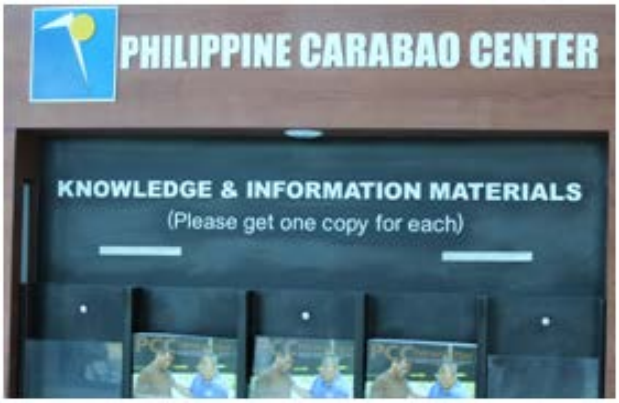
Overseas Practice on（**Field Epidemiology**・Collaborative Research）
report form（For Student）

2016 / 07/ 12 (Year/Month/Day)

Name	Nyamsuren Ochirkhuu
Laboratory	Laboratory of Infectious Diseases
Year (Grade)	D3
Place of practice	The Philippine Carabao Center in Science city of Munoz, the Philippines
Period of practice	2016.06.20 - 2016.06.29
Purpose	To find cattle mixed-infected with several pathogens which cause chronic infection in cattle, such as bovine leukemia virus (BLV), *Mycobacterium avium* subspecies *paratuberculosis*, *Anaplasma marginale*, *Babesia bovis*, *Babesia bigemina*, *Theileria* sp., and *Trypanosoma evansi* from cattle in Luzon island of the Philippines for the clinical trial of chimeric antibody therapy which was established in our laboratory (Laboratory of Infectious Diseases, Graduate School of Veterinary Medicine, Hokkaido University)

1. Departure from Sapporo city and arriving the Philippine Carabao Center (PCC): (20th-21th, June) We left from Sapporo city 20th of June (Monday) and arrived at PCC in Science city of Munoz, which is our host institute. PCC is an attached institute of the Department of Agriculture under the government of the Republic of the Philippines, and the main objectives of PCC are to study and promote the water buffalo in the Philippines as a multi-purpose animal that can be raised for milk, meat, hide and to control their diseases.



Our laboratory has been collaborating with PCC for a long time. In next day, we visited PCC, adjusted our sampling work with PCC researchers, and prepared all necessary items such as vacuum tubes, needles, syringes, mask, gloves and gumboots for sample collection work in the farms.

2. Research activity at the PCC in the Philippines

a. Sample collection: (22th-25th, June) Actual plan for sample collection had been slightly changed due to the accessibility to the distant sampling area from the PCC. I moved to the sampling area with Dr. Satoru Konnai and three research staffs of PCC (Daryl Gonzales Dela

Cruz, Ermyn D Ermitanio, Michelle M. Balbin). The distance from PCC to sampling area was approximately 320 km, and it took about 9 hour by car. After 4-day-working, we collected 158 blood samples from cattle and 46 samples from goat in the Cagayan region of Luzon island, in the Philippine.

Sample collection work from cattle and goats in Cagayan region of Luzon island, in the Philippine

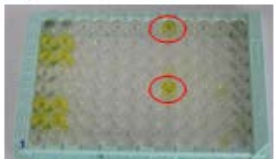
b. DNA extraction and serum separation: (26th, June) After coming back to PCC, we started serum separation and DNA extraction in the laboratory of Animal health, PCC. We extracted 158 DNA samples from cattle blood by using genomic DNA purification kit (Promega) and isolated also 158 tubes serum for the screening of several pathogens at our laboratory.

DNA extraction and serum separation work in the animal health laboratory of PCC

c. Detection of the *M. paratuberculosis* by ELISA assay: (27th, June) All 158 serum samples were screened by ELISA assay for *M. avium* subspecies *paratuberculosis.* Of these, two cattle (C43 and C48), which from same farm, were positive for the infection.

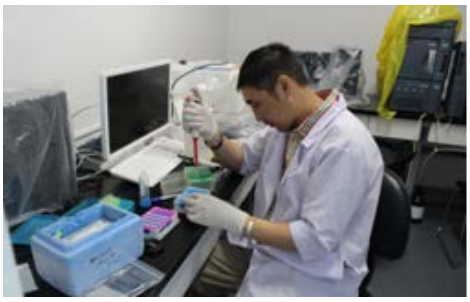
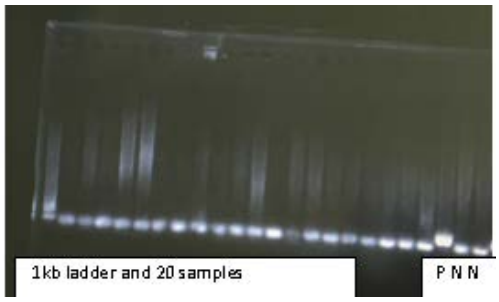
ELISA screening for *M. paratuberculosis*





d. Detection of the Caprine arthritis encephalitis virus (CAEV) in goat samples: (27th, June) We also collected 40 samples to detect CAEV in goat in the Cagayan region of the country. The prevalence of the infection was 12.3 % in 2015 (Padiernos *et al*., 2015), but this was only from three areas of the country. The prevalence of CAEV in the Philippines is not known. Thus, I tried to detect CAEV in the samples by nested PCR assay on 20 goat DNA samples for my training, and none of the samples was found positive for the infection. I will screen the goat samples from Mongolia for CAEV detection in the near future.

nested PCR assay for CAEV detection on 20 goat samples which were collected by us

3. A seminar for students and research staffs: (28th, June) Dr Michelle M. Balbin and her colleagues helped me to organize a special seminar for students and research staffs in the meeting hall at PCC. Title of the presentation was “Study on epidemiological and immunological research for intractable infectious diseases in livestock” and it included a molecular epidemiological survey of vector borne infections in cattle in the Luzon island of the Philippines, which I have done previously. Many professors, research staffs and students attended the seminar and interested in some part of my study.

Open presentation for researcher and students


4. Coming back to Sapporo from the Philippines: (28th, June) We left the PCC at early morning to Ninoy Aquino International Airport in Manila, and came back to Sapporo at 11 pm in the same day.

5. Detection of several pathogens by PCR assays in the samples: (29th, June-8th July) After coming back to Laboratory of Infectious Diseases in Graduate School of Veterinary Medicine, Hokkaido University, we conducted nested and single step PCR assay for the screening of several pathogens in cattle. Of 158 samples, 12 (7.6%) for BLV, 72 (45.6%) for *A. marginale*, 56 (35.4%) for *B. bovis*, 30 (19.0%) for *B. bigemina*, 48 (30.4%) for *Theileria* sp., and 2 (1.3%) for *T. evansi*, were found positive. In addition, many different combinations (50.6%) of mixed infections with 2 and 3 kinds of pathogens were frequently observed in the samples and two cattle (sample ID 99 and 105) have been chosen for the clinical trail of chimeric antibody, which is established in our laboratory.

（Field Epidemiology・Collaborative Research）Evaluation by supervisor

Institution・Official title・Name	Graduate School of Veterinary Medicine, 印 Professor, Kazuhiko Ohashi
According to the schedule, Nyamsuren Ochirkhuu has successfully completed his overseas practice on the epidemiological survey in Philippine Carabao Center (PCC), in the Philippines. During the stay, he had good communication with heads and research staffs of the host institute and farmers of the sampling areas. On this overseas practice, he was able to collect enough numbers of samples to check mixed-infected animals with several pathogens for the clinic trail of chimeric antibody as he mentioned in his report, and share some technique with young staffs of PCC for the detection of Johne's disease and caprine arthritis encephalitis virus by ELISA and PCR assays, respectively. In addition, he had a good opportunity to present his works to the staffs and students of PCC and to discuss about similar studies done at their laboratories, that is helpful to improve his presentation/discussion skills.	

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