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Malaria and dengue vector biology and control in Southeast Asia

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Abstract

This chapter reviews the situation of vector biology and control of both malaria and dengue in the Southeast-Asian region as part of the World Health Organization (WHO/TDR) working-group meeting on strategic planning to bridge laboratory and field research in disease vector control. Many research studies on malaria were related to the survey of malaria vectors and parasites and their spatial and temporal distribution in each country. A few studies demonstrated application of molecular tools to identify sibling species in the vector complexes as well as the genetic structure and gene flow among these complex species. Despite insecticide resistance having been detected in many vector species, insecticide-impregnated bednets are still reported as a cost-effective and efficient way for malaria control. Social-science and socio-economic studies indicate that the level of education and poverty is related to the risk of malaria infection and also emphasize the importance of education as part of successful control programmes. The majority of research on dengue vectors in Southeast Asia involves surveillance for species composition, relative abundance and seasonal distribution of both immature and adult stages. Identification of key breeding containers and patterns of landing/biting of adults are routinely investigated in the study areas. Some aspects of vector ecology and vector biology related to the symbionts of the mosquitoes have been reported. Several studies have pointed out the importance of human transportation as a means for spreading dengue. Recent studies also demonstrated that the disease spread from the larger cities, which serve as the viral reservoirs, to smaller communities in a radial manner. Several socio-economic studies in different countries indicate variations in knowledge and practice related to dengue. Dengue control programmes in Southeast Asia have recently shifted from application of insecticides to integrated vector control strategies using biological control agents, pyrethroid-based insecticides, source reduction and environmental management. However, most of the present vector control measures are not sustainable due to several factors related to both community participation and persistence of public-health vector control programmes. Genetic control using modern molecular technologies may offer novel solutions for future control of vector-borne diseases.

Keywords: *Aedes*; *Anopheles*; dengue; malaria; mosquito; vector; Southeast Asia

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Malaria

Spatial distribution of malaria vectors and parasites

Malaria remains an important health threat in rural areas of Southeast Asia. All four known human malaria parasites, but predominantly *Plasmodium falciparum* and *P. vivax* are present in the region. Members of three species complexes of *Anopheles* mosquitoes, *An. dirus*, *An. maculatus* and *An. minimus*, are the most important vectors. In Vietnam, malaria is found in mountainous and woody areas as well as in coastal regions. The main vector is *An. dirus*, which is found in stagnant and shaded waters in forested areas, whereas *An. minimus*, another vector species, breeds in running streams in hilly areas. In contrast, *An. sundaicus*, a coastal vector species, adjusts to a variety of habitats. *P. falciparum* and *P. vivax* occur at about the same rate except for the woody regions where *P. falciparum* is more prevalent (75%) (Nguyen 1993).

A preliminary survey of *Anopheles* in 8 provinces in Laos showed that out of 19 species collected, *An. aconitus* is the predominant species, especially in the month of December, and only 3 species, i.e. *An. dirus*, *An. maculatus* and *An. minimus*, are infected with oocysts (Vythilingam et al. 2001). Another malaria survey in one of the southeastern provinces of Laos has reported 28 species collected by both human and animal baits. Sporozoites of both malaria parasites were found in *An. dirus* and *An. minimus* as well as in *An. philippinensis*. Four species, *An. notanandai*, *An. sawadwongporni*, *An. willmori* and *An. Hodgkini*, were recently reported for the first time in this region (Toma et al. 2002). A field survey for malaria prevalence in southeastern Laos by PCR assay has shown that the most common malaria parasite is *P. falciparum*, and mixed infection by 2-4 species of parasites was detected in 23.1% of the samples (Toma et al. 2001). Interestingly, there is a report that *P. falciparum*, but not *P. vivax*, is associated with acute malnutrition among youths in Laos (Takakura et al. 2001).

In Myanmar, *An. dirus* is reported to be one of the primary vectors of *P. falciparum* causing cerebral malaria. The positive rate during 1998-2000 in Bago, Mandalay and Tanintharyi Divisions ranged from 9.9% to 34.3% (Oo, Storch and Becker 2003). A recent entomological survey conducted in Vietnam, Laos and Cambodia showed that *An. dirus* A is still an important malaria vector despite its low density, whereas the role of *An. minimus* A in malaria transmission varies both temporally and spatially. The brackish-water-breeding species, *An. sundaicus* occurs in high density due to the recent changing patterns of land use from rice cultivation to shrimp farming (Trung et al. 2004).

In peninsular Malaysia, *An. maculatus* is the main vector of malaria even though its abundance is only 9.1%, which is about half of the average number of the other two dominant species, *An. aconitus* and *An. barbirostris* (Rahman, Adanan and Abu Hassan 2002). In Indonesia, malaria is common throughout the country and *P. vivax* is the most abundant malaria parasite (Rodhain 2000). The primary vector species, especially in forested hilly areas of Java, are *An. maculatus* and *An. balabacensis* (Barcus et al. 2002).

Analysis of the distribution of malaria-endemic areas in the Indochina peninsula has been conducted using GIS/remote-sensing techniques. These are useful for identification of endemic malaria based on the normalized difference vegetation indices (NDVI) (Nihei et al. 2002). GIS analysis was also used to study distribution of the larval stages of *An. flavirostris*, a principle malaria vector in the Philippines. The study has shown that early larval instars are clustered in shady stream-bank areas,

whereas the late instars are weakly related to shade (Foley, Torres and Mueller 2002). The GIS-based spatial patterns of surface slope and wetness were used to identify the distribution of breeding sites of four major malaria vectors, *An. dirus*, *An. maculatus*, *An. minimus* and *An. sawadwongporni* in Northern Thailand (Sithiprasasna et al. 2003b). Spatial and temporal distribution of *Anopheles* mosquitoes in the same area was reported. A total of 21 species were collected and 86% of species biting humans were *An. minimus*, which was found to be infected with both *P. falciparum* and *P. vivax* (Sithiprasasna et al. 2003a). Konchom et al. (2003) studied malaria incidence during 1991-2001 in 30 highly endemic provinces along the Thai borders. They reported the trend of malaria parasite species shifting from *P. falciparum* to *P. vivax* along the western border to Myanmar and northern border to Laos as well as along the eastern border to Cambodia, while the opposite trend of parasite distribution was found in the southern border to Malaysia. There was also a significant difference in annual parasite incidence between border and non-border especially along the border with Myanmar and Cambodia. A survey on malaria among mobile Cambodians at the Thai-Cambodia border reported an overall infection rate of 2.4% with 93.8% of the infections being due to *P. vivax* (Kitvatanachai et al. 2003).

Population genetics and identification of *Anopheles* species complexes

Understanding population genetics of mosquito vectors is important in planning malaria control. In Thailand, microsatellite markers have been developed for studying genetic variations in natural populations of *An. maculatus* (Rongnoparut et al. 1996). A large number of alleles and high polymorphisms have demonstrated the usefulness of these microsatellite markers in studying gene flow and population-genetic structure of this vector species. High levels of genetic diversity in a small population of *An. maculatus* were detected in the above study. Population structure and population history of *An. dirus*, the main vectors of malaria in Southeast Asia, were studied using sequence analysis of the mitochondrial COI gene (Walton et al. 2000). This study reported that *An. dirus* A extends eastward from Thailand to Laos, Cambodia and Vietnam while *An. dirus* D extends westward through Myanmar. Both species are parapatric but there is little genetic differentiation either within or between species. *Anopheles dirus* C has a patchy distribution along the Thai-Myanmar border and also extends southward into peninsular Thailand. However, no gene flow between populations of *An. dirus* C has been detected. Because of greater genetic diversity in species D, it has been hypothesized that population expansion occurred first in this species and subsequently in species A.

In Southeast Asia, the presence of species complexes makes it more difficult to identify the vector species correctly, which may lead to the wrong target in vector control. In general, members of the sibling species usually exhibit behavioural differences. Two species within the *An. minimus* complex, with differences in resting and biting behaviours, have been discovered in Vietnam using isozyme electrophoresis (Van Bortel et al. 1999). In central Vietnam, the misidentification of *An. minimus* as *An. varuna* was a good example of the importance of the correct identification of vector species in order to implement malaria control effectively. Both *Anopheles* species are different in feeding behaviour, i.e., *An. varuna* is highly zoophilic and is not considered to be a vector, whereas *An. minimus* feeds on both animals and humans and has been confirmed as a vector (Van Bortel et al. 2001). Recently, a multiplex PCR assay was developed to identify the members of the *An. minimus* complex as well as other closely related species, i.e., *An. aconitus*, *An.*

pampanai and *An. varuna* (Phuc et al. 2003). This technique can be applied to all life stages and is simpler, quicker and cheaper than previous assays.

Insecticide resistance and behaviour of malaria vectors

Development of insecticide resistance among vector species in the Southeast-Asian region is an important factor leading to failure in malaria control. Insecticide resistance patterns in mosquito vectors in Thailand have been reported by Chareonviriyaphap, Aum-aung and Ratanatham (1999) and Prapanthadara et al. (2000). Behavioural responses of *An. minimus* to DDT, deltamethrin and lambda-cyhalothrin were evaluated using an excito-repellency escape chamber. Both colony-reared and wild populations of *An. minimus* exhibited insecticide-avoidance behaviour, i.e., contact irritancy and non-contact repellency (Chareonviriyaphap et al. 2001). The behavioural avoidance response to insecticides, which was the first sign of insecticide resistance, was detected not only in *An. minimus* but also in three other malaria vectors, i.e. *An. dirus*, *An. maculatus* form B and *An. sawadwongporni*, regardless of insecticide susceptibility, age, nutritional or physiological status (Chareonviriyaphap, Prabaripai and Bangs 2004). Later, the change of feeding behaviour in natural populations of *An. minimus* A and C in responding to DDT spraying was reported. Both species tend to feed on cows rather than humans and there was no preference for indoor, outdoor or forest biting (Rwegoshora et al. 2002). Recently, seasonal abundance and blood-feeding activity of *An. minimus* was studied in Western Thailand (Chareonviriyaphap et al. 2003). Results indicated that this species is more abundant during the wet season and that the human-biting peak in this area is different from other areas suggesting site-specificity in feeding behaviour. This study concluded that site-specific studies were necessary to evaluate vector behaviour accurately as it relates to malaria transmission.

Malaria vector control

Malaria control programmes in Southeast Asia are quite difficult to accomplish due to the presence of vector species complexes and insufficient information on the feeding behaviour of vectors, as well as the resistance of vectors to insecticides. Malaria vector control in this region has recently shifted from routine, residual space-spraying inside houses to the use of pyrethroid-impregnated bednets. In Laos, impregnated bednets have recently been reported to reduce malaria transmission successfully (Kobayashi et al. 2004). Despite a successful reduction of malaria in Thailand, re-emergence of the disease was evident by an increase of the annual parasite indices during 1998 (Chareonviriyaphap, Bangs and Ratanatham 2000). Evaluation of repellency and killing effects of bednets treated with etofenprox, deltamethrin, lambda-cyhalothrin and permethrin was carried out in Northern Thailand and results showed that all four insecticides have a high repellence effect. However, the problems of cross-resistance, persistence of chemicals and types of mosquito-net materials should be considered for further evaluation (Prasittisuk et al. 1996).

An evaluation of malaria control in central Vietnam showed that both spraying of insecticides in and around the houses and the use of insecticide-impregnated bednets were efficient (Nguyen et al. 1996). However, spraying with lambda-cyhalothrin was more effective than with pyrimiphos and DDT. It was recently reported that malaria in Vietnam, as well as in other Southeast-Asian countries, is related to forest activities so control efforts should target forest workers. Experiences of malaria control in refugee camps on the Pakistan-Afghanistan and Thailand-Myanmar borders had concluded that both government and non-government agencies could play a significant role in

solving issues in malaria control. Moreover, integration of research within implementation programmes may result in innovation and sustainable malaria control (Rowland and Nosten 2001).

Social sciences and socio-economics related to malaria

Social factors related to malaria occurrence have been studied in Eastern Thailand (Butraporn, Sornmani and Hungsapruak 1986). This study demonstrated that poor education and low income as well as long residency and frequent forest association led to a high risk of malaria infection. A study on the behaviour dealing with self-prevention of malaria among mobile populations in Eastern Thailand indicated that the age group of 30-39 years old has the highest risk due to periodic movement into the forested areas. Their moderate knowledge of, and attitude to malaria does not enable them to protect themselves against it (Butraporn et al. 1995). A more recent study in Thailand also indicated the importance of both socio-economic and cultural factors affecting malaria control programmes (Panvisavas 2001; Panvisavas, Dendoung and Dendoung 2001). In Laos, a study on knowledge and behaviour of people regarding prevention of malaria, conducted in 1999-2000, showed that the level of malaria prevention was related to the level of education (Uza et al. 2002). Health education in the target community is, therefore, an important component for the success of vector control programmes.

In Thailand, the use of impregnated bednets to prevent malaria in children is high among mothers who have knowledge about the disease (Sri-aroon et al. 1998). The cost-effectiveness of lambda-cyhalothrin-treated bednets was evaluated against the use of DDT spraying and malaria surveillance in Western Thailand. Results showed that the bednet programme was most cost-effective when compared to DDT spraying and malaria surveillance (\$1.54 versus \$1.87 and \$2.50 per case of prevented malaria) (Kamolratanakul et al. 2001). A pilot malaria control programme using DDT-impregnated bednets in Laos during 1995 to 1997 was evaluated. The villages where treatment occurred showed a significant increase of the number of bednets used and a significant decrease of malaria infection when compared to control communities. In addition, this study reported that risk factors were related to occupation, location of the house and use of mosquito nets (Philavong et al. 2000).

Dengue and dengue hemorrhagic fever

Biology and ecology of dengue vectors

In Southeast Asia, both *Aedes aegypti* and *Ae. albopictus* are important vectors of dengue (DF) and dengue hemorrhagic fever (DHF). Several studies on *Aedes* vectors in this region have reported the distribution and abundance of both immature and adult stages. In Sarawak, Indonesia, a survey for larvae of *Ae. aegypti* and *Ae. albopictus* in urban housing indicated that both species shared habitats in houses (9%) and in vacant land (4.5%) (Seng and Jute 1994). In South Sulawesi, Indonesia, *Ae. aegypti* was found mainly in earthen jars indoors while *Ae. albopictus* bred mainly in drum cans in hilly and mountainous areas (Ishak et al. 1997). In Thailand, the biology of both dengue vectors on Samui Island was reported (Thavara et al. 2001). The larval habitats of both species were distinctly separated, i.e., *Ae. aegypti* preferred to breed in earthen jars and concrete water storages while *Ae. albopictus* bred in coconut husks and coconut floral spathes that held rain water. *Aedes aegypti* eggs were not detected in outdoor ovitraps at a distance of 1.5 meters from houses and 75.4% of mosquitoes biting indoors in the daytime were *Ae. aegypti*. The survey for dengue vectors in five

geographical zones of Thailand demonstrated that *Ae. aegypti* predominates in all areas whereas *Ae. albopictus* is restricted to the southern part of the country. As previously reported, water jars are the most important breeding sites of *Ae. aegypti*, while broken cans and plastic containers are the preferred breeding habitats of *Ae. albopictus* (Chareonviriyaphap et al. 2003). An ecological survey of dengue vectors carried out in central Laos during the year 2000 reported that *Ae. aegypti* is dominant among 7 species collected. The key habitats are water jars, cement water tanks, drums and discarded containers, while containers containing *Mesocyclops* do not have *Aedes* larvae (Tsuda et al. 2002).

Longitudinal studies on dengue vectors were conducted in Thailand. Seasonal distribution of *Aedes* larvae in Eastern Thailand reported that even when the larvae are less abundant during the dry season, every part of the studied villages have some of them (Strickman and Kittayapong 2002). This study suggests that vector control in Southeast Asia should concentrate in schools or areas with greatest abundance based on the calculation of larval indices. Another study in Eastern Thailand also showed that breeding containers with high larval nutrients produce large numbers of pupae and large-sized mosquitoes. An estimate of the number of females per house was above the threshold for increasing transmission in all months except from December to February. The number of pupae per house and local temperature were used to calculate transmission risk using Focks' model. Results indicated that the risk is greatest in the months of May and June (Strickman and Kittayapong 2003). Studies on the population dynamics of *Ae. aegypti* in Thailand showed that temperature, but not rainfall, is correlated with female abundance. In addition, high temperature may increase age distribution of young adults and frequent blood feeding due to rapid reduction of energy reserves (Scott et al. 2000b). Blood-feeding behaviour of mosquito vectors is important for the understanding of dengue transmission. Multiple blood feeding and micro-movement to obtain blood sources have been confirmed for *Ae. aegypti* using PCR-based identification of human-blood meals (Chow-Shaffer et al. 2000). In Thailand, it was found that 65% of *Ae. aegypti* feed twice on the same day (Scott et al. 2000a). Mark-release-recapture studies in Thailand indicated that the survival rate of *Ae. aegypti* was age-dependent. Traditional linear regression analysis showed that the survival rate of older females was significantly greater than that of younger ones whereas the more sensitive non-linear regression analysis could not detect differences in the survival rate of both age cohorts in Thailand (Harrington et al. 2001).

Field studies in Thailand concerning the *Wolbachia* endosymbiont of *Ae. albopictus* have been reported. These bacteria are found to infect several species of Southeast-Asian mosquitoes (Kittayapong et al. 2000) and might be used in genetic control through cytoplasmic-incompatibility-induced population replacement. In nature, *Aedes albopictus* is double-infected with these bacteria whereas *Ae. aegypti* has never been reported to be infected. Recently, stable infections of *Wolbachia* in *Ae. aegypti* have been successfully obtained and it was found that these transinfected lines do not exhibit differences in fitness when compared to naturally uninfected populations (Ruang-areerate et al., unpubl. data). Cross-mating between *Wolbachia*-infected and uninfected *Ae. albopictus* has shown that *Wolbachia*-mediated cytoplasmic incompatibility in field-caught and laboratory-reared old-aged *Aedes albopictus* is very strong (Kittayapong et al. 2002). Maternal transmission and field prevalence of 100% in natural populations of *Wolbachia*-double-infected *Ae. albopictus* in Thailand support the potential application of these bacteria as gene-driving mechanism (Kittayapong et al. 2002; Kittayapong, Baimai and O'Neill 2002).

Population genetics, vector competence and dengue transmission

DF and DHF have long been reported as the most common urban diseases in the Southeast-Asian region since the 1950s, before spreading worldwide. All four serotypes of dengue viruses co-circulate in this region (Gubler and Kuno 1997). A study showing the spread of DHF by travellers from East Timor to Townsville, Australia evidenced the importance of humans as vehicles for disease spreading (Hills et al. 2000). There has also been a report on the high risk of DHF in the area where foreigners work for petroleum companies in Indonesia, subsequently returning to their home countries (Mangara et al. 2000). Seroprevalence of dengue virus among German overseas aid workers was found to be 6.4% (43/670), and of these 43, the highest seroprevalence (19.4%) was detected in those returning from Thailand (Eisenhut, Schwarz and Hegenscheid 1999). As reported from the study by Harrington et al. (in press) in Thailand, the human-movement factor may perhaps be more important in the spreading dynamics of the disease than the dispersal and flight range of *Aedes* vectors. This idea is supported by the recent estimates of population-genetic organization and gene flow in *Ae. aegypti* using microsatellite markers (Huber et al. 2004). This study showed that there is less genetic differentiation between mosquito populations from Vietnam (Ho Chi Minh City) and Cambodia (Phnom Penh) than between either of them and Thai populations, suggesting that passive migration through human transportation is the major cause of vector spreading.

Genetic structure of *Ae. aegypti* was studied in Vietnam in relation to vectorial competence and resistance to insecticides (Huber et al. 2003). Estimation of population-genetic organization and gene flow showed that ecological disturbance through urbanization, which had direct impact on sanitation, has a direct impact on the vectorial system. The relationship between genetic differentiation and vector competence for dengue-2 virus has been reported (Huber et al. 2002a). Genetic variations in Ho Chi Minh City and its outskirts were studied using starch-gel electrophoresis and microsatellite markers. Results showed that genetic differentiation is lower in the city when compared to its outskirts, depending on the abundance of breeding sites and human hosts as well as the insecticidal control during dengue outbreaks (Tran et al. 1999; Huber et al. 2002b). Moreover, seasonal and environmental factors also had an effect on the genetic structure of *Aedes* vector populations (Huber et al. 2002c). In Thailand, genetic differentiation was confirmed in *Ae. aegypti* samples collected from different subdistricts. Results may be related to insecticide treatment in these areas (Mousson et al. 2002). In conclusion, further studies on genetic variations of vector populations are required to provide further insights into the understanding of disease epidemiology.

A study on the major mosquito fauna in the forested area undergoing development of an oil palm plantation in Sarawak, Malaysia showed the reduction of the species composition of malaria vectors and the risk of malaria transmission but, on the other hand, an increase of dengue vectors and the risk of dengue transmission (Chang et al. 1997). Evidences for dengue infection in both vector species, i.e., *Ae. aegypti* and *Ae. albopictus*, were reported from Samui Island, Thailand (Thavara et al. 1996). Transovarial transmission of dengue viruses was reported in Singapore. Males of both species were found positive with dengue viruses using a type-specific PCR technique. The serotypes were checked and the results showed a negative correlation between DEN-1 and DEN-4. DEN-1 was higher than DEN-4 in *Ae. aegypti* while the opposite was detected in *Ae. albopictus* (Kow, Koon and Yin 2001).

A study in Selangor, Malaysia revealed a positive correlation between a dengue outbreak and rainfall pattern, which increased the number of breeding habitats of

Aedes vectors (Li et al. 1985). Biological and entomological parameters related to a seasonal pattern of dengue using a mathematical model reported that the strongest influence on the seasonality and pattern of dengue transmission is the duration of infectiousness of the host, vector mortality and biting rates (Bartley, Donnelly and Garnett 2002). Susceptibility to dengue viruses among *Ae. aegypti* mosquitoes collected in different seasons of the year showed no seasonal correlation, even though a seasonal pattern of dengue transmission was observed in Thailand. It was suggested that characteristics of the virus, vector density and frequency of host–vector contact should be considered instead (Thongrunkiat et al. 2003).

Distribution of dengue and Japanese encephalitis among children in rural and sub-rural areas in Thailand has been studied. The results showed that most transmission occurs in residential environments and within a young age group (3-8 years old), which has a significantly higher risk of infection than older children (Strickman et al. 2000). An epidemiological study of DHF in Thailand suggested that vector control activities should concentrate on areas and populations at higher risk (Barbazan, Yoksan and Gonzalez 2002). Integration of geography and pathology for DHF is required to understand the complex epidemiology of the disease, which depends on a variety of factors (Menard 2003). Recently, the spatial and temporal studies of DHF incidences in all 73 provinces of Thailand (1983-1995) could discriminate between seasonal and non-seasonal transmission. The spatial-temporal dynamics of DHF incidence using the data set of 850,000 infections in Thailand from 1983 to 1997 showed that the disease occurred first in Bangkok, the largest city and capital of Thailand, and then moved radially at the speed of 148 km per month. This finding provided a crucial piece of information, namely that the permanent viral reservoir is to be found in large cities; these should therefore be the targets for long-term control of DHF (Cummings et al. 2004).

Dengue vector control

Up until the present, there has been no promising solution for sustainable control of dengue vectors. The trend for dengue vector control in this region has shifted from relying solely on insecticides to biological control, source reduction and environmental management through community participation (Gubler and Kuno 1997). Several countries in the region have recently carried out integration of vector control approaches.

In Vietnam, a survey for *Mesocyclops*, *Micronecta* and fish as biological control agents demonstrated that a large number of breeding containers already contained *Mesocyclops* and the presence of both *Mesocyclops* and *Micronecta* provided some level of control (Nam et al. 2000). A few years later, a successful dengue vector control programme was initiated in three provinces of Northern Vietnam with an application of the biological control agent, *Mesocyclops* spp., and clean-up campaigns through community participation (Kay et al. 2002). Recently, prolonged efficacy in controlling *Aedes* larvae in water containers by a combination of *Bacillus thuringiensis israelensis* (Bti) and copepods was evaluated in Thailand (Kosiyachinda, Bhumiratana and Kittayapong 2003). The enhancement of control activities was observed when rice grains were used as supplementary food for copepods.

From Malaysia, there are few reports on high efficiency of a combination of biological and chemical insecticides (Seleena, Lee and Chiang 2001; Sulaiman et al. 1997; 1999; 2000). The details of dengue vector control and dengue situation in Malaysia were discussed by Poovaneswari (1993), Tham (1993), and Yap et al.

(1994). Singapore has a well-established system for dengue vector surveillance and control. The control strategy integrates case detection, source reduction, health education and law enforcement (Wang 1994). Application of an autocidal ovitrap as a vector control measure against *Ae. aegypti* was developed in Singapore since 1977 by Lok, Kiat and Koh (1977). Recently, a mixture of Bti (Vectobac 12 AS) and pirimiphos-methyl (Actellic 50 EC) applied through thermal fogging at various heights and distances was tested as an efficient approach for simultaneously controlling both larvae and adults of *Ae. aegypti* in Singapore. However, there is a report from Central Java, Indonesia of an unsuccessful vector control trial when *Toxorhynchites* was used as a biological control agent (Annis et al. 1990).

In Thailand, Gratz (1993) pointed out that dengue vector control, as in most other countries, had made little use of the methodologies arising from research. After a long routine application of insecticides as a vector control measure, the trend in Thailand was recently geared toward environmental protection (Chunsuttiwat and Wasakarawa 1994). Several studies were conducted in Thailand regarding the use of repellents, physical and biological control agents. Three types of screen covers were developed for preventing vectors from breeding in water jars, which were the most common and important breeding site of *Ae. aegypti* in Southeast Asia (Kittayapong and Strickman 1993). Thanaka (*Limonia acidissima*) and DEET (di-methyl benzamide) mixture were evaluated for their efficacy as repellents against both *Anopheles* and *Aedes* vectors in Thailand. Almost complete protection up to 6 hours was obtained with a combination of 20% thanaka and 0.5% permethrin. Laboratory bioassays with *Ae. aegypti* indicated that the combination could extend protection from exposure up to 10 hours (Lindsay et al. 1998). The Thai strain of mosquito densovirus infecting both *Ae. aegypti* and *Ae. albopictus* was reported to be efficient in killing *Aedes* larvae and could be developed as a biological control agent (Kittayapong, Baisley and O'Neill 1999). Evaluation of Bti tablets and temephos ZG were conducted in Thailand. Results showed that at the dosage of 0.37 g per 50 l of water could provide control activities between 90 and 112 days while a new formulation of zeolite granules (ZG) of temephos (1%) at the operation rate of 5 g per 50 l of water yielded 100% control for more than 6 months. Both larvicides increased clarity of the water with no unpleasant odour (Mulla et al. 2004).

As synthetic pyrethroids are still used in the public-health system to control vector-borne diseases, the development of insecticide resistance in vectors remains an issue of concern. Some information on vector resistance to pesticides in Thailand was reported by Chareonviriyahpap, Aum-aung and Ratanatham (1999). Brengues et al. (2003) reported the presence of permethrin resistance in a few localities in Southeast-Asian countries, i.e., Indonesia, Thailand and Vietnam. The evidence of both DDT and pyrethroid resistance in Indonesia and Vietnam suggested the presence of a knock-down resistant *kdr*-type mechanism.

Social science and socio-economics related to dengue

It has been reported that health education had an effect on the outcome of a DHF vector control programme in an urban area of Northern Thailand by reducing the Breteau index to about half (from 241 to 126) (Swaddiwudhipong et al. 1992). A recent study on climatic and social risk factors for *Aedes* infestation in rural Thailand reported that factors such as availability of public water wells, existence of transport services and proportion of tin houses, were positively associated with larval indices (Nagao et al. 2003). Socio-economic factors, i.e., per capita gross provincial product (GPPpc) and health-care resources in relation to geographic distribution of malaria

and dengue in Thailand, were also examined. It was recommended from the study that this approach be used for considering resource utilization in integrated control of both diseases (Indaratna et al. 1998). In Northern Thailand, a KAP (Knowledge, Attitudes and Practices) survey reported that 67% of people in the study area had knowledge of dengue, significantly different with respect to age, sex and occupation. Young students had a higher level of knowledge of dengue when compared to older housewives and unemployed persons. In addition, people with knowledge of dengue reported more frequently the use of preventive measures against vectors (Van Benthem et al. 2002). Another KAP survey was carried out among the caretakers of DHF in primary schools in one province in central Thailand. The majority of people in this study area were mothers with primary-school education. Results indicated that they need more understanding of the disease. In general, the caretakers whose children had DHF had higher response in prevention, control and treatment than the group who had healthy children (Kittigul et al. 2003). Both studies demonstrated the importance of educational campaigns to obtain community participation in DHF control. A KAP survey recently reported from Malaysia reflected that most people have a high level of knowledge about dengue and vector control (Hairi et al. 2003). In addition, both were significantly correlated with the attitude towards *Aedes* control. However, there was no correlation between the level of knowledge and the vector control practice, which implied that a high level of knowledge did not necessarily lead to good practice. Therefore, differences in social factors and cultures in each country need to be considered for planning educational programmes.

Conclusions

In conclusion, long-term vector control approaches include source reduction and environmental management, chemical and microbial larvicides, and personal protection using household insecticide products and repellents. Space spray, both thermal fogging and ultra-low volume, is normally used as short-term control measure, especially during disease epidemics. Practical vector control approaches in Southeast Asia rely on persistent efforts by the government sectors as well as communities themselves leading to variations in the success of vector control programmes. Planning and decision-making for resource utilization for malaria and dengue control at both national and regional levels could be more efficient through co-analysis of the disease-epidemic patterns and utilization of health-care resources. Due to the fact that both malaria and dengue vaccines are still under development and the current vector control programmes hardly provide a long-lasting effect, transgenesis-based development of refractory strains of mosquito vectors that are refractory to disease pathogens may offer a new alternative for disease control, which could be successful regardless of the level of community participation. However, genetically modified mosquitoes need to be very efficient in spreading themselves and competing with natural vector populations. In addition, acceptance of the strategy by the community itself will be an important issue. Therefore, the biology and ecology of vectors as well as social and socio-economic factors in different geographic regions need to be investigated in more detail to confirm the possibility of application.

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