Student Free Design Activities (One Health on-site Training)
報告書 Report

報告者 [Reporter]

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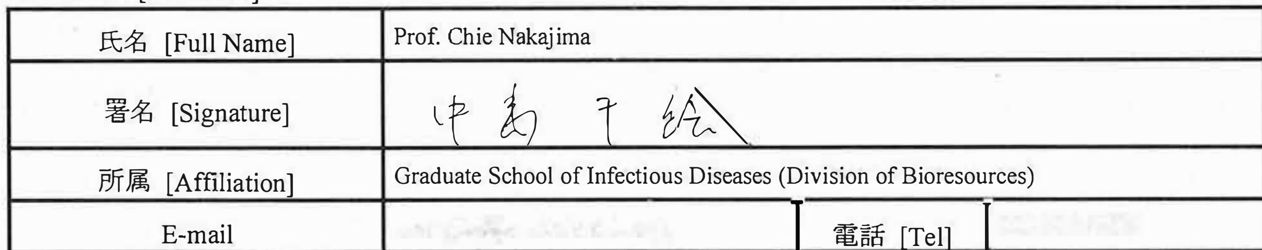

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活動報告 [Activity Report]
※活動内容が判る様な写真や図表を加えて下さい。/ Provide photos, tables and figures that clearly show the activities during the period.

タイトル [Course Title]	M405_Student Free Design Activities (One Health on-site Training)
実施期間 [Periods]	December 2nd – January 8
共同実施者 [Other participants]	None
言語 [Language]	English
実施場所 [Location]	Ghana; Noguchi Memorial Institute for Medical Research - UG

申請時計画の実施報告 [Report how you carried out your plan in the application form]

From the onset the purpose of this SFDA 4 was 3fold: 1. To obtain suspected bovine tuberculosis (BTB) tissue samples from abattoirs in Ghana. 2. Culture the tissue samples obtained and observe growth after 4 weeks. 3. Extract DNA from the cultured isolates which will then be transported to Japan for further molecular analyses as part of my PhD project which aims to investigate the genetic diversity of Mycobacterium strains causing BTB in cattle slaughtered in abattoirs in Ghana. As part of this ongoing project, sample collection across selected abattoirs in Ghana started in June 2023. In this module, I aimed to gain practical experience in both fieldwork and working in a BSL3 facility in Ghana. Although I successfully completed the first two parts of my three-part objective in Ghana - collecting suspected BTB samples from selected abattoirs and culturing the tissue samples, I couldn't proceed to the third part of DNA extraction due to some challenges I faced. The challenges I faced were mainly related to time (owing to fastidious nature of *M. bovis* growth, requiring 4 -8 weeks for growth) and host institutional logistics constraints that prevented me from doing what I had initially planned to do before returning. Despite this, the overall outcome of my trip to Ghana was satisfactory. I'll summarize my report under 4 main headings herewith.

Fig. 1

Lab access documentation, training and examination: As part of the institutional protocols, I was required to complete some documentations undergo a P3 and lab access training, pass an exam on it before being granted access to start my experiments. This I did as scheduled. It was to ensure that I had the necessary skills and knowledge required for working in the laboratory setting. This step was crucial in facilitating my subsequent lab work. The training included but was not restricted to: training on general safety in the laboratory, training on general rules for bsl-3 facility, training

on bsl-3 general practices, training on safety and personal protective equipment, training on laboratory facilities, training on emergency response and evacuation plan: reporting fire and other emergencies, training on lines of communication, training on specific procedures for spills, other emergencies in the bsl-3, training on decontamination procedures, training on hazardous materials management plan, training on equipment usage, training on tuberculosis (TB) tissue handling and processing and lastly exam and evaluation. This I completed and was awarded my certificate of lab access.

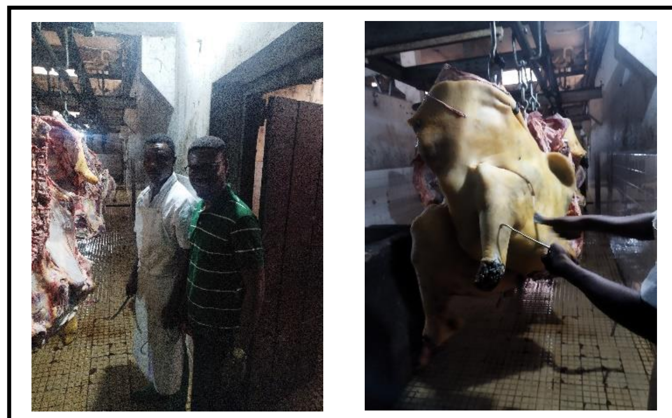
Visits to various abattoirs in Ghana for the purpose of sampling. Through these visits, I collected relevant samples that would contribute to my research on bovine tuberculosis (BTB). These samples would later be analyzed and assessed in the lab. I visited about 10 abattoirs where sampling had already begun across Ghana to have a firsthand experience in the abattoir meat inspection process across the country. As at the time of my trip 143 samples had been collected, I was able to collect about 20 more samples during my trip across the abattoirs putting the total sample figure to 163. Figure 2 and 3 complements this information, marking the geographical distribution of the abattoirs to give a visual representation of the areas covered during my sampling efforts as well as the meat inspection process. Table 1 shows the number of samples collected so far. Of the number samples collected I was able to culture 75 before my departure.

Fig. 2 Sample sites

Table 1

Sample Area	# of Samples
Navrongo	5
Yendi	9
Tamale	69
Kumasi	42
Koforidua	5
James Town	26
Accra	7
Tarkwa	0
Takoradi	0
Kasoa	0
Total	**163**

Fig. 3 Sampling from Abattoirs



LJ Media Preparation: I was engaged in the preparation of a specialized media for mycobacterium culture (LJ media). Two different types of LJ media were prepared. One supplemented with glycerol and another with pyruvate. Each sample was to be cultured on 2 pairs each of LJ media supplemented with glycerol or pyruvate. This process helps distinguish between strains and explain pathogenicity by providing important insights into the metabolic capacity of the bacteria.

Glycerol-supplemented media serve as a metabolic challenge, revealing strains with pyruvate-independence, capable of utilizing alternative pathways or possessing residual pyruvate stores. Pyruvate-supplemented media act as a metabolic safety net, ensuring optimal growth for all strains, regardless of their internal pyruvate production efficiency.

Comparing growth patterns across both media reveals valuable information. Strains demonstrating exclusive growth on pyruvate-supplemented media signify complete pyruvate dependence, potentially harboring mutations affecting pyruvate acquisition or synthesis. Alternatively, strains exhibiting growth on both media suggest varying degrees of pyruvate independence, indicating diverse metabolic adaptations within the M. bovis population.

The preparation included Potassium Phosphate, Magnesium Sulphate, Magnesium Citrate, Asparagine, Sodium Pyruvate, Glycerol, Distilled water, 2% Malachite green, and Egg homogenate, with specific quantities as detailed below in Table 2:

Fig. 4 Media Preparation

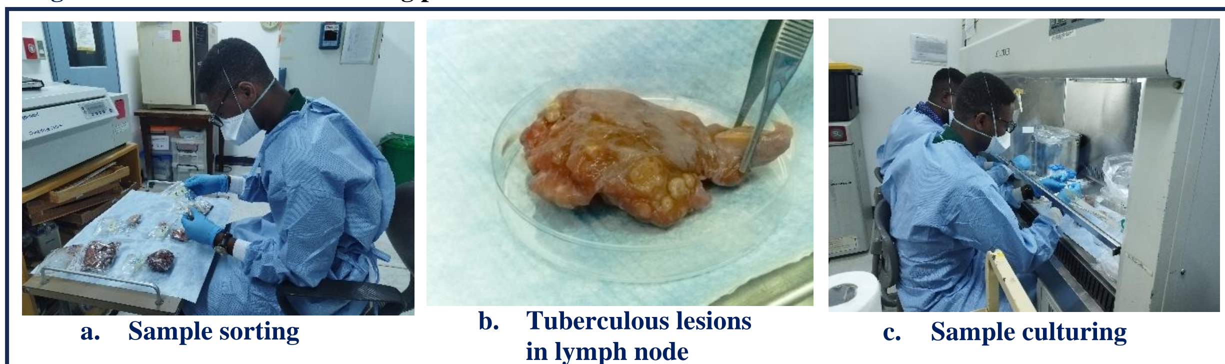
Table 2

Components	***w* (Glycerol)**	***w* (Pyruvate)**
Potassium Phosphate	0.4g	0.4g
Magnesium Sulphate	0.04g	0.04g
Magnesium Citrate	0.1g	0.1g
Asparagine	0.6g	0.6g
Sodium Pyruvate	*	1.3g
Glycerol	2ml	*
Distilled water	100ml	100ml
2% Malachite green	3.3ml	3.3ml
Egg homogenate	167ml	167ml
Final pH of media	7.0	7.0

Suspected *M. bovis* tissue culture and microscopy: The process was done in a BSL 3 laboratory. The tissues were first thawed and sorted out about 30 mins before the start of the procedure. The lesions were then examined carefully. With the aid of a sterile scalpel all adipose tissues were removed and other non-pathological tissues also to expose the characteristic thick solid granulomatous nodules. The nodules were then incised and cut portions of the discrete tubercles were placed into a petri-dish and excess fat or tissues removed. The cut tubercles were homogenized manually in a sterile mortar with a sterile pestle. The homogenized tubercles were then suspended in sterile phosphate buffered saline (PBS) 6.0 ml in a 50 ml Falcon tube for decontamination. Three milliliters (3.0 ml) of each tissue homogenate suspension were decontaminated with equal volumes of 5% (w/v) oxalic acid in labelled 50 mL Falcon tubes with intermittent vortexing every 10 minutes at room temperature for 30 minutes.

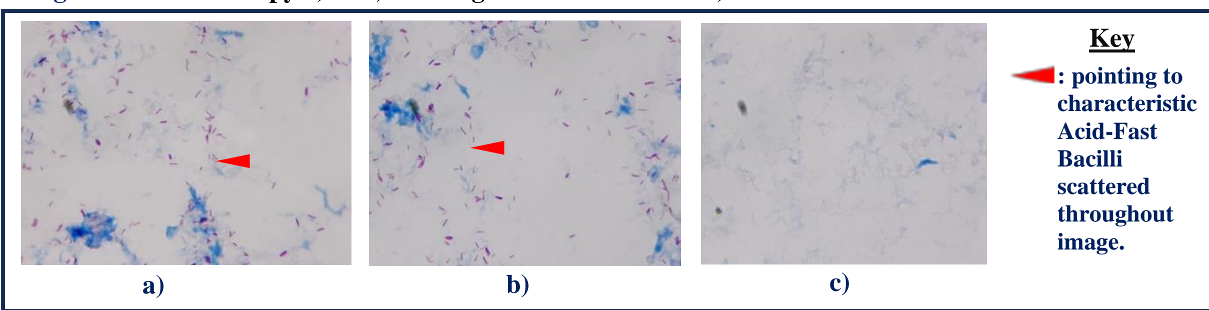
The suspensions were then concentrated by centrifugation at 3000 rpm for 30 minutes to pellet the mycobacteria. The supernatant was carefully decanted afterwards and the pellets were resuspended in 2.0 ml of PBS for smear microscopy and inoculation onto Lowenstein- Jensen (LJ) culture media. Smears were prepared directly from the decontaminated homogenate pellet on clean labelled slides, allowed to air dry, heat fixed and stained with the Ziehl-Neelsen method for acid-fastness. One hundred microliters (100 µl) of the decontaminated tissue homogenate were inoculated onto four (4) labelled Lowenstein- Jensen (L-J) media slants; two supplemented with glycerol and the other two supplemented with 0.4% sodium pyruvate. The inoculated media slants were incubated at 37°C. The slides were then examined for acid fast bacilli (AFB).

Fig. 5. Pictures from the culturing process



a. Sample sorting **b. Tuberculous lesions in lymph node** **c. Sample culturing**

Fig. 6. AFB microscopy a) & b) showing stained bacilli and c) no bacilli observed.



目的達成状況報告 [Report how you achieved your goal/objectives listed in the application form]

Did you achieve all the goals you initially planned? If not, please describe why you failed to fulfill your objectives.

I managed to achieve some of the first objectives, including sample collection, processing and cultivation. However, there was a challenge with the growth phase of *Mycobacterium bovis*, a particularly fastidious organism that requires a prolonged period of 4 to 8 weeks for optimal growth. Unfortunately, my ability to observe significant growth before I left was hampered by this prolonged incubation period and prevented me from returning with heat killed bacteria or DNA to Japan.

In spite of this setback, I was greeted with positive news and accompanying photographs (Fig. 7) showing the successful cultivation of some samples, the post return phase began to unfold positively. The positive outcome not only confirmed the success of the experiment I carried out, but it also provided a sense of relief and hope for further development.

Fig. 7

One Health Approach実践報告 [Report how your activity could link to One Health Approach]

Did you have a chance to experience One Health approach (collaboration with people from other academic areas)? Please describe some of the examples of One Health approach you implemented in your activity. Otherwise, explain the possibility(ies) how you could link this activity to One Health approach for your future.

Certainly, I had the privilege of being able to take an active part in One Health and foster collaboration with professionals from various academic backgrounds. The TB team at the institute unites diverse experts in animal, human, and environmental scientist, alongside skilled data analysts. I particularly valued the perspectives of the social scientists during our interactions, whose insights, like those gleaned from community surveys where TB surveys has been conducted, enriched my understanding of TB transmission beyond the purely biological.

I believe working with human TB researchers in this project can help identify zoonotic TB cases within the region of my study. The data analysis can also pinpoint transmission hotspots within the area. I'm planning on expanding this One Health approach into my next activities by collaborating with social scientist also for questionnaire administration to livestock owners and herders. I want to create a more comprehensive understanding of the health challenges and promote collaboration between experts from diverse fields. I envision such a collaboration of people from diverse backgrounds can help in creating a comprehensive "One Health map" of TB in Ghana, integrating scientific, socio-economic and environmental factors alongside transmission data. Partnering with epidemiologists and social scientists would further deepen this interdisciplinary approach, leading to holistic solutions for TB control in Ghana.

備考 [Remarks]

※ 報告書を作成後、担当教員に確認をお願いし署名をもらってください。PDFファイルとしてVetLogから提出してください。
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