Overseas Practice on（Field Epidemiology・Collaborative Research）report form（For Student） 2016 / 06 / 22 (Year/Month/Day)

| | |
|---|---|
| Name | May June Thu |
| Laboratory | Unit of Risk Analysis and Management |
| Year (Grade) | D 2 student |
| Place of practice | Hokudai Center for Zoonosis Control in Zambia (HUCZCZ), The University of Zambia |
| Period of practice | 29 days from 4th May 2016 to 1st June 2016 |
| Purpose | Field study on ticks and tick-borne diseases in Zambia |

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

（Field Epidemiology・Collaborative Research）Evaluation by supervisor

| | |
|---|---|
| Institution・Official title・Name | Unit of Risk Analysis and Management 印<br>Norikazu ISODA |
| Ms May collected more than 1,500 ticks in several area in Lusaka and completed the morphological survey. Though not all of the samples were investigated for tick-borne pathogens, we obtained the very important information and inspection regarding the genetic diversity of *Rickettsia* spp. through her oversea activity. Under the limited time and resource condition, her results obtained already is beyond my expectation and, moreover, could propose another potential research project to be solved using the methodology of risk assessment. | |

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※2 The Steering Committee of the Leading Program will first confirm the content of this report and the report will be forwarded to the Educational Affairs Committee for credits evaluation.

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# Report of Overseas Practice on Collaborative Research in Zambia

## 1. Purposes

The aims of this field study on ticks and tick-borne diseases in Zambia were to provide a first molecular evidence of tick-borne pathogens in Zambia and to compare the results with those in other African countries as well as Asian counties including Japan.

## 2. Methods

### 2.1. Sample collection sites

A total of 1,509 ixodid ticks were collected from Chipata (the capital city of Eastern Province) and Lusaka (the capital city of Zambia). Argasidae (soft ticks) were collected from Leopard hill cave in Lusaka. Location map was showed in Figure 1.


Figure 1. Location map of sample collection sites.

### 2.2. Collected samples

Ticks were mainly collected from cattle and horses by using tick removers. The questing ticks were collected from vegetation by dragging method. Animal blood was also collected at the same sites.

Figure 2. Collection of ticks from the horses.

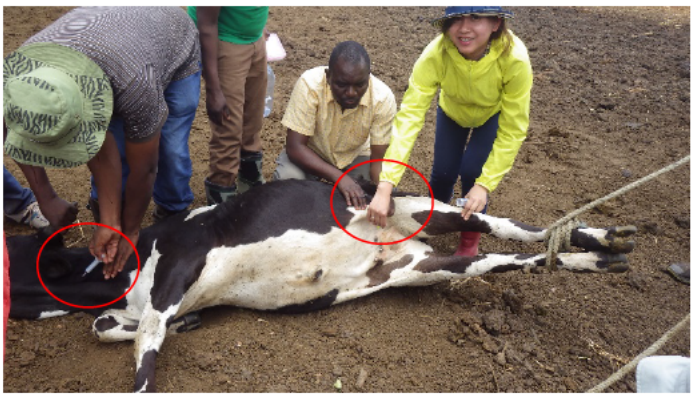

Figure 2. Collection of ticks and blood samples from cattle.

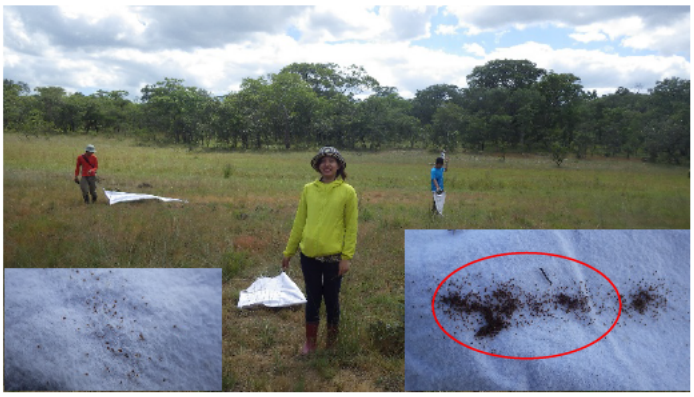

Figure 3. Collection of ticks from vegetation.

### 2.3. Morphological identification of tick species

Tick species were individually identified by a stereomicroscopic examination at the laboratory of HCZCZ (Hokudai Center for Zoonosis Control in Zambia).

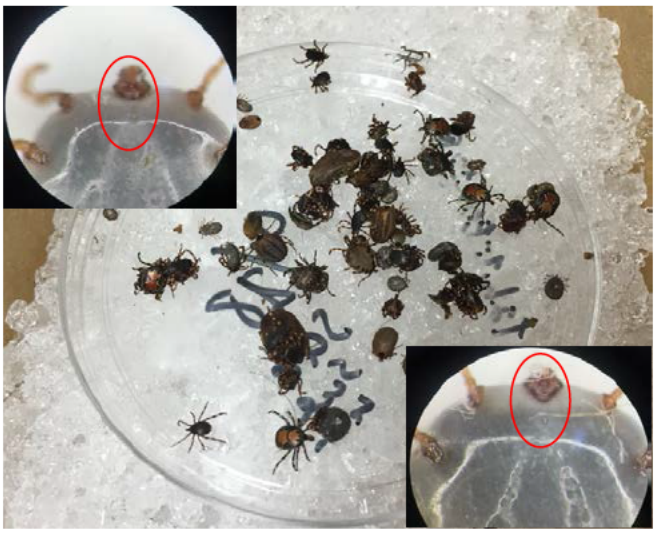

Figure 4. Identification of tick species

### 2.4. DNA extraction and screening of tick-borne pathogens

DNA was extracted from ticks and animal blood by using DNAzol® (Thermo Fisher scientific). PCR targeting several tick-borne pathogens (*Rickettsia* spp. *Ehrlichia spp.* and *Coxiella burnetii*) were employed. To detect *Rickettsia* spp., citrated synthase gene (*gltA*) was amplified. For *Ehrlichia* spp., 16S ribosomal RNA gene (rDNA) was amplified. For *Coxiella burnetii*, isocitrate dehydrogenase (*icd*) gene was amplified. Sequencing analysis was conducted to confirm the amplification of target pathogens. The obtained sequences were compared with the related sequences deposited in GenBank.

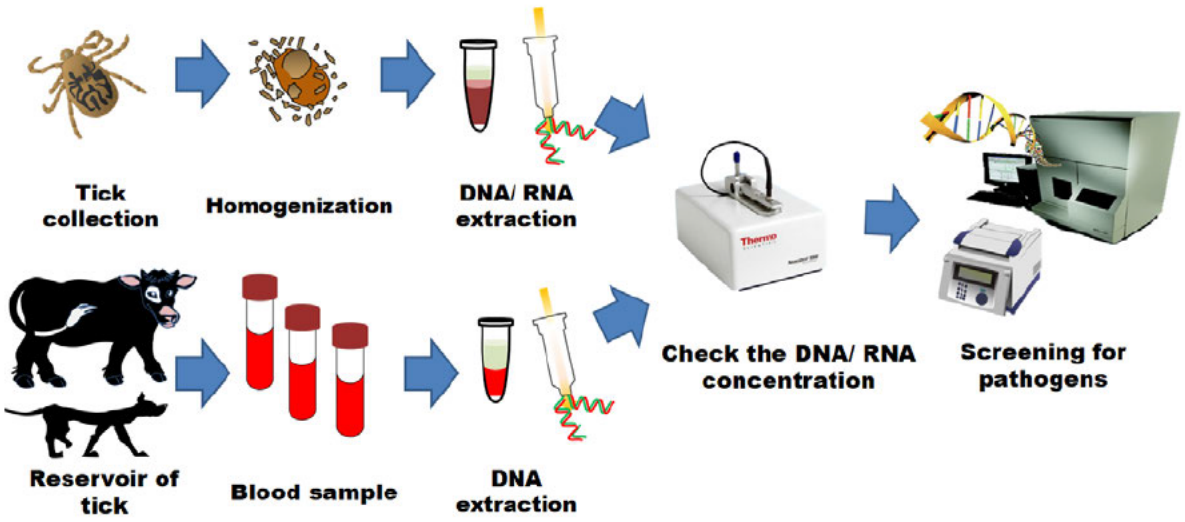


Figure 5. Work flow at the laboratory

## 3. Results

Based on morphological features, three genera and six species (n=1,372) were identified in Chipata. These included *Amblyomma variegatum*, *A. pomposum*, *Rhipicephalus appendiculatuis*, *Rh. Zambeziensis*, *Rh. (Boophilus) microplus* and *Hyalomma truncatum*. Two species (n=137), namely *Rh. decoloratus* and *Rh. geigyi*, were collected from the horses in Lusaka. A total of 105 *Ornithodoros faini* were collected from Leopard hill cave in Lusaka.

Among the ixodid ticks, 24 samples had been tested for tick-borne pathogens. Out of 24 tick DNA samples, seven samples (29.2%) were positive with *Rickettsia* spp. and four samples (16.7%) were positive for *Ehrlichia* spp. by conventional PCR (Figure 6). Five sequences obtained from *A. variegatum* tick showed 100% similarity with *Rickettsia africae* (GenBank no. KJ645939) found in *A. variegatum* tick from Madagascar. The other two sequences obtained from *A. variegatum* showed 99% similarity with *R. africae* (GenBank no. CP001612) from Ethiopia. Three samples detected from *Rh. microplus* and one sample detected from *Rhipicephalus* sp. in Chipata showed 100% similarity with *Ehrlichia* sp. (GenBank no. AF497581) found in Mali and Niger.

Out of 105 soft tick DNA samples, 38 (36.2%) samples were positive for *Rickettsia* spp. by conventional PCR. None of the samples was infected with *C. burnetii*.

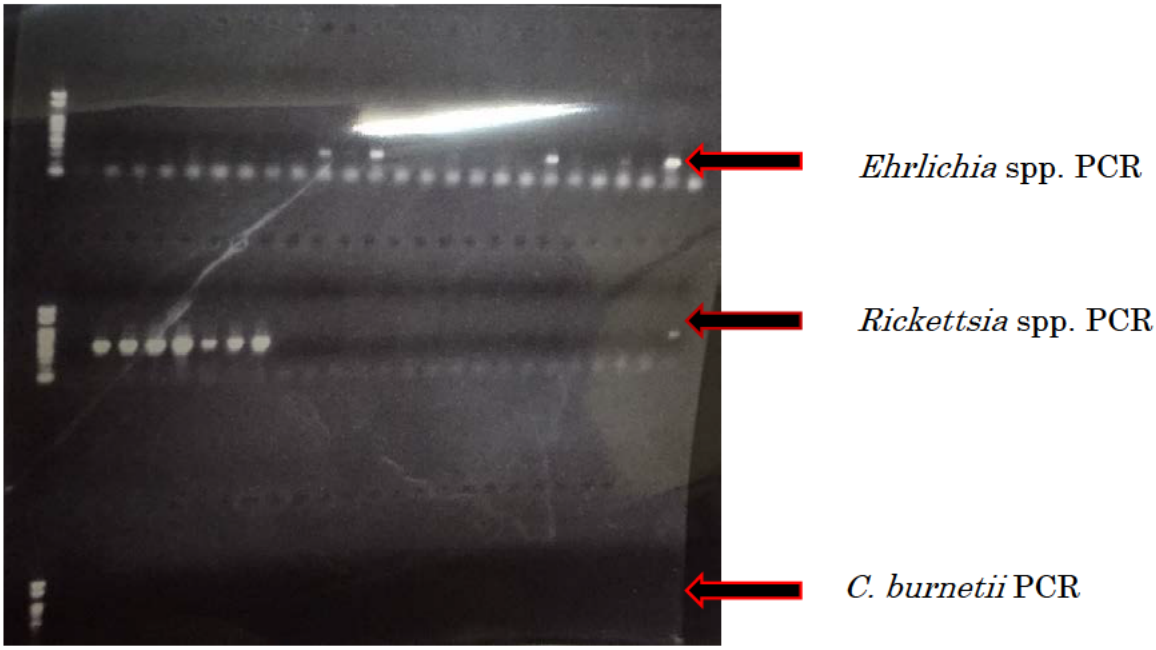


Figure 6. Detection of tick-borne pathogens by PCR

## 4. Conclusions

Due to time limitations, PCR screening of tick-borne pathogens has not been completed during my stay in Zambia, which will be followed up by our collaborators in Zambia. Further studies include isolation of rickettsial bacteria in arthropod cell lines, whole genome analysis using next-generation sequencing technologies, and comparative genome analysis for better understanding of genetic diversity of *Rickettsia* spp. found in Zambia.