Overseas Practice on （Field Epidemiology・~~Collaborative Research~~）

2015/05/11 (Year/Month/Day)

report form（For Student）

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Laboratory	Division of Molecular Pathobiology, Research Center for Zoonosis Control
Year (Grade)	D2
Place of practice	School of Veterinary Medicine, University of Zambia Livingstone and Kazungula District, Southern Province, Republic of Zambia
Period of practice	April 18th-29th, 2015
Purpose	Mosquito sample collection for field epidemiological study of mosquito-borne viral diseases

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

1. Background and objective

The Republic of Zambia (Zambia) is a country located in the southern Africa surrounded by the neighboring countries: Angola, Democratic Republic of Congo, Tanzania, Malawi, Mozambique, Zimbabwe, Botswana, and Namibia. As a landlocked country, the landscape of Zambia is mostly constituted by high plateaus with some hills, mountains, and also river valleys. Zambia has a tropical climate with a dry season (May-October) and a rainy season (November-April), which is suitable for the mosquitoes to live and to breed thus continuously being a risk factor for arbovirus transmission to human although only a few human cases of Rift Valley Fever (RVF) and suspected yellow fever (YF) have been reported in Zambia so far. It is important to continuously investigate the presence of the arboviruses in the mosquitoes in Zambia and such activity has been a part of epidemiological research conducted annually by the Division of Molecular Pathobiology, Research Center for Zoonosis Control (CZC). For this activity, we are targeting the female mosquitoes for screening of arboviruses since only the females that feed on blood and bite the hosts thus the arboviruses are likely to be detected from them.

2. Sampling method

There were two types of mosquito trap used in this study. One was the

Centres for Disease Control miniature light trap (CDC trap) which used light and CO_2 to attract the mosquitoes and was hung around 1.5 m above the ground. The CO_2 was obtained from sugar fermentation by yeast (Figure 1A). The other trap was the Biogents Sentinel trap (Sentinel) which was used in combination with a lure which releases chemical substances such as ammonia, lactic acid, and caproic acid that are also found on human skin (Figure 1B). All of the traps were set up during daytime and were settled for overnight. On the morning of the next day, the mosquitoes were collected from the traps and killed by cold temperature in a refrigerator. The species of the mosquitoes were identified based on their characteristic morphology using a light microscope.

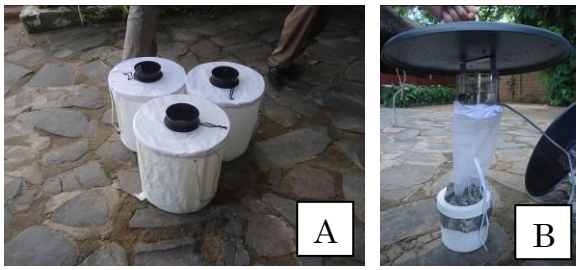

Figure 1. Mosquito traps used during the activity. (A) Sentinel trap. (B) CDC trap.

3. Sampling activities

The sampling locations on the Livingstone and Kazungula District, Southern Province were decided after a discussion with Prof. Hirofumi Sawa and Dr. Yasuko Orba from Division of Molecular Pathobiology, CZC, Dr. Patricia Bwalya from the District Veterinary Office Livingstone and Prof. Bernard M. Hang'ombe from the Department of the Veterinary Medicine, University of Zambia. Generally, the mosquito traps were set up at places near water reservoir, used tires, waste reservoir or plantations that might act as breeding site for mosquitoes.

Livingstone District. The samples were collected in several location including the residential area, the District Veterinary Office of Livingstone, and several sites at the Mosi Oa Tunya National Park (Table 1 and Figure 2). Various mosquito species have been collected. *Culex* mosquitoes were abundant during the collection. There were also a few *Mansonia*, *Aedes*, and *Anopheles* mosquitoes (Figure 3).

Figure 2. Mosquito collection in Livingstone District. (A) CDC trap at residential area (Mamtcho lodge). (B) Sentinel trap at Dry Manzi Picnic Site, Mosi Oa Tunya National Park.

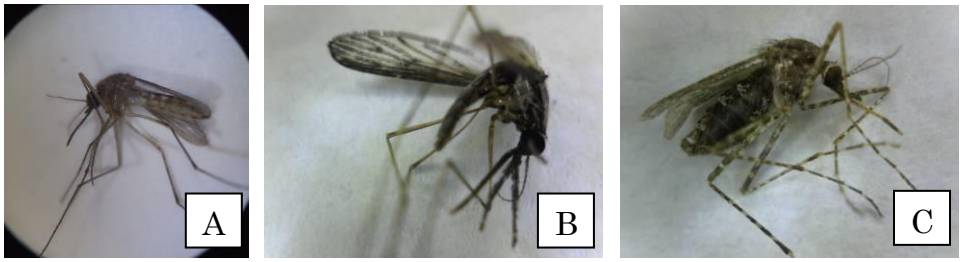

Figure 3. Mosquitoes collected in Livingstone District. (A) *Culex sp.* (B) *Anopheles coustani*. (C) *Mansonia uniformis*.

Kazungula District. The sample collection done in Kazungula District was situated in 7 different locations with the help Ms. Maureen P. Mubanga from the Veterinary Compound Kazungula. From a location called Sikaunzwe, there was a report of children dying after they were bitten by mosquitoes, however the underlying cause of this event is still remain unclear. Based on this report, we then decided to collect the samples from Sikaunzwe area and other locations in Kazungula (Table 1 and Figure 4).

The farthest locations were Kwandu and Kasaya school area. A pond could be found at both locations and the CDC trap was set up within the plantation area near the pond. Interestingly, several species of *Anopheles* mosquitoes could be collected (Figure 5A). A distinct *Aedes* mosquito that is yellow in color, *Aedes ochraceus* (Figure 5B), and another species of mosquito (a scaly mosquito), *Aedeomyia sp.* (Figure 5C), could also be collected from Kasaya School area.

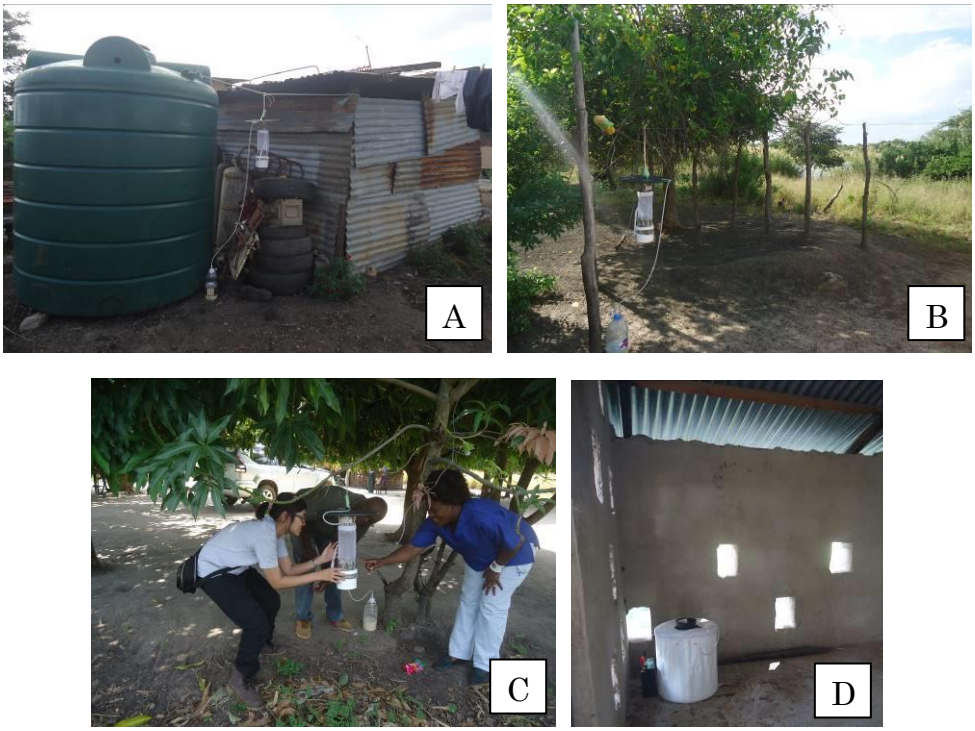

Figure 4. Mosquito collection in Kazungula District. (A) CDC trap at Lumbo village. (B) CDC trap at Kasaya School. (C) CDC trap at Kwandu. (D) Sentinel trap at Sikaunzwe.

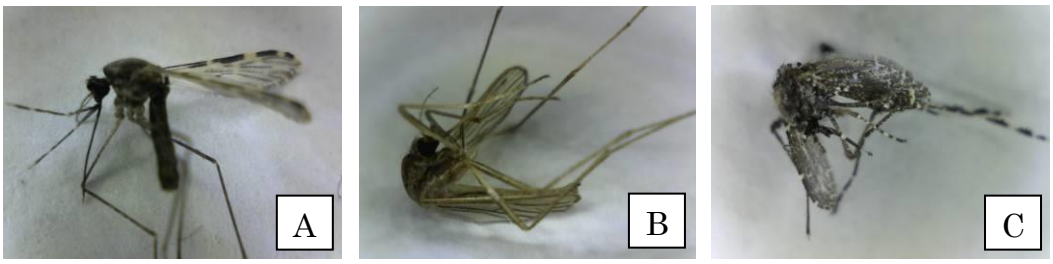

Figure 5. Mosquitoes collected in Kazungula District. (A) *Anopheles funestus*. (B) *Aedes ochraceus*. (C) *Aedeomya sp*.

In summary, 888 female mosquitoes of various species (Table 2) could be collected in this activity, including *Aedes* mosquitoes that are known as the vector for RVFV and YFV. In addition, several species of *Anopheles* mosquitoes that are known as the vector for malaria were also collected during the activity in several sampling locations. The presence of the arboviruses will be further detected by RT-PCR with primers targeting the Pan-Flavivirus, RVFV, Chikungunya virus and also deep sequencing using next generation sequencer.

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

Date	Locations		Type of traps	Number of traps
Day 1 (April 21st)	Livingstone	Tabonina guest house	CDC	1
			Sentinel	1
		Mamtcho lodge	CDC	1
			Sentinel	1
		District Veterinary Office Livingstone	CDC	3
Day 2 (April 22nd)	Livingstone	Gate 1 of Mosi Oa Tunya National Park	CDC	2
			Sentinel	1
		Gate 2 of Mosi Oa Tunya National Park	Sentinel	1
		Gate 3 of Mosi Oa Tunya National Park	CDC	1
		Gate 4 of Mosi Oa Tunya National Park	CDC	1
		Dry Manzi Picnic Site, Mosi Oa Tunya National Park	CDC	1
			Sentinel	1
Day 3 (April 23rd)	Kazungula	Zambezi Sands	CDC	1
		Kazungula border	Sentinel	1
		Lumbo village	CDC	1
		Mambova	Sentinel	1
		Sikaunzwe	CDC	1
			Sentinel	1
		Kasaya School	CDC	1
		Kwandu	CDC	1
Day 4 (April 24th)	Livingstone	Olga's Italian corner	CDC	2
			Sentinel	1
		District Veterinary Office Livingstone	CDC	3
			Sentinel	1

Table 2. Mosquitoes collected in the activity

Mosquito species	Number of mosquitoes
Culex sp.	819
Aedes sp.	16
Anopheles sp.	42
Mansonia sp.	10
Aedeomya sp.	1
Total	888

（Field Epidemiology・Collaborative Research） Evaluation by supervisor

Institution・Official title・Name	Division of Molecular Pathobiology, CZC 印 Professor Hirofumi Sawa
Describe overall evaluation on the applicant's activity in overseas practice. Ms. Anindita has performed good research activities through this Overseas Practice o Field Epidemiology. I am well satisfied her all activities, including preparation of equipment, discussion to the counterparts, identification of captured mosquitoes, and writing a report. I believe that she will become a good Zoonosis Control Expert based on her experiences in this Zoonosis Control Expert Course of the leading Program.	

※1 Send the electronic file to the Leading School section, International Affairs Office, also submit the original print out with seal of supervisor to the Leading School section, International Affairs Office.
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