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DETECTION OF PLASMODIUM VIVAX AND PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE ANTIGEN IN ANOPHELINE MOSQUITOES COLLECTED IN SOUTHERN THAILAND

Rampa, Rattanarithikul

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氏名・（本籍）	RAMPA RATTANARITHIKUL（ランパ ラタナリシクル）　（タイ国）
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審査委員	主査　教授　松村武男 教授　堀田　博　教授　栗原　毅 教授　川端眞人

論文内容の要旨

Malaria vectors, the anopheline mosquitoes, are able to transmit malarial parasites of the genus *Plasmodium* among humans in tropical regions. Therefore, vector control programs are undoubtedly important for prevention of this aisease that causes approximately 2 million deaths around the world each year. Based on this background, we studied the population dynamics of malaria-infected mosquitoes in southern Thailand from 1992 to 1993. This is the first report on malaria studies in this area. The results obtained here demonstrate new entomologic findings concerning transmission of these malarial parasites, which will contribute to the public health in malaria endemic areas in Southeast Asia.

The incidence of malaria in Thailand has been greatly reduced during the past40 years but is still significant. In 1992, the annual incidence of malaria-related illness in all of Thailand, was 134.5 per 100,000 population with a mortality rate of 0.45 %, based on passive case detection that significantly underestimates the incidence. Malaria cases are primarily restricted to provinces that are covered with scrub and forest, and close to international borders. Prachaup Khiri Khan Province in peninsular Thailand on the border with Myanmar, which was chosen as one of our study areas, had the highest annual rate of malaria incidence in southern Thailand in 1992 (299 cases in 100,000 population).

In 1992 and 1993, we conducted studies on the abundance, parity rates, nocturnal biting activity, and host preference of anophelines in southern Thailand (Rattanarithikul et al., *J. Am. Mosg. Cont. Assoc.* 12, 52-57, 1996; *ibid* 12, 75-83, 1996). In these studies, we found that *An. maculatus* Theobald, *An. minimus* Theobald, and *An. sawadwongporni*

Rattanarithikul and Green were the most common *Anopheles* (*Cellia*) species in these villages. To evaluate their contribution to malaria transmission in this region, we tested specimens collected in the previous study for the presence of *Plasmodium falciparum* (Welch) and *P. vivax* (Grassi and Feletti) circumsporozoite (CS) antigens by enzyme-linked immunosorbent assay (ELISA). Using human-biting and parity data, we also calculated the vectorial capacity which represents an estimate of the ability of vector mosquitoes to transmit malaria to humans, and the entomologic inoculation rate which represents the rate, of infected mosquitoes biting humans.

Study areas were five villages of Palao-U, Thasalao, Wangpao, Salui and Phato in Prachaup Khiri Khan, Phetchaburi and Chumphon Provinces in southern Thailand. In general, the environments of the five villages are similar and all are located in valleys in the forested hills that extend along the Thai-Myanmar border. Palao-U is highly endemic for malaria, because it is located in close proximity to intact forests and heavy scrub areas that are suitable sites for *An. minimus, An. maculatus, An. sawadwongporni.*

Details for mosquito collection and examination have been described previously (Rattanarithikul et al., 1996). Briefly, mosquitoes were collected from human-baited, bovid-baited and light traps monthly in Palao-U or quarterly in other four villages during 13 months from October 1992 to October 1993. Anophelines were identified and approximately 50% of the non-blood fed females were tested for the presence of CS antigens of *Plasmodium vivax* and *P. falciparum* by sandwich ELISAs using monoclonal antibodies specific for these antigens. The vectorial capacity was calculated as $C = (ha)ap^n/-Inp$. The inoculation rate, h', was the number of CS antigen-positive mosquitoes collected at human-biting per person per day.

Circumsporozoite antigens were found in 28 out of 7,938 mosquito specimens (eight out of 20 *Anopheles* species), which were distributed in all villages. Infection rates were significantly higher ($p<0.01$) in specimens collected from Palao-U (0.7%; 16 of 2,196) and Thasalao (0.8%; 2 of 244)than in those from Phato (0.3%; 6 of 2,192), Wangpao(0.2%; 2 of 995), and Salui (0.1%; 2 of 2,311), consistent with higher malaria incidence rates in Palao-U. The proportions of infected specimens collected with human-baited (12 of 28), bovid-baited (11 of 28) and light traps (5 of 28) were not significantly different: therefore we combined data of all the mosquitoes collected by three methods for subsequent analyses. In the village of Palao-U, seasonal comparison of total infected mosquitoes indicated that most of the infected mosquitoes were caught during the dry season (November-February) and at the end of the rainy season (March-October).

Plasmodium viva CS antigens were detected in seven specimens and *P. falciparum* CS antigens in 21 specimens. The combined rates of *P. falciparum* and *P. vivax* infection

were 1.4% in *An. dirus* Peyton and Harrison, 1.0% in *An. hyrcanus* (Pallus) group, 0.7% in *An. maculatus*, 0.7% in *An. sawadwongporni*, 0.5% in *An. nivipes* Theobald, 0.4% in *An. barbirostris* Van der Wulp group, 0.3% in *An. minimus*, and0.2% in *An. aconitus* Donitz. Two of these species, *An. minimus* and *An. dirus* have been considered the most important vectors in Thailand, whereas *An. maculatus* has been an important vector in Malaysia. On the other hand, five of the species found to contain CS antigens, *An. barbirostris* group, *An. hyrcanus* group, *An. aconitus*, *An. nivipes* and *An. sawadwongporni*, have been believed to play no rolein the transmission of malaria in Thailand so far. However, this study revealed a potenial of these species to transmit malarial parasites.

The vectorial capacity of *An. minimus* was the highest among all species in the rainy season (1.06) and also in the dry season (3.21). Especially, the value obtained in the dry season was more than two-fold higher than that obtained with *An. maculatus*, the species with the second highest vectorial capacity (1.48). In Palao-U, only *An. minimus* contributed to the transmission of *P. vivax*. In addition, *An. minimus* was the predominant vector of *P. falciparum*, particularly during the dry season. The inoculation rate of *An. minimus* was the highest (0.26), and those of *An. maculatus* and *An. sawadwongporni* were the next highest (0.13) in the dry season. In the rainy season, only a low inoculation rate (0.05) was observed in *An. minimus*, *An. maculatus* and *An. dirus*. Thus, malaria transmission in the northern peninsular appeared to be caused primarily by *An. minimus*, and secondarily by *An. maculatus*, *An. sawadwongporni*, and *An. dirus*.

Surprisingly, the role of *An. dirus* in malaria transmission in this village in southern Thailand seems to be only secondary, different from reports in central and southeastern Thailand. Although the rates of infection with two *Plasmodium* species and the daily survival rates were high and comparable with rates reported in these areas the human-biting rate was very low in our area, thereby reducing the vectorial capacity and the inoculation rate.

In conclusion, we found four new *Anopheles* species which may serve as malaria vectors in southern Thailand. *Anopheles minimus* was considered to be the most important vector, and *An. maculatus*, *An. sawadwongporni* and *An. dirus*, of secondary importance vectors in this area. This finding will be important for further ecologic studies on *Anopheles* mosquitoes as well as *Plasmodium* parasites, and for the control of the malaria vectors as well as human malaria in Thailand and neighboring countries.

論 文 審 査 の 結 果 の 要 旨

マラリアの制圧は，熱帯感染症学の最重要研究テーマの一つである。本申請者は，マラリア原虫媒介蚊（アノフェレス属蚊）の生態・疫学上の調査研究をタイ国南部半島地域にて行い，本地方におけ

るマラリア伝播の動態について有意義な成果を得た。

タイ国は常夏の熱帯地に位置するが，国の東・西・南・北の各地域の気候・生物相が異なる。とくに南部半島地域は生物相が複雑で，マラリア媒介蚊の種類とそれらの生態については研究がほとんどなされていない。そこで先ずこの地方のマラリアの疫学と媒介蚊の調査研究は，蚊の分類学的素養の上に注意深く実施されねばならなかった。その意味でRampa Rattanarithikul氏は東南アジアにおける蚊分類学第１人者であり，近年タイ国産出の蚊について“Illustrated keys to the medically important mosquitoes of Thailand(1994)”を刊行し，またハマダラカ属数種の新種記載をしている研究者であることから，本地域のマラリア媒介蚊調査研究の最適任者であった。

本論文の次の成果は，南部半島地域にて1992年から1993年にかけて採集された蚊を供して得られたものである。

(1)ELISA法によりスポロゾイト保有蚊８種を特定し，ヒト嗜好性の成績から内４種がヒトへのマラリア伝播に関与することを見出した。

(2)媒介能vectorial capacity解析および接種率human inoculation rate解析によって，*Anopheles minimus*が最も高い媒介能力を，ついで*An. maculatus, An. sawadwongporni*が媒介能を持つことが確認された。

(3)*An. dirus*はタイの他の地域では最も重要な媒介蚊となっているにもかかわらず，南部半島地域では媒介能力が低く，二次的な媒介蚊と判定された。

(4)ライトトラップおよび牛おとりの蚊採集法は，人おとりの採集法とほぼ同等の採集効率であることなどが見出された。

本申請者はこれまで，マラリア媒介蚊の明らかでないタイ国南部半島地域において，従来の基本的研究方法に加えて，近年開発された新しい方法を駆使して，媒介蚊の種を決定するという結論を導きだしたもので，本論文は学問的にも価値ある業績であると認められる。さらに本研究で得られた成果は，蚊の季節的消長および宿主嗜好など昆虫学的側面を調べ上げた副論文とともに，この地域のマラリア媒介蚊対策に重要な貢献をなし，マラリア疫学上の必要不可欠の知見を与えるものである。

よって本研究は学位論文としてすぐれたものであり，本申請者は博士（医学）の学位を得る資格があると認める。