NRCPD-OUAVM Joint Research Report

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1. Principal investigator

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Position: Researcher

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2. Project title:

Identification of tick vectors, including novel and genotype-specific species, that transmit *Theileria equi* and *Babesia caballi*

3. Collaborating research group members at NRCPD

Name: Naoaki Yokoyama

Position: Professor (WOAH expert for equine piroplasmosis)

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

04/01/2024 – 03/31/2025, one year

5. Purposes and objectives

Equine piroplasmosis, a leading cause of economic losses in the horse industry, is an infectious disease caused by *Theileria equi* and *Babesia caballi*. Implementing a systematic strategy for tick control, with a focus on specific tick vectors, is crucial for effectively managing equine piroplasmosis. The efficacy of these strategies could be improved by addressing potential new tick vectors and those that transmit the virulent parasite genotypes in endemic countries. Our recent study revealed the prevalence of *T. equi* and *B. caballi* infections among horses in Kyrgyzstan. However, the specific tick vectors transmitting these parasites remain unknown. Therefore, the objectives of this study were to 1) identify specific tick vectors, including potentially novel ones, involved in the transmission of *T. equi* and *B. caballi* in Kyrgyzstan and 2) determine the tick vectors responsible for transmitting the specific parasite genotypes.

6. Outline of research process

Questing ticks were collected from the pastures and vegetation around the horse farms, where our recent study identified *T. equi* and *B. caballi*-positive horses, throughout Kyrgyzstan. The collected

ticks preserved in ethanol and transferred to NRCPD, where they were subjected to species identification based on morphological keys and then to DNA extraction. All of the tick DNA samples were initially screened with specific PCR assays for detecting *T. equi* and *B. caballi*, to identify specific tick vectors transmitting these parasite species. To investigate if the transmission vectors are genotype-specific, positive samples are undergoing further analysis using PCR assays developed at NRCPD that specifically target *T. equi* and *B. caballi* genotypes. Selected PCR amplicons will be sequenced, and the obtained sequences will undergo phylogenetic analyses to validate the findings.

7. Outline of research achievements

We collected a total of 394 questing ticks were using a flagging method from six of seven Kyrgyz provinces, including 69 in Batken, three in Osh, 219 in Jalal-Abad, 21 in Talas, 77 in Chuy, and five in Issyk-Kul. Among them, 329 were adults, two were nymphs, and 63 were larvae. Morphological identification classified the adult ticks into seven species: *Haemaphysalis punctata* (n=318), *H. sulcata* (n=2), *Rhipicephalus sanguineus* (n=2), *Hyalomma dromedarii* (n=2), *H. rufipes* (n=1), *Dermacentor marginatus* (n=3), and *D. silvarum* (n=1). The two nymphs were identified as *H. punctata*. The morphological identification of tick species was confirmed by analysis of *cox1* gene sequences of tick origin. Subsequently, all tick DNA samples were screened with specific PCR assays for detecting *T. equi* and *B. caballi*. The PCR-positive samples are currently being screened with genotypic-specific PCRs, followed by sequencing analyses to verify the findings.

8. Publication of research achievements

Upon completion of this study, a manuscript summarized the findings will be submitted to a peer-reviewed scientific journal.