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Abstract of Dissertation

Applicant

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Student ID Number: 22606

Name of Applicant: Thillaiampalam Sivakumar

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(牛ピロプラズマ症の分子生物学的診断の開発と疫学調査に関する研究)

Abstract

The bovine piroplasmosis represents a serious threat to the livestock industry worldwide. Different species of *Babesia* and *Theileria* parasites often afflict the cattle farming operations. The present study focused on the development of sensitive and specific molecular diagnostic tools for the detection of *Babesia* parasites and on the molecular epidemiology of bovine piroplasms.

Although *Babesia bovis* is the most virulent *Babesia* parasite of cattle, babesiosis caused by the *B. bigemina* becomes fatal if unattended. The widely used *Spe*I-*Ava*I nested PCR (nPCR) assay failed to differentiate *B. bigemina* from *B. ovata*, which is a benign parasite that is morphologically and genetically similar to *B. bigemina*. Therefore, in the present study, a nPCR assay based on the gene that encodes apical membrane antigen-1 (AMA-1) was developed. The highly sensitive *AMA-1* nPCR assay was specific for *B. bigemina*, and no PCR amplicons were observed when DNA samples from several other blood parasites, including *B. ovata*, were tested. When both nPCR assays were employed to screen the Mongolian cattle populations, *AMA-1* nPCR detected only *B. bigemina*. However, *Spe*I-*Ava*I nPCR produced PCR amplicons not only from *B. bigemina* but also from *B. ovata* and two other unknown origins.

Therefore, the *AMA-1* nPCR might be a suitable diagnostic PCR assay that can be used to survey the *B. bigemina*.

On the other hand, there is no PCR assay available for the specific detection of *B. ovata*. In addition, although the coinfection with *B. ovata* and *Theileria orientalis* is common in Japan, the significance of this coinfection was not studied in the past. Therefore, a PCR assay based on *B. ovata AMA-1* gene was developed. The *AMA-1* PCR assay was highly sensitive and specific for *B. ovata*. The PCR assay was later evaluated using over 2000 blood DNA samples sourced from cattle bred in ten different countries. The findings strongly suggested that the *AMA-1* PCR might be useful tool for the universal detection of *B. ovata*. Subsequently, a herd of dairy cattle in Hokkaido, Japan was screened using the *AMA-1* PCR and a previously established PCR (*MPSP* PCR) assays for *B. ovata* and *T. orientalis*, respectively. Essential anemia indicators, red blood cell (RBC) count, hematocrit (HCT), and hemoglobin concentration (Hb), were calculated from all the blood samples that were used to extract DNA for the PCR assays. The findings showed the presence of anemic animals only among the cattle that were either infected with *T. orientalis* or coinfected with both parasites. However, the anemia rate was much higher among coinfected-animals than the *T. orientalis*-infected cattle. In addition, lower mean RBC values were also observed in the animals which were infected with *T. orientalis* and both parasites when compare to that of non-infected category. In contrast, mean Hb and HCT were lower only for the animals which were coinfected with *T. orientalis* and *B. ovata*. These observations suggested that while *B. ovata* does not induce the anemia as a sole agent, this parasite may contribute to the development of anemia in cattle that are coinfceted with *T. orientalis*.

Sri Lanka is an agriculture country and livestock industry is one of the key components of the agriculture sector. However, large sum of foreign currency has been used to import milk and meat products because the local production is insufficient to meet the demand. Among the other reasons, infectious diseases that infect cattle are partly responsible for the low productivity of dairy cattle in this country. Although clinical cases of bovine piroplasmosis have often been detected in Sri Lanka, the genetic characterizations of agents causing this disease have never been reported. Therefore, the present study focused on the genetic diversity analysis of *B. bovis* and *T. orientalis* in Sri Lanka.

B. bovis, which induces a fatal disease in cattle, is endemic in Sri Lanka. Live attenuated vaccine (K-strain) developed in Australia has been introduced into the country more than two decades ago and used to immunize the cattle in selected dairy farms. In Australia, the past studies demonstrated that the population size of *B. bovis*

strains which are resistance to the immune pressure induced by the vaccines might become larger if a particular vaccine is used for a prolong period. Subsequently, these resistance strains might cause a severe babesiosis even among the vaccinated cattle. In addition, it was also observed that the genetic characters of merozoite surface antigens (MSA) of *B. bovis* vaccine strains and outbreak isolates were different from each other. Therefore, in the present study, the genetic diversity of the Sri Lankan *B. bovis* isolates was determined based on the genes that encode MSAs. The findings showed that the *MSAs* (*MSA-2c*, *MSA-2a1*, and *MSA-2b)* sequences were highly diverse in Sri Lanka. In the phylogenetic analyses, Sri Lankan sequences of the respective genes were found in more than one clade. None of the Sri Lankan *MSA* gene sequences was found to be clustered together with the K-strain. Importantly, some of the Sri Lankan sequences were observed in clades where the sequences from Australian K-vaccine outbreak isolates were located. Additionally, the deduced amino acid sequences of *MSA* genes in K-strain shared very low similarities with those from Sri Lankan field isolates. These findings suggest that the Sri Lankan *B. bovis* isolates are genetically diverse and divergent from K-strain. Further studies to determine the antigenicity differences between the field isolates and the K-strains are a priority in Sri Lanka. The findings also warrant animal experiments to evaluate the protection level of K-strain against different field isolates.

T. orientalis occasionally causes disease outbreaks worldwide although the parasite was described to be benign. The parasite population can be divided into well-defined genotypes based on a gene that encodes major piroplasms surface protein (MPSP). Genotype-dependent virulence and immunity have been observed in the infected cattle. Therefore, investigation on the genetic variations of this parasite would not only allow the researchers to predict the clinical relevance of this parasite but also to design possible immune control strategies. The present study examined the genetic diversity of *T. orientalis* detected from Sri Lankan cattle. The findings showed the presence of 4 different MPSP genotypes of *T. orientalis*, types 1, 3, 5, and 7, in Sri Lankan cattle. Subsequently, all the *T. orientalis*-positive DNA samples were subjected to type-specific PCR assays, and the type 7 was found to be the most predominant genotype which was followed by types 5, 3, and 1. The type 7 was involved in several severe clinical theileriosis outbreaks among cattle in India. Therefore, detection of this genotype as the most common MPSP-type in Sri Lankan cattle should alarm the veterinary authorities in Sri Lanka that there might be possible clinical cases due to *T. orientalis* infection in Sri Lanka.

In summary, the present study has developed highly sensitive and specific PCR

assays for the detection of *B. bigemina* and *B. ovata*. In addition, *B. ovata* was found to be associated with the anemia development when coinfected with *T. orientalis* in cattle. The studies conducted in Sri Lankan cattle indicated that the both *B. bovis* and *T. orientalis* populations are genetically diverse.