BIONOMIC STATUS OF *ANOPHELES EPIROTICUS* LINTON & HARBACH, A COASTAL MALARIA VECTOR, IN RAYONG PROVINCE, THAILAND

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Abstract. A longitudinal entomological survey was conducted to provide in-depth information on *An. epiroticus* and determine whether ecological and entomological factors could influence malaria transmission in Rayong Province, Thailand. The mosquitoes were collected monthly from May 2007 to April 2008 by human landing catch technique from 6:00-12:00 PM for 2 consecutive nights, at 3 collection sites. A total of 3,048 mosquitoes within 5 species were captured: *An. epiroticus*, *Culex quinquefasciatus* Say, *Cx. sitiens* Wiedemann, *Aedes aegypti* (L.) and *Ae. albopictus* Skuse. PCR was used for molecular identification of *An. sundaicus* complex, by determination of COI, ITS2, and D3 genes. The target mosquitoes were *An. epiroticus*, which was the predominant species, accounting for 43.8 % of specimens collected. The biting cycle pattern increased during 6:00-8:00 PM and reached a maximum of 6.6 bites/person/hour by 12:00 PM. The mosquitoes varied in population density throughout the year. The highest biting rate was 37.6 bites/person/half night in September and the lowest (10.2 bites/person/half night) in January. Nested PCR and real-time PCR techniques were used to detect the malaria parasite in *An. epiroticus* adult females. Nine of 926 (0.97%) mosquitoes tested were malaria parasite positive: 6 *P. falciparum* and 3 *P. vivax*. The infective mosquitoes were found in the dry and early rainy seasons. The overall annual entomological inoculation rate (EIR) in the village was 76.6. The overall parity rate was 74%. A total of 38 cement tanks were used to characterize the nature of the breeding places of *An. epiroticus*. *An. epiroticus* larvae coexisted with *Aedes* and *Culex* larvae; the maximum larval density was more than 140 larvae per dip in May. Breeding places included fresh, brackish and salt water, typically with full sunlight and mats of green algae on the water surface. The salinity of the water ranged from 0.5 to 119.4 g/l, with a narrow pH range of 8.2-8.7. Dissolved oxygen was highest in November (6.27 mg/l) and lowest in March (3.46 mg/l). The water temperature varied between 24.6 and 32.8ºC.

Key words: *Anopheles epiroticus*, bionomic status, malaria vector, Thailand

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INTRODUCTION

Malaria is transmitted by female anopheline mosquitoes which generally breed in clean, freshwater habitats. In

some coastal areas, malaria is transmitted by brackish-water breeding mosquitoes. In Asia, the most important species are *An. sundaicus s.l.* and *An. subpictus* (WHO, 2005). Malaria is a potential fatal disease found in some hilly-forested areas and in some costal areas of Thailand. In Thailand, the coastal malaria is found annually and reported by the Ministry of Public Health (MOPH, 2008). Unlike along Thailand international borders where malaria vectors have been well-studied, very little is known about the malaria vectors in coastal areas. The bionomics of *An. sundaicus s.l.* in Southeast Asia are poorly known due to little available data. *An. sundaicus s.l.* is considered an efficient malaria vector but its biologic characteristics and vectorial capacity are not clearly understood (Dusfour *et al*, 2004a). *An. sundaicus s.l.* has been incriminated as a secondary vector of malaria in Thailand (Gould *et al*, 1966; Harinasuta *et al*, 1974). Recently, *An. sundaicus* species A of Southeast Asia was formally renamed *An. epiroticus* (Linton *et al*, 2005).

Insufficient information regarding its status, bionomics, species complex and its ability as a malaria vector make vector control in coastal zone of Thailand difficult. The knowledge of *An. epiroticus* obtained from this study is important for understanding its potential to transmit malaria and should be useful for controlling malaria vectors in coastal areas of Thailand.

MATERIALS AND METHODS

Study area

This study was carried out in Pak Nam Village, Mueang Rayong District, Rayong Province, Thailand. Rayong Province is located 179 km east of Bangkok. It has a total population of 4,368. Pak Nam Village is located in southern Mueang Rayong District, Rayong Province, near the coast. The area has fishery and fish sauce production. There are many discarded fish sauce cement tanks. Malaria was first reported in the area in 2002. A malaria outbreak was reported in 2005 of 14 cases, and cases have been reported yearly since then. A total of 85 malaria cases have been reported in Pak Nam Village during 2002 to 2008 with 61 and 24 of Thai and non-Thai cases, respectively. Three cases (4%) were *Plasmodium falciparum* and 82 (96%) were *P. vivax*. All the falciparum malaria cases were non-Thais. Thai patients presented only with *P. vivax* infection.

Adult and larval mosquito collections

A longitudinal study was conducted during May 2007 - April 2008 in Pak Nam Village (Mueang Rayong District, Rayong Province). Mosquitoes were collected monthly for 2 consecutive nights from 6:00 to 12:00 PM by human landing catch method (HLC) outside 3 houses. Mosquito larvae were sampled from 38 cement tanks (2.5 x 2.5 x 2.5 m) by the dipping method to study breeding places close to adult collection sites and nearby patient houses. According to a longitudinal entomological survey, cement tanks containing brackish water in the study area are artificial breeding places for *An. epiroticus*. There are no natural breeding places for *An. epiroticus* in the area. Water quality was estimated using the HACH sension 156 portable multi-parameter instrument (Hach Company, Loveland, CO).

Laboratory processing of mosquitoes

Adult mosquitoes were identified by microscopic examination of morphological characters using established taxonomic keys for Thai anophelines (Rattanarithikul

et al, 2006). PCR amplification of 3 genes: cytochrome oxidase I (COI), internal transcribed spacer 2 (ITS2), and domain-3 (D3) of 28S rRNA were performed for molecular species identification (Dusfour *et al*, 2004b; Alam *et al*, 2006). *An. epiroticus* female ovaries were dissected to determine parity (Detinova, 1962). Malaria infection rates in the *An. epiroticus* female adults were detected by dissecting each individual into two parts: head-thorax and abdomen. *An. epiroticus* DNA was extracted using the QIAamp® DNA Mini Kit (QIAGEN, Germany) following the manufacturer's instructions with slight modification. The DNA was subjected to nested polymerase chain reaction (Nested PCR) (Snounou and Singh, 2002) to detect plasmodium positive samples, then these were examined using real time PCR (Swan *et al*, 2005).

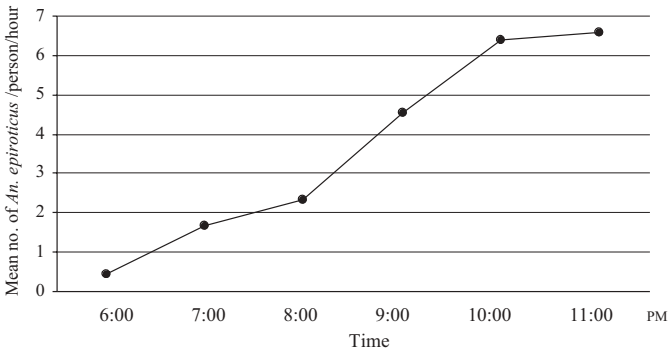

Fig 1–Biting of *An. epiroticus* from 6:00-12:00 PM during May 2007-April 2008.

Data analysis

The human biting rate (HBR) was estimated as the number of mosquitoes captured per person per half night. The parity rate of the famale *An. epiroticus* mosquitoes was calculated using percentages. The sporozoite rate was the proportion of *An. epiroticus* mosquitoes found with malaria parasites divided by the number of *An. epiroticus* mosquitoes tested. The risk for mosquito-borne infection was estimated using the entomological inoculation rate (EIR): the number of bites by an infectious mosquito per person per day (Macdonald, 1957).

RESULTS

Mosquito abundance

A total of 3,048 mosquitoes within 5 species belonging to 3 genera were collected using the human landing catch technique. Morphological and molecular identification revealed *An. epiroticus* or *An. sundaicus* speies A was the only *Anopheles* mosquito found in study area. *An. epiroticus* was the most abundant species (43.8%). Other species collected were *Culex quinquefasciatus* (42.6%), *Cx. sitiens* (8.9%), *Aedes aegypti* (4.63%), and *Ae. albopictus* (0.07%).

Biting cycle of *An. epiroticus*

Determination of biting cycle for *An. epiroticus* was carried out during May 2007-April 2008 from 6:00-8:00 PM. Biting activities are shown in Fig 1. *An. epiroticus* biting gradually increased during the early evening (6:00-8:00 PM) then rapidly increased during the last evening (9:00-12:00 PM). *An. epiroticus* biting was unimodal throughout the year. The peak *An. epiroticus* activity was at midnight (9:00-12:00 PM) with 6.6 mosquitoes per person per hour.

Seasonal abundance of *An. epiroticus*

An. epiroticus was caught throughout the year (Fig 2). The mean number of *An. epiroticus* collected fluctuated from 10.2-37.6 mosquitoes per person per half night. The highest human biting rate was observed in September, a month with high

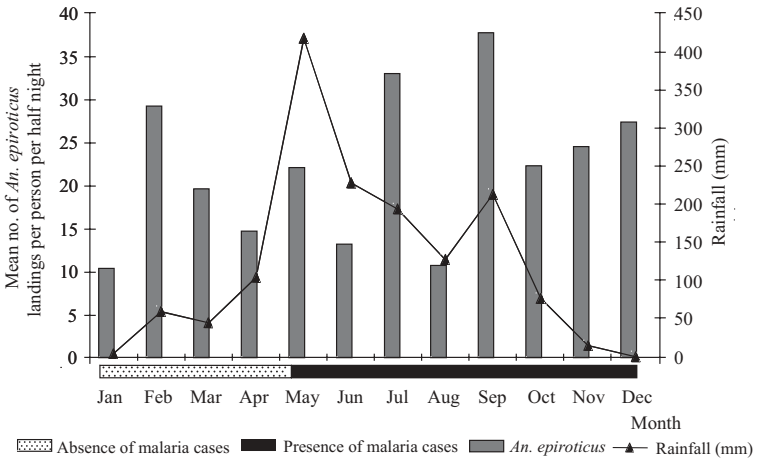

Fig 2–Seasonality of *An. epiroticus* over 12 months compared with malaria cases and rainfall.

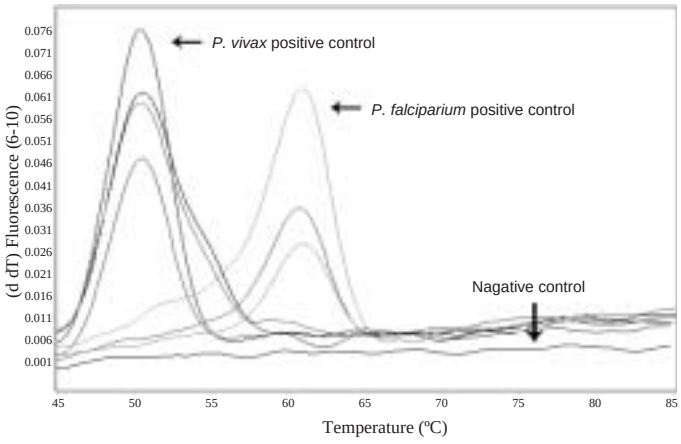

Fig 3–Result of real-time PCR showing melting curve of species-specific plasmodia in *An. epiroticus.* The annealing temperature of *P. vivax* and *P. falciparum* were 51.8 - 55.5 and 60.0 ± 2.0ºC, respectively.

rainfall (212.2 mm), and the lowest rate was found in January. The highest and lowest *An. epiroticus* human biting rates were during the rainy season and dry-cool season, respectively. There appeared to be no effect of rainfall on mosquito density.

Infection rate and entomological inoculation rates (EIR) of *An. epiroticus*

A total of 926 *An. epiroticus* specimens were used to determine plasmodium infection by nested PCR. Nine (0.97%) head-thorax portions were plasmodium positive. Using real-time PCR determination of malaria type in the 9 positive samples, 6 were *P. falciparum* (66.7%), and 3 were *P. vivax* (33.3%) (Fig 3). None of the abdomen portions was positive for plasmodium. The malaria positive specimens were detected in January, February, May, and July with 3, 2, 1, and 3 samples, respectively. Positive samples were found during dry cool season (January and February) and rainy season (May and July). The greatest number of positive samples were in January and July with three positive samples each. In February and May, there were 2 and 1 positive samples, respectively.

The overall annual EIR of *An. epiroticus* in Pak-Nam Village for the study period was estimated at 76.65 positive bites per person per year, or 0.21 positive bites per person per day.

Parity rate of *An. epiroticus*

The parity rate of 1,215 female *An. epiroticus* mosquitoes was determined. Seventy-four percent were parous, the percentages ranged from 61% to 90% throughout the year. The lowest parity rate was found in November at 61%. The parity rate

reached its highest value in January at 90%, showing the proportion of older *An. epiroticus* mosquitoes was greater then.

Larval surveys

Thirty-eight cement tanks were examined to characterize mosquito breeding habitats. The maximum larval density was more than 140 larvae per dip in May with *Aedes* and *Culex* species being the most common found. Breeding places included fresh, brackish, and salt water, with full sunlight and mats of green algae on the water surface. Open sunlit cement ponds and mats of green algae on the water surface provide excellent breeding sites for *An. epiroticus* (Dusfour *et al*, 2004a). There was a broad range of salinity, from 0.5 to 119.4 g/l, with a pH range of 8.2-8.7. Dissolved oxygen was highest in November (6.27 mg/l) and lowest in March (3.46 mg/l). Water temperature varied between 24.6-32.8ºC. Almost all study sites along the coastline were positive for the presence of *An. epiroticus* mosquito larvae.

DISCUSSION

Malaria cases in Pak Nam Village occurred annually during 2002-2008. The most prevalent parasite was *P. vivax* (96%) and a few cases of *P. falciparum* (4%). Both *P. falciparum* and *P. vivax* cases were found in non- Thai patients, while Thai patients only had *P. vivax*.

This study proved *An. sundaicus s.l.* from field collection was *An. epiroticus* (*An. sundaicus* species A) by morphological and molecular identification. *An. epiroticus* was the predominant species in the study area.

Its biting activity occurred throughout the night with the highest peak at 11:00-12:00 PM (6.6 bites/person/hour) when people were presumed to be in bed. *An. epiroticus* was present throughout the year. There appeared to be no correlation between rainfall, relative humidity (RH), and the mean number of *An. epiroticus* in this study. A higher density has been reported in Vietnam with 190 bites/man/night (Trung *et al*, 2004).

This is the first evidence of natural malaria sporozoite infection due to *An. epiroticus* in Thailand. *An. epiroticus* appears to be the sole local malaria vector in the study site. The malaria sporozoite rate of 0.97% in *An. epiroticus* in the present study was greater than that reported in Indonesia (0.07%) by salivary gland dissection (Collins *et al*, 1979) or in Malaysia (0.04%) by salivary gland dissection (Reid, 1968). This may be due to the low sensitivity of dissection. Infective mosquitoes were found mostly in January at the time of the highest parity rate. The present study found the EIR for *An. epiroticus* was 76.65 infective bites/person/year, or one infective bite every five days.

The overall mean parity rate of 74% for *An. epiroticus* in the study area indicates the population has longevity and an ability to be an efficient malaria vector. This parous rate is higher than the 47% found by Trung *et al* (2004). It is similar to the rate for *An. sundaicus* D from India which was 73% (Kumari and Sharma, 1994).

An. epiroticus larvae in our study bred in fresh, brackish and salt water habitats with salinity ranging from 0.5 to 119.4 g/l. This is similar to the findings of Linton *et al* (2001) and Nanda *et al* (2004) who found that *An. sundaicus s.s.* and *An. sundaicus* D from Malaysia and India can breed in brackish and fresh water habitats. *An. epiroticus* larvae coexisted with filamentous algae at a pH range of 8.2-8.7, similar to studies from India, Vietnam, and Indonesia which found a pH range of 7.0-8.5 (Dusfour *et al*, 2004a).

This study clarified the vector status of *An. epiroticus*, its high density, biting rate, seasonal population dynamics, malaria infection and parity, which incriminate it as an important malaria vector in the study area. Moreover, this study proves that coastal malaria occurs in Thailand.

ACKNOWLEDGEMENTS

The authors would like to thank all the personnel at the Vector Borne Control Center 3.3, Rayong Province for their help in the field collecting of mosquitoes. Special thanks to Assistant Professor Mallika Imwong, Department of Clinical Tropical Medicine and Pongruj Rattaprasert, Department of Protozoology, Faculty of Tropical Medicine, Mahidol University for offering valuable suggestions. This research work was financially supported by the Faculty of Graduate Studies, Mahidol University, academic year 2007.

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