（Abroad・Domestic）Internship report form（Student） 2016/05/25 (Year/Month/Day)

Name	Marvin Ardeza Villanueva
Laboratory	Division of Bioresources, Hokkaido University Research Center for Zoonosis Control
Year (Grade)	D4
Internship institution	Department of Population Medicine and Diagnostic Sciences College of Veterinary Medicine, Cornell University
Internship period	Internship period: 04/09/2016 - 05/11/2016
Purpose	The main purpose of this internship activity was to acquire additional and advanced skills/ techniques for me to be able to apply multi-disciplinary approaches when dealing with several infectious diseases of economic concern in my home country. Another aims were to be exposed on other research-oriented environment which I may pick-up some strategies that I can apply as a researcher, and lastly to establish connections with prestigious universities as well as professors for my future career as a post-doctoral fellow in overseas universities or institutes.

The reasons why I chose Cornell University

The reason why I chose Cornell University for my internship was that indeed, it is considered as one of the leading universities in terms of Veterinary Medicine, therefore I have high expectation that I can gain additional research insights aside from what I have learned in Japan in making me a well-rounded researcher for infectious diseases. Another reason was that learning about vaccine development against leptospirosis is essential for our continued efforts for the control and prevention of this zoonosis in the Philippines. I believed that visiting Professor Chang's laboratory can guide me in achieving my goal for developing a vaccine against leptospirosis and other economically important infectious diseases in the future.

Result of the activity

I spent my one month internship activity at the Infectious Disease Research Laboratory/ Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA under the supervision of Professor Yung-Fu Chang. His laboratory is working on a variety of research topics such as

molecular biology of infectious disease, DNA and recombinant sub-unit vaccine development, molecular basis of bacterial pathogenesis, immunopathology of infectious diseases, molecular diagnosis of infectious diseases, host defense mechanisms and comparative genomics and proteomics. I had a discussion first with Professor Chang and we talked about what I want to learn during my stay in his laboratory, which was about focusing more on DNA and recombinant sub-unit vaccine development against leptospirosis and other infectious diseases (see Photo 2). He explained that their current project was on evaluating the leptospiral immunoglobulin-like proteins A and B (LigA, LigB) for vaccine development. Lig antigens have been previously assessed as vaccine candidate since 2002 and were considered the only antigen that had proven protective efficacy to date. Lig proteins were one of the abundant outer membrane proteins (OMPs) of pathogenic leptospires and it is only expressed in vivo. Previous reports showed that the absence of Lig proteins loses its pathogenicity; therefore it is an important virulence factor. Lig proteins are consisted of tandem repeat domains, wherein domains 1-7 are conserved while the remaining tandem repeat domains were unique or variable (see Figure 1). Exploring the immunogenicity of either whole or truncated Lig proteins, both single or in combinations was the current activity of Professor Chang's lab for vaccine development against leptospirosis. Those domains that are highly immunogenic were prepared in different combinations, resulting into fusion or chimeric proteins that will be used for immunizing challenged golden hamsters for vaccine trials. Unfortunately, I was not able to participate in vaccine trial (which will be scheduled on June 2016) and I just mainly participated in the production of recombinant Lig proteins. Nonetheless, I was able to harness my skill in making a fusion or chimeric proteins/ antigens that I can find useful for vaccine development or for diagnostic purposes (see Photos 3-4). I also learned several techniques that are faster than the conventional procedures that I learned in Japan.

Aside from the discussion on leptospirosis for future collaborative research with Prof. Chang's laboratory, we also had a productive exchange of ideas with other economically important infectious diseases in the Philippines such as *Pasteurella multocida* (water buffalo and cattle), *Actinobacillus pleuropneumonia* and *Hemophilus parasuis* (pig) for possible vaccine development.

Other than participating in the usual lab work, I also finished the web-based and classroom training courses that were standard operating procedures of Cornell University's Environmental Health and Safety (EHS) and Occupational Health and Safety Management Systems (OHSAS) rules for those who will be working in the laboratory (see Photo 5). I was also able to attend on several seminars organized at the College of Veterinary Medicine (see

Photo 6).

I was also given a chance to share the knowledge and skills that I've learned here in Japan to one of my fellow PhD student from Pakistan who is also there for his internship. His work is on leptospirosis in horses and I helped him in the epidemiology aspect, making a phylogenetic tree, data analysis and manuscript writing. I also experienced reviewing some papers related to leptospirosis and vaccine development.

Despite the usual laboratory works, I still make time to explore the beauty of Cornell University. The old and magnificent buildings are still well maintained and it resembles the usual scenery in a classic movies. Professor Chang also generously took us to the two state parks within the city. The city of Ithaca has several beautiful parks to visit with, especially nature lovers and to those who wanted to have leisure and having exercise at the same time. The view was breath-taking as it is my first time to see that. I really enjoyed the company of the laboratory members of Professor Chang.

Positive impact on my student life and career path

Visiting Cornell University not only helped me explore other research-based working environment and learned other laboratory techniques essential for my work but also the values and mindset of being a researcher. It made me realized that as a researcher, I should specialize on an area that I will be known for and what I can contribute to my country and study deeper on that chosen field, as Prof. Chang advised. Also, I should be resourceful, given that we have a limited budget in our Institute in the Philippines, so I can apply from other funding institutions or have a strong collaboration in institutes from developing countries. Time is also essential which is why I should not waste it and should continuously make meaningful researches that will ultimately benefit my country. And finally, I should not forget to enjoy my life as a human being and live healthy.

Positive impact on my further research

I am confident that I and my colleague from the PCC had established another strong collaboration with Professor Chang with regards to vaccine development. Currently, I started to make a project proposal on animal leptospirosis in the Philippines which will be a nationwide epidemiological survey. It will also include isolation of leptospires from water buffalo in collaboration with my supervisor in CZC for future analysis using whole genome sequencing as well as application of sensitive, cheap and easy to use diagnostic tests for pathogenic *Leptospira*. And finally - vaccine development if ever we will find that leptospires

to be isolated from water buffalo will be of public health and economic concern. I am happy that I already clear on my path as a researcher when I go back to the Philippines, so I can focus my time and effort to be a better researcher and be able to contribute to my country.

My advice to junior fellows

1. Make a decision on what you really want to do after your study in Japan, and that will give you a hint on where you want to go for your internship.
2. Do the internship as early as possible.
3. Choose the best institute that you want to visit since it is rare to have this kind of opportunity.
4. Make the most out of your visit, learn as much as you can.
5. Bear in mind that you are representing Hokkaido University, so act as a good ambassador
6. Do not forget to relax and enjoy the place.

Approval of supervisor	Institution・Official title・Name Hokkaido University Research Center for Zoonosis Control Professor Yasuhiko Suzuki 印

※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 Attach a copy certificate of the content of internship activity that is prepared by the counterpart at the internship institution (any form with a signature of the counterpart).
※3 The Steering Committee of the Leading Program will first confirm the content of this report and 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

As of Mar 19, 2015

Photo 1. Photo taken at the landmark of Cornell University

Photo 2. Discussion with Prof. Yung-Fu Chang (right) and with Dr. Claro Mingala (PCC).

Photo 3. Pasmid purification as part of protein expression and purification.

Photo 4. Size exclusion chromatography for further purification of protein for vaccine trials.

Photo 5. Attended classroom training as part of Compliance to Cornell University's rules on EHS and OHSAS.

Photo 6. Attended one of the weekly seminars provided by the College of Veterinary Medicine

Photo 7. Outside view of College of Veterinary Medicine At Cornell University.

Photo 8. Entrance of the Equine Hospital

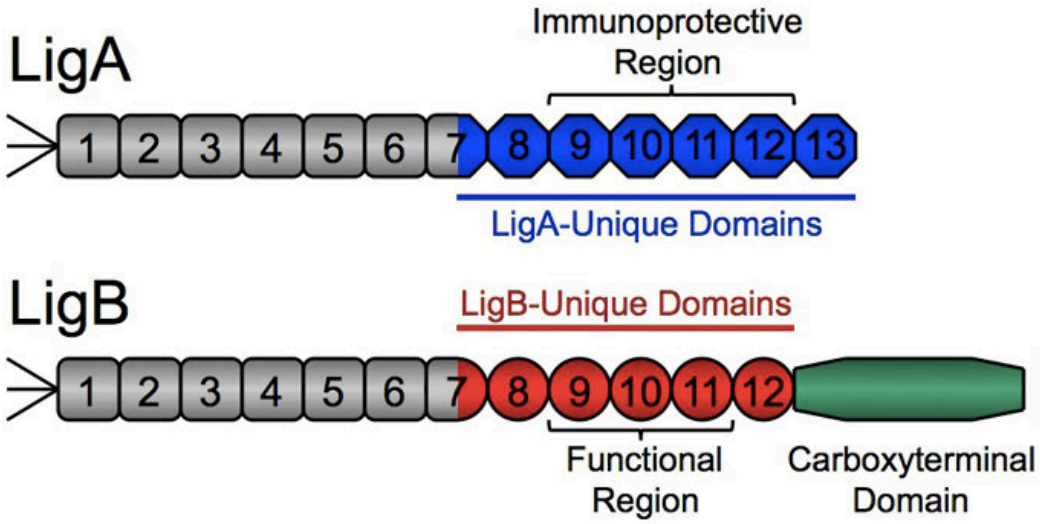

Figure 1. Schematic figure of tandem repeat domains of Lig proteins. (Source: http://id-ucla.org/leptospirosis_research)