

Abstract of Thesis/Dissertation

Applicant

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Graduate School of Animal Husbandry

Obihiro University of Agriculture and Veterinary Medicine

Student ID: 25608Signature of Applicant: Charoonluk Jitapattarasate

Title : Molecular epidemiologic analysis of tick-borne protozoan diseases of beef cattle from different regions of Thailand

(タイの異なる地域における肉牛のマダニ媒介原虫感染症に対する分子疫学調査)

Abstract

Tick-borne diseases (TBDs) are among the leading causes of economic losses in cattle production especially in tropical and subtropical areas. Babesiosis and theileriosis are the most widely distributed, endemic and economically important tick-borne diseases affecting cattle.

Bovine babesiosis is caused mainly by *B. bovis* and *B. bigemina*. Generally, the clinical manifestations of *Babesia* infection include high fever, anorexia, hemoglobinuria and icterus. However, *B. bovis* induces neurological damages and respiratory symptoms in infected animal. *B. bovis* and *B. bigemina* are mainly vectored by one host tick-*Rhipicephalus (Boophilus) microplus* in most part of the world.

Bovine theileriosis is caused by various *Theileria* spp. in different geographic locations in the world. The most severe forms of the disease syndrome are tropical theileriosis and east coast fever caused by *T. annulata* and *T. parva*. The epidemiology of

these diseases are limited by the distribution of the tick vectors. However, benign theileriosis is widely distributed in Southeast Asia and it is caused by *T. orientalis*. Although, *T. orientalis* has been classified as a low pathogenic parasite, infected cattle may present with hemolytic anemia, jaundice and abortion.

Beef cattle are the largest cattle population and local breeds of cattle are the dominant beef cattle in Thailand. Although, their natural tolerance to tick-borne diseases compared to the exotic dairy breed, their productivity is still constrained by babesiosis and theileriosis. The occurrence of bovine babesiosis and benign theileriosis have been reported in different parts of Thailand. However, epidemiological studies on such diseases in beef cattle have been limited.

This study aimed to determine the prevalence of *Babesia* spp. and *T. orientalis* in beef cattle from various parts of Thailand using PCR assay. The risk factors for the occurrence of these parasites were assessed. The genetic markers used for their detection were sequenced to determine their genetic diversities and parasites phylogeny in relation to different countries. The effect of the parasite infections on hematological parameters in naturally infected animals was also evaluated.

A total of 608 blood samples were collected from randomly selected beef cattle in 9 provinces located in north, northeastern and western parts of Thailand between March 2014 and June 2015. The provinces surveyed were Chiangrai, Payao, Maehongson, Khonkan, Mahasarakam, Loei, Kanchanaburi, Rachaburi and Nakhonpathom. Genomic DNA was extracted and examined by standard and nested PCR. *B. bovis* spherical body protein 2 (SBP2), *B. bigemina* rhoptry associated protein-1a (RAP-1a) and *T. orientalis* major piroplasm surface protein (MSPS) were used as genetic markers. The PCR results revealed that overall, 54.3 % of the beef cattle sampled was infected with *B. bovis*, *B. bigemina* or *T.*

orientalis. The highest prevalence of tick-borne diseases was found in the northeastern region followed by the north, western and central regions. *T. orientalis* was the most prevalent (19.2%) parasite in beef cattle followed by *B. bigemina* (10.2%) and *B. bovis* (5.4%). Co-infection with *Babesia* spp. and *T. orientalis* was 19.1%. *B. bigemina* and *T. orientalis* was the most prevalent (8.2%) co-infection although triple infection with the three parasites were observed in 2.0% of the samples.

To identify the risk factors that influenced the prevalence of TBDs in beef cattle farms, univariable and multivariable analysis were applied. The major risk factors for infection with *Babesia* spp. included lack of tick control program, communal grazing and contact with other domestic animals. The risk factors for *T. orientalis* infection in beef cattle were lack of tick control program and tick infestation. The risk factor analysis identified that lack of tick control program was the universal risk factor of the occurrence of *Babesia* spp. and *T. orientalis* in beef cattle farms.

The partial sequences of *B. bovis* SBP2 and *B. bigemina* RAP-1a were cloned and sequenced to determine identities and similarities. Furthermore, *B. bovis* and *B. bigemina* ITS1-5.8s rRNA gene-ITS2 regions and the *T. orientalis* MPSP gene were used as templates for sequencing to determine genetic diversities and their genetic relation between isolates from different countries. Sequencing analysis results revealed that *B. bovis* SBP2 and *B. bigemina* RAP-1a sequences were highly conserved and showed homology with other geographic isolates. On the other hand, The sequences of *B. bovis* region were more diverse in their nucleotide length (30-45 bp) and nucleotide identity (93.1-97.1%) than *B. bigemina* ITS1-5.8s rRNA gene-ITS2 region sequences. In the phylogenetic tree, the sequences of *B. bovis* ITS1-5.8s rRNA gene-ITS2 region formed a monophyletic clade with other known *B. bovis* ITS1-5.8s rRNA gene-ITS2 region sequences. In addition *B. bigemina*

ITS1-5.8s rRNA gene-ITS2 region also clustered in one clade. Phylogenetic analysis showed that the *T. orientalis* MPSP gene from cattle in Thailand was classified into 3 clades (type 3, 5, 7).

All of cattle blood samples (n=608) were evaluated for the hematological profiles. Sixteen samples were excluded from hematological study due to red blood cell hemolysis during processing. All samples were divided into 2 groups based on PCR results as PCR positive and PCR negative groups. Statistical analysis was performed using SPSS version 21.0 for Window (Chicago, USA). The measurements obtained for each group were tested for normality with the Shapiro-Wilk and Kolmonogorov-Smirnov tests. Comparisons between the animals with and without a positive test for single and co-infection in each hematology parameters were performed with the Mann-Whitney U test. All hematological parameter values in both PCR-positive and PCR-negative group were within normal range when compared to the reference values. However, some parameters of infected animals showed significant difference with the non-infected group. The hematocrit values indicated that cattle which had infection with tick-borne pathogens (either single or co-infections) had significantly lower PCV values compared to those of cattle without infections. In contrast, using these parameters (WBC, eosinophil and lymphocyte counts) the leukogram of infected cattle was significantly higher than those of the negative groups. These results were not significantly different in hematological parameters between single and co-infection groups. Although, hemograms of the animal in the present study were indicated within normal range but clinical outcome and its impact on production of the animals should be carried out.

Overall, the present study provides epidemiological information regarding *Babesia* spp. and *T. orientalis* in beef cattle and the genetic diversity of these parasites. Data about

the hematological parameters in natural infection with these parasites under field conditions were also reported. These results are useful in introducing effective strategies for parasite prevention and control in cattle population in Thailand.