Updated Aug. 5, 2019

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（Abroad・Domestic）Internship report form（Student） 2020/03/18 (Year/Month/Day)

Name	Kraisiri KHIDKHAN
Laboratory	Toxicology
Year (Grade)	D4
Internship institution	Department of Pharmacology and Toxicology, and Raptor Center, Faculty of Veterinary Medicine, Kasetsart University, Thailand
Internship period	Internship period: 02/10/2019 - 30/10/2019 (Departure Date from Sapporo: 02/10/2019, Arrival Date in Sapporo: 31/10/2019)
Purpose	- To undertake an internship abroad - To obtain the experience for working as a veterinarian in wildlife unit, especially a raptor center. - To get the knowledge and skill about animal welfare, animal quarantine, the management and create the environments for wildlife. - To gain the experience in toxicological research related to wildlife conservation, the *in vivo* kinetic study and analysis of blood clotting time in raptors exposed to warfarin. - To understand the strategy of working and organizing in the university. - To create the network for job and research collaboration in the future.

- The reason why you chose this institute

After finishing my PhD course, I have plan to be a lecturer at a university, especially Faculty of Veterinary Medicine, Kasetsart University. This faculty is very famous for veterinary teaching, research, animal hospital and farm service facilities in Thailand. The academic position in this faculty is the most important for me because I would like to continue my experimental research, develop the studies on environmental toxicology, and support the specific knowledge of veterinary toxicology to students. Therefore, I chose the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Kasetsart University for my internship.

Assistant Prof. Aksorn Saengtienchai belong to Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Kasetsart University. Her research interests focus on the biomonitoring aspects and the metabolism pathways for drugs and environmental pollutants. Currently, she has the research collaboration with a Raptor Clinic, Kasetsart University Raptor Rehabilitation Unit (KURU) and has interested in the toxicological studies in raptors. I have great interest in raptor biomonitoring studies of warfarin, which is the general

anticoagulant rodenticide in Thailand. Her studies inspire me to study more about the biotransformation pathways of warfarin as well as the effects of warfarin, especially blood clotting time, in migratory birds and raptors.

- Result of the activity

During my internship period, I participated in many interesting events with professors and staffs in Faculty of Veterinary Medicine, Kasetsart University. The Figure 1 show the main contents of my activities in Thailand.

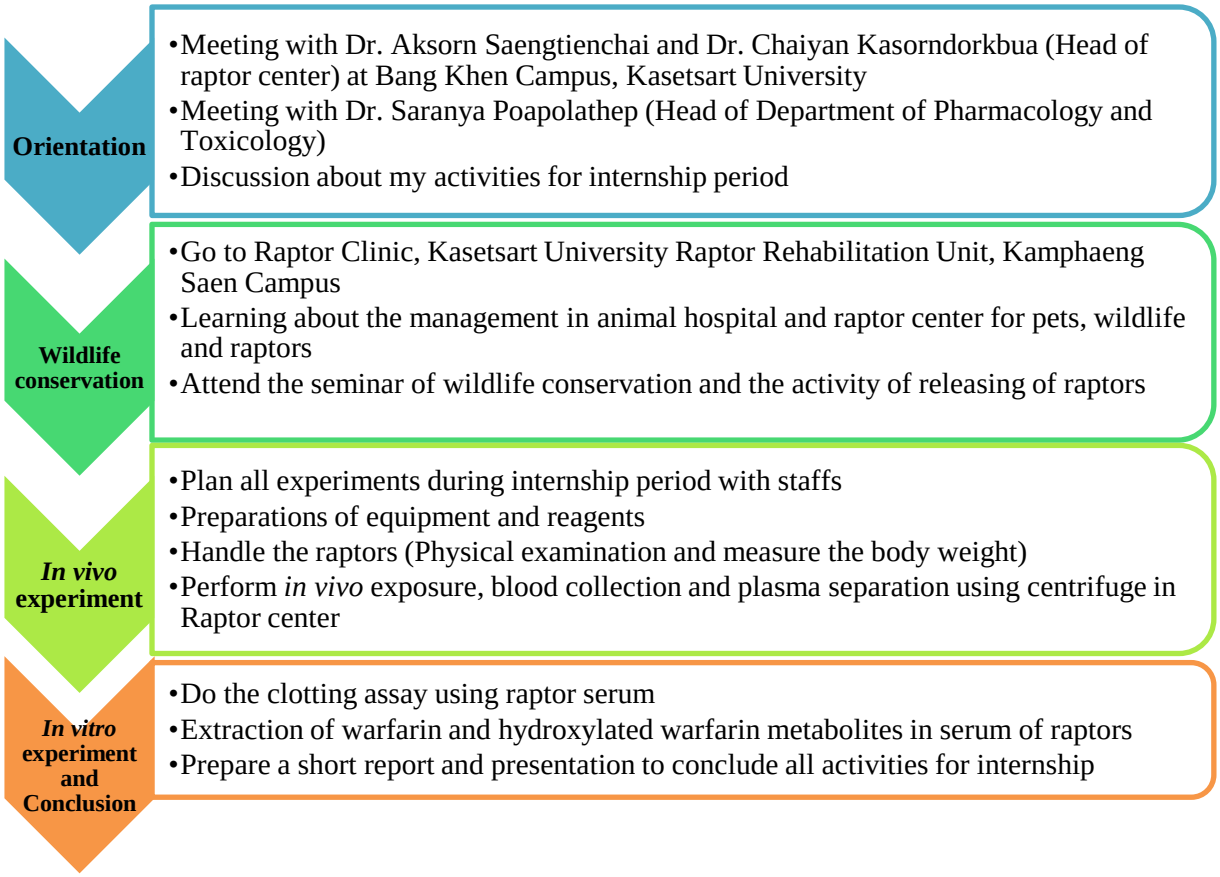

Figure 1. The highlight event during my internship period

According to above lists of my activities, the results and impacts of this internship are include:

1. **Orientation**: After meeting with Assistant Professor Aksorn Saengtienchai at Bangkok, she is very kind and introduced me to Associate Professor Chaiyan Kasorndorkbua (Head of raptor center) at Bang Khen Campus, Kasetsart University. We discussed about the experiment of warfarin exposure in raptors. Because this was my first time for doing the experiment with raptors, they lectured me about the characteristic of each species of raptors, their habitats, foods, seasonal migration. To gain achieve the good results of our research experiment, we discussed

the differences of blood collections between normal birds and raptors. Interestingly, we have established a good relationship; Dr. Chaiyan suggested me do other toxicological experiments in raptors for the future research collaborations. In addition, I met Associate Professor Saranya Poapolathep (Head of Department of Pharmacology and Toxicology) and Associate Professor Amnart Poapolathep (Founder and President of the Association of Mycotoxicology, Thailand). I had a dinner with them (Figure 2). They asked me about my future research and suggested me for getting position in Faculty of Veterinary Medicine, Kasetsart University. I got useful suggestions and very kind support from them.

Figure 2. Dinner with professors of Department of Pharmacology and Toxicology, Kasertsart University.

2. **Wildlife conservation:** After that, I went to Kamphaeng Saen Campus to visit the Kasetsart University Veterinary Teaching Hospital. I was introduced to with staffs and technicians in various units, including small animal (cat, dog, and exotic pets) unit, large animal (cow, goat, horse) unit and wildlife unit. They showed me the separated facilities and equipment for animal treatment and teaching students. In addition, I visited the Raptor Clinic, Kasetsart University Raptor Rehabilitation Unit (KURU). I learned the resource organization, quarantined procedure and maintained system of animal cases from a special technician in this raptor center. It is interesting system that inspire me to establish and develop other systems for other wildlife species. Dr. Chaiyan also invited me to attend the seminar of raptor rehabilitation and wildlife conservation. He presented his research and explained about the treatment or management of several injurious raptors in this center and how to conserve them in the environment. The staffs have the health check program every 1-3 months for taking care of raptors. Furthermore, I got the knowledge about the animal welfare and preparing the suitable environments for not only raptor species, but also other wildlife such as elephant, deer, antelope and wildcat.

After recovering, rehabilitation and blood checking, most raptors will be released to their environment. Fortunately, at that time I could join the activity of releasing of raptors to the nature at Huai Kha Khaeng Wildlife Sanctuary (Figure 3). I learned that the staffs have to consider the suitable place, time, food and their hunters before releasing each raptor species for sustainability. This is a good opportunity to learn for wildlife and conservation medicine that could be relate to the environmental toxicology field in my future research and career.

Figure 3. The special technician in KURU and the releasing raptor to the nature

Figure 4. Raptor Clinic, Kasetsart University Raptor Rehabilitation Unit (KURU) and performing *in vivo* experiment with Assistant Professor Aksorn Saengtienchai and staffs.

3. ***In vivo* experiment**: Before start the experiment at KURU (Figure 4), the technician prepared the raptors, including *Haliastur indus* (n=4), Eastern barn owl *Tyto javanica* (n=1), Black kite *Milvus migans* (n=2), *Buteo burmanicus* (n=1), Black-winged Kite *Elaeneus caeruleus* (n=2), Collared scops owl *Otus lettia* (n=5), Changeable hawk eagle *Nisaetus limeaetus* (n=2) and Crested serpen eagle *Spilornis cheela* (n=2), by checking the body temperature, health status and measuring the body weight. To protect the danger from animals, staffs taught me how to handle them. We also prepared the equipment for handle the raptors, warfarin for oral exposure

and sodium citrate for blood collection. The *in vivo* kinetic study of warfarin in raptors were conducted by oral exposure and the blood was collected by time courses (0h, 0.5h, 1h, 2h, 4h, 6h, 10h, 19h, 24h, 30h, 48h and 72h after warfarin exposure) per each raptor (Figure 5). Those experiment procedures, such as the preparations of equipment and setting up *in vivo* system and time management in this kinetic study, are very useful for me and these competences can be applied for my future research projects.

Figure 5. *In vivo* experiment; warfarin exposure and blood collection in raptors.

Figure 6. *In vitro* experiment; blood centrifugation, clotting time assay and extraction method

4. ***In vitro* experiment**: After blood collection, all blood samples were separated for plasma using centrifuge at 2000g for 5 min (Figure 6). To evaluate the effects of warfarin, the plasma of warfarin exposed raptors (50 μL) was tested for clotting time assay. Since the blood clotting system of raptor was different from other species, the clotting time assay using raptor plasma was failed. In addition, I learned the extraction techniques of warfarin and their hydroxylated metabolites. Briefly, the 10 μL of plasma were mixed with 0.1 M sodium acetate, 1 μM glucuronidase oxazepam (an internal standard of warfarin and an indicator of deconjugation), 1 μM phenyl-d5-7-hydroxywarfarin (an internal standard of hydroxylated warfarin), and 5000 unit of β-glucuronidase. The mixtures were incubated at 37 °C for 3 hours. After incubation, diethyl ether was added and centrifuged at 3000g for 10 min. The organic layer was obtained

and evaporated to dryness under the gentle steam of N_2 gas and re-dissolved by MeOH. Although some of experiments were unsuccessful, this is a good opportunity to build my skills for *in vitro* study that is a significant part of my career path especially in the academic area. I got a lot of techniques and learned how to use the different machines in their laboratory.

5. **Summarize internship activities**: On the day before departure, I presented the results of all experiments and concluded the impacts of this internship to Assistant Prof. Dr. Aksorn Saengtienchai. I could create the good relationship involved in future research collaboration and my career path with her and other professors in Faculty of Veterinary Medicine, Kasetsart University. They gave me some guidelines and advices to get my position in this faculty including writing an application, preparing for interview, and working. This internship is very fruitful for my plans in the toxicology field. More substantially, I can transfer this knowledge and experience gained through this internship to the junior students in our school who are interested in raptors and toxicological studies.

- What do you think the positive impact of the activity will have on your further career path?

The internship activity by visiting the Faculty of Veterinary Medicine, Kasetsart University gave me a great opportunity to expose many research ideas and to learn some specific skills for the toxicology field that I can transfer these experiences and knowledge to my students or colleagues in the future. In addition, learning the management in wildlife unit, creating environment contributed to wildlife as well as treatment of animal cases in the animal hospital and raptor center are useful for my future career path that linked to veterinary toxicology and wildlife conservation. After my doctoral graduation, I would like to work at Faculty of Veterinary Medicine, Kasetsart University, in research and teaching. Also, I plan to work with other faculty members, especially wildlife unit, to strengthen toxicology and wildlife research unit in my department that will be useful in the Asia-pacific region. This internship improved me to learn about the role of lecturer and researcher in the university forward to develop my knowledge in the toxicology area, research skills, logical thinking and teamwork in research fields. This event also encouraged me to build a powerful partnership network relate to my position in this faculty and KURU with experienced staffs, technicians and professors. Moreover, after participating in this program, I can consider my future for the studies and jobs more greatly.

- Advice for your junior fellows

To my junior fellows, this internship will help you to find out the valuable experiences forward to explore your suitable job based on you interests. Your supervisor can help you find the internship place from among his or her collaborative institutions. Please start your

preparations early, especially visa application. Although your preparation is going well, please prepare for suddenly change in your program such as the global spread of coronavirus. Enjoy your internship period and try to do as much as you can to get new competences for your future.

Approval of supervisor	Institution・Official title・Name Laboratory of Toxicology, Hokkaido University Professor Mayumi Ishizuka

※1 Send the electronic file 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.

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