Global Leadership Training Programme in Africa 2018

Activity Report of Field Research

Surveillance and risk analysis of *Trypanosoma* spp. infection in the wildlife-livestock-human interface

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I. Summary

- English Summary

Tsetse flies are distributed in the sub-Saharan African countries. They pose a significant risk to both human and livestock, since they harbor parasites called *Trypanosoma* spp. These *Trypanosoma* spp. parasites cause human African trypanosomiasis (HAT; Sleeping sickness) and African animal trypanosomiasis (AAT; Nagana) to human and cattle, respectively. AAT is estimated to cause livestock producers and consumers a loss of 1.3 billion USD annually.

This study was conducted in the Musungwa chiefdom, located in mid-western Zambia. It is adjacent to Nkala Game Management Area, which acts as a buffer zone for the Kafue National Park. Since tsetse flies favor blood from wildlife, the national park and game management area have high concentration of tsetse flies. This area is also one of the largest cattle-rearing area in Zambia, which raises a concern of high burden of AAT. Therefore, this study aimed to reveal the prevalence of the *Trypanosoma* spp. infection in both tsetse flies and cattle, and to propose control strategies for the villages. Blood sampling from cattle was conducted in five villages. Tsetse flies were captured along and within Nkala Game Management Area. In addition to blood sampling, questionnaire was also conducted against the cattle owners. From the result of the questionnaire, it was assumed that changing the insecticide for tick control would also contribute in tsetse fly prevention. Therefore, during the last days of the fieldwork, workshops were conducted in each village to promote this insecticide method, and to disseminate the general information of trypanosomiasis and the correct prevention and treatment methods.

DNA was extracted from all the tsetse fly and cattle blood samples and transported back to Japan. Analyses to detect *Trypanosoma* spp. infection, genotyping, and risk analysis would be conducted. The results would be given to the cattle owners, and the ultimate goal is to come up with original control strategies for each village.

Livestock is a highly important source of nutrition and income in many low- and middle- income countries. By elucidating the prevalence of AAT and suggesting regional control strategies, this study aims to improve the health in livestock. Therefore, this study will contribute to SDGs goal #1 “End poverty in all its forms everywhere” and SDGs goal #2 “Zero hunger”, and also indirectly contribute to SDGs goal #3 “Ensure healthy lives and promote well-being for all at all ages”.

- 野生動物、家畜、人のインターフェイスにおけるトリパノソーマ原虫感染のサーベイランスとリスク解析

サハラ砂漠以南のアフリカ諸国には、吸血性の昆虫であるツェツェバエが生息している。彼らはトリパノソーマという原虫の媒介昆虫である。そのため人や牛がツェツェバエに噛まれるとトリパノソーマ原虫が感染し、それぞれヒトアフリカトリパノソーマ症(眠り病)や家畜アフリカトリパノソーマ症(ナガナ病)を発症する恐れがある。ナガナ病は貧血や体重減少といった症状を示す消耗性疾患であり、本病による経済的損失は年間 13 億米ドルにも上るといわれている。

本研究は Musungwa chiefdom という地域で行われた。本地域はザンビア中西部に位置し、Kafue National park を取り囲む Nkala Game Management Area に隣接している。このように樹木や野生動物が豊富な地域はツェツェバエの生息密度が高い。また本地域はザンビア有数の牛飼養地域でもあるため、ツェツェバエによる吸血の機会が多いことが予想される。よって本研究ではツェツェバエと牛のトリパノソーマ原虫保有状況を解明し、その予防対策を立案することを目的とした。5 つの village で牛の採血を行い、ツェツェバエが多く生息している Nkala Game Management Area 付近でツェツェバエの捕獲を行った。また、牛の採血を行う際、同時に牛飼養者への質問票調査も行った。その結果、すぐに実践できる予防策として駆虫薬の使用方法を見直すことが挙げられた。この知識伝達のため、フィールドワークの最後にそれぞれの village でワークショップを行い、同時にトリパノソーマ症の基礎知識とその予防・治療方法に関しても説明した。

採材したサンプルすべてから DNA 抽出を行い、帰国した。今後これらの DNA サンプルを用いてトリパノソーマ原虫の検出と遺伝子タイピング、そしてリスク解析を行う予定である。これらの結果はすべて現地の牛飼養者へフィードバックし、それぞれの village に合わせた予防対策を立案することを最終的な目標とする。

家畜は低・中所得国では重要な栄養、および収入源である。本研究は AAT のプレバレンスを調査し、地域に合ったコントロールプログラムを立案することで家畜の健康促進を目指す。よって本研究は持続可能な開発目標#1「貧困をなくそう」、#2「飢餓をゼロに」に貢献する。また、間接的に#3「すべての人に健康と福祉を」に貢献する。

II. Research Activity

1. Introduction

Trypanosoma spp. parasites live inside the blood of vertebrates and are transmitted between vertebrates via the blood-sucking fly: tsetse flies. Various *Trypanosoma* spp. parasites cause African animal trypanosomiasis (AAT) when they infect livestock. AAT is a wasting disease, causing weight loss, anemia and may be fatal if not treated appropriately. Due to the direct loss in livestock products and indirect loss through treatment and prevention efforts, AAT is estimated to cause African livestock producers and consumers US$ 1.3 billion loss annually[1]. Among these *Trypanosoma* spp. parasites, *T. brucei rhodesiense* is a zoonotic pathogen that will infect human and cause human African trypanosomiasis (HAT) in south-east Africa. HAT is one of the neglected tropical diseases; a diverse group of communicable diseases that prevail in tropical and subtropical conditions[2].

In addition to its apparent social-economic impact, there is an arising issue of drug resistance in AAT. Inappropriate use (misuse and overuse) of trypanocide will lead to the emergence of resistant parasites, which will make the treatment of AAT difficult.

Since the parasites that cause both AAT and HAT are transmitted by tsetse flies, revealing the prevalence of the parasites within the tsetse flies is crucial to understanding the ecology of transmission and to succeed in controlling both diseases. However, in most cases the prevalence of trypanosome-infected tsetse flies is unknown.

2. Objectives

The main objective is to construct a model case for regional AAT/HAT control in the Musungwa chiefdom. In order to achieve this, the prevalence of animal and human-infective *Trypanosoma* spp. parasite in cattle and tsetse flies would be investigated.

3. Study Area

This study was performed in the Musungwa chiefdom in the Itezhitezhi area, which is located in the central province of Zambia. This area is adjacent to the Kafue national park and the Nkala game management area, which creates a wildlife-livestock-human interface. It is famous for its high population of cattle, and many villagers witness tsetse flies in their villages. Many farmers claim that their cattle are infected with *Trypanosoma* spp., even though they have not been properly diagnosed.

4. Methodology

Cattle blood samples were collected from the jugular vein with 18G needles and 5 mL syringes. The sampling was conducted in 5 villages within the Musungwa chiefdom: Iyanda, New ngoma,

Ntubia, Kaminza, and Basanga (Appendix Fig 1.), and resulted in collecting 498 blood samples. The samples were immediately used to do micro-hematocrit centrifuge to obtain Packed Cell Volume (PCV) values. Thin blood smears were also made, and Giemsa stained for microscopic observation of parasites. While collecting blood samples, questionnaires were also conducted against the farmers. The questions asked are listed in Appendix Table 1.

Epsilon traps were set inside and at the border of the Nkala game management area and the villages. In areas with an insufficient number of catches, a mobile trap was used to add-up the number of tsetse flies (Appendix Fig 2.). A total of 306 tsetse flies were captured in 12 areas.

Both the cattle blood samples and tsetse fly samples were transported to the laboratory in HUCZCZ, University of Zambia, Lusaka for DNA extraction. The DNA samples were then transported to Japan for further analyses.

5. Research Findings

(1) Questionnaire

It was revealed that the farmers know AAT as "*Luka*", which means "tsetse fly" in the local *Ila* language. 67% among all farmers have seen tsetse flies in their villages before, and 84% had knowledge about AAT and HAT. 91% goes into the GMAs or NPs at least once a year for grazing their cattle. 98% use Samorin (isometamedium chloride) for their cattle, and 100% do some kind of form of tick control, such as spraying and dipping.

From these results, workshops were conducted in each village. The general information of African trypanosomiasis, and some prevention and treatment methods were made into each poster (Appendix Fig 3.). In order to encourage correct drug dosage and hygienic activities, handouts were also distributed during the workshops (Appendix Fig 4.).

(2) PCV and Microscopy (Table 2)

The overall average PCV value was 32% (SD 5.83, 95%CI 31.44-32.47), and the average for each village was 31.75% (SD 4.31, 95%CI 30.83-32.67) for Iyanda, 33.0% (SD 5.63, 95%CI 32.08-34.27) for New ngoma, 29.6% (SD 6.80, 95%CI 28.27-30.87) for Ntubia, 32.5% (SD 5.40, 95%CI 31.39-33.69) for Kaminza, and 33.0% (SD 5.76, 95%CI 31.70-33.80) for Basanga (Appendix Fig 5.). Generally, PCV values below 24% are considered anemic. The number of cattle that had PCV values below 25% was 3, 4, 21, 7, and 6 respectively for Iyanda, New ngoma, Ntubia, Kaminza, and Basanga. The significant difference between the average PCV values between villages was detected by the Kruskal-Wallis test ($p = 0.0002$), and significance between Ntubia vs. Basanga, Ntubia vs. Kaminza, and Ntubia vs. New ngoma was detected by the pairwise comparisons using the Wilcoxon rank sum test (Appendix Table 3).

The microscopic observation is still ongoing. For the preliminary results, the only positive samples were observed in the Ntubia village (7/105, 6.67%).

(3) Molecular detection of trypanosomes using PCR

Several PCR methods will be used to detect and characterize the *Trypanosoma* spp. parasites. First, ITS-PCR (a widely used method for detection of African trypanosomes, amplifying the ITS1 region of the ribosomal RNA genome) would be used to detect and characterize the *Trypanosoma* spp.

parasites to the species level. Secondly, SRA-PCR (a method to detect the serum-resistance associated gene, which is only apparent in *T. brucei rhodesiense*) would be used to detect the human-infective subspecies. After detection, positive samples will be genotyped using next generation sequencing to see the correlation between the *Trypanosoma* spp. parasites seen in cattle and tsetse flies.

6. Discussion

The farmers had a high level of knowledge against tsetse flies and its relationship to the disease in cattle. (The disease was not recognized as AAT, but as "*Luka*"). It is suspected that the assumed high prevalence of AAT in the area is raising the awareness of the disease. Due to this high awareness, many farmers were using trypanocides to their cattle regularly. However, there were many forms of misuses, such as over/under-dosing, using cheap unofficial drugs, and confusion between preventive and treatment drugs. 100% of the cattle owners were conducting tick control but using insecticides that will not kill tsetse flies. Therefore, one of the proposals for AAT prevention was to include insecticides that will kill both ticks and tsetse flies into their tick control practice. This method was introduced to the farmers through the village workshops.

Most of the analyses have not been conducted. From the preliminary results, the Ntubia village had significantly larger number of cattle that was anemic compared with the other villages. In addition, it is the only village that had positive blood smears, which indicates high parasitemia. This suggests that Ntubia has significantly high burden of AAT than other villages. However, further analyses are needed, since whether the anemia was caused by *Trypanosoma* spp. infection is unknown, and the infection would need to be confirmed with ITS-PCR.

7. Conclusion

From the preliminary results, it is suggested that the AAT prevalence is different according to the villages. After conducting further analyses, it is anticipated that we will come up with original prevention strategies against each village. All results would be given back to the farmers through Dr. Delesalle (Melindika Zambia), who is providing community veterinary services in the Musungwa chiefdom.

Through elucidating the prevalence of AAT and suggesting regional control strategies, this study aims to improve the health in livestock. Therefore, this study will contribute to SDGs goal #1 "End poverty in al its forms everywhere" and SDGs goal #2 "Zero hunger", and also indirectly contribute to SDGs goal #3 "Ensure healthy lives and promote well-being for all at all ages".

This study would be included in the doctoral thesis for Hokkaido University.

- Acknowledgement

I am grateful to Dr. Delesalle, who has provided me accommodation during the fieldwork and has supported me for the transportation within the chiefdom, arranging the schedule with the farmers, and advice for blood sampling. I would also like to show my gratitude to Prof. Namangala and Dr. Simuunza from the University of Zambia, who have supported me through the submission of research

permission for the Ministry of Fisheries and Livestock and the ethical clearance of ERES converge, and the members of Hokkaido University Center for Zoonosis Control in Zambia and the Division of Collaboration and Education, Research Center for Zoonosis Control for the support and understanding for this study. Lastly, I am grateful to the Global Leadership Training Programme in Africa for all the support and making this study come true.

- References

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- Appendix

Fig 1.

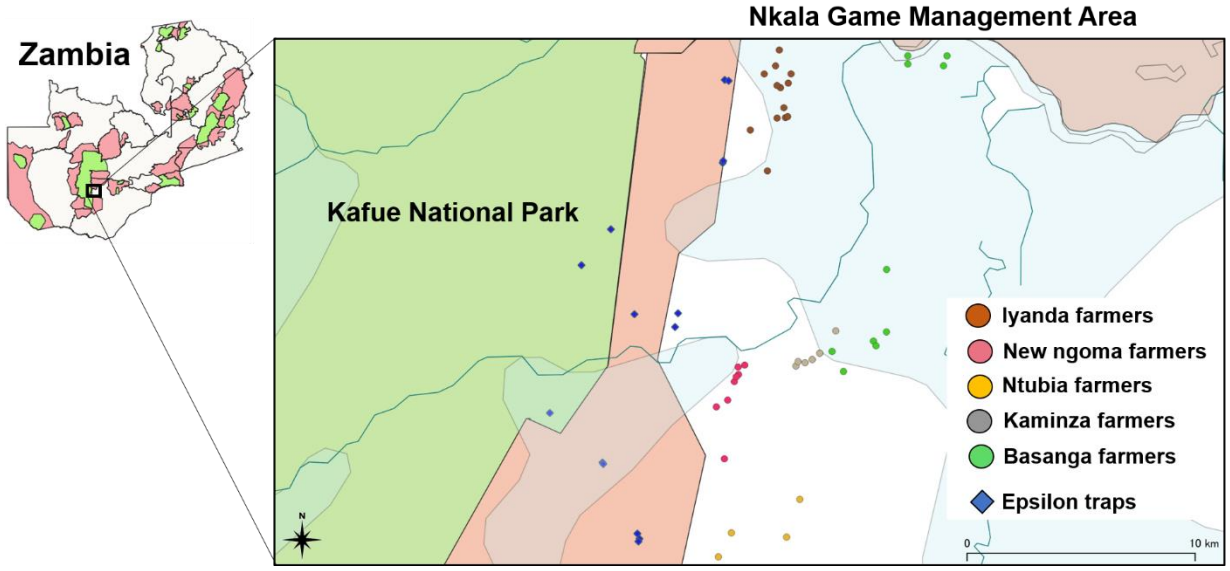


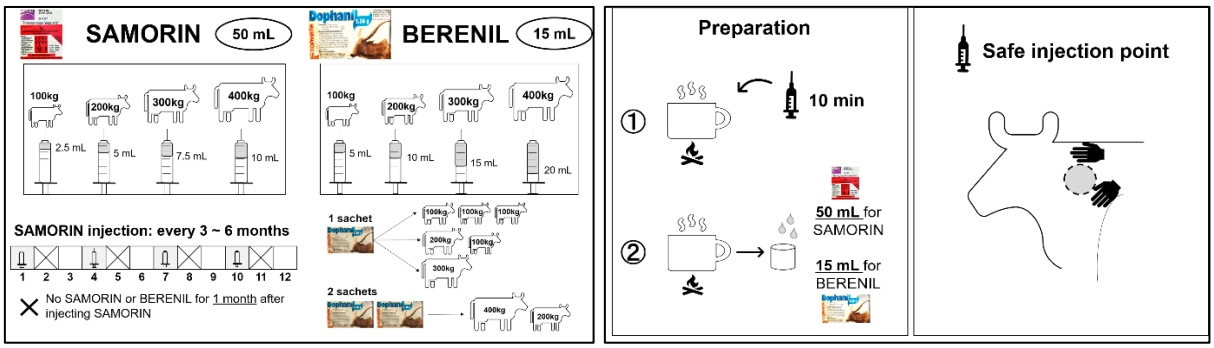
Table 1.

1. How many cattle do you own?
2. Where do you take your cattle for grazing?
3. How often do you go inside the national parks or the game management areas?
4. When was the last visit to the national parks or the game management areas?
5. Have you seen tsetse flies inside your village before?
6. Have you been bitten by tsetse flies before?
7. Do you know that tsetse flies carry disease for both cattle and human?
8. Do you use Samorin (prophylaxis of AAT) and/or Berenil (Treatment of AAT)?
9. How often do you use the drugs?
10. What kind of tick control do you do (spraying, dipping)?
11. How often do you do tick control, and when was the last spraying/dipping?

Fig 2.

Fig 3.

Fig 4.



Village name	Average PCV	Giemsa's stain
Iyanda	**31.8%** (SD 4.31, 95%CI 30.83-32.67)	0 / 90 (0%)
New ngoma	**33.0%** (SD 5.63, 95%CI 32.08-34.27)	Ongoing
Ntubia	**29.6%** (SD 6.80, 95%CI 28.27-30.87)	7 / 105 (6.67%)
Kaminza	**32.5%** (SD 5.40, 95%CI 31.39-33.69)	Ongoing
Basanga	**33.0%** (SD 5.76, 95%CI 31.70-33.80)	Ongoing
Total	**32.0%** (SD 5.83, 95%CI 31.44-32.47)	

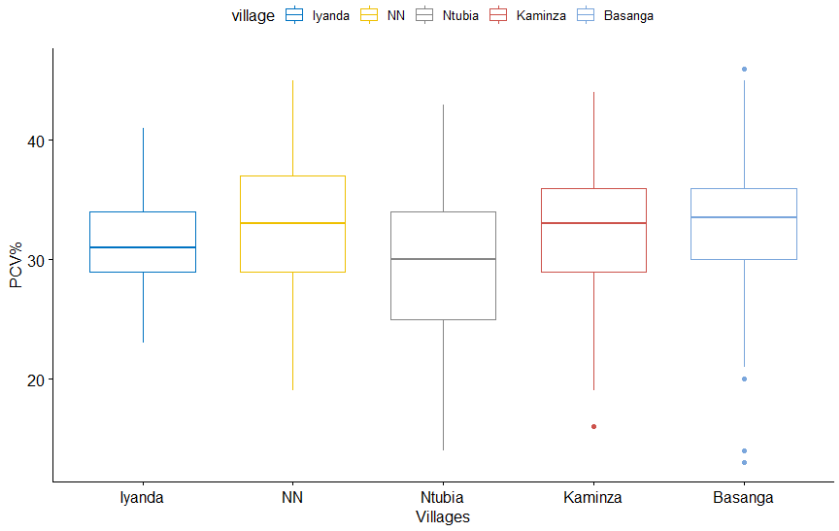
Table 2.

Table 3.

	Basanga	Iyanda	Kaminza	New ngoma
Iyanda	0.0651	-	-	-
Kaminza	0.8835	0.1314	-	-
New ngoma	0.9759	0.1285	0.8835	-
Ntubia	0.0011*	0.0651	0.0038*	0.0011*

*significant level $p < 0.05$ after Benjamini-Hochberg correction

Fig 5.



3. Reflection to the GLTP in Africa

- My motivation to participate in GLTP

  While studying about African trypanosomiasis for my PhD study, I encountered many questions that could not be answered by just the information on books, scientific papers, and internet. The epidemiology of the disease seemed to be highly affected by the ecological, economic and cultural aspects. Therefore, my motivation to participate in GLTP was to conduct a long-term fieldwork where I can directly see and interact with the people and environment where the disease occurs.

- Field experiences

  Although I have a background as a veterinarian, I did not have much experience in clinical veterinary services. The blood collection from the local cattle was a totally new experience for me, and the way the farmers took care of their animals were very inspiring. It was touching to see how each cattle had their own names, and all the family members including the children could identify each individual. It was also a good opportunity to improve my skills in identifying the tsetse fly species and sex, since it was my first time to process such a large number of samples (Pic 1.). Another striking experience was sampling in the plains near the Kafue River. Since there was little rain this year, some farmers have migrated north to the plains to access water earlier than usual. We needed to use canoes to cross the rivers and the whole scenery of the water, grass, and cattle was a beautiful view (Pic 2. – 4.).

- Challenges

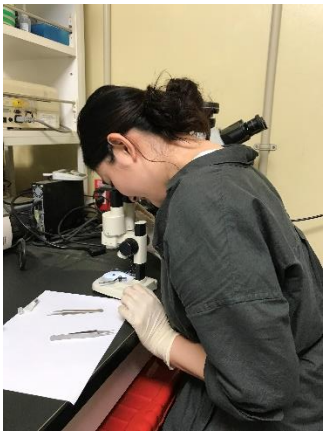
  The first challenge that I experienced was miscommunication with the veterinary assistants. Before arriving at the villages, I have been communicating with the assistants via Dr. Delesalle. The target population size per village was 50, and I was assuming two to three farmers. However, when I arrived at the first village, I was shocked to know that he had included more than 40 farmers for the study! It was a struggle to come up with a plan where all of us can take in. In the end, I decided to increase the sample size to 100 per village and decrease the number of villages to visit. I persuaded the assistants to select up to 15 farmers at most. The second and most frequently encountered challenge was managing the time. The cattle were kept at their barns up to 9 AM and then released during the day for grazing. I needed to reach all of the farmers before this time in order to do the blood collection, but there were many times when the cattle were not there by the time I arrived. The problem of the time also happened during the workshops. Even when I set the time of the workshop to 9 AM, it was usually past 10 AM when the first among all farmers arrives. I gradually understood that the people do not consider the time by the clock, but observes the movement of the sun to estimate the time of the day.

- How to make use of this experience to my future career development

  This experience has inspired me in many ways. I was able to recognize how I really enjoyed and gained motivation against the research by the local farmers. I have not come up with a specific career path, but I would like to be involved in promoting the good health of livestock in low- and middle-income countries. Through this experience, I have also learned the importance of thinking about alternative plans and coming up with flexible ideas. I believe that these skills would help me succeed in any kind of environment.

- Encouragement to other students

GLTP is a perfect program to conduct long-term field activities in Africa. If you are a student who has been doing a lot of lab work, it may be a good opportunity to experience how the preparation, ethical approval, financial applications, and fieldwork is managed. Caution is needed, because many things may take a much longer time than you expected. For example, research permission and ethical clearance may be complicated in many countries. Most of the time, it is easier for the local people to be the one to persuade the authority. In my case, the professors at the University of Zambia have kindly contacted the offices in person, which made the process much easier and faster. My advice is to put a lot of effort in building a good relationship with the African supervisor prior to traveling to Africa. Enjoy!



Pic 1. Identifying tsetse fly species

Pic 2. Having local lunch with the assistants and friends

Pic 3. Using the canoe to cross the river

Pic 4. Cattle grazing at the Lutanga plains