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## NRCPD-OUAVM Joint Research Report

Date: May 31, 2016

Project no: 28-1

### 1. Principal investigator

Name: Dinh Lan Thi Bich

Position: Associate Professor

Affiliation: Institute of Biotechnology, Hue University

### 2. Project title:

Establishment and field evaluation of sero-diagnostic tools applicable to survey the bovine babesiosis in Vietnam

### 3. Collaborating research group members at NRCPD

Name: Naoaki Yokoyama

Position: Professor

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

April 1, 2016 – March 31, 2017

### 5. Purposes and objectives

*Babesia bovis* and *Babesia bigemina* are two economically significant hemoprotozoan parasites of cattle in tropical and subtropical regions of world. Control strategies against these parasite species should essentially be designed based on the rate of exposure, which can be determined by surveying cattle populations using sero-diagnostic tools. Vietnam is an agriculturally rich tropical country. However, the cattle populations in Vietnam were not analyzed to determine the sero-prevalence of *B. bovis* and *B. bigemina*. The objectives of the present study were to 1) detect the and genetically characterize the hemoprotozoan parasites infecting cattle and

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water buffaloes in Vietnam using PCR assays, 2) isolate *B. bovis* and *B. bigemina* rhoptry-associated protein 1 (*rap-1*) gene sequences from parasite-infected Vietnamese cattle and water buffaloes, and 3) develop and evaluate *B. bovis*- and *B. bigemina*-specific ELISA systems using recombinant RAP-1 antigens (rRAP-1) expressed based on gene sequences sourced from Vietnamese cattle.

## 6. Outline of research process

- A PCR-based epidemiological study was conducted to detect and genetically characterize several bovine hemoprotozoan parasites (*B. bigemina*, *B. ovata*, *Theileria annulata*, *T. orientalis*, *Trypanosoma evansi*, and *Tr. theileri*) in cattle and water buffalo bred in Vietnam.
- Type-specific PCR assays were established for *B. bovis msa-1* genotypes prevalent in Asia. The performances of these type-specific PCR assays were then evaluated using *B. bovis*-positive DNA samples that had been previously characterized for *msa* genetic diversity in Asian countries, including Vietnam.
- The *rap-1* gene sequences were isolated from the *B. bovis*- and *B. bigemina*-infected Vietnamese cattle and water buffaloes. The conserved and specific region of these gene sequences were then used to produce *B. bovis* and *B. bigemina* rRAP-1 antigens. Subsequently, rRAP-1 proteins were used as sero-diagnostic antigens to develop ELISAs for detecting *B. bovis*- and *B. bigemina*-specific antibodies from serum samples collected from Vietnamese cattle.

## 7. Outline of research achievements

1. A PCR-based survey detected *B. bigemina*, *T. orientalis*, and *Tr. theileri* among cattle and water buffalo in Vietnam, and a new *Babesia* sp. closely related to *B. ovata* was detected in cattle only. In addition, *T. annulata* and *Tr. evansi* were not detected in both cattle and water buffalo. Phylogenetic analysis detected *T. orientalis mpsp* genotypes 3, 5, 7, and N3 in cattle and 5, 7, N1, and N2 in water buffalo. Additionally, water buffalo-derived *Tr. theileri*

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- catl* sequences clustered together with a previously reported cattle-derived sequence from Vietnam. This is the first report of a new *Babesia* sp. in cattle, and *T. orientalis mpsp* genotype 7 and *Tr. theileri* in water buffalo in Vietnam [1].
2. The genetic diversity of *B. bovis* merozoite surface antigens (MSAs), such as MSA-1, MSA-2b, and MSA-2c, might be linked to altered immune profiles in the host animals. We developed type-specific PCR assays for Asian *msa-1* genotypes, and re-analyzed the genetic diversity of *msa-1* in Sri Lanka, Mongolia, and Vietnam. Specific primers were designed for nine Asian *msa-1* genotypes, which had been detected based on the phylogeny constructed using *msa-1* gene sequences retrieved from the GenBank database. Specificity of the type-specific PCR assays was confirmed using plasmids containing the inserts of *msa-1* gene fragments that represent Asian genotypes. Furthermore, no amplicons were observed by these PCR assays when DNA samples of *B. bigemina*, *B. ovata*, *T. annulata*, *T. orientalis*, *Tr. evansi*, *Tr. theileri*, *Anaplasma marginale*, and *A. bovis*, and non-infected bovine blood were analyzed. In total, 109 *B. bovis*-positive blood DNA samples sourced from Sri Lanka (44 cattle), Mongolia (26 cattle), and Vietnam (23 cattle and 16 water buffaloes) were then screened by the type-specific PCR assays. The sequences derived from all of the PCR amplicons were phylogenetically analyzed. Out of 109 DNA samples, 23 (20 from cattle and 3 from water buffaloes) were positive for at least one genotype. The findings demonstrated that four genotypes, including three novel genotypes, were detected from Mongolia. On the other hand, In agreement with previous studies, five and four different genotypes were detected among the DNA samples from Sri Lanka and Vietnam, respectively. The type-specific PCR assays were also effective in detecting multiple genotypes in co-infected animals. The sequences of the PCR amplicons clustered phylogenetically within the corresponding clades. These findings indicated that the type-specific PCR assays are useful for the determination of genotypic diversity of the *B. bovis msa-1* gene in Asia, including Vietnam [2].
  3. The *rap-1* gene sequences were isolated from cattle and water buffalo infected with *B. bovis* (n=8) and *B. bigemina* (n=19). The percent similarity among the translated amino acid sequences of *rap-1* gene sequences were 96.7 – 100% and 96.5 – 100%, respectively. Based on the conserved and

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specific regions of these gene sequences, *B. bovis* and *B. bigemina* rRAP-1 antigens were produced in *Escherichia coli*. The rRAP-1 antigens were then successfully used as sero-diagnostic antigens in *B. bovis*- and *B. bigemina*-specific ELISA systems. The subsequent sero-surveys conducted in Vietnam using the newly developed ELISAs demonstrated that 71.9 and 78.1% of Vietnamese cattle in the surveyed area were exposed to *B. bovis* and *B. bigemina*, respectively. The findings indicated that the ELISAs that we developed are useful to determine the sero-prevalence of *B. bovis* and *B. bigemina* in Vietnam [Manuscript in preparation].

#### 8. Publication of research achievements

1. Weerasooriya, G., Sivakumar, T., Lan, D.T., Long, P.T., Takemae, H., Igarashi, I., Inoue, N., and Yokoyama, N.: Epidemiology of bovine hemoprotozoa parasites in cattle and water buffalo in Vietnam. *J. Vet. Med. Sci.*, 78: 1361-1367, 2016.
2. Liyanagunawardena, N., Sivakumar, T., Kothalawala, H., Silva, S.S., Battsetseg, B., Lan, D.T., Inoue, N., Igarashi, I., and Yokoyama, N.: Type-specific PCR assays for *Babesia bovis* msa-1 genotypes in Asia: Revisiting the genetic diversity in Sri Lanka, Mongolia, and Vietnam. *Infect. Genet. Evol.*, 37:64-69, 2016.