SATREPS Project: Project for Development of Innovative Research Techniques in the Genetic Epidemiology of Malaria and Other Parasitic Diseases in the Lao PDR for Containment of Their Expanding Endemicity

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Background

Malaria, Schistosomiasis *(Schistosoma mekongi)*, and Opisthorchiasis *(Opisthorchis viverrini)* are a tremendous health burden on the people of the Lao PDR. Although significant reductions in malaria transmission have been reported due to the large-scale distribution of insecticide-treated bed nets (ITNs) through the Global Fund to Fight AIDS, Tuberculosis and Malaria, strategies based on the scientific evidence have not been developed to deal with the genetic variations in parasite and vector populations and drug-

resistant malaria. Recently, artemisinin-resistant malaria was reported in southern provinces [1, 2]. Therefore, it is necessary to survey other provinces, especially the five southern provinces of the country, to monitor and contain the spread of drug-resistant malaria. The Lao Ministry of Health and the WHO has set a goal of eliminating malaria by 2030. To achieve this goal, we have to understand the real malaria situation, including drug-resistant malaria, the prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency, the prevalence of asymptomatic *Plasmodium* carriers, malaria situation among mobile and migrant populations, and develop effective elimination strategies.

Significant progress has been made in the past decades in the reduction of the prevalence of Schistosomiasis (*S. mekongi*) in the endemic areas (approximately 200 villages) in Khong district and Mounlapamok district, Champasak province, Lao PDR, through preventive chemotherapy using praziquantel as the drug of choice once a year as well as health education in the community. However, current monitoring methods rely on the Kato- Katz stool examination whose sensitivity may not be sufficient to detect light intensity *S. mekongi* infections. In 2017, WHO adopted a new strategy that accelerates the elimination of Asian schistosomiasis in the Western Pacific Region, i.e., transmission interruption by 2025 and eradication by 2030.

One criteria of transmission interruption are *"no new case of animal infection*." To achieve this goal, we need sensitive detection methods, such as DNA diagnostic methods (PCR and LAMP) and serological methods (ELISA), to monitor the prevalence of the disease precisely in the endemic areas.

Since Opisthorchiasis (*O. viverrini*) is localized to the Lao PDR and surrounding countries, it is recognized as a neglected tropical disease. Nevertheless, the prevalence of opisthorchiasis is estimated to be as high as 15–54% in the Lao PDR. Little information on the molecular/ genetic

epidemiology of opisthorchiasis is available to develop effective measures for prevention and diagnosis of the disease.

The government of the Lao PDR requested the Japan International Cooperation Agency (JICA) to establish the Lao–Japan Joint Laboratory within Institut Pasteur du Laos (IPL) to conduct highly technological research on malaria parasites (*Plasmodium falciparum, P. vivax,* and the monkey malaria parasite *P. knowlesi*) and human trematodiases (*S. mekongi and O. viverrini)*. The joint research will concentrate on genetic epidemiological studies to detect and control the emergence and dissemination of these parasitic diseases. The project also contributes to the capacity development of researchers and technicians in the Lao PDR through training in field and lab work, seminars, and career development.

In order to carry out this project, the IPL in collaborating with the National Center for Global Health and Medicine (NCGM), Tokyo, Japan, Tokyo Medical and Dental University (TMDU), University of the Rukyus, Okinawa, Japan, the Center of Malariology, Parasitology and Entomology (CMPE), the Lao Tropical and Public Health Institute (Lao TPHI), and other Departments of the Ministry of Health, Lao PDR.

Objectives

The objectives of this project are (1) to develop more convenient and accurate methods (PCR methods, LAMP methods, etc.) for diagnosis of the diseases, (2) to monitor the temporal and spatial epidemiological situations of pathogens and vectors of the diseases, (3) to analyze mechanisms of emergence and expansion of drug-resistant malaria, especially artemisinin resistance, and (4) to analyze the G6PD activity of the Lao population for evaluation of the possible usage of primaquine [3, 4], utilizing molecular biological techniques. Based on the scientific evidence obtained by this project, health education for the people will be strengthened and the

endemicity of the diseases will be monitored together with the local Lao Ministry of Health officials. Research results will also be utilized in government services for the sustainable development of the Lao PDR.

The study period of the project
Five years (May 2014 to April 2019)

Project study sites

Malaria: Savannakhet province, Salavan province, Sekong province, Attapeu province, Champasak province, Khammouane province, Phongsaly province, Luang Prabang province.

Schistosomiasis *(S. mekongi):*
Khong district and Mounlapamok district, Champasak province.
Opisthorchiasis *(O. viverrini):*
Khammouane province, Champasak province.

Ethical clearance

The SATREPS project was approved by the National Ethic Committee for Health Research, the Ministry of Health, Lao PDR from 2014 to present (extended each year).

Activities and Results in November 2018 to April 2019

Prevalence and distribution of artemisinin-resistant *P. falciparum* in Lao PDR

Artemisinin-based combination therapies (ACTs) have been used as a first-line treatment for uncomplicated malaria in Lao PDR since 2005. However, artemisinin-resistant P. falciparum was first reported in 2013 and is indeed threatening malaria elimination by 2030. It is reported that mutations of *the K13* gene in *P. falciparum* are associated with artemisinin resistance and can be used as a molecular marker for monitoring artemisinin-resistant *P. falciparum*. The aim of the

study is to assess the prevalence and distribution of the *K13* mutations in Lao PDR.

Malaria patient blood samples were collected from malaria patients or malaria suspected patients in 156 healthcare facilities in the five southern provinces from October 2015 to June 2017, and the northern most province from November to December 2017 (Table 1). In 2015, only malaria positive samples were collected, whereas in 2016 and 2017, both malaria positive and negative samples were collected. “Malaria positive” means that patients were diagnosed as malaria by microscopy or malaria rapid diagnostic test (RDT). On the other hand, “malaria negative” means that patients were not diagnosed as malaria by microscopy or malaria RDT, although they showed malaria-like signs or symptoms.

Real-time nested PCR was performed to identify Plasmodium species using malaria positive samples (Table 2). The proportion of *Plasmodium* species was heterogeneous by province or by year. For example, in Savannakhet, *P. falciparum* was predominant species while in Sekong, *P. vivax* was predominant species. Direct DNA sequencing was performed to detect the *K13* mutations in isolates of *P. falciparum* (Table 3). Data on the *K13* mutations of *P. falciparum* in the five southern provinces from October 2015 to April 2016 and the northernmost province in 2017 was published in Malaria Journal (Iwagami et al., 2018). Recently, the *K13* mutation analysis of *P. falciparum* collected in the five southern provinces from November 2016 to May (or June) 2017 has just finished. Most of the mutations were C580Y mutation. Surprisingly, the prevalence of artemisinin-resistant mutation decreased from 2015 to 2017, except for Champasak.

However, caution is needed for these results because the sample sizes drastically decreased by year and a sampling bias might exist. For example, in Champasak, the sample sizes drastically decreased although the reported number of malaria cases in Champasak did not drastically change

Summary of malaria patient blood sampling from public health care facilities in 2019

The objective of malaria patient blood sampling is to monitor the distribution and frequency of drug-resistant malaria in Lao PDR. We collected 2,870 dried blood samples on filter papers (FTATM Classic Card, GE Healthcare Life Sciences, WhatmanTM, UK) in 2019. The number of blood samples and the sampling periods in each province are shown in Table 1. The dried blood samples (n=2,472) were collected from malaria patients who visited 156 public health care facilities in the five southern provinces (Savannakhet, Salavan, Sekong, Attapeu, and Champasak) on February 2019. Malaria patients mean that the patients were diagnosed as malaria by either the RDTs or microscopy at health care facilities. On the contrary, the dried blood samples (n=398) were collected from malaria suspected patients who visited eight public health care facilities in Phongsaly, the northernmost province in January 2019. Malaria suspected patients consist of both malaria patients confirmed by either the RDTs or microscopy and malaria negative people confirmed by the tests even though they manifested malaria-like signs and symptoms.

The blood samples are now being analyzed by nested real-time PCR for the identification of *Plasmodium* species. Mutation(s) of the artemisinin-resistant gene (the K13 gene) is also being examined using *P. falciparum* positive samples at IPL and NCGM.

Training course on basic and molecular parasitology at the National Center for Global Health Medicine and (NCGM), Tokyo, Japan

In 2019, two Junior Scientists in Parasitology lab attended a training course on basic and molecular parasitology at NCGM, Tokyo, and Tokyo Medical and Dental University (TMDU), Tokyo, Japan. Dr. Phonepadith KATTIGNAVING attended the training course on 13th January to 2nd February 2019 and Dr. Phoyphaylinh

PRASAYASITH attended the training course on 13th January to 16th February 2019. At NCGM, they studied malaria diagnosis by microscopy (all four human malaria species and one simian malaria parasite: *P. knowlesi*) and DNA diagnosis by PCR, LAMP and DNA sequencing including drug-resistant gene analysis. Dr. Phoyphaylinh also studied how to design PCR primers and the technique of cloning PCR products using plasmid DNA and competent cell (*E. coli*). At TMDU, they studied ELISA technique for *Schistosoma mekongi* and how to maintain *S. japonicum* in a laboratory setting using mice and snail host: *Oncomelania hupensis nosophora*

Completed PhD course on public health at University of the Ryukyus

Mr. Phoutnalong VILAY, CMPE completed PhD course on public health and obtained his PhD from the University of the Ryukyus, Okinawa, Japan under the supervision of Professor Jun KOBAYASHI and Associate Professor Daisuke NONAKA on September 2019. The SATREPS project supported his study in Japan through JICA. He conducted a malaria study among Lao Military personnel in Attapeu and Champasak provinces (Vilay et al., Trop Med Health, 47:11, 2019). His malaria PCR was conducted at IPL under the supervision of Parasitology lab staff. Ms. Emiri TAKAHASHI also completed PhD course on public health and obtained her PhD from the University of the Ryukyus, Okinawa, Japan under the supervision of Professor Jun KOBAYASHI and Associate Professor Daisuke NONAKA in 2019. She conducted a malaria study about medication adherence in Savannakhet province (Takahashi et al., Trop Med Health, 46:44, 2018).

The SATREPS Project Final Meeting

On 2nd April 2019, JICA/AMED SATREPS Project Final Dissemination Meeting was held at the Lao Plaza Hotel. Dr. Shigeyuki KANO, chief advisor of the SATREPS project and Dr. Rattanaxay PHETSOUVANH, Director General of Department of

Communicable Disease Control (DCDC), Ministry of Health were cochaired the meeting. Dr. Bouasy HONGVANTHONG, Project Director of the SATREPS, Former Director of Center of Malariology, Parasitology, and Entomology (CMPE), Ministry of Health, Dr. Viengxay VANISAVETH, Acting Director of CMPE, Dr. Paul BREY, Project Manager of the SATREPS, Director of IPL, and other members of the SATREPS project both Japan and Laos participated in the meeting. Staff members from the Provincial Health Department, WHO Laos Office, JICA Laos Office and Japan Embassy were also attended. Project members of the Parasitology lab presented seven presentations about outcomes of the five-years project including malaria, schistosomiasis and opisthorchiasis. Finally, Dr. Rattanaxay summarized the outcomes of the SATREPS project.

A handover ceremony of Eiken LAMP machine was also held at the end of the meeting. Eiken Chemical, Co. Ltd, Japan donated five LAMP machines for Lao PDR to accelerate malaria elimination by 2030. The five LAMP machines will be installed in health care facilities (District Hospitals) in malaria high endemic districts in the five southern provinces.

Partners

• Center of Malariology, Parasitology and Entomology (CMPE), Ministry of Health, Vientiane Capital, Lao PDR
• Lao Tropical and Public Health Institute (Lao TPHI), Ministry of Health, Vientiane Capital, Lao PDR
• Department of Communicable Diseases Control (DCDC), Ministry of Health, Vientiane Capital, Lao PDR
• National Center for Laboratory and Epidemiology (NCLE), Ministry of Health, Vientiane Capital, Lao PDR
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Acknowledgements / Funding

We wish to thank Dr. Bouasy HONGVANTHONG, Former Director of the Center of Malariology, Parasitology and Entomology (CMPE), the Ministry of Health, Lao PDR, and Project Director of SATREPS for his kind support of this project. We thank Dr. Tiengkham PONGVONGSA, Deputy Director, Savannakhet Provincial Health Department, Lao PDR. We also thank the staff of the CMPE, the Lao TPHI, Provincial Health Departments, Provincial Hospitals, District Hospitals, and Health Centers, the Ministry of Health, for supporting our field surveys and training courses.

This work is financially supported by the SATREPS (Science and Technology Research Partnership for Sustainable Development) project from the Japan International Cooperation Agency (JICA) and the Japan Agency for Medical Research and Development (AMED).

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