Research Activity Report
Supported by "Leading Graduate Program in Primatology and Wildlife Science"
(Please be sure to submit this report after the trip that supported by PWS.)

	2017. 05. 31
Affiliation/Position	Indian Institute of Science/Project Assistant
Name	Upasana Sarraju

1. Country/location of visit

Japan/Yakushima

2. Research project

Parasites of wild mammals in Yakushima; Identity, Abundance and Distribution

3. Date (departing from Japan)

2017. 05. 08 – 2017. 06. 09

4. Main host researcher and affiliation

Dr.Goro Hanya, Prof.Shiro Kohshima, Wildlife Research Centre, Kyoto University

5. Progress and results of your research/activity (You can attach extra pages if needed)

Please insert one or more pictures (to be publicly released). Below each picture, please provide a brief description.

During this visit to Yakushima, I was part of the Parasite Research Group consisting of four other students, led by Dr.Nariaki Nonaka of University of Miyazaki, Dr. Munehiro Okamoto, PRI, Kyoto University, Dr.Akiko Sawada Takakazu Yumoto-san. We conducted research on ectoparasites and endoprasites of wild deer, wild field mice and ground vegetation in three different locations in Yakushima. I used this opportunity to learn to sample for, isolate, collect and identify ectoparasites (ticks and deer ked) and endoparasites (nematodes, trematodes and protozoa). I also learnt techniques for effective sampling of live mammals such as field mice and for internal parasites such as gastrointestinal parasites of wild deer.

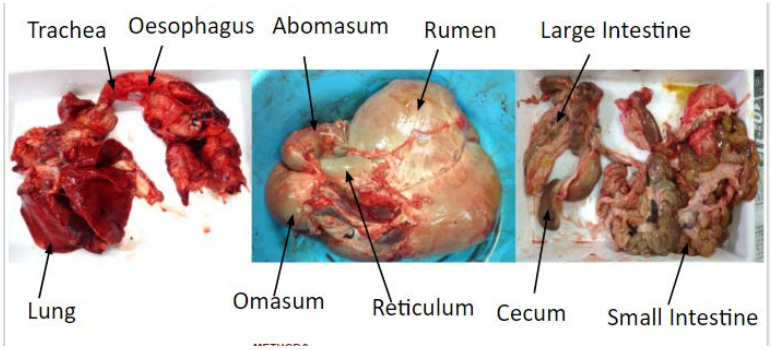
On the day of arrival to Yakushima, we, along with the other research groups were treated to a wonderful traditional lunch at a local restaurant. We were then driven to the Nagoya field station by the sensei. After dropping off our luggage in our assigned rooms, the sensei guided us for a walk in Yakushima National Park. We had the opportunity to observe some macaques and deer, as well as some f the very interesting vegetation found on both sides of the walking path. It was here that we first came across some of the fruit trees than monkeys feed on and deer glean under. As it started to turn dark, we returned to the field station where we had an early dinner and a brief introduction and orientation session, followed by team discussion. During this discussion, Dr.Nonaka and Dr.Okamoto introduced us to the techniques and principles of gastrointestinal parasite study through a Powerpoint presentation and preserved specimens of parasites.

I learnt that the rationale for our study was the fact that gastrointestinal parasites were found in same species (*Cervus nippon*) of Sika deer as the Yakushima deer (*Cervus Nippon yakushimae*) which is a different subspecies endemic to Yakuhsima island. Parasites have not been previously reported in Yakushima deer.

The next morning we began work in our respective research teams.

Dr.Nonaka and Dr.Okamoto had previously obtained the viscera of wild deer. On the first day of the study, we were taught to dissect the deer viscera by first identifying all the organs, removing them from the visceral mass and using surgical scissors to severe the connection tissue and fat between each organ. Thus, we had isolated the esophagus, trachea, lungs, rumen, abomasum, large intestine, small intestine and cecum.

Research Activity Report
Supported by "Leading Graduate Program in Primatology and Wildlife Science"
(Please be sure to submit this report after the trip that supported by PWS.)



Following isolation of each organ, we then proceeded to dissect each organ in order ot observe for Gastrointestinal parasites. Each organ was to be examined and treated in a different based on the kind of parasite that is expected to be present in it. For example, the esophagus and trachea were bisected and the inner surfaces were visually examined for nematodes. The rumen was opened, the contents removed and visually examined. The lungs and pancreas were visually examined as well but cut into smaller pieces and left to soak in saline following with the saline was examined under the microscope. In case of the small- and large intestines, both the inner surfaces and the contents were evaluated for presence of parasites.
The contents of abomasum, small intestine and large intestine were collected and the tissues were washed with saline. The run-off was also collected and mixed into the contents. The contents were then filtered through a series of sieves with decreasing mesh sizes in order ot first remove larger particulate matter and then trap parasites. The filter deposits of the sieves were collected into saline and stored for simple sedimentation and the supernatant was discarded. The volume of the sediments was them made up to 400ml using saline and aliquots of this solution were examined under the microscope.

The size of the meshes in the sieves used depended upon the sizes of parasites ad parasite eggs expected to be found in that tissue.

From the viscera of the first deer we were able to isolate lung worms as well as other nematodes from the abomasums. Once the process was finished, the remaining biological waste was buried manually.

After appropriate labeling of all collected samples, we proceeded to examine the viscera of a second deer, r his time to ensure we had a

T mples. The next day we w n faster than the previous d ghterhouse to observe the slaughtering process of recently hunted deer. We also had the opportunity of examining and bisecting the heart and kidney. Before the deer were slaughtered, we collected ticks and other ectoparasites from the skin of two dead deer into glass bottles containing ethanol. We were also able to identify and collect a deer ked.

Research Activity Report
Supported by "Leading Graduate Program in Primatology and Wildlife Science"
(Please be sure to submit this report after the trip that supported by PWS.)

We obtained three more deer viscera sets which we took back to our lab at the field station and worked on. Among these three deer, only one nematode was found: a lung worm. We examined the ticks collected under the microscope to identify the genera based on head morphology.

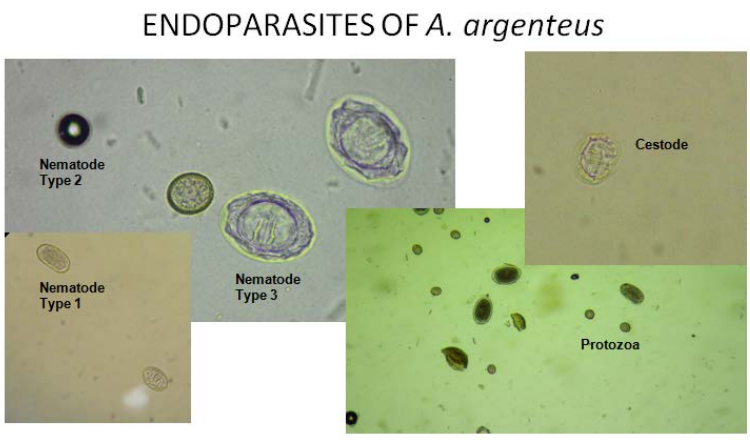
The next day we visited Oko-No-Taki Waterfall for a brief break. We then went to the Yodogawa trail in order to set up ten Sherman traps each containing either peanuts or sunflower seeds as bait for Japanese field mice. We found locations which were likely to be the paths of field mice and placed our traps in those locations and marked the areas with a plastic tag. Once this was completed, we drove back toward Nagoya and stopped at a few areas of heave vegetation to perform Tick Dragging in order to collect ticks that are present on the underside of leaves. As it was raining, we were unable to collect many ticks.

We then returned to the lab and continued examining the collected deer ticks and vegetation ticks under the microscope for genera identification. I identified a new species of *Haemaphysalis* tick with confirmation from Nonaka-sensei. We referred to this as *Haemaphysalis species 2* while the other ticks we came across were labeled *Haemaphysalis species 1*. We also came across a deer ked and unknown ectoparasites.

We left early next morning to Yodogawa trail entrance to collect our traps. On the way, we held a brief discussion regarding the presentation of our field work results.

When we checked our traps, we discovered that we had successfully trapped 11 field mice, about half of which had sunflower seeds as bait, demonstrating no mice-preference for bait. From these trapped mice, we collected ticks, fecal samples and perianal samples. We also recorded the sex and species of the mice. All the mice were *Apodemus argenteus.*

After sampling, we released the mice at the same spot as the trapping. That afternoon, we went for a trail walk at Yakusugi Land. We returned to the lab and identified the mice ticks (all belonging to the *Ixodes* genus, nymph stage), as well as processed the fecal samples. Microscopic observation of perianal samples revealed no parasites. Fecal samples contained many kinds and number of gastrointestinal parasites and eggs.



Research Activity Report
Supported by "Leading Graduate Program in Primatology and Wildlife Science"
(Please be sure to submit this report after the trip that supported by PWS.)

We worked on our group presentation through the night and till the next morning. The presentation was held on May 18, afternoon, and all three research teams presented their work in the Yakushima Field Course.

To celebrate our week together, we had a barbecue at the field station that evening.

The next morning, we collectively cleaned the field station and left for a trail walk at Shiratani Usuikyo Ravine, before departing for Kyoto.

6. Others

I am grateful for the support of PWS in making this visit and academic exchange possible. I look forward to an opportunity to visit again in order to complete the work we began.