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Malaria Vectors in the Mekong Countries: a Complex Interaction between Vectors, Environment and Human Behaviour

by

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1. Introduction

After many decades of control efforts, the malaria situation in the Mekong region deteriorated dramatically in the late eighties of last century. In these years, political isolation of Vietnam, Cambodia and Laos was responsible for reduced access to efficient drugs and insecticides. In Vietnam, the peak of resurgence of malaria with devastating epidemics was reached in 1991 when over one million cases and about 5,000 deaths were reported. Today, the malaria burden has decreased drastically due to the intensive control programme deployed over the past fifteen years (SCHUFTAN 2000, NIMPE 2005). However, the burden of malaria is largely underestimated in the region as the registration system relies essentially on passive case detection by weak health facilities (SNOW *et al*. 2005, ERHART *et al*. 2006). Malaria is at its lowest level ever recorded and control activities could be progressively phased out. However, active transmission is still occurring in remote forested foci and human migration from these endemic foci to non-transmission areas can reintroduce the disease in areas now free of malaria (VERLE *et al*. 1998). The control of these foci will be essential to obtain a sustained reduction of malaria in the region.

A crucial determinant of malaria transmission is the survival of the vector. Mosquitoes will be able to transmit malaria only if they survive, after an

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infectious blood meal, the period required for the malaria parasite to develop into infectious stages, which are sporozoites in the salivary glands of the vector. Furthermore, transmission is determined by the frequency of man-vector contact, which depends on the feeding habits of the mosquito, and the density of vector population. Vector control aims at decreasing malaria transmission by tackling one of the above mentioned transmission related factors (*i.e.* vector survival, man-vector contact or density) using indoor spraying with residual insecticides (IRS) or insecticide-treated bed nets (ITN) (Snow & Gilles 2002). These factors are influenced by vector population characteristics which, in turn, are influenced by environmental and human related factors (tab. 1). The foci where malaria is still occurring are characterised by a complex interaction between vectors, man and environment. National Malaria Control Programmes in Southeast Asia must deal with this complex situation in a changing environment whereby a clear understanding of the vector biology in relation to human behaviour and environment is essential for developing appropriate vector control.

2. Biodiversity of Anophelines in SE Asia and their Taxonomic Status

Anopheles species diversity in Southeast Asia is considerable higher compared to Africa. Beside the major vectors, many anopheline species can be found in domestic environments. Table 2 gives an overview of the *Anopheles* species that are regularly found in the vicinity of human dwellings and up to 20 different *Anopheles* species have been found in one village (Van Bortel *et al.* 2004). The taxonomic status of many of these species is not well established. Moreover, many of the species are difficult to identify on the basis of morphology, particularly in field conditions. The taxonomic status of the major vectors (the *An. minimus*, *An. dirus* and *An. sundaicus* complexes) has been recently explored and new molecular identification methods were developed to distinguish the species of these complexes, thus allowing further investigations into the biology of the individual members of each species complex (Van Bortel *et al.* 1999, 2000; Garros *et al.* 2004ab; Walton *et al.* 1999; Manguin *et al.* 2002; Dusfour *et al.* 2004b). Furthermore, molecular tools were developed to identify all the species belonging to the Funestus group that are present in the region, *i.e.* eight species including the *An. minimus*, *An. culicifacies* and *An. aconitus* subgroups (Van Bortel *et al.* 2000, Garros *et al.* 2004a, b). Molecular methods are under development for the correct identification of species belonging to *An. hyrcanus*, *An. maculatus* and *An. annularis* groups. The molecular identification

Table 1

Factors determining the vectorial capacity of vectors and conditions required for effective vector control measures

	To be effective ***It is assumed that:***	Characteristics of the vector populations	Human and environment factors that influence vector population characteristics
Vector	• **To be present**	• **Habitat/ adaptation capacity** • **Density (+)**	• **(Micro)Environment, changes, agriculture practices, season, climate**
	• **To bite on man**	• **Anthropophily/ Zoophily (+++)**	• **Alternative hosts (cattle)**
	• **To live long enough**	• **Longevity (+++++)**	• **Seasons, climate**
Impregnated bed nets	• **To sleep under the net at the time the vector is biting**	• **Biting cycle (early/late)** • **Bite in/outside the house (endo/exophagy)**	• **Moon, season** • **Sleeping habits** • **Night outdoor activities**
Indoor spraying	• **Vectors rest indoors**	• **Rest in/outside the house (endo/exophily)**	• **Type of house**
Insecticides	• **Vectors are susceptible**	• **Population dynamics** • **Gene flow**	• **Domestic and agricultural insecticide pressure**

allows a quality control on the morphological identification of species usually performed under difficult field conditions. This proved to be useful in Vietnam and Cambodia where the vector *An. minimus* was confused with the none vector species *An. varuna* and *An. culicifacies* B (VAN BORTEL *et al.* 2001, 2002).

Table 2
List of *Anopheles* species observed from 2001 to 2005
in domestic environments in Cambodia, Laos and Vietnam
(classification following HARBACH 2004, LINTON *et al.* 2005, GARROS *et al.* 2006)

Genus (subgenus)	**Section/series**	**Group**	**subgroup**	***species***
Anopheles	Myzorhynchus	Barbirostris		*barbirostris*
				campestris
			Vanus	*barbumrosus*
		Hyrcanus		*argyropus*
				hyrcanus
				nimpe
				sinensis
		Hyrcanus	Lesteri	*paraliae*
				crowfordi
				peditaeniatus
		Umbrosus	Umbrosus	*umbrosus*
Cellia	Myzomyia	Funestus	Minimus	*minimus A*
				minimus C
			Culicifacies	*culicifacies* B
			Aconitus	*varuna*
				pampanai
				aconitus
				jeyporiensis
	Neocellia	Annularis		*annularis*
				philippinesis
				nivipes
				pallidus
		Jamesii		*jamesii*
				splendidus
		Maculatus		*pseudowillmori*
				willmori
			Maculatus	*maculatus*
			Sawadwongporni	*notanandai*

Genus (subgenus)	**Section/series**	**Group**	**subgroup**	***species***
				sawadwongporni
				karwari
	Neomyzomyia	Kochi		*kochi*
		Leucosphyrus	Leucosphyrus	*dirus sensu stricto*
		Tessalatus		*tessalatus*
	Pyretophorus		Sundaicus	*epiroticus*
				subpictus
				indefinitus
				vagus

3. Major Vectors: Species and Association with Landscape

In Vietnam, Cambodia and Laos, three major malaria vectors are recognised. They belong to the species complexes of Dirus, Minimus and Sundaicus. These vectors have a wide geographical distribution where their role in malaria transmission is obvious, at least before the enormous control efforts of the last fifteen years.

The classic breeding places for *An.minimus sensu lato* are margins partially shaded with grass of moderated sized clear-water streams. Larvae can also be found in rock or ground pools and irrigation drains. These habitats are commonly found in foothills. Three species are recognised within the *An.minimus* complex (Harbach 2004) of which two are found in the region: *An.minimus* A and C. Both species occur in sympatry in a large area that includes northern Vietnam, southern China and northern Laos (Garros *et al.* 2006). So far only *An.minimus* A was detected in Cambodia (Van Bortel *et al.* 2004, Trung *et al.* 2004). In the province of Hoa Binh (northern Vietnam), the relative proportion of both species varies from one village to the other (from 2 to 65 % of species C) suggesting different environmental requirements (Van Bortel *et al.* 1999). In central Vietnam (Khanh Hoa Province) high densities of *An.minimus* A were observed in a village until April 1998 after which this species disappeared from the collections (Trung *et al.* 2004, Van Bortel *et al.* 2004) probably due to the wide use of ITNs (Garros *et al.* 2005). *An.minimus s.l.* reappeared abundantly in 2002, but species C became predominant (98 %) suggesting an unknown mechanism of interspecific competition between both species (Garros *et al.* 2005). More recently Garros *et al.* 2006 suggested that species C is associated with new agro-ecosystems in deforested areas (*e.g.* maize) whereas species A occurs in less disturbed forested areas with traditional rice agrosystems. However, it was species A that was found in the suburbs of Hanoi where it breeds in water tanks near houses (VAN BORTEL *et al.* 2003) but was not found in other parts of the Red River Delta (Trung, pers. comm.). Although density of *An.minimus s.l.* was negatively related to landscape fragmentation due to human exploitation (OVERGAARD *et al.* 2003) it seems that the members of the species complex are able to adapt to new man-made environments.

The Dirus complex includes seven species (SALLUM *et al.* 2005) but so far only *An.dirus s.s.* (formarly *An.dirus* A) was found in Laos, Cambodia and Vietnam and is among the most efficient vectors in Southeast Asia. *An.dirus s.l.* is not reported in northern Vietnam, although it is present in southern China, northern Thailand and Laos. Several members of the complex occur sympatric in Thailand, and Myanmar (WALTON *et al.* 1999). In the western part, from Bangladesh to Myanmar, *An.baimaii* (formerly *An.dirus* D) is the most efficient vector. *An.dirus s.l.* is confined to forested areas where it breeds in small temporary shaded pools widely spread in forest, in forest fringe areas around the villages, and in the exploited forest areas, such as rubber plantations or even orchards. The abundance is often the highest in the late rainy season as a consequence of available breeding sites. Different observations in the western distribution of *An.dirus s.l.* revealed an expansion of the mosquito population during the rainy season from mother foci in deep forests to secondary breeding sites at the periphery (KONDRACHIN *et al.* 1991). This may explain the low densities of this forest-dwelling species observed in villages near the forest. Moreover, forest fragmentation has probably direct consequences on the maintenance of the *An.dirus s.l.* populations.

Species of the *An.sundaicus* complex are brackish water breeders on islands and along coastal areas of Southeast Asia. Breeding places are sunlit bodies of stagnant saline water with floating algae (DUFOUR *et al.* 2004a). Recent studies showed that only one species of the complex is present on the mainland and was formally named *An.epiroticus* whereas *An. sundaicus s.s.* occurs only in Borneo (LINTON *et al.* 2005). In Vietnam, *An.epiroticus'* distribution is confined to the Mekong Delta. In one study site of the Mekong Delta, the density increased drastically in two years (from 3,1 bites per man per night in 1998 to 190 bites/man night in 2000). The recent changes in land use from rice cultivation to shrimp farming probably explain this sudden nuisance of mosquitoes (TRUNG *et al.* 2004). Low densities are reported on the Cambodian and Thai Coast (Tho Sochanta, V. Baimai, pers. comm.).

4. Inter- and Intra-specific Behaviour of Vectors

Behavioural traits often exhibit geographical patterns and the variation is often indicative of underlying genetic differentiation or even cryptic species. However, environment factors may also influence the behavioural patterns at the intraspecific level, which are relevant for disease transmission and control (Van Bortel *et al.* 2004). By analysing the ratios of outdoor human-to-cattle landing rates and indoor-to-outdoor human landing the trends for respectively antropophilic and endophagic behaviour was estimated. While *An.dirus* s.s. and to a less extent *An. epiroticus* bite almost exclusively on man, different trends where observed according to the locality for *An.minimus* A, *An.sinensis*, *An.philippinensis* (fig. 1) and other species in relation to the availability of cattle in the villages (Trung *et al.* 2005). The biting activity inside the houses was relatively low for most species except in one site of central Vietnam (fig. 2). This site is a forest village of a minority group living in houses with incomplete walls of split bamboo and very large eaves allowing easy entry of the mosquitoes (fig. 3). Outdoor biting mosquitoes are more difficult to control and this behaviour may explain the persistence of malaria transmission after vector control (Meek 1995).

Insecticide treated nets are mainly effective on late biting vectors. In figure 4 we can see that most anopheline species in the area are early biters by which they will avoid the contact with ITN. However, the main vectors bite later in the night and can be well controlled by ITNs, except in villages of central Vietnam with open construction. In these villages ITN used after 22 p.m. will prevent only 50 % of the bites of *An.dirus s.s.* (Trung *et al.* 2005).

After blood feeding, mosquitoes spent most of their time to rest. If they rest inside the houses (endophilic) conventional interventions based on residual indoor spraying of insecticide can be very effective to control malaria. However the major vector *An.dirus s.s.* rarely rests inside the houses making this kind of intervention not appropriate. *An. minimus s.l.* and *An. epiroticus* have been characterised to be endophilic species, however Trung *et al.* (2005) found both species and all other anopheline species to be highly exophilic. Only in northern Vietnam, *An. minimus* A exhibited a moderate degree of preference to rest indoors but not *An. minimus* C (Van Bortel *et al.* 1999, Van Bortel *et al.* 2004, Trung *et al.* 2005). However, differences in behaviour between species A and C was only studied in one site and more studies over the sympatric range of distribution are needed to understand the range of interspecific divergences in behaviour.

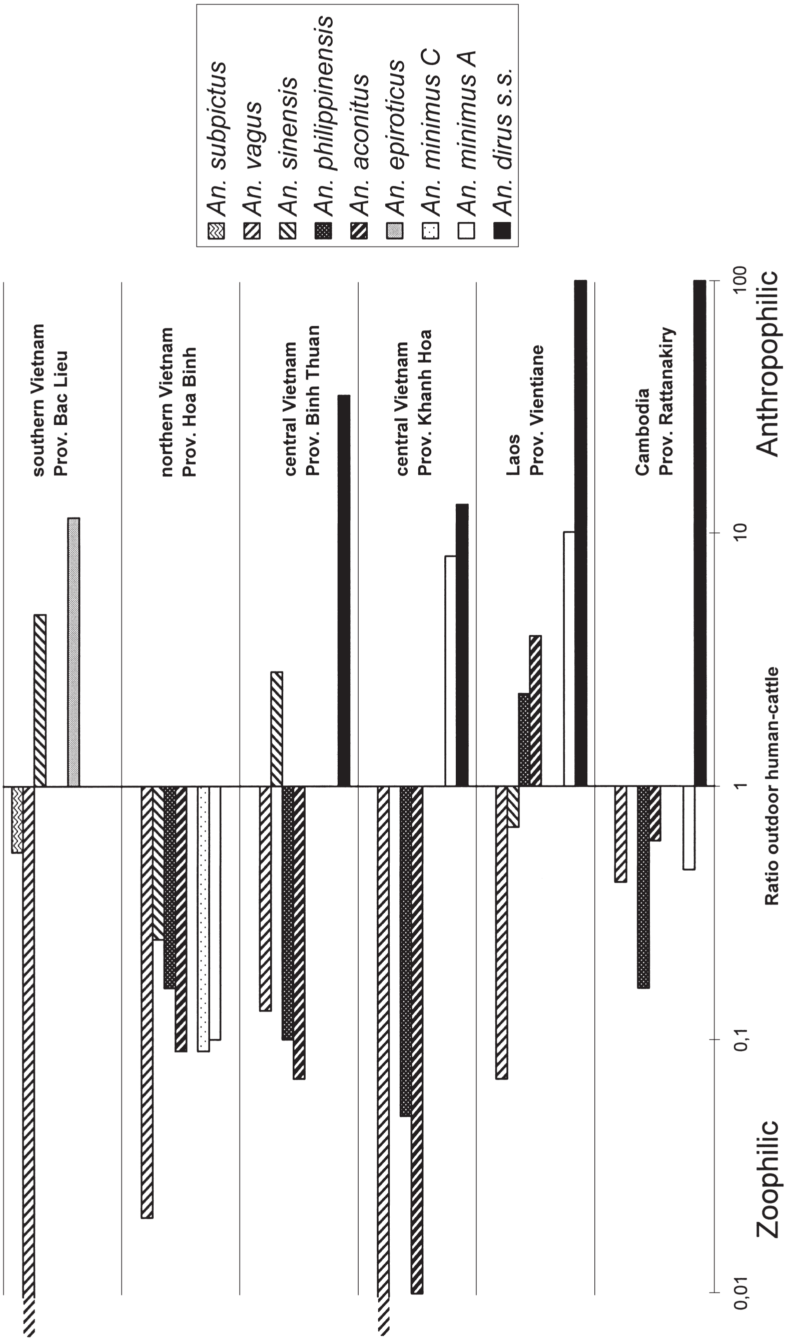

Fig. 1. — Trends of *Anopheles* species in preferring human host to cattle based on the ratios of outdoor human to cattle landing rates (after TRUNG *et al.* 2005). The degree of anthropophily increases from the left to the right.

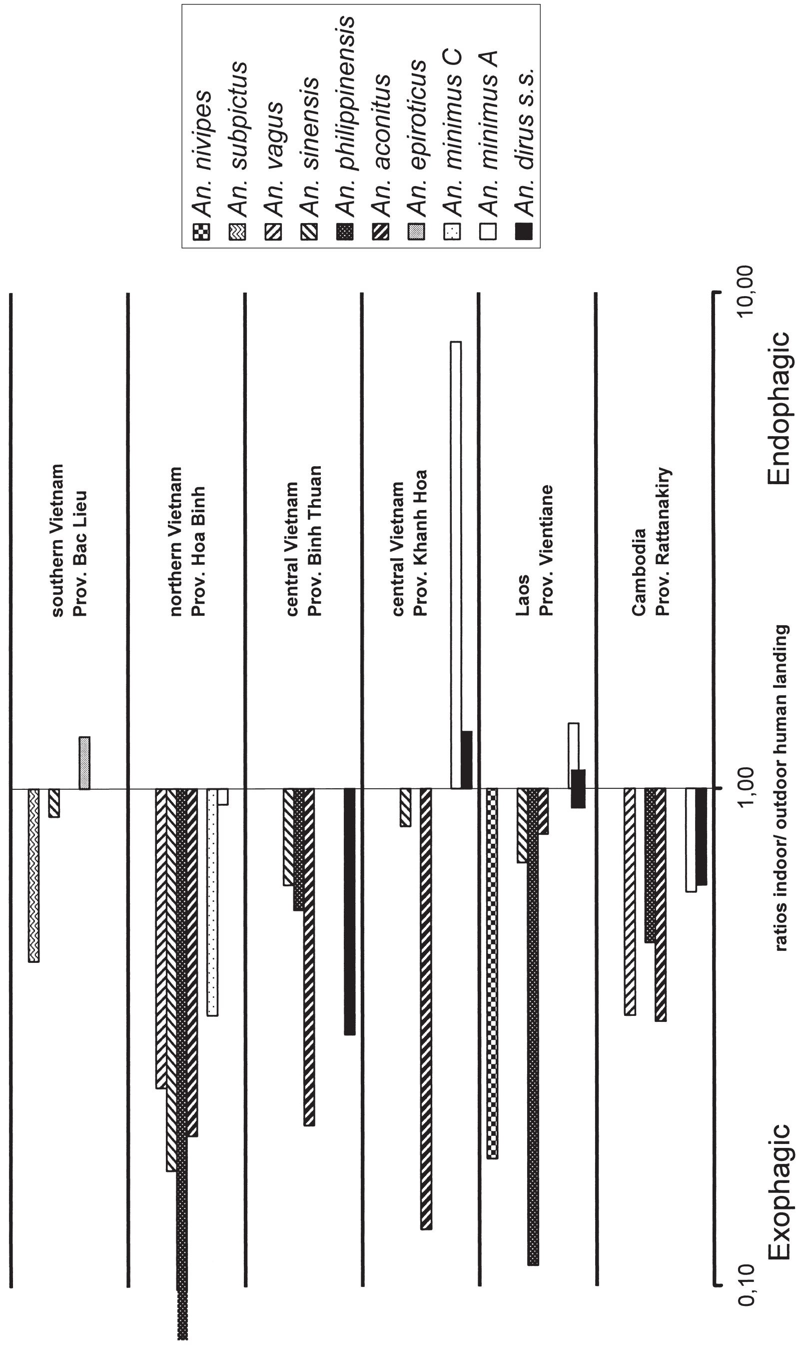

Fig. 2. — Trends of *Anopheles* species in preferring to bite indoor to outdoor based on ratios of indoor to outdoor human landing (after TRUNG *et al.* 2005). The degree of endophagy increases from the left to the right.

Fig. 3. — Open house construction of the ethnic group of Rac Ray in the Province Khan Hoa.

5. Malaria Transmission in Relation to Vector Distribution

In the region, malaria risk is not evenly distributed and depends on the occurrence of vectors, human behaviour and the performances of the control programmes.

In northern Vietnam, the major malaria vector, *An.minimus s.l.*, is confined to the hilly areas. Consequently lowlands, mountain and coastal areas have been almost preserved of malaria in the past. Malaria pressure on human populations occurred during centuries on the foothills and traces of this are still present in some ethnic minority groups. Indeed, the G6PD deficiency trait, conferring resistance to *Plasmodium falciparum*, is high (up to 34 %) in ethnic groups (*e.g.* Muong, Thai, Tho, Dao) that traditionally lived in the malaria endemic foothills, whereas it is almost absent among groups (ea, Mong, Kinh) that lived in the past in mountainous or lowland areas (VERLE *et al.* 2000). The intensive control programme, based on improved case-management and vector control, has been very successful in this region. In 1992 in the province of Hoa Binh, the health services reported 32,673 cases. Five years later the number of reported cases had dropped by 88 %. Only 59 cases could be confirmed by the laboratory and half of them had been infected in

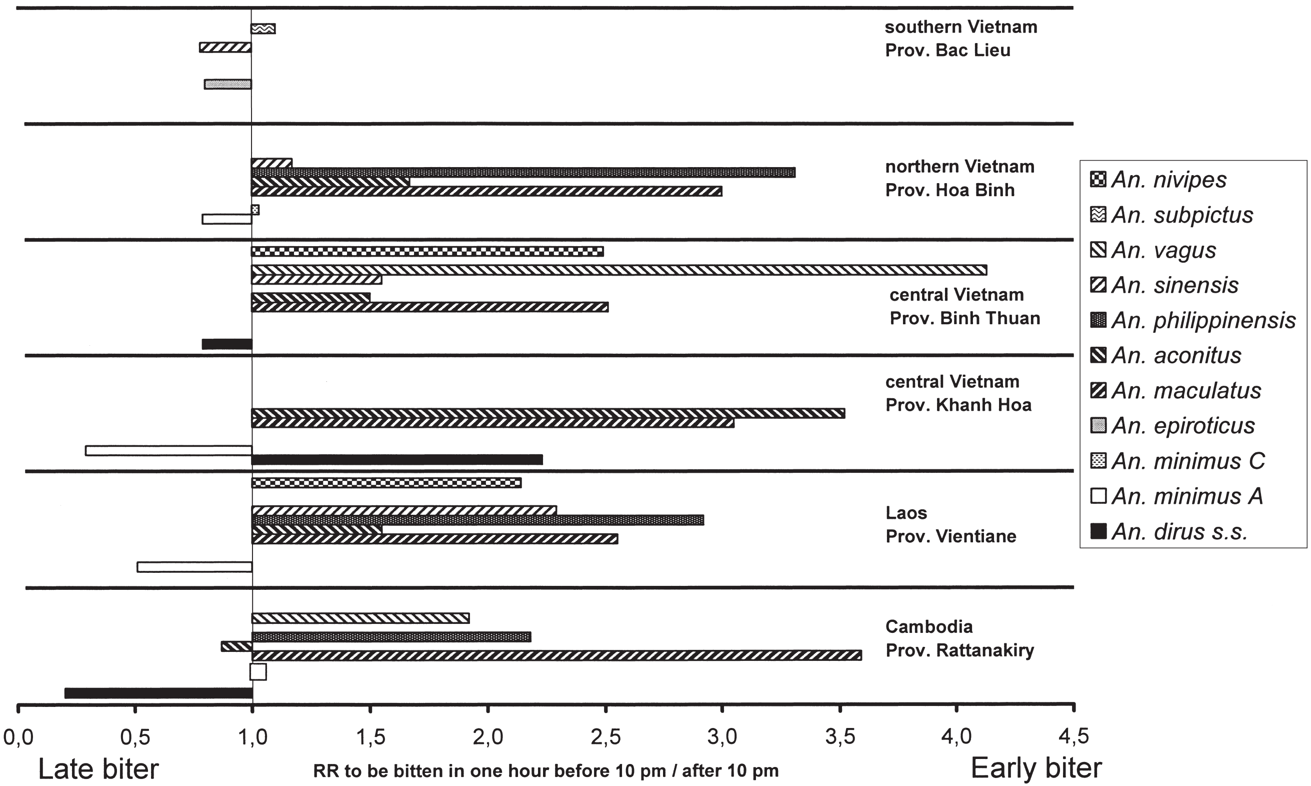

Fig. 4. — Trends for early biting activity based on the relative risk of being bitten in one hour before 10 p.m. compared with one hour after 10 p.m. (after TRUNG *et al.* 2005). The degree of early biting increases from the left to the right.

the southern provinces (VERLE *et al.* 1998). The northern part of the country is now almost free of malaria. However, *An.minimus A* and C are still present. Their high longevity suggests that transmission can occur at almost any time if parasite reservoirs are reintroduced (TRUNG *et al.* 2004). This could explain the reactivation of transmission in some small foci at the border of Laos and China facing uncontrolled migration (NIMPE 2006).

The situation is more complex in the tropical forested hilly areas of central Vietnam, Laos and Cambodia where two major malaria vectors can be found (*An.minimus* A and *An.dirus s.s.). An.minimus s.l.* is mainly present during the dry season (January-July), as during the rainy season larvae of this species are flushed away from their river breeding places by the heavy showers. If *An.minimus* A is clearly involved in malaria transmission (TRUNG *et al.* 2004), the vectorial status of species C needs still to be clarified (GARROS *et al.* 2006). *An. dirus s.s.* is mainly present during the second part of the rainy season once its breeding sites, small rain fed pools, are omnipresent. When both species are present, the successive occurrence of both vectors leads to perennial transmission. *An.dirus s.s.* is certainly the most effective vector in the region: it is highly susceptible to human *Plasmodium*, long living, and almost exclusively anthropohilic, even if they feed primarily on monkeys in the tree-tops (SALLUM *et al.* 2005). It is than not surprising that high levels of transmission can be maintained at low vector densities (TRUNG *et al.* 2004). However, the control of these species is a real challenge for the control programmes regarding the exophagic and exophilic behaviour of this vector. Moreover, a large proportion of forest villagers contract the disease by the bites of this vector during their night activity in the forest (ERHART *et al.* 2004b, 2005).

In the Mekong Delta and along the coast of Cambodia the main vector is *An. epiroticus*, a brackish water-breeding mosquito. This vector was described as being an efficient vector in Vietnam (with sporozoite rates from 0.18 to 4.4%) and in Cambodia (sporozoite rate 0.4%) (DUFOUR *et al.* 2004a). In a village of the Province of Bac Lieu, none of the 11,000 mosquitoes that were examined for the presence of sporozoites, were found positive (TRUNG *et al.* 2004). For explaining the present low incidence of malaria in this site (2.6 case/100 person /year) (ERHART *et al.* 2004a) we should have analysed more than 300,000 specimens! This shows the difficulty of incriminating vectors when the incidence decreased due to interventions. The vector status of the species belonging to the *An. sundaicus* complex may vary in time and from place to place (in DUFOUR *et al.* 2004) which can be responsible for potential risk of malaria outbreak as consequence of environmental changes or natural catastrophes, *e.g.* tsunami (KRISHNAMOORTHY *et al.* 2005).

Beside the major vectors many anopheline species have been suspected or incriminated as malaria vectors in the area. Species such as *An.maculatus s.l.*, *An.aconitus*, *An.subpictus*, *An.philippinensis*, *An.vagus*, *An. sinensis*, *An.campestris* are listed as vectors in the Oriental Region (Mouchet *et al.* 2004) and occur in the Mekong countries. In Vietnam, more than ten species were incriminated as secondary vectors (Phan 1998) but their current importance in malaria transmission is unknown. In Laos, *An. nivipes*, breeding in flooded pools of rice fields settings, is suspected to be responsible for the malaria transmission in settlements other than in foothills or forest (Kobyashi *et al.* 2000). This species was found positive for sporozoites in the central plain of Thailand (Harbach *et al.* 1987). In Sekong province of Laos, Vythilingam *et al.* (2003) found *An.maculatus s.l.* and *An.jeyporiensis* positive with the ELISA test alongside *An. dirus s.s.* This test is however not the most appropriate test to incriminate vectors and additional confirmation may be required. The study of impact of land use, environmental changes and human behaviour on the distribution and abundance of the secondary vectors is important for understanding the complexity of malaria transmission in Southeast Asia and will allow the risk of malaria transmission to be tracked in a fast changing environment.

6. Human Behaviour Patterns as Risk Factors for Malaria

Variability in malaria endemicity is not only linked to a complex interaction between environment, parasites, and vectors but also to human behaviour, a determinant factor in the occurrence of the disease. Some ethnic minorities are high-risk groups because of agricultural practices near or in the forest, and poverty.

Housing conditions will influence the behaviour of mosquitoes. Open construction such as found in minority groups allows anthrophilic mosquitoes to easily detect attractant stimuli from human hosts (Trung *et al.* 2004). The proximity of the houses to the forest edge is a considerable risk factor for malaria. The presence of cattle around or under the houses deviate part of the vectors so that the contact man-vector is lower compared to other places where cattle is almost absent (Van Bortel *et al.* 2004, Trung *et al.* 2005).

Human sleeping behaviour is important in determining man-vector contact considering that anopheline mosquitoes bite only during the night. The introduction of electricity in the villages, sometimes using ingenious ways like small dynamos on rivers, determined an increase in outdoor activities during the evening and a delay in bedtime. In these conditions the period of protection for insecticide-treated bed nets is decreased, particular when early biters and exphagic anopheline species are involved in malaria transmission.

Malaria is an occupational disease affecting people during their temporary stay in the forest like forest workers, gem miners, plantation workers. Farmers may stay away from their village to cultivate their cut-and-burn plot in the forest. They live in temporary houses often without walls. During their temporary stay in the forest, people sleep in hammocks and bed nets are useless. Regular forest activity increases by 4-fold and 10-fold the odds of disease and infection, respectively (ERHART *et al*. 2004). Adapted control tools such as long lasting impregnated hammocks could be appropriate in such environment.

7. Insecticide Resistance

Knowledge of vector resistance and changing trends of resistance in target species are basic requirements to guide insecticide use in malaria control programmes. Only long lasting pyrethroids can be used for impregnation of bed nets, which is nowadays the most effective tool to be protected against malaria. A network (MALVECASIA [1][*]) was set up for the monitoring of insecticide resistance in Cambodia, Vietnam, Laos and Thailand. After three years of intense insecticide resistance monitoring in more than hundred twenty sites, a clear picture of the status of insecticide resistance of malaria vectors was achieved. In Laos and Thailand, resistance is almost absent. In Cambodia, only *An. vagus*, a non-vector species, was found resistant to DDT and different pyrethroid insecticides. In southern Vietnam, high levels of resistance in *An. epiroticus* were detected for all pyrethroids that were tested (MALVECASIA 2005). In northern Vietnam, at the border with China, *An. minimus* species A and C were found resistant to pyrethroids. But, insecticide resistance in Vietnam was only observed in low or transmission free areas so that there is no need to change the malaria control strategy that is currently implemented in Vietnam.

However, due to the high levels of insecticide resistance observed in southern Vietnam and potential risk for epidemics, there is an urgent need to assess the operational consequences of the observed insecticide resistance.

[*] The number in brackets [ ] refers to the note p. 566.

For this purpose, experimental houses will be constructed to evaluate the efficacy of existing vector control tools (*e.g.* insecticide impregnated bed nets) on wild resistant populations of vector species.

8. Conclusions

The goal of malaria control is not to eradicate malaria mosquitoes but anophelism without malaria has been achieved in temperate countries and in large areas of northern Vietnam. Relatively high incidence is still observed in the remaining forested areas, the biotope of *An.dirus s.l.* Transmission still occurs inside the villages located close to the forest edge, but the burden of the disease is mainly associated with forest activity (*e.g.* logging, farming, and mining). Moreover anopheline species other than main vector species could be involved in malaria transmission, hence, intensified research efforts on these so called 'secondary vectors' in different ecological settings is needed. Impact of land use, environmental and human behavioural changes on the distribution and abundance of vectors should be further explored to understand the complexity of malaria transmission in the Mekong Countries. This will allow to progressively assessing the risk of malaria transmission in a fast changing environment. For this purpose a Geographical Information System (GIS) is now under development to analyze the distribution of *Anopheles* species to environmental parameters. All the information has been integrated into a simple visualization tool, SEAGIS.

The most efficient vector, *An. dirus s.s.*, is exophilic, exophagic and bites early (before 22:00) so that ITNs and IRS have little impact on transmission. Thus, innovative interventions targeted to forest workers and taking into account the vector bionomics are urgently needed (ERHART *et al.* 2006). Insecticide resistance observed in the Mekong Delta and to a less extent in northern Vietnam is not yet a major issue as it occurs in very low or non-endemic areas. However further monitoring of insecticide resistance is recommended and operational implication of existing resistance should be assessed.

The most difficult factor to control is certainly human migration. Seasonal workers from non endemic areas with low immunity against malaria work in the endemic foci of forested provinces. When they go back to the malaria free areas, these potential malaria reservoirs can again contaminate the local malaria vectors and annihilate years of control efforts. Therefore, the maintenance of a good surveillance system including the most remote areas, in particular forested borders, is crucial for the future (VERLE *et al.* 1998, ERHART *et al.* 2006).

NOTE

[1] MALVECASIA is a research network studying the distribution and insecticide resistance of malaria vectors in Southeast Asia (Vietnam, Cambodia, Laos and Thailand). Partners are the Institute of Malariology, Parasitology and Entomology (Vietnam), the Centre of Malariology, Parasitology and Entomology (Laos), the National Center for Malaria Control, Parasitology and Entomology (Cambodia), the Mahidol University (Thailand), the Liverpool School of Tropical Medicine (UK), the Institut de Recherche au Développement (France), The Natural History Museum (UK) and the network is coordinated by the Institute of Tropical Medicine (Belgium). The project is supported by the INCO-DC programme of the European Commission (IC4-CT-2002-10041) and by the Belgian Cooperation.

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