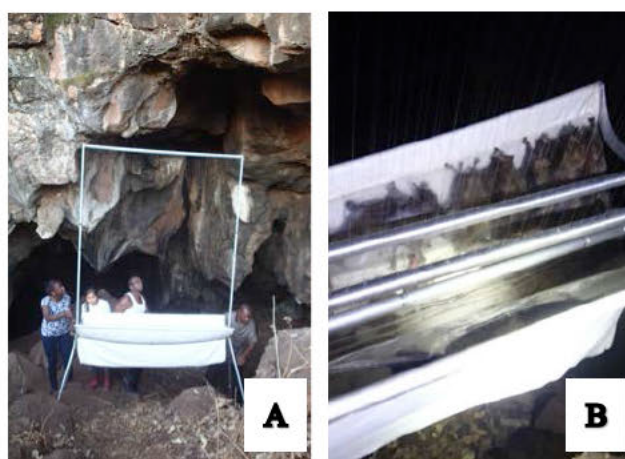


Figure 6: Harp trap: the trap was installed at the entrance of cave (A). Captured bat in the trap's collecting chamber (B)



Figure 7: Length of head to body, wingspan and body weight of each bat was measured.

-Sampling activities

The sampling location at the Suesueman cave, Chongwe district, Lusaka Province was performed under supervision by Prof. Hirofumi Sawa, Dr. Yasuko Orba and Dr. Masahiro Kajihara from the CZC, and Dr. Yong Jin Qiu from the Hokudai Center for Zoonosis Control in Zambia (HUCZCZ), Dr. Bernard M. Hang'ombe from the Department of the Veterinary Medicine, University of Zambia. The cave which is 58.7 km far from the University of Zambia, Lusaka, is habitat of bats, including insectivorous bats and Egyptian fruit bats.

The tick collection activity was performed using the methods as previously described. For the Egyptian fruit bat trapping, harp traps were set up at the north and south entrances of the cave (Figure 6). Sampling of blood and tissue from the Egyptian fruit bat were performed at the HUCZCZ.

One hundred four of soft ticks (*Ornithodoros graingeri*) were collected from the cave. The tick samples will be used for metagenomic analysis to investigate tick-borne pathogens. Eight male and 12 female of Egyptian fruit bats were captured from the cave. Blood, feces and tissue samples of each bat will be used to investigate viral pathogens.

References:

- Liwewe M *et.al*, 2014, “First sero-prevalence of dengue fever specific immunoglobulin G antibodies in Western and North-Western provinces of Zambia: a population based cross sectional study”, *Virology*, 11:135-142.
- Masaninga F *et.al*, 2015, “Distribution of Yellow fever vectors in Northwestern and Western provinces, Zambia”, *Asian Pac J Trop Med*, 7:S88-S92.
- Ndumba I *et.al*, 2015, “Seroprevalence of West Nile virus specific IgG and IgM antibodies in North-Western and Western provinces of Zambia”, *Afr Health Sci*, 15:803-809.
- Towner J *et.al*, 2009, “Isolation of genetically diverse Marburg viruses from Egyptian fruit bats”, *PLoS Pathog*, 5(7):e1000536.

(Field Epidemiology • Collaborative Research) Evaluation by supervisor

Institution • Official title • Name	Division of Molecular Pathobiology, CZC Professor Hirofumi Sawa	印
<p>Describe overall evaluation on the applicant's activity in overseas practice.</p> <p>Ms. Phongphaew who visited Zambia with us to join the epidemiological research activities targeting arboviruses has performed good activities. In this activities, it was the first time to visit Africa for her. She had worked hard to prepare equipment for sampling, collection of mosquitoes, identification of the species of mosquitoes by the light microscopy, dissection of tissues from captured bats and so on. I believe that Ms. Phongphaew has learned many knowledge and skills for epidemiological research in Africa and that she will become a good Zoonosis Control Experts in this Zoonosis Control Expert Course of the Leading Program.</p>		

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