Updated Aug. 5, 2019

This report should be submitted within 2 weeks after you return to Japan. Please do not change the formatting

（Abroad）Internship report form（Student） 2019/12/14 (Year/Month/Day)

Name	Thoko Flav Kapalamula
Laboratory	Division of Bioresources
Year (Grade)	D3
Internship institution	National Tuberculosis Reference Laboratory (NTRL)
Internship period	Internship period: 11/04/2019 - 11/29/2019 (Departure Date from Sapporo: 11/01/2019, Arrival Date in Sapporo: 12/04/2019)
Purpose	1. To learn about the situation, challenges and control strategies of tuberculosis (TB) in Malawi. 2. To initiate collaboration links between Malawi counterparts and Research Center for Zoonosis Control, Hokkaido University. 3. To conduct interview at LUANAR for the future job as a lecturer after graduation 4. To conduct part of my study and collect DNA samples

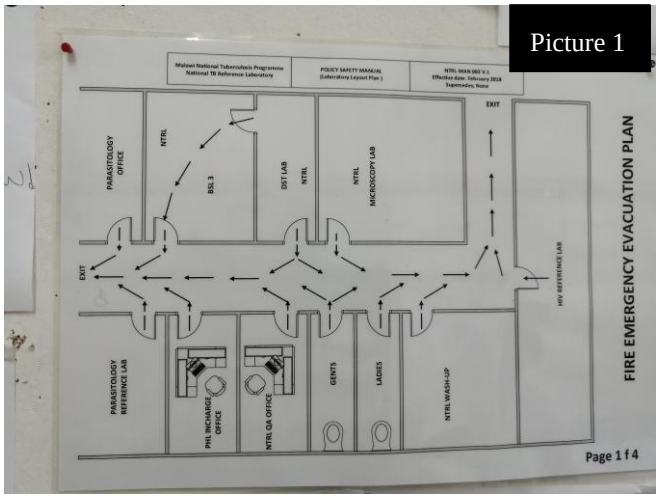
- The reason (s) why you chose this institute

1. I chose NTRL because is the main reference tuberculosis (TB) laboratory in Malawi and is responsible for coordinating TB laboratory network and provision of technical guidance to the network laboratories therefore I thought I could learn more about TB caused by *M. tuberculosis* and *M. bovis* in human.
2. Also I wanted to gain work experience in a government institution like NTRL because I didn't have any exposure of working at a government laboratory of NTRL caliber.
3. I chose NTRL in order to conduct my study because NTRL has equipment that could help me carry out my experiments. Also NTRL keeps TB isolates in biobank therefore, I had access to samples that I could use for my studies and for further studies in future.

- Result of the activity （about 800 words、provide photos, tables and figures that clearly show the activities during the period）

During the first week, I had orientation to NTRL and also introduction to members of the lab. I learnt about management of NTRL. NTRL is under the Public Health reference laboratory directorate of Ministry of Health in Malawi. Under this directorate there are six laboratories namely; HIV Reference Laboratory, Nutrition Laboratory, National TB Reference Laboratory, Biochemistry, Microbiology and Parasitology. NTRL is under the national TB Control Programme in Malawi and Dr. Nyenje is the manager. NTRL is housed within the TB control

Programme premises in Area 3, Mtunthama Drive in Lilongwe Malawi. It shares the building with the parasitology laboratory and HIV/AIDs reference laboratory. The flow plan of the building is as in Picture 1.



NTRL has modern laboratory equipment such as -80^{o}C freezer (Picture 2), biosafety cabins (Picture 3), cold room (Picture 4) and many others. However, because of electricity problems the time I was there the freezer temperature had dropped to -1^{o}C hence they had to shift all samples to another -80^{o}C freezer at microbiology laboratory).

On TB situation in Malawi, I learnt that TB is a major public health threat in Malawi. Currently, TB prevalence is 1014/100,000. TB in Malawi is controlled by treatment regimens. However, there are more cases of HIV coinfections. TB caused by *M. tuberculosis* is well documented as compared to *M. bovis* TB. There are no tools for detecting *M. bovis* TB.

I had the opportunity to conduct part of my study **Genetic epidemiology of bovine TB in Malawi**. In this study, I would like to know the molecular epidemiology of bovine TB in

Malawi. The part conducted in Malawi involved; Collection of cattle tissue specimen suspected to have TB during postmortem at a slaughterhouse and processing it. Every morning I was going to the abattoir and alongside the abattoir veterinarian conduct meat inspection (Picture 5). Therefore, I was collecting the tissues and bring them to NTRL where I was processing them. Briefly, samples were minced using a pair of scissors and folceps then homogenized using a grinder. Tissues were then decontaminated using 4% NaOH for 20 minutes. The samples contents were then neutralized using phosphate buffer saline (PBS) before centrifugation at 3200rpm for 20 minutes. Then discard the supernatant and add 1.5ml PBS to the pellets. DNA was extracted from this and part of it was grown on Mycobacterial Growth Indicator Tube (MGIT) and Löwenstein–Jensen medium with pyruvate and with glycerol. Figure 2 shows the protocol that I used at NTRL to collect samples from the slaughterhouse and process.

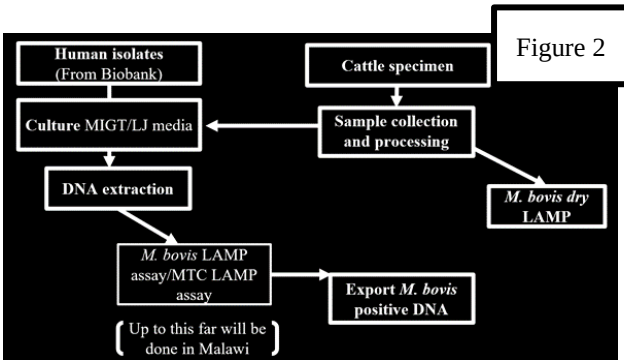
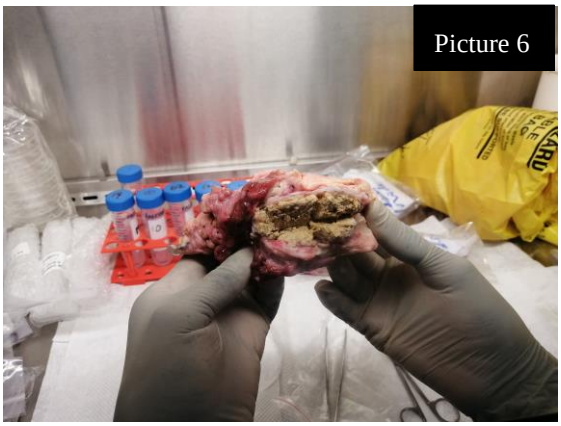


Figure 2

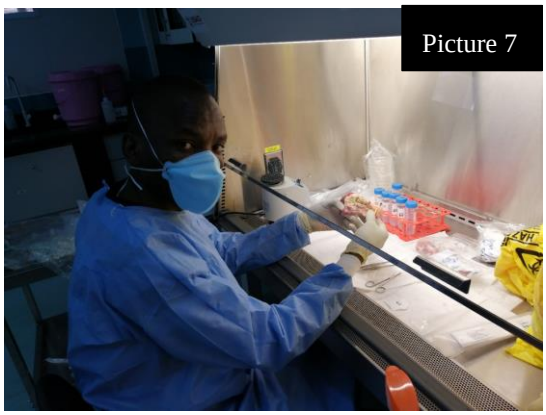
Picture 5 shows collection of specimen at a slaughter house. I was targeting tissues with TB like lesions.

Picture 5

Picture 6



Picture 6 shows the collected cattle specimen in the safety cabin. Picture 7 shows me working on the samples and picture 8 shows me with NTRL technicians.


Picture 7


Picture 8

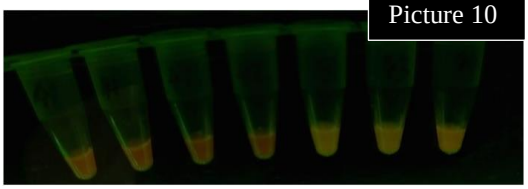
I managed to get results of my experiments (Table 1). Out of the 63 cattle specimens collected, 44 were processed and 37were positive for *M. bovis* by Dry LAMP using crude extracted DNA. After culturing on MGIT, from the positive culture DNA was extracted and *M. bovis* dry LAMP was performed as well. 43 were positive and exported DNA to Japan after getting DNA permit export.

Table 1.	**Results**	
	Cattle samples	**Human isolates**
Processed	43	86
Positive on crude DNA (dry LAMP)	37	Not tested
Negative on crude DNA	6	Not tested
Cultured	43	86
Positive on MGIT	43	5
Positive on dry LAMP	42	1

During this internship activity I had the opportunity to evaluate Dry Loop Mediated Isothermal Amplification (LAMP) assay for detection of *Mycobacterium bovis* from cattle and humans. I developed the assay and the results are shown in table 1. So far there are no rapid, simple and low cost tools for the detection of *M. bovis* used in Malawi. Picture 9 shows the manually made system for leading LAMP results.


Picture 9

Picture 10



N N N N P P P

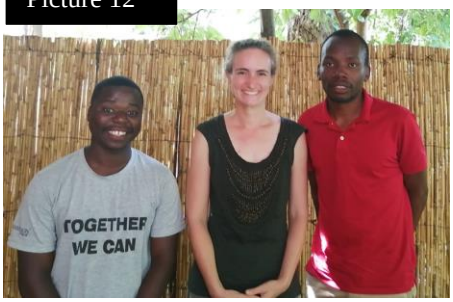
P= Positive, N= Negative

The LED illuminator was placed in an empty glove box and connected to an outside source of batteries. Picture 10 shows some of Dry LAMP results, results were detected following color change of colori-fluorometric indicator (CFI).

I had an opportunity to discuss with NTRL manager and National TB control programme Director about research collaborations. I also discussed with The Acting Dean, School of Veterinary Medicine, LUANAR, Lilongwe (picture 11) about collaborations. I also held discussions with Dr. Catherine and Misheck (Veterinary student) about research collaborations because the student is working on bovine TB as well (picture 12).

Picture 11

Picture 12



Lastly, I had an opportunity to conduct an interview for lecture job at LUANAR, school of Veterinary Medicine. I got the job.

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

This internship has helped me map my future career path as a lecturer/ researcher and an expert of infectious disease control in Malawi.

Scientific skills: I have broadened my knowledge on TB research as result from the fruitful activities during my internship. I have learnt about TB situation in Malawi and control strategies used in Malawi. I have been exposed to the latest information on how Malawi is dealing with TB. I have learn more about zoonotic TB.

Networking: I have had the chance to share and exchange information about CZC with the management at NTRL, The Director of Livestock Department, Microbiology lab at College of Medicine and Dean of Veterinary Medicine School (LUANAR). I am glad that soon CZC will sign MOU with these Malawian institutions

Student life: It has help me interact with technicians and learn other skills.

Career path plan: I have gotten familiar with the nature of institution working culture. My future career path goal is to become a Professor in Malawi one day. I am glad that I had interview with LUANAR as a Lecturer and got employment.

- Advice for your junior fellows

Never stop trying. Never stop believing. Never give up. All things are possible.

Acknowledgements

I would like to thank Leading Progress, Maki san, Professor Suzuki, Professor Nakajima and all Division of Bioresources members for everyone's unique contribution, Thank you very much.

Approval of supervisor	Institution・Official title・Name CZC Professor Yasuhiko Suzuki

※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.

Submit to：VETLOG

Ext: 9545 e-mail: leading@vetmed.hokudai.ac.jp