NRCPD-OUAVM Joint Research Report

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1. Principal investigator

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Position: Lecturer

Affiliation: Rajamangala University of Technology Srivijaya

2. Project title:

Isolation of *Babesia bovis* of Thai origin and characterization of the VESA gene family encoding its virulent factors

3. Collaborating research group members at NRCPD

Name: ASADA Masahito

Position: Associate Professor

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

April 1, 2024 – March 31, 2025

5. Purposes and objectives

This study aims to establish cryostabilates of Babesia parasites and genetically characterize genes related to virulent factors, specifically the VESA gene family, from Asian isolates of Babesia bovis, providing valuable insights into parasite biology. The parasite cryostabilates acquired in this study will be valuable for future research, including genome-wide studies, as well as the development of drugs.

6. Outline of research process

Research in Thailand

1. Blood sample collection:

Blood samples were collected from 15 cattle that exhibited Babesia-like clinical signs, including depression, loss of appetite, anemia, dark-colored urine, and a history of contact with ticks, the vector of Babesia bovis. The blood was added cryopreserve reagent and stored in liquid nitrogen.

2. Blood smear screening:

Blood samples were smeared onto slides, fixed with methanol, and stained with 10% Giemsa solution. The slides were then examined under a light microscope (1000x magnification) to identify the piriform

shape, which is characteristic of Babesia.

3. DNA extraction:

DNA was extracted from 200 µL of each blood sample using a commercial kit (NucleoSpin Blood Extraction Kit).

4. PCR screening:

Fifteen samples were screened by PCR using the primers BTH 18S 1st F (GTGAAACTGCGAATGGCTCATTAC) and BTH 18S 1st R (AAGTGATAAGGTTCACAAAACTTCC) for the first round of PCR. The second round of PCR used primers BTH 18S 2nd F (GGCTCATTACAACAGTTATAGTTTATTTG) and BTH 18S 2nd R (CGGTCCGAATAATTCACCGGAT), following the protocol described by Masatani et al. (2017).

Only PCR-positive samples had their first-round PCR products prepared and sent to the National Research Centre for Protozoan Diseases (NRCPD) at Obihiro University of Agriculture and Veterinary Medicine, Japan, for further analysis.

Research at NRCPD

1. DNA Sequencing:

PCR products were re-amplified using the second-round primers and a larger reaction volume (50 µL). The predicted PCR bands were excised and purified using a gel purification kit (NucleoSpin PCR Clean-up and Gel Purification Kit). The purified products were then subjected to sequencing.

2. Nucleotide Analysis:

The obtained sequences were edited using BioEdit software and compared against known sequences using the nBLAST tool.

7. Outline of research achievements

Out of 15 samples, 6 samples were positive from PCR (about 1,500 bp). The result from sequencing revealed that three samples were *B. bovis*, two samples were *B. bigemina* and one sample was *T. orientalis*. There were two tubes of *B. bovis* of Thai strain were stored in liquid nitrogen.

8. Publication of research achievements

Reference

Masatani, Tatsunori, et al. "Detection and molecular characterization of Babesia, Theileria, and Hepatozoon species in hard ticks collected from Kagoshima, the southern region in Japan." Ticks and tick-borne diseases 8.4 (2017): 581-587.