Australian Government
Australian Centre for International Agricultural Research

Final report

Small research and development activity

project	**Establishing a network for surveillance of emerging zoonotic malaria in Indonesia (ZOOMAL)**
project number	LS/2018/214
date published	16th Sept 2021
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final report number	FR2021-045
ISBN	978-1-922635-52-5
published by	ACIAR GPO Box 1571 Canberra ACT 2601 Australia

1 Acknowledgments

We would like to acknowledge the major role of our international collaborators at Eijkman Institute for Molecular Biology in Jakarta, Indonesia led by Dr Rintis Noviyanti, and Universitas Sumatera Utara, Medan, Indonesia led by Dr Inke Lubis, including their enthusiastic team members.

In addition, we appreciate the assistance and permission of the Ministry of Health, Indonesia, and the National Malaria Control Program to conduct this research.

2 Executive summary

Substantial gains have been made over the past decade towards the elimination of the human malaria species *Plasmodium falciparum* and *P. viva*x malaria in Southeast Asia. However, alongside these gains, there has been an increase in incidence of human malaria from the zoonotic parasite *P. knowlesi* [1, 2]. *P. knowlesi* is a parasite of the long-tailed (*Macaca fasicularis*) and pig-tailed (*M. nemestrina*) macaques, transmitted by the *Anopheles leucosphyrus* group of mosquitoes. Human malaria from *P. knowlesi* has now been reported throughout South-East Asia, in all countries where these macaque hosts and mosquito vectors are found [3], with most cases occurring in agricultural workers.

However, *P. knowlesi* and other zoonotic malaria infections are commonly misdiagnosed with conventional diagnostic tools, with more accurate molecular screening in Indonesia not previously implemented. The aim of this pilot project (LS/2018/214) was to develop and implement sensitive and specific molecular detection tools such as PCR to provide important information regarding the incidence of zoonotic malaria in Indonesia, and establish a network for molecular epidemiological surveillance for *P. knowlesi* and other zoonotic *Plasmodium* species in Kalimantan, Sumatra and Sabang, Indonesia. A better understanding of the spread and distribution of zoonotic malaria species in Indonesia will enable public health systems to strengthen surveillance and protect at-risk populations in agricultural areas of Indonesia. Outputs from the project are also useful to Indonesia's National Malaria Control Program (NMCP) to improve malaria surveillance, prevention, diagnostics and treatment. Building on existing collaborations between Menzies and Indonesian partners, this pilot project aimed to establish the initial Indonesian zoonotic malaria surveillance in partnership with the University of Sumatera Utara (USU) and the Eijkman Institute for Molecular Biology (EIMB).

Specific objectives of this study were:

1. ***Build capacity in Indonesia to use ultrasensitive multi-species screening molecular assays for low-level single or mixed species infections***.

The project built on an existing collaboration with Dr Noviyanti and Dr Coutrier from the Eijkman Institute for Molecular Biology, who led the Nth Kalimantan and Aceh studies. A new collaboration also commenced with Dr Lubis at the University of Sumatera Utara, who led the Nth Sumatra studies. In total 42 staff members were trained over 13 sites across western Indonesia, comprising 38 females (90%). The laboratory at USU had critical PCR infrastructure purchased and molecular laboratory workflow improvements. A gold-standard molecular malaria surveillance protocol encompassing blood collection in field optimal DNA/RNA shield media, an ultra-sensitive *P. genus* screening RT-PCR targeting the 18S rRNA gene, and nested PCR for *P. knowlesi*, human and simian malaria species was validated and implemented.

2. ***Establish pilot malaria surveillance activities at health facilities in North Kalimantan, North Sumatra and Sabang, Aceh*.**

Early engagement with key Ministry of Health stakeholders including health facility staff and site visits allowed development of the final harmonised surveillance protocol. Relevant institutional agreements and ethical approvals were obtained. Multiple site visits, including by Australian investigators in 2019 and 2020, were conducted. Data collection tools, electronic real-time database and study SOPs were developed. Laboratory quality assurance and ongoing training activities were conducted throughout. Feedback to the National Malaria Control program included engagement through the Asia-Pacific Malaria Elimination Network.

3. ***Evaluate the incidence of zoonotic* Plasmodium *species (including mixed infections) among patients diagnosed with malaria by microscopy at health***

facilities in north and east Kalimantan, north Sumatra and Sabang, and among febrile controls.

In total, 1440 patients were enrolled in the study, including 21 (1.4%) with *P. knowlesi*, 151 (11%) with *P. vivax*, 64 (4%) with *P. falciparum*, and 198 (14%) positive for *P. genus* without a species-specific identification. *P. knowlesi* incidence had marked spatial variation, with Malinau in Nth Kalimantan having 10/81 (12.3%) febrile cases positive for *P. knowlesi*, resulting in the highest incidence of 47.9 infections per 100,000 people per year. No *P. knowlesi* cases were reported on mainland Nth Sumatra in densely populated coastal towns, in contrast to the island of Mursala where 7/64 (10.9%) were positive. No PCR confirmed infections with rarer simian malaria including *P. cynomolgi* were recorded.

4. ***Evaluate clinical and epidemiological characteristics of patients with malaria due to* P. knowlesi *or other zoonotic species in patients presenting to health facilities in north and east Kalimantan, north Sumatra and Sabang.***

P. knowlesi cases were older than other malaria species (median age 39 years [range 19-72]). All *P. knowlesi* cases from Malinau and Sabang were male, in contrast to Mursala where 57% *P. knowlesi* cases were female and included asymptomatic infections. Routine microscopy for *P. knowlesi* detection demonstrated 77% sensitivity (95%CI 50-93), lower than the 90% WHO mandated limit. Anaemia was present in 33% of *P. knowlesi* cases. Epidemiological factors associated with increased risk of *P. knowlesi* acquisition included age >30 years (aOR 3.6), male gender (aOR 5.3), sleeping outside (aOR 6.0), forest activities including gathering wood (aOR 8.7) and burning charcoal (aOR 15.4), and household location near rubber plantations (aOR 7.4) and intact forest (aOR 5.0).

Future work: This project achieved the aim to determine the relevant sampling and diagnostic protocols, obtain ethical permits and build relevant capacity in order to implement the subsequent large ZOOMAL multidisciplinary One Health project (LS/2019/116) to evaluate broader associations with agricultural/forestry land use in Indonesia, including geospatial modelling, macaque, and mosquito components. Additional molecular work to identify the *P. genus* positive samples will also be conducted.