Overseas Practice on（Field Epidemiology・Collaborative Research） 2019.01.21

report form（For Student） (Year/Month/Day)

Name	Hla Myet Chel
Laboratory	Lab. of Parasitology
Year (Grade)	D2
Place of practice	University of Veterinary Science (UVS), Yezin, Myanmar
Period of practice	24 days from 7th to 30th December, 2018
Purpose	Morphological and molecular identification of gastrointestinal parasites in Asian elephants of Myanmar

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

Report of overseas practice on field epidemiology in Myanmar

1. Purpose

There is limited information about the parasites of Asian elephants, including captive elephants in Myanmar. This study aimed to morphologically and molecularly identify the gastrointestinal parasites in Asian elephants of Myanmar.

2. Methods

2.1 Sample collection sites

Among more than 50 captive Asian elephant camps of Myanma Timber Enterprise (MTE), two camps were selected to collect fecal samples. One camp is Hmaw Yaw Gyi (HYG) camp in Bago region and the other is Taung Kya (TK) camp in Shan state of Myanmar (Figure 1).

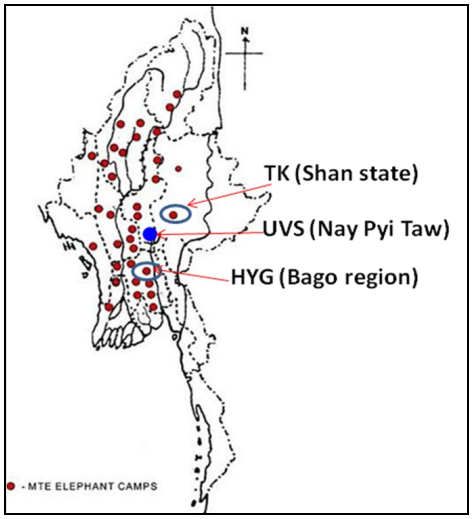

Figure 1. The map of sample collection sites

2.2 Collection of gastrointestinal parasites from elephant feces

One day after deworming with anthelmintics, fecal samples were collected from a total of 19 elephants (8 from HYG and 11 from TK camp). Gastrointestinal worms observed by naked eyes in the feces were picked up by forceps into petri dishes (Figures 2-5).

Figure 2. Collection of feces from elephants.

Figure 3. Collection of worms in the elephant feces. Dewormed parasites were seen in the red circles.

2.3 Morphological identification

Adult nematode worms were distinguished into males and females under a compound microscope and measured their sizes individually (Figure 4). Some adult worms were dissected into anterior, middle and posterior parts (Figure 5). Anterior and posterior parts were treated with lactophenol and glycerol, and observed their morphological characterizations under a stereomicroscope at the Laboratory of Parasitology in UVS.

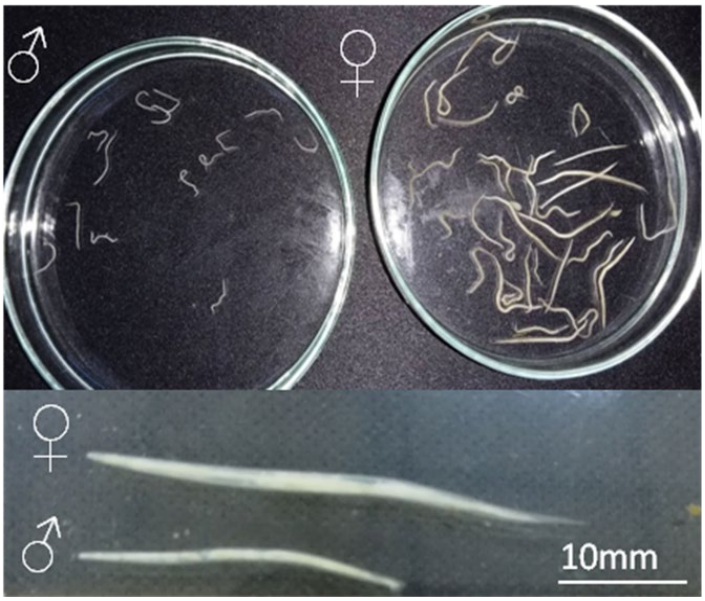

Figure 4. Male and female nematode worms

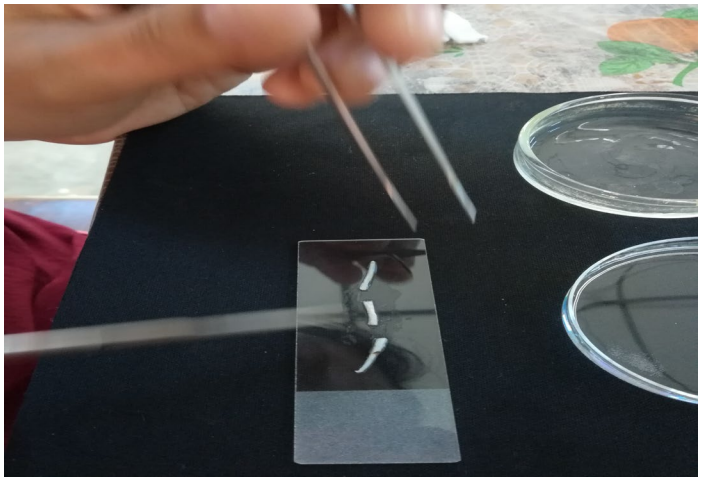

Figure 5. Dissection of a nematode worm

Figure 6. Trematode worms

2.4 DNA extraction and molecular characterization

The middle parts of individual worm were processed for DNA extraction using DNeasy Blood and Tissue kit (Qiagen). The internal transcribed spacer (ITS) region and mitochondrial cytochrome c oxidase subunit I (*cox*1) genes were amplified by PCR in UVS. Resultant PCR products were subjected to sequencing in Japan.

3. Results

The parasites (nematodes and trematodes) were detected in the feces of 13 out of 19 elephants (68.4%). A total of 350 adult nematodes (150 males and 200 females) and 15 trematodes were collected. According to the morphological keys, 27 nematodes examined during the stay in Myanmar were identified as strongyle parasites, and divided into two genera, *Quilonia* and *Murshidia* (Figures 7 and 8). Meanwhile, trematodes appeared to be amphistome parasites (Figure 6).

Among 27 DNA samples examined, PCR amplification of *cox*1 gene and ITS region was succeeded for 26 and 18 samples, respectively (Figure 9). Based on nucleotide sequence analysis of ITS and *cox*1, some parasites showed 92–97% identity to *Quilonia africana,* while others revealed 94–97% identity to *Murshidia linstowi*. These species have been reported in African elephants. Furthermore, preliminary phylogenetic analysis of the *cox*1 gene of 25 samples suggested that Asian elephants of MTE have mixed infection with different genotypes of each parasite species (Figure 10).

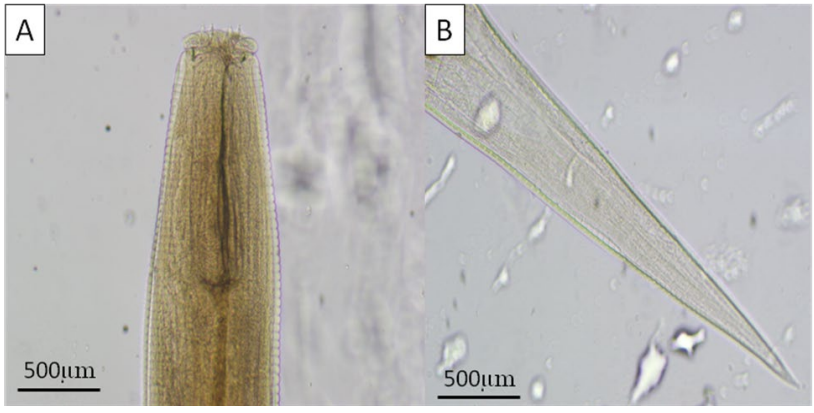

Figure 7. Female anterior (A) and posterior end (B) of *Quilonia* sp.

Figure 8. Male anterior (A) and posterior end (B) of *Murshidia* sp.

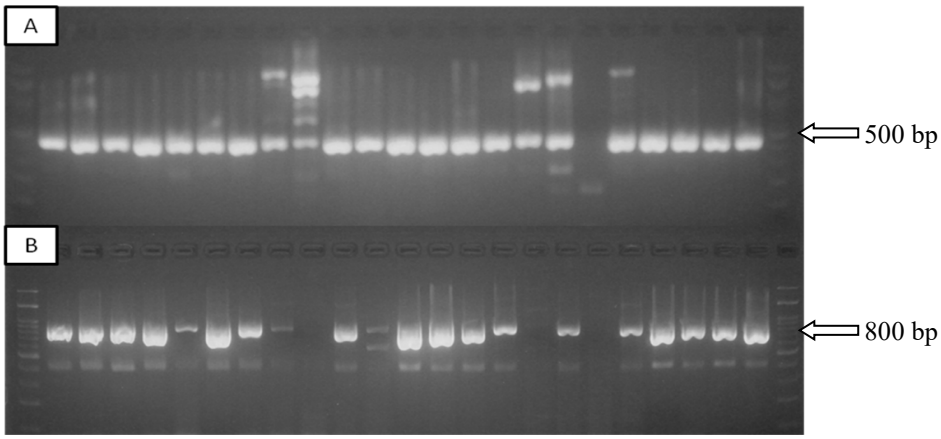

Figure 9. PCR amplification of *cox1* gene (A) and ITS region (B)

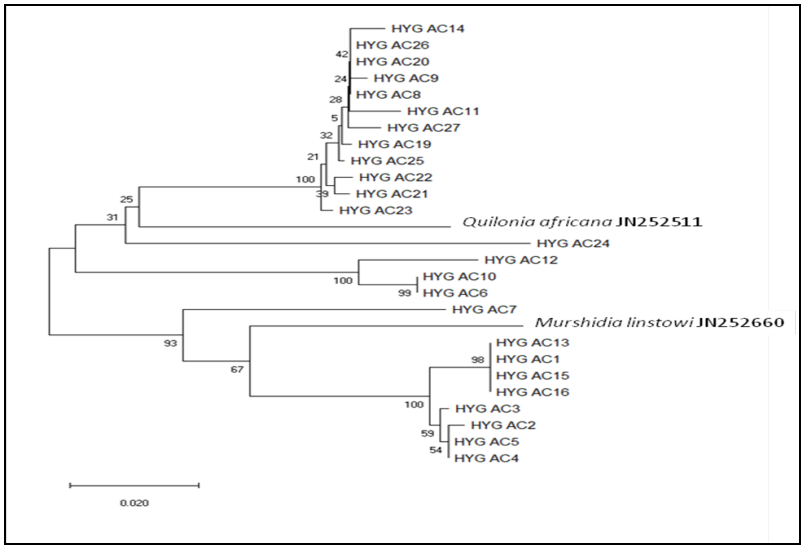

Figure 10. Neighbor Joining phylogenetic tree constructed from sequences (450 bp) of the *cox 1* genes, including *Quilonia africana* and *Murshidia linstowi* from African elephants, and 25 worm sequences from Asian elephants of Myanmar.

4. Conclusions

According to morphological and molecular identification, at least two different genera of strongyle nematodes, *Quilonia* and *Murshidia,* were infected in Asian elephants of Myanmar. Since only 27 nematodes were examined so far, the remaining worms including amphistome trematode will be proceeded to further morphological and molecular characterization. This study will fulfill the lack of genetic information of gastrointestinal parasites in Asian elephants.

（Field Epidemiology・Collaborative Research） Evaluation by supervisor

Institution・Official title・Name	印
Describe overall evaluation on the applicant’s activity in overseas practice.	

※1 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.

Submit to：VETLOG

Ext: 9545 e-mail: leading@vetmed.hokudai.ac.jp