（Abroad）Official trip report form（Student） 13/07/29

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Laboratory	Division of Global Epidemiology, Research Center for Zoonosis Control
Year (Grade)	First Year PhD student
Destination	Philippines
Period of trip	June 2, 2013 – July 6, 2013
Purpose of trip	Conduct of ELISA test sample collection for the research study in Japan

My research study here in Hokkaido University is focused on bacterial zoonotic diseases in livestock in the Philippines. The first disease that I am dealing with is leptospirosis caused by *Leptospira* sp. which is considered to be endemic in the Philippines. Wild and domestic mammals harbor the *Leptospira* sp. and served as reservoir host which is the source of human infection. Numerous studies have been conducted on rodents and human infection but still limited on other animals. Recently, water buffalo was found positive to polymerase chain reaction test (PCR) but remains asymptomatic. We produced recombinant proteins LipL32 and LipL41 for checking the antibody titers of water buffaloes against *Leptospira* sp. these outer membrane proteins are abundant in the outer membrane and are found only among pathogenic species. The objectives of going the Philippines for one month are (1) performed ELISA in testing water buffalo serum samples for *Leptospira* research, (2) collected urine samples from different animals for *Leptospira* research and (3) collecting samples as well as assisting one private cattle farm for the diagnosis of bovine tuberculosis.

The activities during the first week of stay were identifying the negative control for the ELISA assay (this was done in Philippines since transport of serum samples in each country is prohibited). We successfully identified sample that we can use as negative control through fetal calf sera (Figure 1). We also attended a meeting at the WHO Western Pacific Region (WPRO) with Dr. Li Ailan (Director of WPRO), Dr. Tomoe Shimada (Medical Officer, Emerging Disease Surveillance and Response Unit) and Dr. Norizaku Isoda (Technical Officer, Emerging Disease Surveillance and Response Unit) to discuss about the proposed project on leptospirosis in the Philippines (Pictures 1 and 2). Another meeting that we attended was at University of Philippines (UP-Manila), College of Public Health in Manila with Dr. Nina G. Gloriani (Dean, College of Public Health), Dr. Yasutake Yanagihara (Shizuoka University) and Dr. Lolita Cavinta (UP-Manila) discussing about another project on *Leptospira* research (Picture 4). I was also given the chance to present my study during the monthly technical caucus conducted at the Philippine Carabao Center National Headquarters and Genepool at Nueva Ecija, Philippines (Picture 3).

The next three weeks was devoted more on the conduct of the enzyme-linked immunosorbent assay (ELISA) in water buffalo samples (Picture 5). A total of 180 serum samples collected from different locations in the Philippines were tested. Initial analysis of the result showed that 100% of samples tested have high titers against *Leptospira* (Figure 2, Table). This suggests that the disease is circulating and maintained within the herd. Urine collection on water buffaloes and goats were also performed (Picture 8). Extraction of DNA was done at the same day and stored at -20ºC to be used for molecular-based assay. This is also the time when we visited the private cattle farm and assisted them in the diagnosis of bovine intradermal tuberculin test-reactive animals (Picture 7). We collected blood and milk samples for Interferon Gamma Assay and conventional polymerase chain reaction, respectively. We also collected urine samples for leptospirosis research. Interferon Gamma assay was performed using QuantiFERON®-TB Gold In-Tube test (Cellestis Inc., California, USA) and Bovine IFN-γ VetSet™ ELISA Development Kit (Kingfisher Biotech, Inc., USA). Based on the result (Figure 3), only sample 35 gave the expected results for the three tubes (Nil/negative control, TB antigen and Mitogen/positive control) while the rest showed unusual results (Figure 3). Representative result from sample 9 showed high OD value similar to TB antigen and Mitogen control. Explanation for this is the possible presence of heterophile antibodies or non-specific IFN-γ production from other inflammatory conditions. On the other hand, Mitogen/positive control has a lower OD value compared to TB antigen, as shown in sample 32. This might be caused by delayed incubation of the samples or inappropriate temperature prior to processing in the laboratory. Another reason is that the phytoagglutinin A (PHA), the positive control used in human tuberculosis IFN-γ assay gave an inconsistent result in stimulating IFN-γ production using cattle and sheep whole blood (Robbe-Austerman *et al*., 2006). Nevertheless, results from this test will still be useful in conducting other tests for the diagnosis of *Mycobacterium bovis* infection on these animals.

During the last week of stay in the Philippines, collection of urine samples from pigs was performed. This will be used for testing the presence of *Leptospira* sp. among pigs. ELISA testing was also performed in pig serum samples using the rOMP's in checking the antibody titer against *Leptospira*. As shown in Figure 4, most of the pig serum samples (98%) tested has antibodies against *Leptospira*, similar with the findings on water buffaloes. It also showed that the recombinant proteins produced reacted well on serum from other species.

Future activities for leptospirosis study will be dealing on molecular-based diagnostic techniques such as Loop-mediated isothermal amplification (LAMP) assay and polymerase chain reaction for detecting the presence of the bacteria and its identification to species level, respectively. Multilocus sequence typing (MLST) will also be employed for further epidemiological investigation through possible transmission of pathogenic *Leptospira* spp. within and outside the Philippines.

Pictures

Picture1. Meeting at the WHO Western Pacific Region, Manila. Discussion with Dr. Tomoe Shimada (left) and Dr. Norizaku Isoda (next) about the proposed project on zoonotic diseases in the Philippines.

Picture 2. Meeting with Dr. Li Ailan (far left), Director of WHO-WPRO.

Picture 3. Awarding of certificate after the presentation of research study during the monthly Technical Caucus at the Philippine Carabao Center.

Picture 4. Meeting at University of the Philippines-Manila with Dr. Nina Gloriani (far right), Dr. Lolita Cavinta (next) and Prof. Yasutake Yanagihara (center) discussing about the proposed project on leptospirosis in the Philippines.

Picture 5. Performing ELISA test on water buffalo serum samples for checking the antibody titer against *Leptospira*.

Picture 6. Decontamination of milk samples for tuberculosis research.

Picture 7. Blood collection in one private cattle farm in Laguna, Philippines.

Picture 8. Urine collection on water buffaloes kept at Philippine Carabao Center.

Tables

Table 1. Urine samples collected for *Leptospira* research.

Animal	Date	Number of samples	Location
Water buffalo	May 28-29, 2013	130	Nueva Ecija, Philippines
	June 25, 2013	25	Nueva Ecija, Philippines
	June 29, 2013	40	Nueva Ecija, Philippines
Cattle	June 21, 2013	30	Laguna, Philippines
Goat	June 29, 2013	8	Nueva Ecija, Philippines
Pig	July 3, 2013	27	Nueva Ecija, Philippines

Table 2. Milk samples collected from tuberculin test-reactive cattle for tuberculosis research.

Animal	Date	Number of samples	Location
Cattle	June 21, 2013	46	Laguna, Philippines

Figures

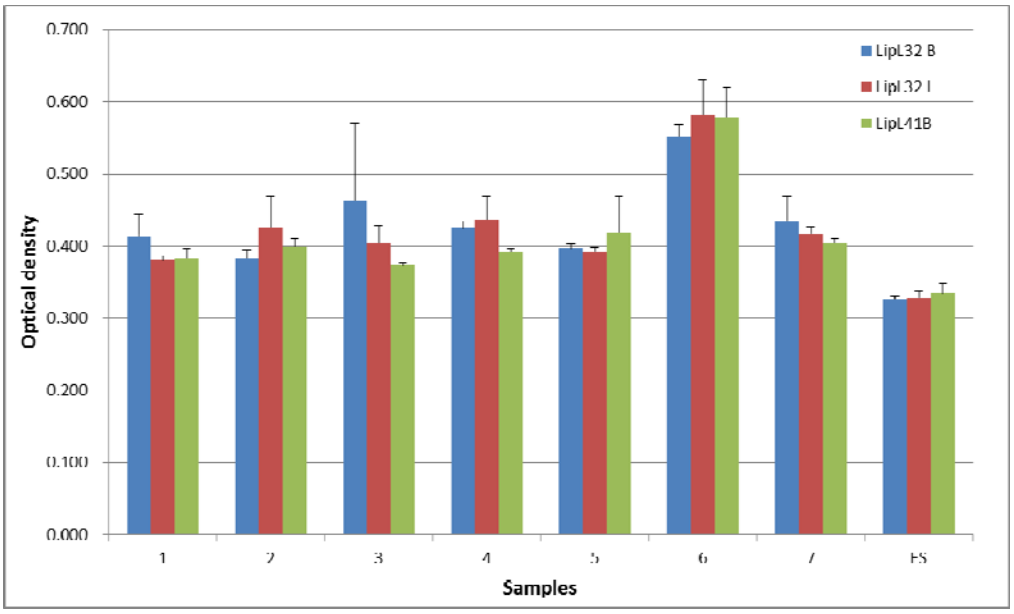

Figure 1. Testing of fetal serum (FS) as negative control for the ELISA test.

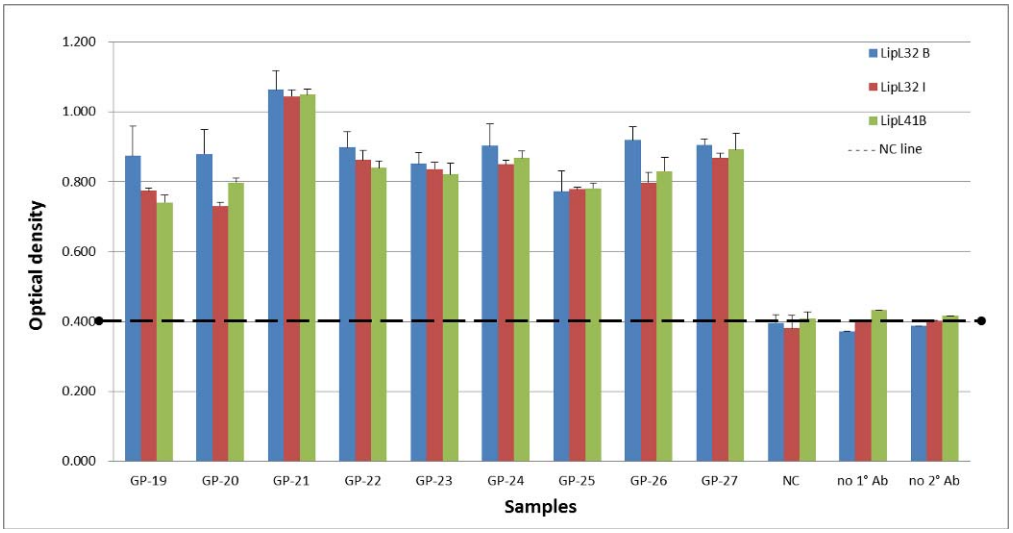

Figure 2. Representative result of water buffalo serum samples tested for ELISA assay using rOMP's..

Table 3. Water buffalo serum samples used for the ELISA test.

Location	Number of serum samples	Number of reactive serum samples to rOMP's (ELISA)
Philippine Carabao Center (PCC) National Headquarters and Genepool, Muñoz, Nueva Ecija, Philippines (GP)	50	50/50 (100%)
PCC at Central Luzon State University, San Jose City, Nueva Ecija, Philippines (CLSU)	50	50/50 (100%)
PCC at University of the Philippines-Los Baños, Los Baños City, Laguna, Philippines (UPLB)	50	50/50 (100%)
PCC at Cagayan State University, Piat, Cagayan, Philippines (CSU)	14	14/14 (100%)
PCC at Mariano Marcos State University, Batac, Ilocos Norte, Philippines (MMSU)	16	16/16 (100%)
Total	180	180/180 (100%)

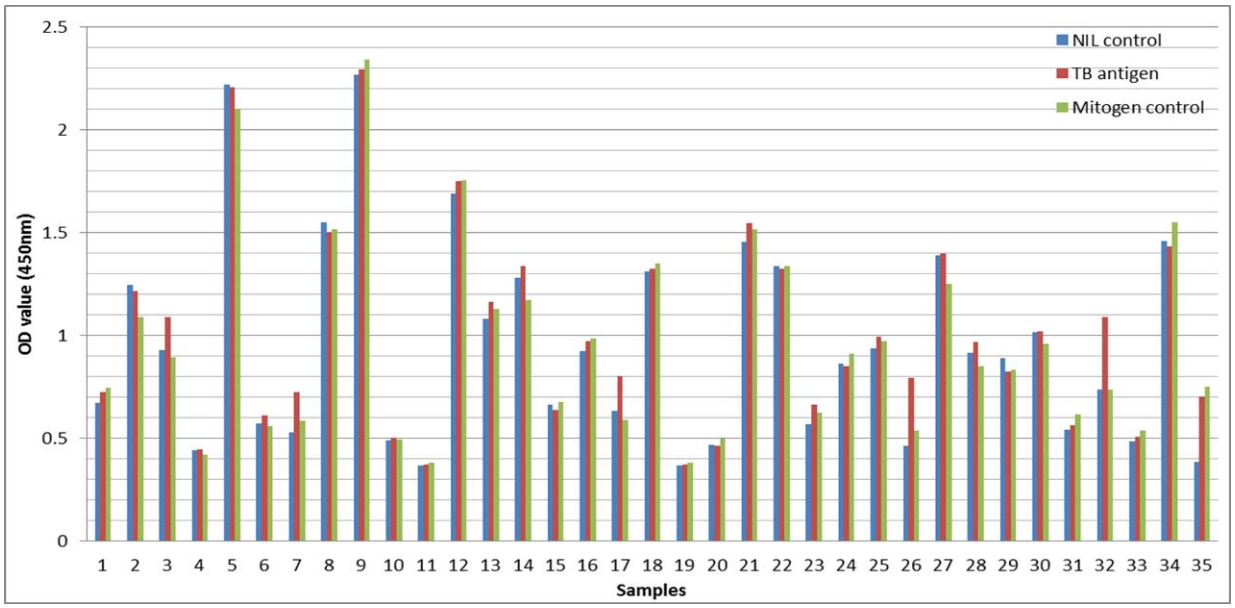

Figure 3. Result of bovine Interferon Gamma Assay for tuberculin test-reactive cattle.

Table 4. Whole blood samples collected from tuberculin test-reactive cattle for bovine interferon gamma assay.

Animal	Date	Number of samples	Location
Cattle	June 21, 2013	35	Laguna, Philippines

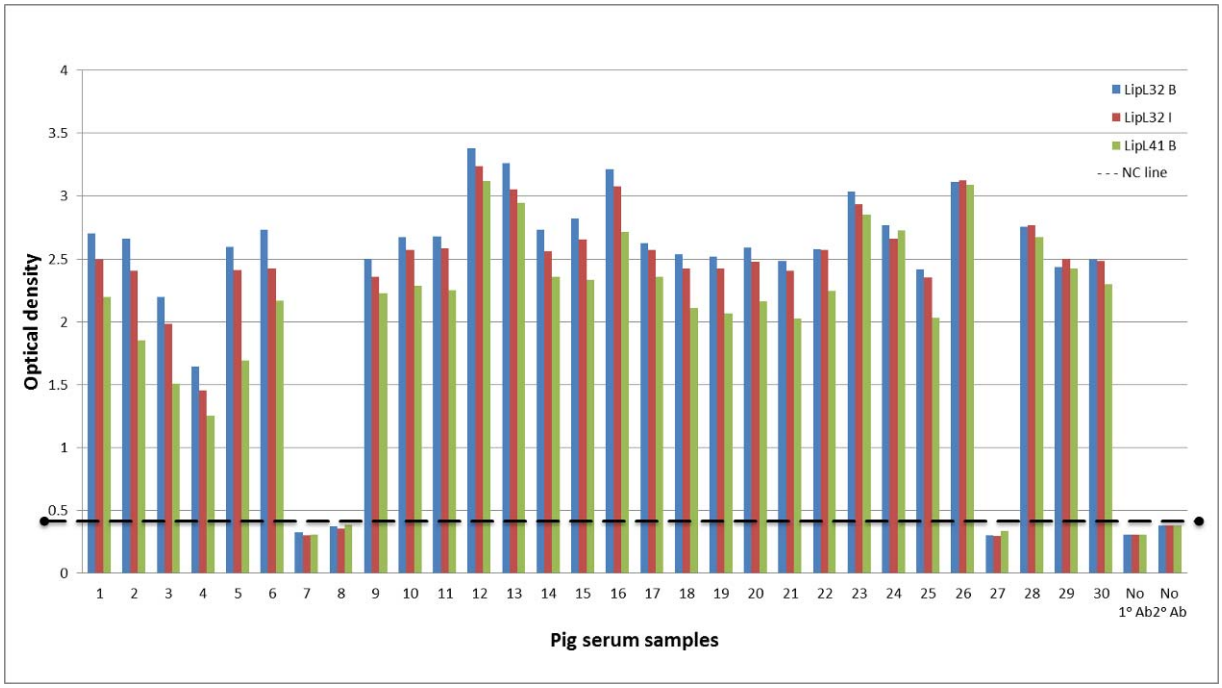

Figure 4. Representative result of pig serum samples tested for ELISA assay using rOMP's.

Table 5. Pig serum samples used for ELISA test.

Animal	Number of serum samples	Number of reactive serum samples to rOMP's (ELISA)
Pig	140	137/140 (98%)