Form No.5

Abstract of Thesis/Dissertation

Applicant

Master's/Doctoral Program of Veterinary Science

Graduate School of Animal and Veterinary Sciences and Agriculture

Obihiro University of Agriculture and Veterinary Medicine

Student ID: 19185008

Name of Applicant: ATCHARAPHAN WANLOP

Signature of Applicant: Atcharaphan Wanlop

Title : Studies on modification of miracidium hatching technique (MHT) for preparation of single-genome DNA for use in population structure analysis of *Schistosoma japonicum* and development of ELISA for diagnosis of *S. mekongi* infection in humans
(日本住血吸虫の集団構造解析で使用するシングルゲノム DNA の調整を目的としたミラシジウムふ化法の改良およびメコン住血吸虫症の患者の診断を目的とした ELISA の開発に関する研究)

Abstract

Schistosomiasis is a disease caused by blood flukes belonging to the genus *Schistosoma*. These parasites are endemic in 78 countries throughout the world wherein more than 240 million people suffer from the infection among over 700 million people at risk. Asian zoonotic schistosomiasis is caused by *S. japonicum* and *S. mekongi* infections. Their zoonotic nature leads to enhanced disease transmission, making schistosomiasis management challenging. Understanding the epidemiology and dynamics of the parasite's transmission between humans and reservoir animal hosts in the field will be aided by knowledge of the genetic diversity of *S. japonicum*. In addition, there is no vaccine available for schistosomiasis, and thus, disease control is mainly based on MDA with praziquantel. Diagnostic tools with high sensitivity and specificity are needed to accurately assess the

status of the endemicity within a community and to monitor the efficacy of the MDA program. The general aim of this study is to develop a means to obtain this information to promote MDA towards elimination of Asian zoonotic schistosomiasis.

In Chapter 1, the previous miracidium hatching technique (MHT) was modified to apply the technique for preparation of *S. japonicum* single-genome DNA. The information regarding the genetic diversity of *S. japonicum* is indispensable to obtaining an accurate understanding of the epidemiology of schistosomiasis and the transmission dynamics of schistosomes among their definitive hosts. DNA samples originating from a single genome should be prepared to accurately examine the parasite's genetic diversity. The miracidium, a larval stage of the parasite, can be useful material for single-genome DNA preparation. In this study, the previous protocol for the MHT was modified with 96-well plastic ELISA plates to individually collect a miracidium for single-genome DNA preparation. In addition, the effects of light conditions on hatching rates were evaluated. The results showed that the highest hatching rate was observed under the sunlight condition (92.4%), followed by fluorescent light (88.0%). The lowest hatching rate was recorded under the dark condition (4.7%). These results indicated for the first time, to our knowledge, that sunlight was the most efficient light source for the MHT. Furthermore, the study confirmed that a 0.85% NaCl solution and the dark condition prevented miracidium hatching and could be used to store the eggs until the MHT is conducted. In addition, successful amplification of 18S rRNA gene and microsatellite markers from DNA isolated from a single miracidium also confirmed the quality of the single-genome DNA for subsequent application.

In Chapter 2, recombinant antigen-based serology from *S.japonicum*was evaluated

for the diagnosis of *S. mekongi* human infections. The Asian schistosomiasis mekongi is mainly diagnosed by stool microscopy. However, serodiagnosis like enzyme-linked immunosorbent assay (ELISA) with soluble egg antigen (SEA) has been shown to have better sensitivity compared to the stool examination, especially in the settings with low intensity of infection. To date, no recombinant antigen has been assessed using ELISA for the detection of *S. mekongi* infection due to the lack of genome information of this schistosome species. Thus, the objective of this study is to evaluate several recombinant *S. japonicum* antigens that had been developed in our laboratory for the detection of *S.mekongi* infection. Crude antigen SjSEA and recombinant antigens Sj7TR, SjPCS, SjPRx-4 and SjChi-3 were evaluated in indirect ELISA using serum samples positive for *S. mekongi* infection. Cross-reaction was checked using sera positive for *Opisthorchis viverrini*. ELISA results showed that *S. japonicum* SEA at low concentration showed better diagnostic performance than the recombinant antigens tested using the archived serum samples from Cambodia. Because recombinant antigen has several advantages over crude antigen in the application with ELISA, further optimization of the recombinant antigens should be done in future studies to improve their diagnostic performance for detecting *S. mekongi* infection in humans.

In Chapter 3, recombinant antigen of *S. mekongi* TPx-1 (rSmTPx-1) was expressed, and the antigen was evaluated for its performance in detecting *S. mekongi* infection in humans by ELISA. Decades of use of numerous control measures, which include MDA using praziquantel, have resulted in a decrease in the prevalence of schistosomiasis mekongi. This, however, has led to a decrease in sensitivity of the Kato-Katz stool

examination, which is considered the gold standard in the diagnosis of schistosomiasis mekongi. In this study, a gene coding for *S. mekongi* TPx-1 was cloned, and the recombinant antigen (rSmTPx-1) was expressed to develop a serological assay with high sensitivity and specificity that could replace the Kato-Katz technique. Diagnostic performance of rSmTPx-1 as an antigen in ELISA for detecting human schistosomiasis was compared with that of the recombinant antigen of *S. japonicum* TPx-1 (rSjTPx-1) using a panel of serum samples collected from endemic foci in Cambodia. The sensitivity and specificity of rSmTPx-1 in ELISA were 89.3% and 93.3%, respectively, whereas those of rSjTpx-1 were 71.4% and 66.7%, respectively. In addition, the the higher Kappa value of 0.82, which was calculated between ELISA with rSmTPx-1 and Kato-Katz, confirmed better agreement between them than between ELISA with rSjTPx-1 and Kato-Katz (Kappa value 0.38). These results suggested that ELISA with rSmTPx-1 could be a potential diagnostic method for detecting an active human *S. mekongi* infection.

In conclusion, this study has proposed two useful tools for disease surveillance and assessment of the efficacy of an MDA program that can lead to the elimination of schistosomiasis. The modified MHT can be used in field studies for recovering miracidium to prepare single-genome DNA for population genetic studies in the future. Application of additional recombinant *S. mekongi* antigens with ELISA might have a potential role in developing a reliable and accurate diagnostic test for detecting *S. mekongi* infections in humans and reservoir animals in the future.