CALL FOR PROPOSALS

To support Cambodian Masters degree or DES

Title of the project:

Mapping of *Plasmodium falciparum histidine-rich protein 2/3* gene deletions in Cambodia

Investigator:
Dr KHIM Nimol, PhD, researcher at Institut Pasteur du Cambodge (IPC)

Supervisor of a second year Master student:
At IPC: Dr KHIM Nimol, PhD

Duration: 12 months (January to December 2017)

Place : Institut Pasteur du Cambodge (IPC)

សេចក្តីសង្ខេប

ជម្ងឺគ្រុនចាញ់វាបង្កឡើងដោយប៉ារ៉ាសីតប្លាស់ស្មូដ្យូម ហើយនៅតែជាជម្ងឺមួយដែលប្រឈមដល់សុខភាពសាធារណៈនៅក្នុងប្រទេសកម្ពុជា។ យោងតាមរបាយការណ៍នៃមជ្ឈមណ្ឌលជាតិប្រយុទ្ធនឹងជម្ងឺគ្រុនចាញ់ ក្នុងឆ្នាំ ២០១៥៖ អ្នកឈឺដោយជម្ងឺគ្រុនចាញ់នេះមានរហូតដល់ ៥៥០០០នាក់ នៃប្រជាជនកម្ពុជា។ តេស្ត (Rapid Diagnostic Tests"RDT") ត្រូវបានអនុវត្តតាមទំលាប់ជាលក្ខណៈទូលំទូលាយ នៅតាមកំរិតសហគមន៍ ដើម្បីរកវត្តមានមេរោគគ្រុនចាញ់នៅលើអ្នកជម្ងឺដែលសង្ស័យថាមានជម្ងឺ គ្រុនចាញ់មុននឹងផ្តល់ថ្នាំដល់អ្នកមានជម្ងឺគ្រុនចាញ់។ ប៉ុន្តែវាហាក់ដូចជាមានភាពមិនប្រកតីកើតឡើងរវាងលទ្ធផលវិជ្ជមាននៅលើកញ្ចក់ឈាម និងលទ្ធផលអវិជ្ជមានតាមរយៈតេស្ត RDT វិភាគរកហ្សែន *Pfhrp2* ជារើយៗ។ មូលហេតុដែលនាំឲ្យកើតមានកំហុសឆ្គងលើលទ្ធផលអវិជ្ជមាននេះ នៅមិនទាន់មានដំណោះស្រាយនៅឡើយ ហើយវាអាចជះឥទ្ធិពលដល់អនុសាសន៍ក្នុងការលុបបំបាត់ជម្ងឺគ្រុនចាញ់នៅក្នុងប្រទេសកម្ពុជា នាឆ្នាំ ២០២៥។

ហេតុដូច្នេះ ការសិក្សាស្រាវជ្រាវដើម្បីរកការលុបបំបាត់ដោយឯកឯងរបស់ហ្សែន *Pfhrp2/Pfhrp3* នៃប៉ារ៉ាសីតប្លាស់ស្មូដ្យូមគួរតែបានបង្កើតឡើង។

ដើម្បីអនុវត្តនូវគោលការណ៍នេះ ការសិក្សាលើ ១២០០ សំណាកឈាមកំពុងមានស្រាប់ ដែលបានរក្សានៅមជ្ឈមណ្ឌលវិភាគវេជ្ជសាស្ត្រនៃមន្ទីរពិសោធន៍ផ្នែកជម្ងឺគ្រុនចាញ់ នៃវិទ្យាស្ថានប៉ាស្ទ័រកម្ពុជាដែលនឹងត្រូវបានជ្រើសរើសដោយផ្អែកទៅលើលទ្ធផលអវិជ្ជមាន RDT ពីតាមតំបន់នៃភាគខាងលិច ខាងកើត ខាងជើង និងខាងត្បូងនៃប្រទេសកម្ពុជា។ សំណាកឈាមទាំងអស់នេះនឹងត្រូវវិភាគរកវត្តមានប៉ារ៉ាសីតប្លាស់ស្មូដ្យូមហ្វាស៊ីប៉ារ៉ូម។ បន្ទាប់មក ការវិភាគរកវត្តមានហ្សែន *Pfhrp2/Pfhrp3* នឹងត្រូវអនុវត្តឡើង ចំពោះវត្ថុសំណាកដែលមានលទ្ធផលវិជ្ជមានប្លាស់ស្មូដ្យូមហ្វាល់ស៊ីប៉ារ៉ូម។

យើងរំពឹងទុកថា វិធីសាស្ត្រទាំងនេះនឹងអាចចូលរួមចំណែកដល់មជ្ឈមណ្ឌលជាតិប្រយុទ្ធនឹងជម្ងឺគ្រុនចាញ់ក្នុងការកែលំអរយុទ្ធសាស្ត្រអនុវត្តក្នុងការលុបបំបាត់ជម្ងឺគ្រុនចាញ់។

Summary

Malaria, is a parasitic disease, remains one of the public health burdens resulted in more than 55 000 clinical cases in Cambodian people (CNM 2015). The Malaria Rapid Diagnostic Tests (RDT) are routinely and widely used at community levels to screen malaria infection among malaria suspected people before giving the treatment. However, it seems that the discordant results of RDT based on *Pfhrp2* detection and microscopy have been more encountered. The cause of suspected false-negative results is not well established which would be one of the concerns in the context of government's commitment to eliminate malaria in Cambodia in 2025.

In this context, investigations of more studies to determine the deletion of *Pfhrp2/Pfhrp3* genes in parasites should be developed.

To assess the performance of this promising tool, a retrospective study will be conducted on 1200 samples having negative-RDT results from symptomatic and asymptomatic individuals collected in different part of the country. Positive PCR results will be screened for *Plasmodium falciparum* species. The *Pfhrp2/ Pfhrp3* genes will be amplified on *Plasmodium falciparum* samples.

Our approaches would be supported the National Malaria Control Program to improve strategies implemented in order to achieve the ambitious goal of malaria elimination.

Context and challenge:

Malaria is a protozoan parasite *Plasmodium* and transmitted by female *Anopheles* mosquitoes, which bite mainly between sunset and sunrise. This disease persists one of the worldwide public health concerns. Responsible each year over 214 million clinical episodes leading to more than 438 000 deaths [1], 90% of the cases occur in sub-Saharan Africa [2, 3].

Five *Plasmodium* species infected in human have been documented in Cambodia. Despite major efforts made by national and international health organizations to implement effective strategies to control malaria, the number of *Plasmodium falciparum* infected has been decreases dramatically.

Malaria Rapid Diagnostic Test (RDTs), which has been recommended by World Health Organization, are available in remotes areas of malaria endemic settings to diagnose all febrile patient suspect to have malaria. The current commercialized Rapid Diagnostic Test (RDT) are based on the detection of *P. falciparum* antigens such as *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP2), *Plasmodium lactate dehydrogenase* (pLDH), or aldolase (PfAldolase), [4, 5].

Nevertheless, *Pfhrp3* which belong also to the HRP gene family, PfHRP3 might cross-react with PfHRP2 because of the resemblance of their amino acid sequence. However, recent studies have described that some parasites may deleted their *Pfhrp2/Pfhrp3* genes [6]. The prevalence of such parasites (which are not detected by the PfHRP2-based RDT) are frequent in South America and in at least four African countries including Eritrea and Ghana [7]. To date, no studies have been conducted in Cambodia to establish the causality of less accuracy of commercialized RDT in symptomatic malaria infection while PfHRP2-based RDT are frequently used at community and in the health center levels.

Purpose of the project:

In this context, our purpose is to assess the frequency of parasites with *Pfhrp2/Pfhrp3* deletion in different areas of Malaria transmission in Cambodia.

Specific objectives of the project:

Our objectives are firstly to define the absence and presence of *Pfhrp2/ Pfhrp3* among the patients who are negative in current *Pfhrp2*-based RDT and positive malaria infection identified by PCR then to do a countrywide map of *Plasmodium falciparum* histidine- rich protein 2/3 gene deletions.

Output results and impacts of the project:

Firstly, we would explore the prevalence of *Pfhrp2/ Pfhrp3* genes in patient with negative *Pfhrp2*-based RDT by molecular approaches (PCR/Sanger sequencing). Our research would straightening the strategically plan in particular regions in the framework of malaria elimination in Cambodia.

Methodology:

Sample collection

1200 isolates from venipuncture blood/dried blood spots, stored in BioBank of Malaria Molecular Epidemiology Unit, Institut Pasteur in Cambodia, collected from symptomatic patients or asymptomatic individuals between 2008 and 2014 at different sites in Cambodia will be tested. All specimens were found negative by RDT and positive by microscopy or PCR.

Activities of the project and implementations:

1. Development of real-time PCR for the detection of *Plasmodium falciparum* histidine-rich protein 2/3 genes
    - DNA extraction by using Qiagen mini blood kit from 3D7-calibrate blood samples
    - Preparation of a serial DNA concentration of 3D7 ranged from 1ng/uL to 0.0001 ng/uL.
    - Identification and determination of the threshold of detection for *Plasmodium falciparum histidine-rich protein* 2/3 genes from each DNA concentration analyzed.
2. Evaluation of the distribution of parasites with *Pfhrp2/ Pfhrp3* genes deletion
    - Detection of *Plasmodium falciparum* infection from 1200 specimens (400, 250, 400 and 150 obtained from Western, Eastern Northern and Southern of country) [8]
    - Amplification of *Pfhrp2/Pfhrp3* genes
    - Mapping the *Pfhrp2/Pfhrp3* genes deletions

Investigators:

- PI: Khim Nimol, PhD, Researcher, Malaria Molecular Epidemiology Unit
- Advisor: Didier Ménard, PhD, Head of Malaria Molecular Epidemiology Unit

Institut Pasteur du Cambodge (IPC)

Supervisors:

At IPC: Khim Nimol, PhD

Duration:

12 months: 12 months (January to December 2017)

Place:

Malaria Molecular Epidemiology Unit, Institut Pasteur du Cambodge

Candidates

The candidates, who study in University of Health Sciences or in University of Agriculture or in University of Royal Phnom Penh or in Institute of Technology of Cambodia, have been finished their 1st year of their Master degree courses for academic year 2015-2016. They are now enrolled in the second year of academic year 2016-2017.

The candidates have to submit their CV, their cover letter, 2 reference letters including one from their Dean or their rector of University. They have to be interviewed.

There will have an Agreement between our co-supervisor and their students.

References

1. WHO: **World Malaria Report 2015.** 2015.
2. Breman JG, Alilio MS, Mills A: **Conquering the intolerable burden of malaria: what's new, what's needed: a summary.** *Am J Trop Med Hyg* 2004, **71:**1-15.
3. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI: **The global distribution of clinical episodes of Plasmodium falciparum malaria.** *Nature* 2005, **434:**214-217.
4. Banoo S, Bell D, Bossuyt P, Herring A, Mabey D, Poole F, Smith PG, Sriram N, Wongsrichanalai C, Linke R, et al: **Evaluation of diagnostic tests for infectious diseases: general principles.** *Nat Rev Microbiol* 2006, **4:**S21-31.
5. Bell D, Peeling RW, Pacific/TDR WH-ROftW: **Evaluation of rapid diagnostic tests: malaria.** *Nat Rev Microbiol* 2006, **4:**S34-38.
6. Cheng Q, Gatton ML, Barnwell J, Chiodini P, McCarthy J, Bell D, Cunningham J: **Plasmodium falciparum parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting.** *Malar J* 2014, **13:**283.
7. WHO: **False-negative RDT results and implications of new reports of P. falciparum histidine-rich protein 2/3 gene deletions.** 2016.
8. Canier L, Khim N, Kim S, Sluydts V, Heng S, Dourng D, Eam R, Chy S, Khean C, Loch K, et al: **An innovative tool for moving malaria PCR detection of parasite reservoir into the field.** *Malar J* 2013, **12:**405.