NRCPD-OUAVM Joint Research Report

Date: May 16, 2018

Project no: 29-7

1. Principal investigator

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2. Project title:

Development of immunochromatographic (ICT) assays applicable for the field survey of bovine babesiosis in Vietnam

3. Collaborating research group members at NRCPD

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

April 1, 2017 – March 31, 2018

5. Purposes and objectives

Bovine babesiosis is one of the economically significant infectious diseases in cattle. Among *Babesia* species that cause bovine babesiosis, *Babesia bovis* and *Babesia bigemina* are known to be of high virulence, as these parasite species induce severe clinical disease, characterized by high fever, hemoglobinuria, anemia, jaundice and sometimes death. Thus, control of *B. bovis* and *B. bigemina* is vital for successful cattle farming. Unlike the genetic diagnostic tools that detect only active infections, sero-diagnostic methods determine the rate of exposure with *B. bovis* and *B. bigemina*. Therefore, sero-surveys are preferred when estimating risk of *B. bovis* and *B. bigemina* infections in cattle.

Immunochromatographic (ICT) assays are now widely used to investigate sero-prevalence of various infectious diseases, as they are useful on-site diagnostic tools. Vietnam, a south-east Asian country, is endemic for both *B. bovis* and *B. bigemina*. However, the cattle populations of this country were never analyzed to determine sero-prevalence of *B. bovis* and *B. bigemina*. Therefore, our aim was to develop ICT assays for the sero-diagnosis of *B. bovis* and *B. bigemina* in cattle in Vietnam. Recently, we prepared *B. bovis* and *B. bigemina*-specific recombinant forms of RAP-1 antigens for the development of ICT assays. However, the antigens should be evaluated in ELISAs before using them to develop ICT assays. Therefore, in the present study, we evaluated these antigens in ELISA systems using serum samples from cattle native to Vietnam and those imported from Thailand for the detection of animals sero-positive to *B. bovis* and *B. bigemina*.

6. Outline of research process

At a slaughter house located in Hue, blood samples were collected from 101 Vietnamese yellow cattle and 54 Brahman cattle (males) that had been imported from Thailand during September to December, 2016. The animals were apparently healthy during the sampling. The Vietnamese animals were 1 – 6 years old (98 and 3 animals were 1 – 3.5 and 4 – 6 years old, respectively), while the Thai cattle were 1.5 – 3.5 years old. Blood samples were subjected to serum separation and DNA extraction. The serum samples were analyzed by the *B. bovis*- and *B. bigemina*-specific ELISAs, while the DNA samples were subjected to *B. bovis*- and *B. bigemina*-specific PCR assays

7. Outline of research achievements

In the Vietnamese cattle, *B. bovis*- and *B. bigemina*- ELISA positivity rates (73.3 and 77.2%, respectively) were higher than those in the Thai cattle (42.6 and 55.6%, respectively). The PCR-positivity rate of *B. bigemina* was also higher in the Vietnamese cattle (30.7%) as compared to the Thai cattle (7.4%), but the *B. bovis* PCR-positive rates were comparable between the Vietnamese and Thai cattle (15.8 and 5.6%, respectively). Co-infections with *B. bovis* and *B. bigemina* were detected by ELISA and PCR in both the Vietnamese (61 and 7 animals, respectively) and Thai cattle (17 and 1 animals, respectively). Of the 16

Vietnamese cattle positive for *B. bovis* by PCR, 12 were positive by ELISA, while the remaining four PCR-positive animals were negative by ELISA. In addition, of the 31 Vietnamese cattle that were positive for *B. bigemina* by PCR, 22 were ELISA-positive, whereas the remaining PCR-positive samples were negative by ELISA. The PCR-positive but ELISA-negative results indicate that these animals were infected recently and were sampled before they developed any ELISA-detectable antibodies. On the other hand, all the Thai cattle positive for *B. bovis* and *B. bigemina* by PCR were ELISA-positive, suggesting that the imported Thai cattle did not receive the infections recently.

The importation of *B. bovis*- and *B. bigemina*-infected cattle might introduce new parasite strains, which might complicate the development of immune control strategies in Vietnam. For instance, the genetic variations of merozoite surface antigens, such as merozoite surface antigen-1 (*msa-1*), might result in altered immune profiles in infected animals. The recent investigations indicated that the genotypic distribution of *B. bovis* merozoite surface antigen-1 (*msa-1*) is different between Vietnam and Thailand. Thus, the introduction of Thai strains of *B. bovis* might be a stumbling block for designing control strategies in Vietnam. Thai cattle, which usually arrive in Hue after 1 – 3 weeks from departing from Thailand, are occasionally allowed to graze before slaughtering. Therefore, entry of *Babesia*-infected ticks together with the imported cattle into Vietnam and transmission of *B. bovis* and *B. bigemina* from infected-Thai cattle to ticks in Vietnamese pasture cannot be ruled out. Therefore, steps should be taken to import *Babesia*-free cattle from Thailand into Vietnam.

The findings of the present study also confirm that the recombinant RAP-1 proteins that we prepared are useful sero-diagnostic antigens. Therefore, we will use these RAP-1 antigens to develop ICT assays capable of detecting *B. bovis* and *B. bigemina* infections in cattle in Vietnam.

8. Publication of research achievements

Sivakumar T, Lan DTB, Long PT, Viet LQ, Weerasooriya G, Kume A, Suganuma K, Igarashi I, Yokoyama N. 2018. Serological and molecular surveys of *Babesia bovis* and *Babesia bigemina* among native cattle and cattle imported from Thailand in Hue, Vietnam. J. Vet. Med. Sci., 80: 333–336.