ANOPHELES BARBIROSTRIS/CAMPESTRIS AS A PROBABLE VECTOR OF MALARIA IN ARANYAPRATHET, SA KAEO PROVINCE

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Abstract. As a result of dramatic increase in malaria cases in Sa Kaeo Province from 666 cases in 1995 to 4,381 in 1997, a brief entomological study was carried out during January 1998 to December,1999 in Pa Rai subdistrict where most malaria cases were reported. Of fourteen species of mosquitos found, only *Anopheles barbirostris* group was the most abundant species throughout the year. Adult identification was not able to confirm species within *An. barbirostris* group, particularly between *An. barbirostris* and *An. campestris* because of morphological resemblance. Therefore, the *barbirostris* group captured in this study is reported to be either *An. barbirostris* or *An. campestris*. The seasonal prevalence of *barbirostris/campestris* was bimodal in distribution (September and November) and coincided well with malaria occurrence in this area. Human landing collections revealed high adult densities with 20 bites/person-night for mean indoor density and 53.5 bites/person-night for mean outdoor density. The biting peak was during 21.00-24.00 hours. Among 223 *barbirostris/campestris* dissected for oocysts and sporozoites only one gut from the outdoor collection in November was infected with oocysts. There were no sporozoites detected in salivary glands of all mosquitos collected. This appears to indicate that in the absence of major vectors local species may serve as potential transmitters of malaria in Thailand.

INTRODUCTION

Malaria has been recognized as a major disease problem in Thailand since 1918 with an estimate of 40,000 to 50,000 deaths annually. Malaria was the leading cause of death (271.7-194.9 per 100,000) until 1950 (Division of Malaria Centre, 1973). The establishment of the National Malaria Control Program with a variety of activities including expansion of primary health care delivery and malaria clinics significantly reduced the mortality rate from 351 per 100,000 population in 1947 to 1.26 per 100,000 population in 1998 (Vijaykadga, 1999). At present malaria transmission is largely confined to forested rural areas principally along the borders with Myanmar and Cambodia, an epidemiology characterized by multidrug resistant *Plasmodium falciparum*, exophagic and exophilic behavior of the major anopheline vectors (*Anopheles dirus* and *An. minimus*) and intensive population movement. However, there has been a resurgence of malaria in some areas of the country where malaria incidence was previously at a low level, attributable to population migration due to economic, demographic and ecological factors (Vijaykadga, 1999). In 1998 malaria transmission occurred in several provinces of southern Thailand; Chumphon, Ranong, Phuket but particularly in Surat Thani where malaria cases were highest (17,302 cases) - an increase of nearly 300% from the previous year. Among these, 91.2% were associated with agricultural activity. Thailand's economic crisis resulted in a movement of people from urban centers to rural areas to exploit forests for agriculture

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purposes areas where malaria vectors were abundant. In Surat Thani, over 1,600 acres were exploited. Indigenous as well as imported cases were reported within the country.

Malaria transmission in Sa Kaeo Province, an eastern boundary province with Cambodia was also atypical. Malaria cases increased from 666 cases in 1995 to 1,066 cases in 1996 and 4,381 in 1997; 88.5% of cases were due *P. vivax*. Infection was thought to be introduced by migrants. The major vectors, *An. dirus, An. minimus* and *An. maculatus* s.l. were found occasionally. This study presents the entomological data obtained from Pa Rai, a subdistrict of Aranyaprathet, Sa Kaeo Province. The objectives of the study was to demonstrate potential of the *An. barbirostris* group in malaria transmission.

MATERIALS AND METHODS

Study area

Sa Kaeo is a newly established province upgraded from Sa Kaeo district of Prachin Buri Province in 1993. The province is located 236 km from Bangkok. It is administratively divided into 6 districts: Muang, Watthana Nakhon, Aranyaprathet, Ta Phraya, Wang Nam Yen, and Khlong Hat. According to the annual report of Malaria Division, Sa Kaeo was regarded as a low-risk area until 1996. Henceforth malaria transmission in Sa Kaeo was among in the highest ten in the country. The highest transmission (1,498 cases in 1998) of the country occurred in Aranyaprathet on the Thai-Cambodian border, 70 km away from Sa Kaeo town. This border town is a marketplace where products from Cambodia are on sale at Ban Khlong Luk, a village 6 km away. Aranyaprathet has a total population of 81,835 with a population density of 75.60 persons/km^2, the highest in the province. Three mosquito collection stations were located in Pa Rai, a subdistrict with the highest number of malaria cases in Aranyaprathet (281 cases in 1998). Pa Rai covers an area of 31 km^2 with a population of 3,899 in 1999 distributed among 8 villages and 847 households. In terms of land use, 81.68% of the total area was devoted cultivation of rice (49.5%), maize (33.39%), cassava (31.2%), sugarcane (9.6%) and other cash crops.

Field investigations

A longitudinal study was conducted from January 1998 to December, 1999 in Pa Rai subdistrict (Aranyaprathet district, Sa Kaeo Province). Mosquito collections to assess biting activity were made monthly by indoor and outdoor landing captures in three collection stations from 18.00-05.00 hours for two nights. Although cattle were scarcely found in the study area, however, a single cow-baited collection was made incidentally in October of 1999 when the mosquitos were most abundant. The collected mosquitos were identified and examined for parity based on tracheation of the ovaries of mosquitos (Detinova, 1962). Midguts and salivary glands were examined by dissection for the presence of oocysts and sporozoites, respectively. Larval surveys were carried out monthly by dipping from all available water collections to determine presence of anopheline mosquitos. Immatures stages were brought to the insectary and raised to obtain pupal skins for identification of members of the *barbirostris* group. Blood samples were examined monthly for malarial parasites by optical microsopy. Climatological data were provided by the Meteorological Department, Ministry of Communications, and were reported as mean monthly temperatures and total rainfall.

RESULTS AND DISCUSSION

Malaria situation in Aranyaprathet

Malaria situation in Aranyaprathet was less than 600 cases annually until 1996. Between 1997 and 1999, there was a dramatic increase of malaria with 1,496-1,956 cases reported of which more than 95% were due to *P. vivax* and the rest, to *P. falciparum* (Fig 1). Fig 2 demonstrates malaria transmission was apparently unimodal starting in July, reaching a peak in December and declining after December to June.

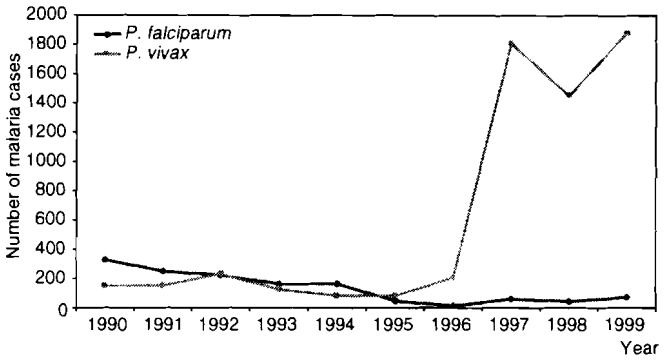

Fig 1–Malaria cases in Aranyaprathet during 1990-1999.

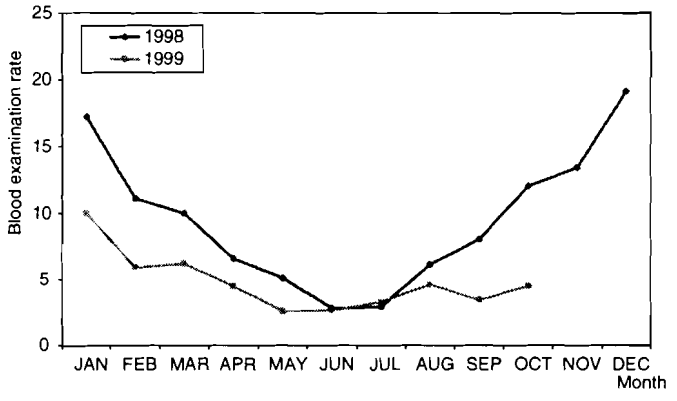

Fig 2–Seasonal variation of slide positive rates in Aranyaprathet.

Mosquito abundance

Fourteen species of mosquitos were collected by means of human landing catches and a cow-baited trap. Identification of female adults of members of the *barbirostris* group was inaccurate, particularly between *An. barbirostris* and *An. campestris* because of their similar and overlapping characters. Precise identification of this species group is based on the examination of the pupal skins (Harrison and Scanlon, 1975). In this study the designation *An. barbirostris/campestris* is meant to represent either the species *barbirostris* or *campestris*. The species composition of the mosquitos found feeding on human and cattles was consisted of *Aedes mediolineatus, Ae. vexans, Anopheles aconitus, An. barbirostris/campestris, An. nigerrimus, An. nivipes, An. peditaeniatus, An. tessellatus, Culex bitaeniorhynchus, Cx. gelidus, Cx. quinquefasciatus, Cx. tritaeniorhynchus,* and *Mansonia uniformis*.

A total of 1,990 anopheline mosquitos belonging to 6 species were captured by human landing catches. The most abundant species (66.2%) was *An. barbirostris/campestris*. *An. aconitus*, another suspected vector was also caught in relatively smaller numbers (9.7% of total collections). Other *Anopheles* species collected were *An. hyrcanus* group (*An. peditaeniatus* and *An. nigerrimus*) (13.6%), *An. nivipes* (5.1%), *An. tessellatus* (4.4%), and *An. vagus* (1.0%). The major vectors, *An. dirus, An. minimus* and *An. maculatus* s.l. were not found during the study period. Based on the annual reports of Malaria Division, these main vectors have occurred in low numbers and have even been absent from the study sites since 1980.

In contrast to the cow-baited collection, *An. barbirostris/campestris* occurred in small numbers. A total of 1,108 collected mosquitos was comprised of 65.3% *An. peditaeniatus*, 21.5% *Cx. tritaeniorhynchus*, 7.1% *An. barbirostris/campestris* and 1.5% *An. aconitus*. The data revealed that adult *barbirostris/campestris* at least from this area were anthropophilic.

Infection rate

All anopheline mosquitos were dissected for oocysts and sporozoites. Only *An. barbirostris/campestris* was found infected by malarial parasites. Out of 223 dissected guts of *An. barbirostris/campestris* from the outdoor collection in November, only one gut was found infected with 67 oocysts (0.45% infection rate). There were no sporozoites detected in salivary glands of the mosquitos collected.

To date member species of *An. barbirostris* group has not been incriminated as a vector of malaria in Thailand, however, *barbirostris* was reported several times to be infected by malarial oocysts and sporozoites. Gingrich *et al* (1986) determined potential *Anopheles* spp vectors throughout Thailand by ELISA sporozoite analyses and ranked *An. barbirostris* as

the second highest positive rates for *P. falciparum* and *P.vivax* following *An. dirus*. Griffith (1955) reported malarial oocysts from a single *barbirostris* from Chiang Mai Province. In addition the presence of *barbirostris* has been widely acknowledged by our field workers because its actual distribution is common throughout the country in contrast to those of the other members of *barbirostris* group. In view of biological aspect, however, there is a little potential for *barbirostris* being a malaria vector because the adult *barbirostris* are generally zoophilic in Thailand (Harrison and Scanlon, 1975). The identity of *An. barbirostris* in these studies is inconclusive and needs confirmation. The possibility of *campestris* being a vector of malaria is more plausible than that of *barbirostris* as regarded by Reid (1968) that *An. campestris* is highly anthropophilic and ranked as the third species of *Anopheles* in Southeast Asia most attracted to man. Owing to the unknown distribution and feeding habits of Thai *campestris* coupled with the problem of adult identification, its vector status for malaria in Thailand is problematical at present.

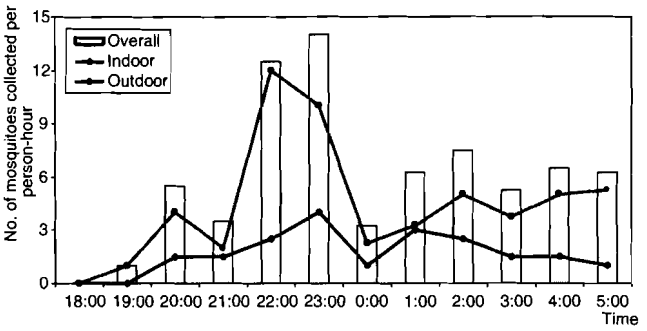

Fig 3–Biting activity of *An. barbirostris/campestris* collected indoors and outdoors during October-November, 1999.

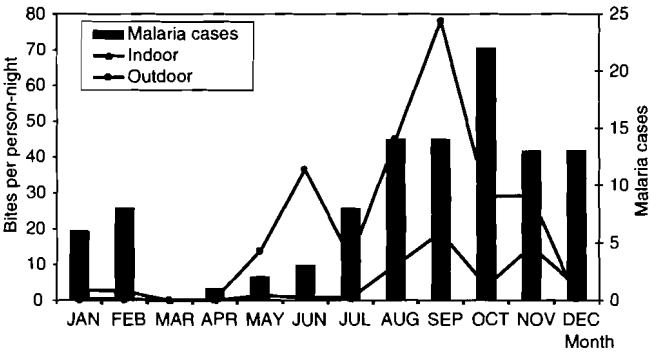

Fig 4–Indigenous cases and *An. barbirostris/campestris* density in Pa Rai Subsistrict, Aranyaprathet (1998).

Parity rate

Among 941 *An. barbirostris/campestris* dissected for parity based on the coiling of ovarian tracheoles, the parous rate was moderately high *ie*, 60.7%.

Bionomics of *An. barbirostris/campestris*

Biting cycle: Determination of biting cycle of *An. barbirostris/campestris* was carried out during October to November when population densities were highest. Biting activities were between 19.00-05.00 hours as shown in Fig 3. The biting patterns similarly fluctuated throughout the night from 21.00-05.00 hours with the highest peaks between 22.00-23.00 hours and smaller peaks after 01.00 hour. The biting activity was similar to that described by Gould *et al* (1965) which found biting peak of *An. campestris* from human-baited collections during 19.00-24.00 hours in Pathum Thani Province and by Moorhouse and Wharton (1965) which was between 20.00-02.00 hours in Malaysia. More mosquitos were collected from outdoor catches. The highest density of 12 bites/person-hour occurred at 22.00 hours and 4 bites/person-hour at 23.00 hours for outdoor and indoor catches, respectively. In comparison to the major malaria vectors, *An. dirus, An. maculatus* s.l. and *An. minimus,* reported in various areas of Thailand, *An. barbirostris/campestris* were active in higher densities (20 bites/person-night for mean indoor density and 53.5 bites/person-night for mean outdoor density).

Seasonal prevalence

The seasonal abundance of *An. barbirostris/campestris* was determined by night observations with human landing catches. The sea-

sonal trend showed a bimodal distribution with peak biting rates of 19 and 15 bites/person-night in September and November, respectively for indoor populations and 36.5 and 78 bites/person-night in June and September, respectively for outdoor populations (Fig 4). The mosquito abundance synchronised with malaria occurrence in this area. The outdoor density of *An. barbirostris/campestris* from May to June was possibly responsible for an initial rising in malaria cases from June to July followed by a big peak of malaria cases from July to February which was synchronous with indoor and outdoor peaks of *An. barbirostris/campestris* abundance from July to December.

Malaria transmission in the study area appears to be seasonal possibly associated with sugarcane cultivation which covered an area of 18,190 rai (29.1 km^2). The sugarcane season was during May-February. Cultivation started during May to July and was harvested during January to February. It was estimated that 1 person/rai/day was needed for planting and 5 persons/rai/day for cutting. This labor force was mostly immigrants from Cambodia. According to an immigration officer in Aranyaprathet, some 3,000 Cambodians cross daily via Wattana Nakhon district. Reports showed that 6.7% of 2,669 immigrants examined for malaria parasites were found infected; of these165 (6.2%) blood smears were positive for *P. vivax*. It was observed that many immigrants working for sugarcane cultivation slept overnight in the temporary huts in sugarcane fields with no means of protection from mosquito bites. This constituted a suitable source of malaria infection in the study area.

Larval surveys

Six species of *Anopheles* including *aconitus, campestris, hyrcanus* group, *nivipes* and *vagus* were collected commonly on the periphery of villages in semi-permanent pools, ponds and rice fields and in pits and marshes within villages. Most contained water from 5-30 cm deep, algae, low grasses and other aquatic plants common around the periphery with partial shade. All immatures of *barbirostris* group collected in these breeding habitats were identified as *An. campestris* by associated pupal skins. *An. campestris* and *An. hyrcanus* group were consistently detected in the same sites throughout the year.

SUMMARY

An. barbirostris/campestris is a probable malaria vector based upon its density, biting habits, seasonal population dynamics, the finding of an infected mosquito and the absence of other main vectors of malaria. Unpublished works detected *An. barbirostris/campestris* positive with sporozoites by either ELISA or dissecting technique, however, inaccurate identification of the adults made it impossible to incriminate any of these species as an additional malaria vector of Thailand. Although only one *barbirostris/campestris* was found infected with oocysts in this investigation, the findings seem to indicate that malaria in Pa Rai may be transmitted by its local mosquito species (*An. barbirostris/campestris*) in the absence of the main vectors. Further investigation on the species confirmation and bionomics of *An. barbirostris/campestris* are needed to clarify its vector status.

ACKNOWLEDGEMENTS

We wish to thank Professor David Molyneux, Liverpool School of Tropical Medicine for critical reviewing the manuscript. This study is a collaboration of the Division of Malaria Control, Department of Communicable Disease Control, Ministry of Public Health, Thailand and the Faculty of Tropical Medicine, Mahidol University.

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