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

report form（For Student）

2017/01/16(Year/Month/Day)

Name	Rabin KADARIYA
Laboratory	Laboratory of Wildlife Biology and Medicine
Year (Grade)	Second year (D2)
Place of practice	1. Field sample collection: Annapurna Conservation Area in collaboration with National Trust for Nature Conservation, Nepal 2. Diet analysis: Institute of Forestry, Pokhara, Nepal 3. DNA extraction: Nepal Anti-Tuberculosis Association/German-Nepal Tuberculosis Project (GENETUP), Kathmandu, Nepal
Period of practice	27 June 2016 - 3 January 2017
Purpose	Non invasive sample collection for genetic and ecology study of Asiatic black bears

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

A collaborative study on "genetic variation and ecology of Asiatic black bears (*Ursus thibetanus*)" in Annapurna Conservation Area of Nepal was conducted from June 27, 2016 to January 3, 2017. The non-invasive samples of bears were collected from different sites of Annapurna Conservation Area whereas DNA was extracted in genetic lab of GENETUP, Kathmandu and diet of bears was analyzed in zoological lab of Institute of Forestry, Pokhara. Series of meetings were organized with related stakeholders for research permission, field coordination, logistic arrangement and lab work. First, Meeting was organized with Dr. Maheshowar Dhakal and Mr. Gopal Prakash Bhattarai, Deputy Director General and Mr. Laxman Prasad Paudel, Ecologist of Department of National Park (DNPWC) to obtain legal permission of study. Similar type of meeting was also organized with Mr. Ganga Jung Thapa, Executive Director and Dr. Naresh Subedi, Coordinator, National Trust for Nature Conservation (NTNC) for seeking field support as NTNC has located field stations in different parts of Annapurna Conservation Area. Similarly, meeting was organized with Dr. Bhagawan Maharjhan and Dr. Bhawana Shrestha of GENETUP for seeking support of lab facility to extract DNA. After getting research permission from DNPWC, I traveled to Pokhara where I got permission from Annapurna Conservation Area Project (ACAP) for fieldwork. Mr. Lal Prasad Gurung, Project Director and his staffs from ACAP had fully supported me for the organization of fieldwork in different sites of conservation area. Without support of ACAP central and field team, It was almost impossible to organize field work in remote mountain terrain. Fieldwork was organized after first discussion with staffs of field station and discussion with community leaders **(Photo 1).**

Annapurna conservation area **(Photo 2)** is the largest conservation area of Nepal and well known tourist destination for mountain trekking and cultural tourism. Mixed evergreen forest and conifer forest of southern part provides habitat for Asiatic black bears. Field visit were organized in seven different sites of Annapurna conservation to represent variation in sample after the confirmation of bear existence from stakeholders, field staff, local people and final confirmation with shepherd to save our energy, money and time. Five and two units were selected from southern and trans-himalayan region of conservation area respectively. Non-invasive samples especially fecal were searched in summer (July-September) and pre-winter (October-December) season. First, my team searched bear trail, footprint, climbing scratches, ground/log digging sites, bear food platforms (**Photo 3**) to find the existence of bears in the particular sites following the recent information from livestock herders and villagers (**Photo 4**) who frequently visit the forest. If the above-mentioned signs observed, we intensified searching for fresh feces (≤4 days) for genetics and any age of feces for diet of bears (**Photo 5**). The age of the feces were estimated based on freshness of feces, other bear sign in surroundings and local villagers knowledge. The surfaces of fresh feces were rubbed using a sterile cotton swab and preserved in tube having 99.5% ethanol (**Photo 6**). 99 fresh fecal samples were collected from seven different sites within Annapurna Conservation. Of which 46 were collected in July-September and 53 were collected in November-December period. Similarly, 46 and 53 feces were found in agricultural land and wild habitat respectively (**Table 1**).

I also expected to collect hair sample that is non-invasive too but DNA quality is always good so I set up hair trap (**Photo 7**) in four different places of Mustang district with the aim to extend in other sites by observing its effectiveness. A hair trap consisted of a single barbed wired strand approximately 20 m long wrapped around at least 4 trees, 40-50 com above the ground. Rotten flesh meat was baited in the center of trap at 3 m above ground level to attract bears. The trap was closely monitored by automatic camera capture (**Photo 8**). Unfortunately, I could not get any bears signs from the trap located in 4 locations. Fortunately, 11 hair samples were collected from barbed fences (**Photo 9**) which was set up to protect maize land and one hair sample was collected from broken branches. The GPS location, sample characteristics, and habitat descriptions were noted in detail.

Genomic DNA was extracted using Qiagen's QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) for fecal samples and Wako DNA Extractor FM Kit (Wako, Osaka, Japan) for hair samples following manufacturer's protocol with slight modification by considering equipments available in the genetic lab of GENETUP (**Photo 10**). Microsatellite and mitochondrial DNA will be analyzed in future to know the genetic diversity and phylogenetic relationship of Asiatic black bears in Nepal.

Similarly, all fecal samples were soften in water and washed them through 2 mm sieves with the help of under graduate students form Institute of Forestry to separate individual food items (**Photo 11**). All items were identified to the finest taxonomic resolution possible. I visually estimated the relative volume of each food item and assigned an exact volume. Items found in trace amounts were given an arbitrary volume of 1% or only noted,

but bear hairs, dry leave, and wood particles were excluded before estimating volume. I identified wild fruits, bamboo shoots, grasses, agriculture crop (maize, rice and apple), mammal's hair and insects in the bear feces. I will calculate percent frequency of occurrence (FO) and percent volume (PV) to identify contribution of each food item of bear.

Photo 1: a) Interaction with ACAP field staff and community members in Sikesh. b) Community meeting in Mustang

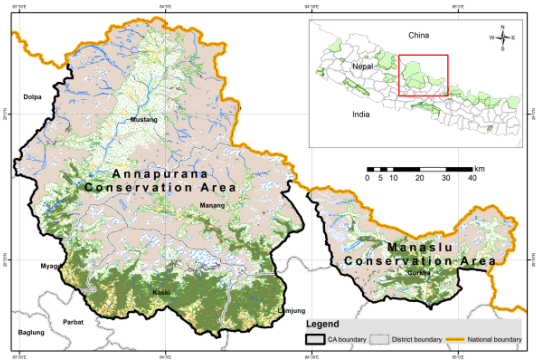

Photo 2: Map of Annapurna Conservation Area

Photo 3: Claw mark of bear on tree

Photo 4: A livestock owner who spend his whole life in forest (also attack by bear)

photo 5: bear feces found in forest

photo 6: collection of fecal sample for genetic study from maize land

photo 7: Hair trap set up

photo 8: Camera for monitoring trap site

Photo 9: Bear hair in barbed wire fence

Photo 10: Extraction of DNA from bear feces

Photo 11: a) Reference of bear food items

Photo11: b) washing of bear feces to identify food items

photo: Bear habitat landscape in Annapurna Conservation area

Table 1: Details of sample collection

S.N.	Site (place)	July - September	October - December	Total	Remarks (Agriculture land /Forest)
1	Mustang (6)	30 (11)*	3	33 (11)*	31/2
2	Mygdi (2)	6	6	12	6/6
3	Ghandruk (2)	(1)*	2	2 (1)*	0/2
4	Siding (3)	3	11	14	3/11
5	Karuwa (2)	1	7	8	0/8
6	Siklesh (3)	1	24	25	1/24
7	Manag (3)	5		5	5/0
Total		46 (12)*	53	99 (12)*	46/53

*hair samples

（~~Field Epidemiology~~・**Collaborative Research**）Evaluation by supervisor

Institution・Official title・Name	Graduate School of Veterinary Medicine 印 Professor Toshio Tsubota
Describe overall evaluation on the applicant's activity in overseas practice. Rabin Kadariya had gotten a success of collaborative research with some organization of Nepal such as DNPWC, NTNC, ACAP and GENETUP to collect DNA samples of Asiatic black bears in the Annapurna Conservation Area of Nepal. His effort and accomplishment of this collaborative research with difficult fieldwork and negotiation for permission was great and evaluated as the Superior. I hope valuable findings on genetic variation of Asiatic black bears in Nepal will come from his further studies.	

※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.

※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.

Submit to：Leading School section, International Affairs Office

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