NRCPD-OUAVM Joint Research Report

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1. Principal investigator

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Position: Lecturer
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2. Project title:

Molecular detection and genetic characterization of *Babesia caballi* and *Theileria equi* in horses and donkeys in Malawi.

3. Collaborating research group members at NRCPD

Name: Naoaki Yokoyama
Position: Professor

4. Research period (in mm/dd/yyyy, and total number of years)

01/04/2023 -31/03/2024: one year

5. Purposes and objectives

The purpose of this study was to detect and genetically characterize *Babesia caballi* and *Theileria equi* infecting donkeys and horses in Malawi. Secondly, our study aimed at identifying the vector ticks of *B. caballi* and *T. equi* in Malawi.

The specific objectives were to 1) detect *B. caballi* and *T. equi* in blood samples collected from donkeys and horses, using microscopy and species-specific PCR assays, 2) identify the genotypes of *B. caballi* and *T. equi*, and 3) identify the tick vectors transmitting *B. caballi* and *T. equi* to donkeys and horses in Malawi.

6. Outline of research process

Research in Malawi

Blood sampling: Blood samples were collected from a total of 185 equines, consisting of 178 donkeys and 7 horses, using EDTA-coated vacutainer tubes in Dedza and Lilongwe districts in central Malawi.

Preparation of blood smears: Thin blood smears were prepared from all of the 185 samples collected. The blood smears were fixed with methanol, and then stained with Giemsa.

DNA extraction: Approximately 125 µL of blood from each animal was transferred onto an FTA card, and allowed to dry at room temperature. The genomic DNA samples were then extracted from the FTA cards, and preserved at -20^{o}C until use.

The prepared blood smears and DNA samples were then transferred to National Research Centre for Protozoan Diseases (NRCPD) at the Obihiro University of Agriculture and Veterinary Medicine, Japan.

Research at the NRCPD

Microscopy: Blood smears were observed under a light microscope for detecting *T. equi* and *B. caballi* parasites within the infected erythrocytes.

PCR screening: The DNA samples were subjected to previously described *T. equi*- and *B. caballi*-specific PCR assays.

Genotyping of *T. equi*: The DNA samples that had tested positive for *T. equi* in the screening PCR assay were analyzed with the type-specific PCR assays for detecting the genotypes of *T. equi*.

Cloning, sequencing, and phylogenetic analyses: Amplicons obtained from the *T. equi* genotype-specific PCR assays were randomly selected, cloned, and then sequenced. The resultant sequences were subjected to phylogenetic analysis to verify the PCR results.

7. Outline of research achievements

The present study analyzed a total of 185 equines bred in Malawi for the infections of *T. equi* and *B. caballi* using microscopy and PCR. The microscopy results showed that 91 (49.2%) animals were positive for *T. equi*, but none for *B. caballi*. Our results from the PCR assays demonstrated that 156 (84.3%) animals were positive for *T. equi* infection. In contrast, all surveyed animals were PCR-negative for *B. caballi*. Subsequently, the *T. equi*-positive DNA samples were screened with the type-specific PCR assays for detecting the *T. equi*-genotypes A, B, C, D, and E. We found that the surveyed equines were infected with all five genotypes (A – E) of *T. equi*. Our findings indicate that the infection of *T. equi* is common among equines in Malawi, highlighting the need for strategies to control equine piroplasmosis in this country. Our findings additionally imply that such control strategies should be developed in light of the genotypic diversity of *T. equi*.

A manuscript summarizing these findings and research achievements has been prepared and will soon be submitted for publication in a peer-reviewed scientific journal. With the necessary permission, I transferred some of the reagents acquired for this project to Malawi, where they will be used for my future research activities and capacity building. As the next step, we have planned to collect tick samples from the equine farms investigated in this study, in order to identify the tick vectors capable of transmitting *T. equi* in Malawi.

8. Publication of research achievements

In progress