VOLUME 9 NO 2 PP 230–237 FEBRUARY 2004

Malaria transmission and major malaria vectors in different geographical areas of Southeast Asia

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Summary During the last decade, major progress in malaria control has been achieved in Vietnam, Laos and Cambodia. However, malaria is still a potentially fatal disease in some hilly-forested areas and continues to be endemic in a few coastal foci. To estimate the risk that stems from the major vectors after a decade of intensive malaria control, an entomological study based on human landing collections was conducted between April 1998 and November 2000 in six study villages (four in Vietnam, one in Cambodia and one in Laos) located in different physio-geographical areas. Five villages were selected in places where new cases of malaria still occurred. In the sixth village, in the northern hilly area of Vietnam, no case of malaria was detected during the past 3 years. In three study villages of the hilly forested areas of Cambodia and central Vietnam, *Anopheles dirus* A still played an important role in malaria transmission and maintain perennial transmission inside the villages despite its low density. *Anopheles minimus* A was found in all study villages except in the southern coastal village of Vietnam. Its role in malaria transmission, however, varied between localities and surveys. In one study village of central Vietnam it was almost absent (one specimen collected over 480 man nights), and in another village sporozoite positive specimens (2.8%) were only observed during the first two surveys whereas this species disappeared from the collections from November 1998 onwards (six surveys: 360 man nights). In the northern study site *An. minimus* A and C were found in all collections, but no local malaria transmission occurred. However, the constant presence of these two species associated with a high longevity (parous rate up around 80% and 65%, respectively), suggests that transmission can occur at almost any time if parasite reservoirs are reintroduced in the area. The proper management of malaria cases and population movement is, therefore, important to prevent outbreaks and the reintroduction of malaria in northern Vietnam. In the study site of the Mekong delta, *An. sundaicus* occurred at high densities (up to 190 bites/man/night). The recent changes in land use from rice cultivation to shrimp farming probably explains the increase of this brackish water breeding species during the study period. However, none of the 11 002 specimens was positive for *Plasmodium* circumsporozoite protein (CSP). The relative low survival rate as estimated by the parous rate (around 47 %) may reflect its low vectorial status that could explain the very low malaria incidence (1.9 case/100 persons/year) in this study site. A calculated sporozoite rate of maximum 1/300 000 is enough to explain this low malaria incidence. Despite the successes in malaria control, the vector *An. dirus* A continues to play an important role in malaria transmission, whereas *An. minimus* A showed temporal and spatial variation in its role as vector. The role of *An. sundaicus* as vector could not be confirmed because of the low incidence in the coastal study village. Other *Anopheles* species may be locally involved, but in the five study villages where malaria is still present they probably do not contribute significantly to malaria transmission. The study also points towards the fact that in Southeast Asia it will become increasingly difficult to incriminate *Anopheles* species in malaria transmission while the risk for malaria transmission still persist.

keywords *Anopheles dirus*, *Anopheles minimus*, *Anopheles sundaicus*, malaria, transmission, Southeast Asia

Introduction

The economic and social implications of malaria have prompted the governments of Vietnam, Laos and Cambodia to consider malaria control as one of the priority programmes in public health. Nowadays, the malaria burden has decreased drastically in the region, especially in Vietnam, owing to the intensive control activities conducted during the last decade in which vector control has played a central role. However, if malaria has disappeared in many areas of northern Vietnam, it continues to be an important health problem in hilly-forested areas where *Anopheles dirus sensu lato* and *An. minimus s.l.* are the main vectors (Hung *et al.* 2002). In the southern coastal areas, the incidence of malaria decreased to a low level and transmission in the remaining foci is attributed to the brackish water breeder *An. sundaicus* (Phan 1998). Even if the programmes have been successful so far, the remaining malaria foci may compromise the achievements in the long-term because of intense human migrations from endemic areas to areas where malaria is under control but where vectors are still present (Verlé *et al.* 1998).

In Vietnam, criteria to define the population at risk have been based on previous occurrence of malaria and epidemics and the presence of the malaria vectors. *Anopheles dirus*, *An. minimus* and *An. sundaicus* are still considered as the main vectors but other *Anopheles* species have been incriminated in malaria transmission in different parts of Southeast Asia (Harrison 1980; Kobayashi *et al.* 2000; Vythilingam *et al.* 2003). However, little updated information is available on the role of local anopheline species in malaria transmission (Rattanarithikul *et al.* 1996a) while recent environmental changes, including modified land use, may considerably affect vector distribution and dynamics of malaria transmission (Meek 1995). Hence, after a decade of malaria control, an updated knowledge on vector capacity, in the first place of the major vectors, is needed. Consequently, we conducted an entomological study in Vietnam, Laos and Cambodia in order to estimate the risk that stems from the major malaria vectors occurring in different ecological areas. This study answers the needs expressed by the National Malaria Control Programmes that are confronted with the complex vector situation in a changing environment where evaluation and possible reassessment of control practices is essential. The findings of this study will help to establish effective and appropriate vector control measures fitting with new challenges and contributing to sustain the success of malaria control programmes in Vietnam, Laos and Cambodia.

Materials and methods

Study sites

Mosquitoes were collected in four villages in Vietnam and in one village in both Laos and Cambodia. Van Duc A village (9°11′N, 105°19′E; southern Vietnam) is located in a coastal area of the Mekong delta. Canals, rice fields and brackish water surrounding this village are used for shrimp farming. The vegetation consists mainly of nipa trees growing along the rivers. Houses are built directly on the ground and are mainly made of leaves of nipa trees. The remaining five villages are situated in hilly forests. Houses in Khoi (20°38′N, 105°10′E; northern Vietnam), Na Ang (18°33′N, 101°59′E; Laos) and Cha Ong Chan (13°43′N, 106°59′E; Cambodia) are built on stilts about 1–2 m above ground. In village 3 (10°50′N, 107°40′E) and Lang Nhot (12°14′N, 108°56′E), both located in central Vietnam, the majority of houses are built directly on the ground. Houses in these five villages are commonly made of thatch or tile for the roofs and wood or bamboo for the walls. In Lang Nhot, most houses are small, largely open with incomplete walls and very large eaves.

The climate is tropical humid in all study sites except in the northern village of Khoi where a cold winter period lasts for 3 months (December–February, monthly mean temperature of 17 °C). The rainy season generally lasts from May to October, with average annual rainfalls in 1998 of 1750 mm in Khoi, 2700 mm in Lang Nhot, 2800 mm in Van Duc A, 1780 mm in Na Ang, 3300 mm in Cha Ong Chan and 2100 mm in village 3.

In Khoi village, outbreaks were common at the end of the 1980s and the beginning of the 1990s, but no local malaria case was detected since 1995 (A. Erhart, unpublished observation; Verlé *et al.* 1998). Malaria is still an important health problem in Lang Nhot, village 3 and Cha Ong Chan. The incidence rate of malaria disease in Lang Nhot was 63 per 100 persons/year in 1998 (Erhart, unpublished observation) and 22 per 100 persons/year in village 3 for the period 2000–2001 (Erhart *et al.* 2003). A parasitological survey in December 1998 revealed that 44.5% of children under 9 years of age in Cha Ong Chan were infected with *Plasmodium falciparum* (T. Sochantha, unpublished observation). In Na Ang and Van Duc A malaria incidence is low. In 1998 the incidence rate of malaria disease in Van Duc A was 1.9 per 100 persons/year (Erhart, unpublished observation). During the entomological study period and the last 2 years preceding our mosquito collections, no vector control measures based on insecticides had been implemented to our knowledge in the study villages.

Mosquito collections

In the four study villages of Vietnam, eight entomological surveys were conducted concomitantly (April, August and November 1998; April and November 1999; April, August and November 2000). In Laos and Cambodia, only three surveys took place (March, July and October 1999).

For each entomological survey, human landing collections were carried out during 10 successive nights. Two houses were selected per study village with one collector indoors and two collectors outdoors. A first team of collectors worked from 18 : 00 to 24 : 00 hours, and a second from 24 : 00 to 6 : 00 hours. The houses for collections were the same throughout the study period.

Mosquito processing

After morphological species identification by using an illustrated key for anophelines of Southeast Asia [modified from Institute of Malariology, Parasitology & Entomology (IMPE 1987)], specimens of *An. minimus s.l.*, *An. dirus s.l.* and *An. sundaicus* were stored individually in liquid nitrogen or silica gel. The members of the *An. minimus* complex were identified using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay developed by Van Bortel *et al.* (2000). The results of identification of the collected specimens of the *An. dirus* complex have been presented elsewhere. Only species A of the *An. dirus* complex was found to be present in our samples of Vietnam, Laos and Cambodia (Manguin *et al.* 2002).

Almost all collected specimens of *An. minimus* A, *An. dirus* A and *An. sundaicus* were subjected to enzyme-linked immunosorbent assay (ELISA) to detect *Plasmodium falciparum*, *P. vivax* 210 and *P. vivax* 247 circumsporozoite protein (CSP) in the head–thoracic portion of mosquitoes (Burkot *et al.* 1984; Wirtz *et al.* 1985, 1992). As no local malaria case occurred in Khoi village and in the whole province of Hoa Binh since 1995, specimens of both *An. minimus* A and *An. minimus* C collected in this village were not tested by ELISA for CSP detection. In order to get an idea of the transmission potential of the *Anopheles* vectors occurring in areas where transmission is low, and where direct observation of sporozoites in head–thorax portion does not give conclusive results, ovarian tracheoles were examined to estimate the parous rate (Detinova 1962). These parous rates are indirect estimates of the survival rate that is the most important factor of the vectorial capacity (Davidson 1954). The percentage of parous female mosquitoes was estimated on mosquitoes collected during the last four surveys (November 1999–2000).

Calculations of entomological risk

The annual entomological inoculation rate (AEIR) was estimated and used to calculate the entomological risk according to Krafsur (1977): $1 - \exp(-\text{AEIR})$. The entomological risk is the probability of receiving at least one positive bite per year and was compared with the incidence rates from Erhart *et al.* (2003). For one site, Van Duc A, the entomological risk was used to calculate the maximal sporozoite rate that is needed to explain the observed incidence. Therefore we assumed that the observed incidence of malaria was equal to the entomological risk.

Results

Distribution of major malaria vectors and human landing rates

Anopheles minimus A was collected in the five study villages located in hilly forested areas in Laos (Na Nang), Cambodia (Cha Ong Chan), central Vietnam (village 3 and Lang Nhot) and northern Vietnam (Khoi), whereas species C of the *An. minimus* complex was only found in the study site of northern Vietnam. *Anopheles dirus* A was present in the same villages except in Khoi. In the coastal village of the Mekong delta (Van Duc A), *An. sundaicus* was the only major vector captured.

The in- and outdoor human landing rates of both *An. minimus* A and *An. dirus* A in Cha Ong Chan (Cambodia) were very low, never exceeding one mosquito per man per night (0–0.58). In Na Ang (Laos), *An. dirus* A was only collected in July 1999 at very low densities (0.15), whereas, *An. minimus* A was found at high human landing rates (from 10 to 68 landings/man/night) in all three surveys (Table 1).

In Khoi village (northern Vietnam), both *An. minimus* A and *An. minimus* C were caught in all eight surveys (Table 2). The highest human landing rates were recorded at the end of the rainy season (November) and in April 1998. This was more pronounced for *An. minimus* A. In Lang Nhot (central Vietnam), the human landing rates of *An. minimus* A were high in April 1998, then sharply decreased and this species was not found anymore in this village from November 1998 onwards. In village 3 (central Vietnam) only one specimen of *An. minimus* A was collected by human landing collections during the entire study period. Low human landing rates of *An. dirus* A were recorded in both Lang Nhot and village 3, rarely more than one mosquito per man night. However, *An. dirus* A was collected in all eight surveys in these two villages. In Van Duc A, village of the Mekong delta, *An. sundaicus*

Table 1 Indoor* and outdoor† human landing rates (number of mosquitoes per man per night) of *Anopheles minimus* A and *An. dirus* A in Cha Ong Chan (Rattanakiry, Cambodia) and Na Ang (Vientiane, Laos)

Village	Species	Human landing	March 1999	July 1999	October 1999
Cha Ong Chan (Rattanakiry, Cambodia)	*An. minimus* A	Indoor	0.15	0.05	0.25
		Outdoor	0.30	0.25	0.18
	An. dirus A	Indoor	0.00	0.40	0.00
		Outdoor	0.00	0.58	0.05
Na Ang (Vientiane, Laos)	*An. minimus* A	Indoor	25.35	68.25	18.15
		Outdoor	36.43	35.98	9.98
	An. dirus A	Indoor	0.00	0.15	0.00
		Outdoor	0.00	0.15	0.00

* 20 man nights/survey.
† 40 man nights/survey.

Table 2 Indoor* and outdoor† human landing rates (number of mosquitoes per man per night) of *Anopheles minimus* A, *An. minimus* C, *An. dirus* A, *An. sundaicus* in four study villages in Vietnam

Village	Species	Human landing	April 1998	August 1998	November 1998	April 1999	November 1999	April 2000	August 2000	November 2000
Khoi (Hoa Binh)	*An. minimus* A	Indoor	1.90	1.25	2.40	0.60	1.20	0.80	1.60	1.80
		Outdoor	1.48	0.88	1.18	0.55	1.55	0.38	1.53	4.20
	An. minimus C	Indoor	1.15	0.05	0.30	0.10	0.05	0.00	0.10	0.05
		Outdoor	1.25	0.60	0.95	0.65	0.78	0.25	0.18	0.45
Lang Nhot (Khanh Hoa)	*An. minimus* A	Indoor	16.90	0.20	0.00	0.00	0.00	0.00	0.00	0.00
		Outdoor	1.98	0.18	0.00	0.00	0.00	0.00	0.00	0.00
	An. dirus A	Indoor	0.10	0.10	0.70	0.05	0.60	0.30	1.05	1.05
		Outdoor	0.13	0.05	0.43	0.08	0.43	0.20	1.08	0.68
Village 3 (Binh Thuan)	*An. minimus* A	Indoor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Outdoor	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	An. dirus A	Indoor	0.00	0.15	0.15	0.05	0.10	0.00	0.45	0.30
		Outdoor	0.03	0.85	0.65	0.18	0.58	0.10	0.75	0.53
Van Duc A (Bac Lieu)	*An. sundaicus*	Indoor	3.10	27.95	16.00	153.40	71.90	189.35	117.20	77.10
		Outdoor	3.30	12.23	17.70	103.35	58.95	149.73	101.20	68.85

* 20 man nights/survey.
† 40 man nights/survey.

was abundant (12–189 landings/man/night) except in the first survey of April 1998 (Table 2).

Plasmodium CSP rates

Anopheles dirus A in village 3 (central Vietnam), and both *An. minimus* A and *An. dirus* A collected in Lang Nhot (central Vietnam) and Cha Ong Chan (northeast Cambodia) were positive for CSP. All specimens of *An. minimus* A and *An. dirus* A in Na Ang (Laos) and *An. sundaicus* in Van Duc A (Mekong delta) were CSP-negative (Table 3).

Parous rates

No significant differences in proportion of parous mosquitoes were observed between in- and outdoor human landing collections of the different vectors. Therefore, mosquitoes collected in- and outdoors were pooled to estimate the parous rates (Table 4). The parous rate of *An. dirus* A in Lang Nhot was not significantly different from that of *An. dirus* A in village 3 (Mantel–Haenszel stratified by survey M–H, $P = 0.27565$). In order to get an idea of the transmission potential of *An. minimus* A and C in Khoi and of *An. sundaicus* in Van Duc A, the proportions of parous mosquitoes of these species were compared with those of pooled samples of *An. dirus* A. No significant difference in proportion of parous mosquitoes was observed between *An. minimus* C (in Khoi) and *An. dirus* A (M–H, weighted odds ratio = 1.16, $P = 0.70297$), whereas the parous rate for *An. minimus* A (in Khoi) was higher (M–H, weighted odds ratio = 2.15, $P < 0.0005$) and that of *An. sundaicus* was lower (M–H, weighted odds ratio = 0.45, $P < 0.0005$) than *An. dirus* A. In Khoi, the parity rate of *An. minimus* C was lower than that of *An. minimus* A (M–H, weighted odds ratio = 0.37, $P < 0.005$).

Table 3 Circumsporozoite protein (CSP) rates of *Anopheles minimus* A, *An. dirus* A and *An. sundaicus* collected in five study villages

Village	Species	Number of mosquitoes tested	Number of mosquitoes positive with *P. falciparum* (P.f)	*P. vivax* (P.v.) 210	*P. vivax* 247	P.v 210 – P.v 247	Percentage (P.f + P.v)
Lang Nhot (Khanh Hoa)	*An. minimus* A	361	7	0	2	1	2.8
	An. dirus A	189	1	0	0	1	1.1
Village 3 (Binh Thuan)	*An. dirus* A	242	3	0	0	0	1.2
Cha Ong Chan (Rattanakiry)	*An. minimus* A	72	1	0	0	0	1.4
	An. dirus A	28	2	1	0	0	10.7
Na Ang (Vientiane)	*An. minimus* A	5683	0	0	0	0	0.0
	An. dirus A	8	0	0	0	0	0.0
Van Duc A (Bac Lieu)	*An. sundaicus*	11 002	0	0	0	0	0.0

Table 4 Percentages of parous mosquitoes of *Anopheles minimus* A, *An. minimus* C, *An. dirus* A and *An. sundaicus* in four study villages in Vietnam (number of mosquitoes examined in brackets)

Species/village	November 1999	April 2000	August 2000	November 2000	Total
An. minimus C (Khoi, Hoa Binh)	68.7 (32)	50.0 (10)	33.3 (9)	78.9 (19)	64.3 (70)
An. minimus A (Khoi, Hoa Binh)	83.3 (83)	90.3 (31)	66.3 (92)	85.0 (200)	80.8 (406)
An. dirus A (Lang Nhot, Khanh Hoa)	55.2 (29)	61.5 (13)	71.4 (63)	51.1 (47)	61.2 (152)
An. dirus A (Village 3, Binh Thuan)	44.0 (25)	75.0 (4)	62.5 (32)	96.0 (25)	67.4 (86)
An. sundaicus (Van Duc A, Bac Lieu)	47.1 (384)	61.2 (874)	50.9 (965)	28.1 (807)	47.3 (3030)

Table 5 Comparison of annual entomological inoculation rates, entomological risks and malaria incidences

	Annual entomological inoculation rate (AEIR) *An. minimus*	*An. dirus*	*An. sundaicus*	All species	Entomological risk*	Malaria incidence**
Lang Nhot (1998)	58.254	1.095	–	59.349	1.000	0.630
Cha Ong Chan (1999)	0.767	5.207	–	5.974	0.997	45%
Village 3 (2000)	–	1.095	–	1.095	0.665	0.220
Van Duc A (1998)	–	–	0***	0.000	0.000	0.019

* Probability of receiving at least one positive bite per year (1 − exp(−AEIR).

** Number of malaria attacks/man/year (Erhart *et al.* 2003). Only parasite rate was available in Cha Ong Chan (T. Sochanta, unpublished observation).

*** Observed sporozoite rate 0/11 002; calculated sporozoite rate = 1/300 000 (using the entomological risk equal to the malaria incidence).

Inoculation rates and malaria incidence

Annual entomological inoculation rates are presented in Table 5 for villages and years with a known incidence of the disease or parasitic prevalence. The risk of receiving one or more sporozoite inoculation per year is compared with the malaria incidence. This estimated risk is higher than the incidence of the disease but this is not surprising as not all infective bites result in a malaria attack. As in Van Duc A no positive *An. sundaicus* mosquitoes were found for ELISA sporozoite detection (0/11 002) although malaria is still present, we calculated the theoretical sporozoite rate that is needed to explain the observed incidence. A sporozoite rate of maximal 1/300 000 is enough to explain the low incidence.

Discussion

As human hosts are sporadically infected, a sufficient number of malaria vectors feeding on humans is necessary to maintain malaria transmission (Molineaux 1988). Despite the low human biting rates in our study villages of central Vietnam and Rattanakiry Province of Cambodia, *An. dirus* A had CSP rates above 1%, which clearly indicates that this species is still a very efficient vector and plays an important role in the malaria transmission in these areas. This confirms previous observations showing that a small population of *An. dirus s.l.* can maintain focally high level of malaria endemicity (Rosenberg *et al.* 1990). On the contrary, high biting rates were observed for *An. minimus* A (10–70 bites/man/night) in the study village of Laos and for *An. sundaicus* (up to 190 bites/man/night) in the Mekong delta, but none contained CSP. Potential malaria vectors occurring at high densities have often a survival rate which is too low for assuring effective malaria transmission (Coosemans *et al.* 1992). This was confirmed in our present study by the low parity rate of *An. sundaicus* (around 47%) in Van Duc A (Bac Lieu Province, Mekong delta). It is, therefore, worth emphasizing that the density of a vector species should not be used as the first indicator for estimating the risk of malaria transmission. Hence, suspecting anopheline species as vectors based on their relative abundance (Kobayashi *et al.* 2000) can be misleading.

In hilly forested areas of Southeast Asia, malaria transmission is perennial because of the presence of both *An. dirus s.l.* and *An. minimus s.l.*, the first being present mainly during the rainy season, the second during drier periods of the year (Ismail *et al.* 1978; Harbach *et al.* 1987; Rattanarithikul *et al.* 1996b; Phan 1998). This was the case in Cha Ong Chan (Cambodia). However, the role of *An. minimus* A as malaria vector was not always obvious: important variations in time and space were observed. In village 3 (Binh Thuan Province) it was almost absent (one specimen collected over 480 man nights), and in Lang Nhot (Khanh Hoa Province) sporozoite positive specimens were only observed during the first two surveys whereas this species was not collected from November 1998 to November 2000 (six surveys: 360 man nights). In the northern study site *An. minimus* A and C were found in all collections, but no local malaria transmission occurred. On the contrary, the constant presence of *An. dirus* A assured a perennial transmission of malaria in the study villages of central Vietnam and Cambodia.

The use of insecticides, mainly dichloro-diphenyl-trichloroethane (DDT), for house spraying in malaria control programmes was one of the main factors causing the disappearance of *An. minimus s.l.* in many areas in Asia (Harrison 1980; Parajuli *et al.* 1981; Rosenberg *et al.* 1990). Vector control activities, as mosquito net impregnation appreciated by the people in Vietnam, cannot be excluded in the present study although it was not officially planned. However, the causes of the disappearance of *An. minimus s.l.* are probably complex. *Anopheles minimus s.l.* breeds along grassy streams in clear, unpolluted water (Harrison 1980). Changes in water velocity and vegetation, including emergent aquatic plants and those growing on the shores, may affect the abundance of this mosquito. Furthermore, pollution of the streams by pesticides used in agriculture and other pollutants, such as laundry detergents released in the streams, may even more affect the persistence of *An. minimus s.l.* (Overgaard *et al.* 2002). *Anopheles dirus s.l.* has the ability to adapt to changing environmental conditions from its natural forest habitats (Singhasivanon *et al.* 1999). Currently there is ample evidence to indicate that *An. dirus s.l.* is able to adapt to peripheral areas where natural forests were replaced with orchards, tea, coffee, and rubber plantation (Gingrich *et al.* 1990; Rosenberg *et al.* 1990). Therefore we can assume that the very anthropophilic malaria vector, *An. dirus* A, will probably persist despite forest fragmentation in central Vietnam. This means that the vector control in this area should focus firstly on *An. dirus* A. However, *An. dirus s.l.* is difficult to control by indoor spraying using residual insecticides because of the highly exophilic behaviour (Scanlon & Sandhinand 1965; Meek 1995). Moreover, the efficacy of insecticide-treated nets or hammocks against this species is also questionable. Indeed in Lang Nhot, more than 50% of the bites of *An. dirus* A occurred before 22 h (H. D. Trung, unpublished observation).

The observation in Lang Nhot, where 2.8% of the collected *An. minimus* A specimens were infected by *Plasmodium* sporzoites, clearly shows that this species is still efficient in transmitting malaria and that *An. minimus* A should therefore be carefully followed-up, also in places where actual transmission is absent. Since 1995, no local malaria case occurred in Khoi village and only imported cases are reported for the whole province of Hoa Binh (northern Vietnam). However, the risk for malaria outbreaks is still present (Verlé *et al.* 1998). The return of malaria infected villagers who had recently stayed in malaria endemic areas in central and southern Vietnam, and the constant presence of *An. minimus* A and *An. minimus* C with high parity rates (around 80% and 65%, respectively) suggest that malaria transmission can restart at any time. The proper management of the malaria cases and movement of villagers are, therefore, important to prevent epidemics and the reintroduction of malaria in northern Vietnam. The higher degree of anthropophily (Van Bortel *et al.* 1999) and parity rates, an indirect estimation of the survival rate (Davidson 1954), of *An. minimus* A suggests that this species has a higher potential of transmission than species C.

In the study site of the Mekong delta, the increase of *An. sundaicus* population, accompanied by the disappearance of *An. subpictus* from our collections (data not shown) may be explained by the changes in land use. Freshwater surfaces used for rice fields decreased progressively during the study period for shrimp farming using brackish water. This favoured *An. sundaicus* instead of *An. subpictus* as the latter is less tolerant to salinity (Anno *et al.* 2000). A census on the changes in land use in this area should be performed to confirm this hypothesis. The absence of CSP-positive *An. sundaicus* combined with low parity rate may reflect its low vectorial status. We calculated that a sporozoite index of 1/300 000 or even lower can explain the observed incidence of malaria cases in Bac Lieu. With such low level of malaria incidence it becomes extremely difficult to identify the species responsible for the transmission. However, in this coastal environment, other species than *An. sundaicus*, like *An. nimpe* and *An. subpictus*, have been suspected as malaria vector (Hinh *et al.* 1997).

Despite the successes in malaria control, *An. dirus* A continues to play a dominant role in malaria transmission whereas *An. minimus* A showed temporal and spatial variation in its vector status. The risk of malaria transmission is not limited to endemic areas, but concerns also the areas currently free from malaria where malaria vectors still exist. Infectious vectors collected in the villages located in hilly forested areas of Cambodia and central Vietnam indicate that the risk to get malaria is not limited to forest activities but exists also in the villages. Beside the three majors vectors other anopheline species were collected on man. In one of the study villages (village 3 Suoi Kiet), we found 17 species to land on man (Van Bortel *et al.* 2001). Some of these anopheline species may be locally involved in malaria transmission, but considering the malaria incidence that can be attributed to the main vectors, we can assume that their role as malaria vectors in the study villages is almost negligible. The study also points towards the fact that in Southeast Asia it will become increasingly difficult to incriminate *Anopheles* species in malaria transmission while the risk for malaria transmission still persist.

Acknowledgements

We express our sincere thanks to staff of Anopheles Unit of the National Institute of Malariology Parasitology and Entomology (NIMPE, Hanoi, Vietnam), Sub-Institute of Malariology Parasitology and Entomology Hochiminh City, Antimalaria Centres of Hoa Binh, Khanh Hoa, Binh Thuan and Bac Lieu Provinces (Vietnam), National Center for Malaria Control, Parasitology and Entomology (Phnom Penh) and Health Department of Rattanakiry Province (Cambodia), Center of Malariology Parasitology and Entomology (Vientiane) and Health Department of Vientiane Province (Laos) for their excellent technical support in field works. We are grateful to P. Roelants and D. Schrijvers for their laboratory assistance. This work was carried out within the framework of the INCO-DC research project ERBIC18CT970211, the Institutional Collaboration between NIMPE-Vietnam and the Institute of Tropical Medicine, Belgium, supported by the Belgian Co-operation (DGDC) and a PhD grant from Belgian Technical Co-operation (BTC). The authors thank Prof. Umberto D'Alessandro for his comments.

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