

NRCPD-OUAVM Joint Research Report

Date: May 21, 2023
Project no: 2022-joint-20

1. Principal investigator

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Position: Associate Professor

Affiliation: Faculty of Veterinary Science, Chulalongkorn University

2. Project title:

Identification of mosquitoes in goat farms and molecular screening of malaria parasite in mosquitoes.

3. Collaborating research group members at NRCPD

Name: Masahito Asada

Position: Associate Professor

4. Research period (in mm/dd/yyyy, and total number of years)

April 1, 2022 – March 31, 2023

5. Purposes and objectives

Mosquitoes play a key role in transmitting many infectious diseases including malaria. Ungulate malaria parasites are known to be transmitted by the mosquitoes belonging to the genus *Anopheles*. There have been a few reports about mosquitoes responsible for malaria transmission in mouse deer and white-tailed deer while mosquito vector for goat malaria transmission remains unknown. The objective of this study was to identify the vectors responsible for transmitting the goat malaria parasite, *Plasmodium caprae*.

6. Outline of research process

The mosquitoes were collected from goat farms located in six districts across four provinces in northern and western Thailand. Each location has been visited only once. From each district, one or two specific localities were chosen as follows: Lao Khwan District (14° 28' 33.4" N 99° 48' 25.0" E and 14° 28' 51.1" N 99° 48' 11.7" E) in Kanchanaburi, Wiang Sa District (18° 31' 56.9" N 100° 37' 50.2" E) and Mueang Nan District (18° 49' 07.0" N 100° 46' 36.9" E) in Nan, Ban Kha District (13° 17' 32.3" N 99° 25' 06.8" E) in Ratchaburi, Kaeng Krachan District (12° 53' 45.7" N 99° 42' 45.4" E) in Phetchaburi, and Ban Mai District in Kanchanaburi (13° 53' 27.3" N 99° 37' 16.2" E and 13° 54' 40.8" N 99° 37' 52.4" E). Mosquitoes were sorted out under the stereomicroscope for unfed, blood-fed, half-gravid and gravid status as well as its group or species levels based on pictorial identification key. Then, DNA extraction

was conducted in a pool of 1-3 mosquitoes, followed by screening for *Plasmodium* spp. by PCR. Mosquito species and Plasmodium-positive samples were sequenced for confirmation.

7. Outline of research achievements

A total of 1,019 anopheline and 133 non-anopheline mosquitoes were collected from goat farms in Thailand, where goats infected with *P. caprae* were found. Molecular biological methods targeting the cytochrome c oxidase subunit 1 (cox1), cytochrome c oxidase subunit 2 (cox2) genes, and the internal transcribed spacer 2 (ITS2) region were used to identify anopheline mosquitoes. To detect *P. caprae*, both pooled and individual mosquitoes were tested using the head-thorax parts containing the salivary glands. Primers specific to three genetic markers, namely cytochrome b, cytochrome c oxidase subunit 1, and 18S small subunit ribosomal RNA genes, were employed. Furthermore, blood samples were collected from goats during the mosquito surveys to determine their malaria infection status. The study unveiled six groups comprising nine mosquito species found on goat farms, namely Hyrcanus, Barbirostris, Subpictus, Funestus, Tessellatus, and Annularis. *Anopheles subpictus* and *Anopheles aconitus* were identified as carriers of *P. caprae* DNA. This marks the first time that *An. subpictus* and *An. aconitus* have been implicated as potential vectors for *P. caprae*.

8. Publication of research achievements

8.1 Nguyen AHL, Nugraheni YR, Nguyen TT, Aung A, Narapakdeesakul D, Kaewlamun W, **Asada M, Kaewthamasorn M. 2023.** Molecular characterization of anopheline mosquitoes from the goat malaria-endemic areas of Thailand. *Med Vet Entomol.* 37(2):381-395. doi: 10.1111/mve.12638.

8.2 Nguyen AHL, Pattaradilokrat S, Kaewlamun W, Kaneko O, **Asada M, Kaewthamasorn M. 2023.** Myzomyia and Pyretophorus series of *Anopheles* mosquitoes acting as probable vectors of the goat malaria parasite *Plasmodium caprae* in Thailand. *Sci Rep.* 13(1):145. doi: 10.1038/s41598-022-26833-4.

Attach reference materials as necessary.

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ORIGINAL ARTICLE

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Molecular characterization of anopheline mosquitoes from the goat malaria-endemic areas of Thailand

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OPEN **Myzomyia and Pyretophorus series of *Anopheles* mosquitoes acting as probable vectors of the goat malaria parasite *Plasmodium caprae* in Thailand**

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