Name	Sarad Paudel
Laboratory	Wildlife Biology and Medicine
Year (Grade)	D2
Destination	Nepal (Chitwan National Park, Parsa Wildlife Reserve and Koshi Tappu
	Wildlife Reserve)
Period of trip	May 7 to June 9, 2013
Purpose of trip	For the research on "Prevalence of tuberculosis among the elephant
	handlers of protected areas of Nepal"

Abroad Official trip report form

## Summary of activities

A trip was made to Nepal from May 7 to June 9, 2013 for the study on the prevalence of tuberculosis among the elephant handlers of protected areas of Nepal.

I was accompanied by my supervisor Prof Toshio Tsubota in the beginning of the field work. Like during the previous visits, we had some important meetings in Kathmandu and Chitwan with the experts working in the field of human tuberculosis and wildlife conservation. On May 8, we met with Dr Bhawana Shrestha and Mr. Bhagwan Maharjan at Tuberculosis lab of German Nepal Tuberculosis Project (GENETUP) in Kathmandu. We did the Material Transfer Agreement (MTA) for the transfer of the DNA extraction from the isolates of elephants and the elephant handlers between GENETUP and Graduate School of Veterinary Medicine, Hokkaido University. We also discussed my plan of tuberculosis research in the elephant handlers and use of their lab for the culture. The meeting was fruitful. On the same day, we had a meeting with Dr Maheshwar Dhakal, Ecologist of Department of National Parks and Wildlife Conservation (DNPWC) in his office at Kahtmandu. We explained the purpose and objectives of my study strip. On May 9, we also had meetings with Mr Ganga Jung Thapa and Dr Naresh Subedi at National Trust for the Nature Conservatino (NTNC) in Kathmandu regarding the future collaboration for the bear research in Nepal. We also had some meetings with Senior Veterinary Officer and other officials at Chitwan National Park. We explained our purpose of the study.

After completion of series of meetings and organization of the supplies, I started my field study. The study was carried out in three protected areas of Nepal viz. Chitwan National Park (CNP), Parsa Wildlife Reserve (PWR) and Koshi Tappu Wildlife Reserve (KTWR).

There are 50 captive elephants diagnosed with tuberculosis using DPP VetTB Assay (Chembio Diagnositcs., USA) in different protected areas of Nepal. Each elephant has generally three handlers. As the handlers are transferred from one elephant to another after some duration, so the sampling was also done from the handlers who previously worked in tuberculosis positive elephants too. 308 sputum samples were collected in total from 153 handlers (CNP – 130, PWR – 15, KTWR – 8). Early morning sputum was collected for a two day in a row. Due to some unavoidable circumstances, only one sample was collected from two handlers and 3 samples from 4 handlers. The written consent was taken from each handler before the participation in the study. A short questionnaire survey was done with each elephant handler. The sputum sample was run at the tuberculosis lab of German Nepal Tuberculosis Project (GENETUP) within 36 hours of the sputum collection. The decontamination was done using NALC solution. The microscopy was done in acid-fast stain using Auramine. The culture of the processed sputum sample was done in the L-J media.

The questionnaire survey showed that about 14% of handlers complained some minor symptoms like chest pain and coughing. Two handlers had successfully completed their TB treatment. 6% handlers had someone in their family with TB sometime back. The microscopy result showed that all the sputum samples were negative on the acid-fast (AF) staining. The culture results will be known after eight weeks i.e. from July 14 (inoculation on May 14) to August 2 (inoculation done on June 2). The DNA will be extracted from the isolates and the genetic analyses including Multi-locus Variable Number Tandem Repeat Analysis (MLVA) and spoligotyping will be done at Center for Zoonosis Control, Hokkaido University.



Fig.1 Briefing how to collect sputum samples to the handlers



Fig.2 Questionnaire survey with handlers

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Fig.3 Sample collection from handlers



Fig.4 Inoculation of processed sample in L-J Media at TB lab in Kathmandu