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Vector Biology and Malaria Transmission in Southeast Asia

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1. Introduction

There are two primary geographical divisions within an area collectively called Southeast Asia, Mainland SEA and Maritime SEA. Mainland SEA includes Cambodia, Lao PDR, Myanmar, Thailand, Vietnam and Peninsular Malaysia. The first five countries including Yunnan Province, southern China is referred to as the Greater Mekong Subregion (GMS). Maritime SEA consists of Eastern Malaysia (Sarawak and Sabah States located on Borneo Island), Brunei Darussalam, Indonesia, Philippines, Singapore and East Timor (Timor-Leste). Most of these areas are at risk for a variety of vector-borne diseases, especially malaria, one of the most important diseases transmitted by mosquitoes in the genus *Anopheles*.

Despite over 100 years of scientific investigation, malaria remains the leading cause of death among children living in Sub-Saharan Africa and every year is responsible for more than 200 million clinical infections worldwide. The World Malaria Report in 2011 estimated that the number of malaria cases rose from 233 million in 2000 to 244 million in 2005 then dropped to 225 and 219 million in 2009 and 2010, respectively [1, 2].However, mortality from malaria has decreased by over 26% globally since 2000 due to the increased availability of long-lasting insecticide-treated nets, indoor residual spraying, and better access to diagnostic and effective treatment using artemisinin-based therapies (ACTs) [3]. In Thailand, the malaria incidence has markedly decreased over the past 60 years in response to organized malaria control programs [4, 5] and other countries like Vietnam have made great strides in reducing both incidence and mortality in recent decades [6,7]. During the past two decades, significant reduction in malaria

cases has also been reported in Cambodia, Laos, and eastern Malaysia, [8, 9,10]. During this same period, Myanmar and East Timor reported either no change or an increase in the number of cases; however the coverage of control activities appeared to be limited in relation to the total population at risk. The confirmed malaria cases in Myanmar increased by more than 16-fold between 2000 and 2009, primarily the result of an increased availability of parasitological diagnosis by both microscopy and RDTs [1, 9, 10]. Several countries have advanced a great deal in tackling malaria transmission and providing ready access to diagnosis and treatment using artemisinin-based combination therapies (ACTs) against *Plasmodium falciparum*, the most deadly form of malaria parasite, with treatment success >90% of cases. However, resistance to artemisinin-based compounds has already emerged along the Thai-Cambodia border, a similar pattern of resistance that begun with chloroquine, followed by sulfadoxine-pyrimethamine and mefloquine, common drug treatments used in malaria control years ago [11,12].

Malaria transmission continues with high risk in refractory foci, especially areas near the international borders between countries, such areas are commonly associated with rural, forested, undeveloped and sylvan environments compounded by frequent uncontrolled human population movement across shared borders for economic and socio-political reasons [13,14,15,16,17,18]. Other contributing epidemiological factors have either maintained or even enhanced transmission potential in certain areas including various factors that contribute to malaria mosquito vector distribution, vector competency and capacity for transmission, bionomics, adult behavior, and abundance. Contributing factors also include physical and topographical changes such as new development projects including dam and road construc-tion, mining, reforestation, deforestation and commercial plantations (e.g., rubber, palm oil). Deforestation is particularly severe and widespread in Southeast Asia, the highest relative rate of deforestation of any major tropical region in the world. By year 2100, it is estimated that over three quarters of the original forests and up to 42% of the associated biodiversity will result in massive species declines and outright extinctions [19].

Outdoor transmission and biting immediately after dusk and early morning hours continue to pose a major prevention and control challenge. Additionally, population movement and congregation increase the likelihood of exposure to malaria and reintroduction of transmission in receptive areas. To understand malaria risk in an area, the *Anopheles* fauna and bionomics of the important species including those composed of complexes must be better understood. Unfortunately, there are only a few recent studies in each country which cannot provide a complete picture on malaria vectors in this region. Because the accurate identification of vector species and knowledge of their ecology and behavior is essential for epidemiologic studies and the design and implementation of vector control strategies, a major challenge in most countries in the region is the lack of trained entomologists and budgets supporting essential field and laboratory work. Our aim in this chapter is to provide an overview on the malaria vectors of the Greater Mekong Subregion, in which 6 countries are reviewed. Thailand represents the epicenter of the Mekong countries from northwest to southwest (Myanmar), the eastern border (Cambodia & Vietnam), northeast (Lao PDR) and the southern border (Malaysia). The focus will be on reviewing the current malaria transmission in relation to the

various malaria vectors, with an emphasis on the geographic variation, vector biology and ecology of each species and how these factors promote malaria transmission in the region.

2. Malaria transmission and primary vectors in mainland Southeast Asia

Review of mosquito biogeography has shown that the greatest mosquito biodiversity occurs in the SEA region and the Neotropics, with high species richness in Indonesia, Malaysia and Thailand [20, 21, 22]. The basic malaria transmission equation (model) indicates a positive correlation between vector density (and life span) in relation to attack on humans and number of malaria cases; however, even small changes in vector density can result in substantial changes in the proportion of humans infected [23]. This is more apparent in areas of relatively lower transmission than those with stable high attack rates. Malaria stability over time is generally greater in areas with highly efficient vector(s) and those having multiple primary vector species present throughout the year or alternating activity patterns based on seasonal changes and local conditions. However, the primary inter-dependent relationship between Human – Vector – Pathogen is influenced by a fourth set of factors, namely demography (human placement and movement), numerous environmental factors, landscape (vector habitat), socioeconomic conditions, that can greatly impact malaria transmission in each country and specific locations (foci) [24, 25, 26, 27, 28, 29, 30,16]. In general, SEA is faced with a complex vector system whose members are difficult to distinguish morphologically that often include a diverse array of non-vectors, potential vectors and malaria vectors [31, 32]. As members of a species complex usually exhibit significant behavioral differences, understanding the biological, behavioral and ecological characteristics of each species will be relevant to the epidemiology and disease control methods used. Three main malaria vectors are recognized on the SEA mainland: *Anopheles dirus* sensu lato (s.l.) (Dirus Complex), *An. minimus* s.l. (Minimus Complex), *An. sundaicus* s.l. (Sundaicus Complex). The Minimus Complex comprises of three sibling species; *An. minimus* (formerly species A), *An. harrisoni* (species C) and *An. yaeyamaensis* (species E). Whereas the latter species is found only in Japan, *An.minimus* and *An. harrisoni* have a broad distribution in SEA and are known vectors of malaria throughout their respective distributions [33, 34]. *An. minimus* s.l. is widespread in the hill forested areas, utilizing mainly margins of slow running streamsunder partial shade and grassy margins [35, 36, 37, 38, 7, 34].

In these forested areas of SEA, malaria transmission can be perennial because of the presence of both *An. dirus* s.l. during rainy season and *An. minimus* s.l. during the drier periods of the year. The Dirus Complex currently includes eight species [39, 40]. Among them two main malaria vectors, *An. dirus* and *An. baimaii* which are considered forest and forest-fringe malaria vectors with an anthropophilic and exophagic behaviors. Their reproduction takes place in and near forested areas (primary and secondary evergreen, deciduous and bamboo forests) with plentiful rain water pools, puddles, as well as artificial containers. Both species are also found in dense mono-agricultural environments, in particular rubber, fruit, and manioc/cassava plantations [18, 32, 33, 41, 42]. One of the factors that make *An. dirus* an important and efficient malaria vector is its strong attraction to humans [32, 43]. The Sundaicus Complex

comprises 4 members, however only *An. epiroticus* is reported on the SEA mainland [44]. These four species are coastal vectors, developing primarily in brackish water while some populations can exist in freshwater habitats. *An. epiroticus*, has adapted to a diverse array of biotopes, but also share some common features such as brackish water (optimum 1-7 g NaCl/litre), moderate sun exposure, stagnant or slightly moving water, with floating green algae and presence of vegetation [44, 45]. *Anopheles epiroticus* exhibits both endo- and exophagy while being mainly endophilic and anthropophilic in resting and feeding preference, respectively, although both exophily and zoophily have also been demonstrated [7, 32, 46].

New insights into malaria vectors, in terms of vector bionomics and malaria transmission, are detailed within each country and are framed by the inherent complexity of the epidemiology and the current challenges faced in SEA for implementation of appropriate vector control as one of the key approaches of integrated control for eventual malaria elimination in the region.

2.1. Cambodia

2.1.1. Overview

The Kingdom of Cambodia covers an area of approximately 181,000 km² with 15 million inhabitants, comprised mainly of ethnic Khmer (90%), along with Vietnamese, Chinese and other minorities. This country is bounded on the north by Thailand and Lao PDR, on the east and southeast by Vietnam, and on the west by Thailand and the Gulf of Thailand. Much of the country's topography consists of rolling plains. Dominant geo-physical features include the large, centrally located, Tonle Sap (Great Lake) and the Mekong River, which traverses the entire country from north to south. The climate is monsoonal and has marked wet and dry seasons of relatively equal length. Both ambient air temperatures and relative humidity generally are high throughout the year. Forest covers about two-thirds of the country, but it has been degraded in the more readily accessible areas by burning (slash-and-burn agriculture), and by traditional shifting agricultural practices. Approximately 44% of the population live in high malaria risk areas among which approximately half (~3 million people) live in or around forested areas where there is potentially intense transmission [2]. *Plasmodium falciparum* is the dominant malaria infection reported (63%) followed by *P. vivax* [3]. Between 2001-2009, the number of reported cases detected by the official health system in Cambodia (confirmed cases by MOH) fell from 121,612 to 80,644 and further declined to 44,659 in 2010 [47, 1]. The main provinces with endemic malaria are Battambang, Kampong Speu, Pursat, Peah Vihear, Mondulkiri, Rattanakiri, Pailin and Siem Reab [10, 48]. Malaria transmission is seasonal with a peak occurring during May–July and October–November in the forested and forest-fringe areas of the north, west and northeast, and also in the rubber plantations located in the east and northeast parts of the country. In the rice growing areas of the south and central regions, transmission is typically low or non-existent. There is no reported endemic transmission in urban areas. Low intensity transmission is found focally in coastal areas. Malaria incidence is highest in the eastern provinces of Mondulkiri and Rattanakiri where the disease disproportionately affects ethnic minorities and migrants [8]. According to the Health Management Information System (HMIS), confirmed malaria cases is predominantly observed

in males aged 15-49 years (51%), and regarded an occupational risk [49]. Because of the decades long civil war, including the brutal genocide in the 1970's and systematic destruction of infrastructure under the Khmer Rouge regime, Cambodia was left with a very limited health infrastructure and capacity, particularly in rural areas. In recent years, this situation has seen a remarkable rebound, with the public sector providing the majority of diagnosis and treatment through both community-based and government health centers. Over the last decade, many of Cambodia's key health indicators have improved dramatically with the increased resources. Universal diagnostic testing for malaria, primarily using malaria microscopy and Rapid diagnostic test (RDTs) formats, is now common practice in the majority of Cambodian public sector facilities [50]. In addition, with both Global Fund against HIV/AIDS, Tuberculosis and Malaria and USAID support, village malaria workers and mobile malaria workers have been trained and equipped with RDTs and artemisinin-based combination treatments (ACTs) to more accurately diagnose and effectively treat malaria, thereby improving access to these services in remote rural communities. In spite of this, the quality of malaria microscopy in many facilities is regarded sub-optimal, particularly in remote locations. In facilities where both microscopy and RDTs are available, the staff prefers using RDTs because of the ease of use. Additionally, the majority of persons with fever are reported to go to private sector providers where the availability of high-quality diagnostic testing is limited and where there is a financial incentive to provide treatment (sometimes outdated, ineffective chemotherapies) to a patient with a negative test. Another challenge is that an increased prevalence of *Plasmodium vivax* would have implications on the severity of illness, risk of death, and provision of optimal drug therapies to eliminate latent, relapsing forms of the parasite; therefore identifying the parasite species is crucial for case management [51]. Further progress in reducing the burden of the disease will require improved access to reliable diagnosis and effective treatment of both blood-stage and latent parasites and more detailed characterization of the epidemiology, morbidity and economic impact of vivax malaria.

2.1.2. Malaria vectors and biodiversity of Anopheles in Cambodia

In 1975, the list of anophelines known from Cambodia was revised to include 37 species [52]. Between 1959-1963, *An. dirus* s.l., *An. minimus* s.l., *An. maculatus* and *An. sundaicus* s.l. were reported as main malaria vectors in Cambodia [53, 34]. However, there has been no record of entomology activities in the following 25 years due to socio-political issues in the country. In 1997, two years of vector surveys reported 19 and 25 species of anopheline mosquitoes in Kompong Speu and Kratie Provinces, respectively in which *An. dirus* s.l, *An. minimus* s.l and *An. maculatus* were included [53]. With molecular techniques having been developed for identifying members within the species complexes, a significant increase of anopheline species have been recorded in Cambodia. *An. minimus* has been the only species of the Minimus Complex recorded in Cambodia [54, 55, 31]. *An. minimus* was recorded as a late evening biter and more anthropophilic where cattle were scarce with the ratio of indoor to outdoor human landing collections ranging between 0.62 and 7.95 [32]. *Anopheles* specimens were found sporozoite positive by ELISA tests for the detection of circumsporozoite protein (CSP) of *Plasmodium falciparum* and *P. vivax* [7, 32]. Distribution and abundance of this primary malaria vector has changed in response to land-use modifications, deforestation, climate change, and
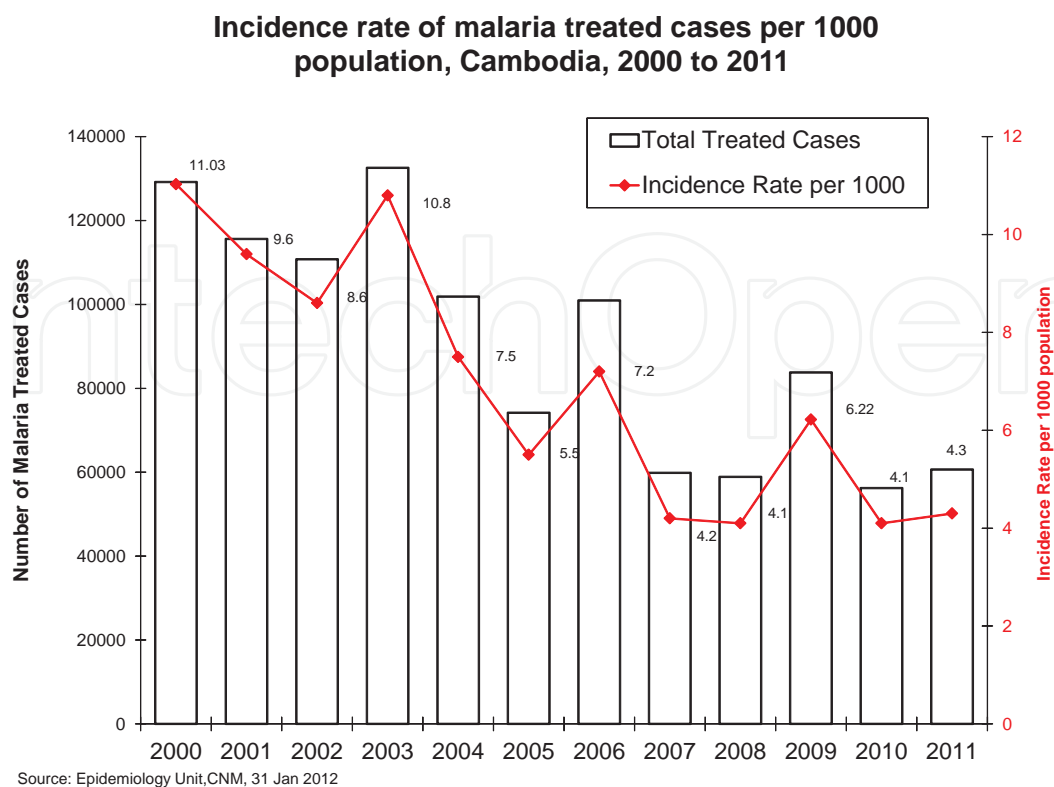
Figure 1. Malaria Incidence Rate per 1,000 population (solid line) and a total treated cases (bar) in Cambodia between 2000 and 2011. Source: Meeting on Outdoor Malaria Transmission in the Mekong Countries for 13 countries during 12-13 March 2012, Bangkok, Thailand. [http://www.rbm.who.int/partnership/wg/wg_itn/ppt/ws2/m4SivSovannar-oth.pdf.]

possibly due to insecticides used as part of vector control in malaria endemic areas [35, 34, 38, 27, 56, 41, 57]. The Dirus Complex in Cambodia is represented by *An. dirus* only which plays an important role in malaria transmission [31] with CSP rates having been reported above 1% [7]. *An. sundaicus* s.l. has been recorded along the southern coastal areas of Cambodia [58] and later identified as *An. epiroticus* (*An. sundaicus* A). Larvae of *An. epiroticus* are found in large open stagnant brackish water areas, sunlit pools, and often occurring in distinct foci along the coast [59]. In Cambodia, suspected and potential malaria vectors include *An. annularis* s.l., *An. barbirostris* s.l, *An. culicifacies* B although this latter species is mostly considered as a poor or non-vector (collected in Rattanakiri Province, northeast of Cambodia), *An. nivipes*, *An. philippinensis*, *An. sinensis*, and *An. subpictus* s.l. [54, 60]. Within the Maculatus Group, a recent study recorded for the first time *An. sawadwongporni* in the Kampong Spoe Province [31], yet its vector status in Cambodia is unknown. The Subpictus Complex has a coastal distribution in southern Cambodia [59].

2.1.3. Distribution of malaria vectors and behavior of Anopheles species in Cambodia

Forest cover is a very strong determinant of malaria risk. In SEA, forest malaria remains a big challenge for malaria control and in Cambodia malaria risk has increased within 2 to 3 km from the forest border. It is important to note that forest-related malaria covers a wide

epidemiological spectrum regard varying vector species and bionomics, human demographics and behavior and control [61]. In Cambodia, malaria transmission is closely associated with two primary malaria vectors that inhabit the forest and forest fringe, *An.dirus* which inhabits predominantly forested areas, and *An. minimus*, a relatively less efficient malaria vector, that occurs in and around rice fields near the forest fringe [7,34,31]. *An. dirus*, *An. minimus* and *An. maculatus* are mainly outdoor biters [32]. This exophagic tendency of vectors is associated with the persistence of malaria transmission among populations with outdoor activities during night time. Intraspecific behavior differences have been observed among different populations of *Anopheles* species. However, in Cambodia, *An. dirus* has shown a higher degree of anthropophily than other malaria vector species [32]. The inoculation rate of *An. dirus* has been recorded over 1% in Rattanakiri Province indicating this species is a very efficient vector and plays an important role for perennial malaria transmission [7]. *Anopheles minimus* has been found less anthropophilic, preferentially attacking animals more than humans, whereas *An. dirus* showed a higher degree of anthropophily and early biting before 22.00 hr [32]. The host and temporal feeding patterns of malaria vectors are important factors in determining the vector status of *Anopheles* species, both influenced by host availability and location (indoors or outside)[62]. The abundance of malaria vectors in Cambodia is site-specific, for example in Pailin Province, among the three main malaria vectors, *An.minimus* (67.2%) was found more predominant than *An. maculatus* (20.6%) and *An. dirus* (9.9%), while in Pursat Province, 52% of the vector species were *An. dirus*, probably influenced by the suitability of the local environmental conditions and topography [63].

The current vector control methods against indoor feeding and resting vectors include indoor residual spraying (IRS) and insecticide-treated nets (ITNs), but where the vectors primarily feed and rest outdoors, these vector control methods are ineffective, except possibly in those cases where the insecticide used has a high spatial repellent effect [64, 65]. A recent study showed a nearly 45% reduction of blood feeding *An. minimus* in two villages after introduction of long-lasting insecticide-treated hammocks (LLIH) in study sites in Pailin and Pursat Provinces [63]. The obvious risk of regular insecticide use is the development of insecticide resistance in the vector populations. However, so far insecticide resistance has not been a major problem for the primary malaria vectors, *An. dirus* and *An. minimus*. Both species remain susceptible to permethrin, only one site study in Cambodia found *An. dirus* DDT resistant, but this was only based on 23 specimens tested [56]. *Anopheles epiroticus* remains susceptible to permethrin but shown some evidence of possible deltamethrin resistance. The monitoring of the susceptibility status of *Anopheles* to insecticides should be performed regularly as this provides essential information for the correct choice of insecticide to be most effective in vector control. Most studies suggest that ITNs can provide a fair degree of protection if properly used [66, 63, 67, 68, 69]. Therefore, Cambodia has actively distributed ITNs to many at-risk populations. Overall, ITNs ownership improved from 43% in high risk areas in 2007 to 75% in 2011 [63, 3]. Cambodia has recently drafted a new strategic plan following the Prime Minister's announcement that Cambodia's goal would be to eliminate malaria by 2025 [70, 48].

2.1.4. Implication of changing social and environment conditions on vectors and transmission

Environmental factors can have a pronounced impact on the distribution and behavior of malaria vectors [71]. *Anopheles dirus* occurs in forest areas but has an ability to adapt to changing environmental conditions from natural forest habitats to cultivated forests, such as rubber and tea plantations and various types fruit orchards [72, 73, 27]. Deforestation is one of the most potent factors either promoting or reducing infectious diseases, in particular malaria in SEA [74, 75, 57]. Deforestation is caused by a wide variety of human activities, including logging, land clearance for agricultural development, transmigration programs, road construction, mining and hydropower development [76, 77]. Globally, estimates of deforestation range from 36,000-69,000 km²/year. Deforestation in SEA has been extensive with the mean annual rate of deforestation of 0.71 to 0.79% of land cover and is higher than reported in Latin America (0.33%-0.51%) or Africa (0.34%-0.36%) [78].The forest vector species that transmit malaria are among the most sensitive to environmental changes [27]. The extensive clearing of forests has had enormous impact on local natural ecosystems, in particular dramatically altering micro-climates by reducing shade, humidity, and rainfall patterns [38, 79]. For anopheline species that use shaded water bodies, deforestation can reduce larval habitats, thus their propagation and adult densities [38]. In Cambodia, the forest area was reduced from 93,000 km² in 2003 to 66,959 km² in 2005 [57], and this possibly has had a direct influence on the richness of ano-pheline mosquito fauna including some malaria vectors.

2.2. Lao People Democratic Republic (Lao PDR, Laos)

2.2.1. Overview

Lao PDR is a land-locked country, which borders five countries, China, Vietnam, Cambodia, Thailand and Myanmar, respectively. Most of the western border of Laos is demarcated by the Mekong River, which is an important artery for transportation and commerce. Two-thirds of Laos is covered by primary and secondary forests with a mountainous landscape and an abundance of rivers and natural resources which remain intact. The country has a tropical climate with high humidity throughout the year. The Mekong has not been an obstacle but a facilitator for communication between Laos and northeast Thai society (same people, same language) reflecting the close contact that has existed along the river for centuries.

Malaria is considered endemic throughout the country, but intensity of transmission is known to vary between different ecological zones; from relatively low transmission in the plains near the Mekong River and in areas of high altitude, to intense transmission in more remote, hilly and forested areas. Malaria has long been a leading cause of mortality and morbidity in the country. Transmission of malaria is perennial, but with large seasonal and regional variations. Peak transmission occurs between May and October, coinciding with the hot and rainy season. Malaria is also a problem in the dry season in certain areas of Laos [80]. In 1992, *P. falciparum* was the predominant species accounting for 95% of all recorded malaria cases [81] and remains so with 93% of all reported cases [3] representing leading cause of morbidity and mortality in Laos. A field survey for malaria prevalence in southern Laos using molecular-based parasite detection assay showed that mixed species infections were common with all 4 human plas-

modia species detected among 23.1% of positive samples [82]. A recent national survey of the malaria distribution revealed that approximately 41% of the country's population is living in areas of no malaria transmission, particularly large areas in the central regions of the country while malaria incidence of more than 1 per 1,000 population is occurring in seven provinces, Saravane, Savannakhet, Sekong, Attapeu, Champasack, Khammouan, Phongsaly, collectively representing 36% of the Lao population [3, 69]. Significant reductions have been reported following investments in malaria control, in particular the large-scale introduction of artemi sinin-based combination therapy (ACT) beginning in 2004, ITNs introduced in 2000, and IRS in 2010, in conjunction with socio-economic and environmental changes [3]. In 2008, only 11 deaths among 18,743 confirmed malaria cases were reported (population ~6 million), com- pared with 600 deaths and 70,000 confirmed cases in 1997 (Center for Malariology, Parasitology and Entomology [CMPE] unpublished data). However, malaria still continues to be a serious public health problem in some focal areas such as remote areas in southern Laos [8].

Between 2005 and 2008, the National Malaria Control Programme introduced a new strategy to improve case management at the community level, which involved training of 12,404 village health volunteers (VHVs) in 6,202 villages in the use of *P. falciparum*-specific malaria rapid diagnostic tests and to guide administration of ACT to infected patients. The VHVs represent the most peripheral level of the public health care system in Laos. Volunteers are selected by villagers and a village committee to provide primary health care services, including diagnosis and management of respiratory diseases, diarrhea, and uncomplicated malaria. Activities also include performing health education, assist in vaccination campaigns, and report morbidity and mortality data to the local health center or the district health office [69]. In Laos, insecticide- impregnated bednets have been reported to reduce malaria transmission successfully [68]. Much of the support has focused on the distribution of ITNs. The CMPE is now in the process of scaling up bed net coverage with a projected target of 3.6 million units reaching the most vulnerable ethnic minority groups, other persons at risk, and together with implementing appropriate diagnosis and effective treatment programs. Improving access to effective malaria treatment has become one of the greatest challenges. In recent years, artemisinin-derivative combination therapy (ACT; artemether-lumefantrine) has been adopted as the first-line treatment for uncomplicated malaria in many countries including Lao [83, 84, 85]. Recent data has shown that 89% of patients with malaria received a parasitological-confirmed diagnosis and were treated with an ACT [69, 86]. Furthermore, as the government public health system in Laos provides the vast bulk of primary health care, a private system for health access is growing, especially in the peripheral areas.

2.2.2. Malaria vectors and biodiversity of Anopheles in Laos

South-East Asia is one of the world's richest regions in terms of biodiversity. The species distribution and factors shaping it are not well understood, yet essential for identifying conservation priorities for the region's highly threatened flora and fauna. Several malaria vectors belong to sibling species that may greatly differ in their biology, behavior and other characteristics of epidemiological importance, such as resistance to insecticides. The sibling
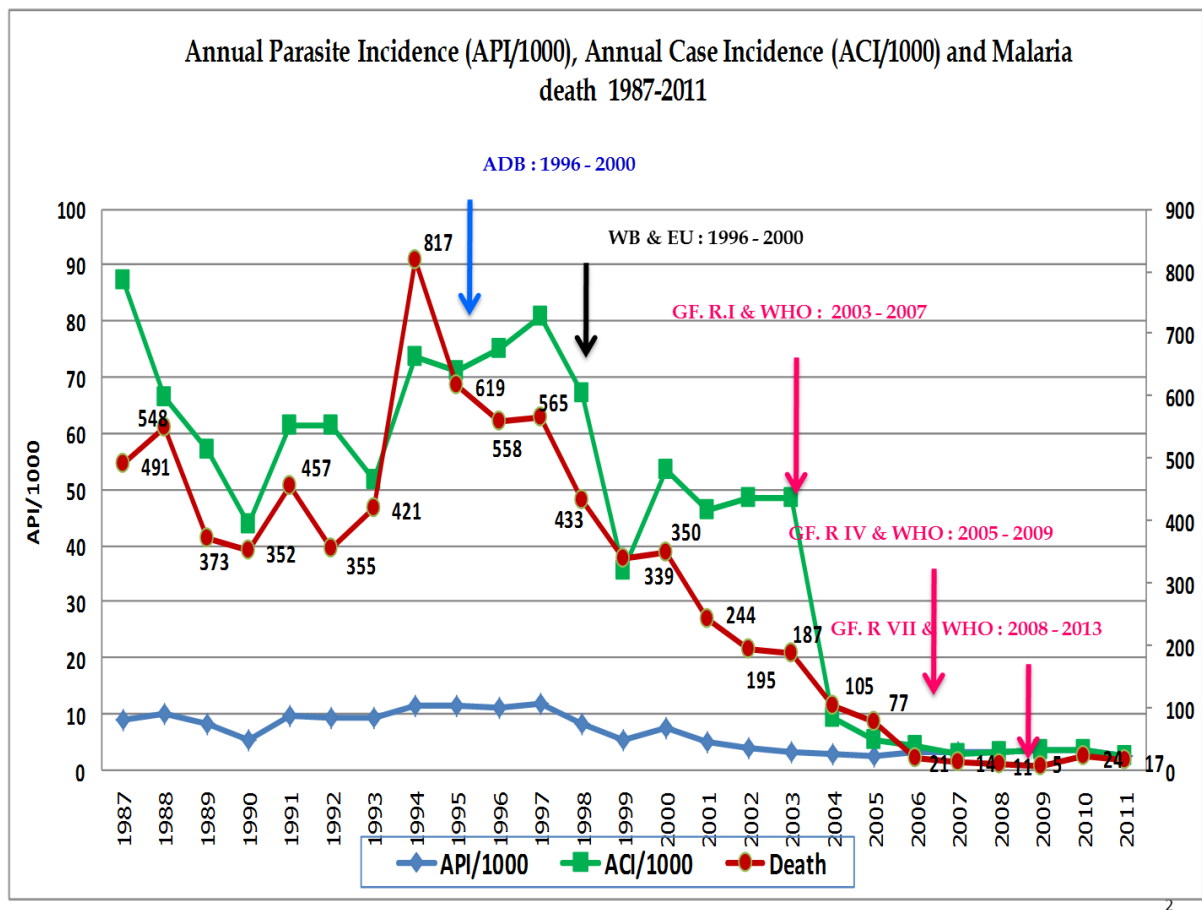
[http://www.rbm.who.int/partnership/wg/wg_itn/ppt/ws2/m4LaoPDR.pdf.]

Figure 2. Annual Parasite Incidence (API/1,000 population), Annual Case Incidence (ACI/1,000 population) and malaria deaths in Laos from 1987 to 2011. Source: Meeting on Outdoor Malaria Transmission in the Mekong Countries for 13 countries during 12-13 March 2012, Bangkok, Thailand.

species have been described as having individual distribution patterns depending on the landscape and seasonal environmental changes.

There are four recognized malaria vectors in Laos: *An. dirus, An. minimus s.l., An. maculatus s.l.,* and *An. jeyporiensis.* Among these *An. dirus* and *An. minimus* are considered the primary vector species. The anopheline situation in Laos is regarded as complex because of taxonomic and ecological variations that affect malaria transmission in the country [80,86]. *Anopheles minimus* and *An. harrisoni* are known to occur largely in sympatry (i.e., occurring together in the same area) in northern Laos [34]. Anopheline abundance and species composition are site-specific and can vary throughout the year depending on conditions. A mosquito survey in Khammouane in 1996 and 1999-2000 found 19 and 28 different anopheline species, respectively. Studies have shown that the vectorial capacity (a transmission probability index) of *An. dirus* was 0.009-0.428, while *An. minimus* s.l was 0.048-0.186, *An. vagus, An. philippinensis, An. nivipes* were predominant species but mostly zoophilic [87, 88]. Three other species belonged to *An. maculatus* Group, including *An. notanandai, An. sawadwongporni,* and *An. willmori* along

with *An. hodgkini* (Barbirostris Subgroup), a species reported for first time in Khammouane Province [88]. In 1999, an entomological survey covering 8 provinces, found that out of 19 anopheline species collected, *An. aconitus* was the predominant one, especially in the month of December, yet only 3 species, *An. dirus*, *An. maculatus* s.l. and *An. minimus* s.l. were found infected with malaria oocysts [86]. In 2000-2001, 16 anopheline species from Sekong Province were captured with only *An. dirus*, *An. maculatus* s.l. and *An. jeyporiensis* found positive for human malaria sporozoites [89]. *Anopheles dirus* was found to be the primary vector and sporozoite rates were highest during the transitional dry season. Two years of mosquito surveys, from 2002-2004, were conducted in Attapeu, the southern-most province bordering Vietnam and Cambodia, and a town located in a large valley surrounded with forest. It is one of the endemic malaria provinces which documented 8,945 mosquitoes belonging to 14 genera and 57 species, of which 21 species were *Anopheles*. Maculatus Group, *An. sawadwongporni* and *An. notanandai*, were found in large numbers but only *An. minimus* was found malaria sporozoite positive [90, 91]. There is very limited information about adult behavior and breeding habitats of anophelines in Laos. Recently, information has also been provided on non-vector species, for example, *An. annularis* s.l., *An. philippinensis,* and *An. sinensis* [60].

2.2.3. Distribution of malaria vectors and behavior of Anopheles in Laos

An. minimus s.l. is widespread in the country and has been identified in all malaria endemic provinces in Laos. It primarily breeds in slow running streams closely associated with forested hilly areas, irrigation ditches, and rice fields. The mosquito feeds predominantly on humans but also on cattle and other animals and is regarded as primarily endophagic and endophilic. A recent study found both *An. minimus* and *An. harrisoni* present in northern Laos [56]. While *An. dirus* is most common in the central and southern parts of the country, it is considered rare in the north. *Anopheles dirus* is the most important malaria vector in the southern part of Laos. It breeds preferentially in stagnant and shaded waters (e.g. hoof prints, small rain-fed ground pools) in the rainforest, forested foothills and agricultural plantations, but has also been found to breed in scrub lands with lower vegetation. Population densities for this species typically increase during the wet season of the year while also having higher sporozoite infective rates at the end of the rainy season [89, 90]. The species is predominantly anthropophilic making it an ideal vector, but it will also feed on domestic animals with an indoor: outdoor blood feeding ratio of 1.6 [90]. The biting cycle of *An. dirus* has been documented to begin early evening, from 19:00 and remaining active through the night until 06:00, with peak activity around 22:00 [90, 92].

2.2.4. Implications of changing social and environment conditions on vector and transmission

Anopheles dirus is the most capable and dangerous malaria vector in Laos, particularly in southern Laos associated with forest-related habitats. This species has also become well adapted to human-induced environmental change, for example utilization of disturbed scrub areas containing low standing vegetation [90]. Laos' national forest coverage has dropped from 70% in 1940, at around 17 million hectares, to 41% in 2001, when a ban on timber exports was enacted, yet illegal deforestation has remained rampant over the past decade. From 2002 to

2010, central Laos's forestry cover decreased by 3.5%, while 9% of the southern forests disappeared [(http://www.nationmultimedia.com/home/Laos-to-increase-forest-cover-age-30145391html (December 2010)]. The government plans to increase forestry cover in Laos to 65% by 2015 and 70% by 2020 (The National Assembly, seventh five-year economic plan for 2011-2015). The current reforestation programmes have concentrated on allowing investments in large rubber plantations in Laos' border regions with southern China and Vietnam. For example, 10,000 hectares have been allocated for rubber plantation development in one area, and this has attracted populations from the Laos highlands to migrate to the plains, especially in Sanamxay District, to work in the rubber and sugar cane plantations. From October to December 2011, a total of 11,833 persons tested for malaria found 3,091 infected as reported from all facilities in the area including Attapeu Province villages. Up to the end of January 2012, 8 deaths due to malaria were reported from Attapeu.This outbreak of malaria has been attributed to the large scale development projects in the province, mainly concentrated in Phuvong and Sanamxay Districts, and the resulting population movements into the areas. In Phuvong District, extensive land clearing for Nam Kong 2 and 3 hydroelectric dams have been completed with dam construction beginning in 2013. The surge in logging activities associated with land clearing, primarily for the prized 'MaiKhayung' (rosewood), has attracted both local populations as well as people from other provinces to Attapeu. Most malaria patients admitted to provincial and district hospitals have been from other provinces or neighboring countries. In Phuvong District, from October to December 2011, 68% of the non-local malaria cases were from Vietnam and approximately 10% of cases were seen in children under the age of 5 years. This should be the lesson for other neighboring malaria-endemic provinces of Savannakhet, Saravane, Sekong and Champasack in southern Laos, where significant development projects are also planned, as well as other neighboring countries that are either initiating, planning or contemplating major development projects that would create extensive environment changes to design strategies to prevent or mitigate the occurrence of disease outbreaks as a result.

2.3. Malaysia

2.3.1. Overview

The Federation of Malaysia, a federal constitutional monarchy in Southeast Asia, consists of thirteen states and three federal territories and has a total landmass of 329,847 km² separated by the South China Sea into two similarly sized areas, Peninsular Malaysia located on mainland SEA and Malaysian Borneo. National borders are shared with Thailand, Indonesia, and Brunei, and maritime borders exist with Singapore, Vietnam, and the Philippines. Malaysia is a multiracial country consisting of Malays, Chinese, Indians, Ibans, Kadazans and smaller ethnic groups with total population of approximately 28.3 million [93]. Several vector-borne diseases remain serious concerns in Malaysia, including malaria.

During the 1960s, the number of malaria cases were estimated at 300,000 annually before the Malaria Eradication Program (MEP) was launched. The program was successful in dramatically reducing malaria transmission with number of cases decreasing from 181,495 at the start of MEP in 1967 to 44,226 cases at the end of the program in 1980. In 1983, the country changed

strategy to one focused on 'control' by adopting the Malaria Control Program (MCP). The MCP continued the fight against malaria before reorganizing to the Vector-Borne Disease Control Program (VBDCP) in 2010. The key objective of the current program is to continue the reduction of malaria morbidity and mortality and to prevent the recurrence of malaria in non-endemic areas. The VBDCP also includes activities for the prevention and control of other vector-borne diseases like dengue fever and lymphatic filariasis [94, 95]. The MCP activities had been successful in reducing the number of malaria cases in Malaysia from 48,007 cases in 1986 to 7,010 cases in 2009 [96, 97].

Currently, malaria is still one of the most important vector-borne diseases in the country, primarily in Malaysian Borneo (Sarawak and Sabah states), although only 4% of the population is living in areas within active malaria transmission foci [3]. These refractory areas are partly attributed to anti-malarial drug resistance, insecticide resistance and cross border migration. In 2005, there were almost two million legal migrant workers in Malaysia. Most of these foreigners came from malaria endemic countries, a majority being from Indonesia (68.9%), followed by Nepal (9.9%), India (6.9%) and Myanmar (4.6%) [98,99]. In addition, the risk of malaria is high among the aboriginal groups such as Orang Asli, who lived in the interior of Peninsular Malaysia in remote hilly, cleared jungle areas [96]. In 2009, 7,010 malaria cases were reported in the country with approximately 57.2% of cases occurring in Sabah, 26% in Sarawak and 16.8% in Peninsular Malaysia. Most cases were caused by *Plasmodium vivax* (48.15%), followed by *P. falciparum* (26.75%), *P. knowlesi* (13.01%), *P. malariae* (8.37%) and mixed species infections (3.68%) [97,2]. *Plasmodium knowlesi* has more recently been recognized as an important zoonotic malaria species in eastern Malaysia (Borneo) and outbreaks have been found primarily in Borneo, Sarawak and Sabah and West Malaysia, [100] as well as other countries in SEA (see the Chapter by Vythilingam & Hii). In Malaysia, *An. latens* and *An. cracens* (both members of the *An. leucosphyrus* Subgroup) have been incriminated as vectors of *P. knowlesi* [101, 102, 103].

Malaysia has launched a national vector control program to include use of targeted indoor residual spraying (IRS), ITN distribution, artemisinin-based combination anti-malarial drugs, larviciding aquatic habitats harboring immature stages of vector species, environmental management measures, and personal protection methods [104]. After years of insecticide use to control vectors, development of physiological resistance to insecticides has been detected in some malaria vectors. Hii (1984) reported that *An. balabacensis* was tolerant to DDT and years later that several other anopheline species had also developed resistance to DDT and perme-thrin [105].

2.3.2. Malaria vectors in Malaysia

Seventy-five species of *Anopheles* have been recorded in the country, only 9 of which are reported as malaria vectors to include *An balabacensis* and *An. latens* (both Leucosphyrus Complex), *An. cracens* (Dirus Complex), *An. maculatus* (Maculatus Group), *An. letifer*, *An. campestris*, *An. sundaicus* and *An. epiroticus* (both Sundaicus Complex), *An. donaldi*, and *An. flavirostris* [96]. Each species is considered a malaria vector in various areas of the country, sometimes existing in sympatry (Table 1).

Anopheline species	Peninsular Malaysia	Sarawak and Sabah
An. balabacensis		+
An. campestris	+	
An. cracens (=*An. dirus* B)	+	
An. donaldi	+	+
An. flavirostris		+
An. letifer	+	+
An. latens (=*An. leucosphyrus* A)		+
An. maculatus	+	+
An. epiroticus/An. sundaicus	+	+

Table 1. Anopheline vectors in Malaysia [33,59]

Anopheles maculatus is within a species group that comprises at least nine genetically-related species [39]. Historically, *An. maculatus* has been the principal vector of malaria in West Malaysia, particularly in hilly areas not covered with dense forest [106,107,108]. This species prefers to breed in pools formed along the still banks of rivers and small streams. The larval breeding habitats include shallow pools (5-15cm depth) of clear water, with muddy substrate and plants or flotage [109]. In Borneo, this species appears to be more zoophilic and is not regarded a malaria vector of any importance [106].

Anopheles campestris belongs to the Barbirostris Subgroup (subgenus *Anopheles*) and is a potential vector of malaria and filariasis, particularly along the west coast of Peninsular Malaysia [110]. The larvae commonly breed in rice fields, burrow pits, stagnant ditches in coconut plantations, earthen wells, and sometimes in slightly brackish water [111]. Reid (1968) reported that this species could be found in deep water with some vegetation and light shade. Adults are generally anthropophilic, will enter houses to blood feed and rest.

Anopheles cracens (formerly *An. dirus* species B), is a member of the Dirus Complex, found exclusively in the Thai-Malaysian peninsular area of mainland SEA. *An. cracens* is the vector of *P. knowlesi* in Kuala Lipis of peninsular Malaysia [102]. Larvae typically inhabit small, usually temporary, shaded bodies of fresh, stagnant water, including ground pools, puddles, animal footprints, and wells. This species is found in hilly and mountainous areas containing primary or secondary evergreen and deciduous forests, bamboo, and fruit and rubber plantations [112, 113, 114].

Anopheles letifer larvae prefer to breed in stagnant dark-brown (often acidic) water found in peat swamps, especially in jungle clearings along forest edges, with or without shade. Oil palm cultivation areas are also habitats for *An. letifer* associated with open and blocked swamps [115]. In peninsular Malaysia, *An. letifer* is regarded a vector of human malaria and Bancroftian filariasis [106, 96, 116], particularly at low elevations on the coastal plains.

Anopheles epiroticus (formerly *An. sundaicus* species A) and *An. sundaicus* s.s. are members of the Sundaicus Complex [117] and considered important vectors of malaria in coastal areas [106, 118, 44]. In Peninsular Malaysia, *An. epiroticus* occurs mostly along coastal areas while *An. epiroticus* is found in Sarawak (Borneo) [46]. The immature stages are typically found in sunlit pools of brackish water, containing filamentous and floating algal mats, and sparse vegetation. Particularly favorable habitats include ponds, swamps, lagoons, open mangrove, rock pools and abandoned or poorly maintained coastal shrimp and fish ponds [46]. Adults rest by day both outdoors and indoors and readily bite people indoors. Sporozoite rates can often be relatively low but are compensated by large adult densities [106].

Anopheles donaldi is one of the primary malaria vectors in Sarawak with a reported sporozoite rate of 0.23% [119]. This species prefers small streams and ground pools, containing clean and shaded fresh water, occasionally rice fields and open marshlands [106,115]. The adults are found in forest fringes in hilly areas and near tree-covered swamps in the lowlands [106]. *Anopheles balabacensis*, a member of the Leucosphyrus Complex, is regarded as the main vector of malaria in Sabah [120,111]. This species occurs in forested area of Malaysian Borneo (eastern Sarawak and Sabah). The immature stages are principally found in shaded temporary pools of stagnant fresh water, including ground puddles, animal footprints, wheel tracks, ditches and rock pools [59]. In addition, *An. balabacensis* is also a vector of *Wuchereria bancrofti* responsible for lymphatic filariasis [121,116]. In most areas, this species is very anthropophilic and will readily enter houses to blood feed.

Anopheles flavirostris is a malaria vector in Sabah along the eastern coast [111] belonging to the Minimus Subgroup [122]. This species demonstrates anthropophilic and endophagic behaviors in Sabah [121]. Characteristically, *An. flavirostris* larvae are found in clear, slow-moving freshwater stream habitats that are partly shaded by over hanging vegetation and margins containing emergent plants or grasses [123]. *Anopheles latens* (formerly *An. leucosphyrus* A), a member of the Leucosphyrus Group, is a primary vector of human malaria in Sarawak. Additionally, *An. latens* also transmits the monkey malaria parasite, *P. knowlesi* to humans in Sarawak [101]. Like all members in the group, this is a forest mosquito and larval habitats of *An. latens* are primarily found in shaded, temporary ground pools, small pools on margins of forest streams, and natural containers of clear or turbid water in forested areas [59]. In Sarawak, [124] this species was commonly found in shaded pools, a forest stream and swampy patches. Adults will enter houses in the evening to bite, generally delaying activity until after 2200 hr.

2.3.3. Effects of changing environmental conditions on malaria vectors and transmission

In Malaysia, malaria transmission appears more strongly associated with land development rather than water development projects [125]. Land use changes, such as deforestation, increased urbanization and agriculture can directly impact mosquito abundance, species biodiversity, biting behavior, and vector competence [77]. For example, the effect of forest clearance for rubber plantations exposes land and streams to direct sunlight and thus increased and expanded the available breeding habitats for *An. maculatus*, which further led to a marked increase in the incidence and severity of malaria [126]. Vythilingam et al. [119] found that *An. donaldi* appears to have replaced *An. balabacensis* as the main vector in Kinabatangan of Sabah

as a result deforestation and malaria control activities. Similarly, the clearing of mangroves and swamps for fish aquaculture or mining resulted in an increase in suitable larval habitats of filariasis vectors and *An. epiroticus* followed by malaria outbreaks [76,119, 127].

2.4. Myanmar

2.4.1. Overview

Myanmar (formerly Burma) has a total land area of 678,500 km². The extent of border areas with the 5 surrounding countries include 193 km with Bangladesh; 2,185 km with China; 1,463 km with India; 1,800 km with Thailand, and a relatively small stretch with Laos. Administratively, the nation is divided into 14 states and divisions, 65 districts, 325 townships. The climate is tropical with the southwest monsoon occurring from June to September and a northeast monsoon from December to April.

Migration across international borders through specific points of entry from Myanmar includes, Tachilek, Myawaddy and Kawthaung, Thailand; Muse, Namkhan and Khukok, China; Tamu, India; and Maungdaw, Bangladesh. There are also other less important points of entry into Thailand where Thai and Burmese citizens normally need only a valid border pass to cross at official check points. At Mae Sai, approximately 60,000 Thais and 30,000 Burmese nationals crossed the border in 1997. That is one important reason why malaria morbidity and mortality along the Thai-Myanmar border is especially high and refractory to most control methods [127] and why the disease peaked in intensity between 1988 and 1991 [128].

Malaria is a severe public health problem in Myanmar, in particular along parts of international borders [129]. Confirmed malaria cases in Myanmar increased from 120, 029 in 2000 to 447,073 cases in 2008. The 2009 World Malaria Report (WMR) stated that Myanmar (Burma), with a population of over 50 million, had 17% of all malaria cases recorded in Southeast Asia, the highest percentage in the region [47]. There were 400,000 confirmed malaria cases in the country and about 1,100 deaths due to malaria in 2008, occurring in 284 out of 324 townships [85].

In 2008, Chin State reported the highest morbidity rate of 44.7 per 1,000 inhabitants, whereas the highest number of malaria cases was reported in the Rakhine State, followed by Sagaing State (Figure 4) [130,131].

Generally, malaria transmission peaks just before and after the monsoon rains which normally occur between June and September. The populations most at risk include: 1) people who live or migrate into high malaria risk areas, especially along the borders; 2) international migrants or laborers involved in mining, agriculture (e.g., rubber plantations), the construction of dams, roads, and irrigation projects; 3) those who farm or related work near or in forests and along forest fringes such as wood and bamboo cutters; 4) pregnant women and children under five years old; and 5) ethnic minorities residing in more remote areas with poorer access to primary health care. Out of a total population of 60 million, the proportion of residents living under some degree of malaria risk or none is as follows: high risk 37%, low risk 23% and no risk 40% [3]. Overall 36 townships had higher than 4% mortality in cases diagnosed [132]. Significant numbers of ethnic minorities (approximately 100,000) live in semi-permanent refugee camps

Figure 3. Malaria morbidity and mortality rate in Myanmar during 1988-2008. (http://www.actmalaria.net/home/vector_control.php#base)

along the Thai-Myanmar border where malaria transmission is rampant. The Thai government's policy is to eventually repatriate Shan and other minorities back to Myanmar.

All four species of human plasmodia (*P. falciparum, P. vivax, P. malariae* and *P. ovale*), exist in Myanmar. In 2008, the NMCP Myanmar reported 391,461 *P. falciparum* cases (87.6% of all malaria infections) followed by *P. vivax* at 52,256 (11.7%), while *P. malariae* and *P. ovale* were seen in only 283 and 5 cases, respectively. Currently, *P. falciparum* is still the predominate species at 68% of all cases detected [3]. Additionally, one human infection with *P. knowlesi* was found in a Burmese worker at Ranong Province of Thailand. This zoonotic infection may have been acquired in Kawthoung District, Myanmar, a district close to Ranong Province [133]. *Plasmodium falciparum* resistance to antimalarial drugs is a primary concern in the country. Chloroquine and sulfadoxine-pyrimethamine (S-P) resistance at various levels is now common. Also, well documented report of resistance in small case series has appeared. Resistance to chloroquine by *P. vivax* has been reported [134,135].

2.4.2. Malaria vectors and species diversity

Due to Myanmar's diverse geography, there is a relatively large number of dominant malaria vector species. Out of 36 *Anopheles* species distributed in the country, 10 species at 16 locations have been found infected with malarial parasites [136]. In Myanmar, the primary vectors responsible for the majority of infections are *An. dirus* s.l. and *An. minimus* s.l. [59]. Other anopheline species, predominantly zoophilic feeders, may also, under ideal conditions, feed
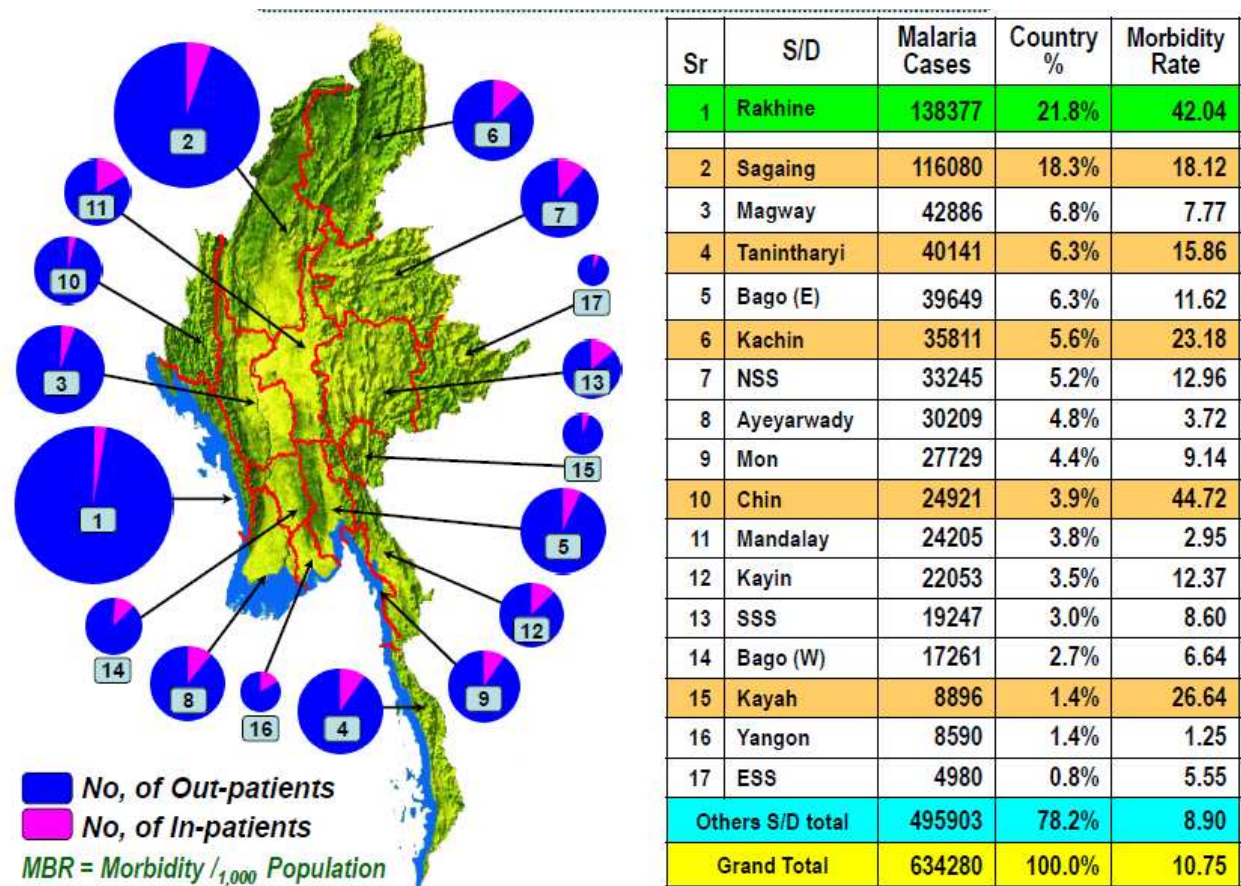
Sr	S/D	Malaria Cases	Country %	Morbidity Rate
1	Rakhine	138377	21.8%	42.04
2	Sagaing	116080	18.3%	18.12
3	Magway	42886	6.8%	7.77
4	Tanintharyi	40141	6.3%	15.86
5	Bago (E)	39649	6.3%	11.62
6	Kachin	35811	5.6%	23.18
7	NSS	33245	5.2%	12.96
8	Ayeyarwady	30209	4.8%	3.72
9	Mon	27729	4.4%	9.14
10	Chin	24921	3.9%	44.72
11	Mandalay	24205	3.8%	2.95
12	Kayin	22053	3.5%	12.37
13	SSS	19247	3.0%	8.60
14	Bago (W)	17261	2.7%	6.64
15	Kayah	8896	1.4%	26.64
16	Yangon	8590	1.4%	1.25
17	ESS	4980	0.8%	5.55
	Others S/D total	495903	78.2%	8.90
	Grand Total	634280	100.0%	10.75

No, of Out-patients
No, of In-patients
MBR = Morbidity /$_{1,000}$ Population

Figure 4. Malaria morbidity rate in States/Divisions of Myanmar in 2008. (http://www.actmalaria.net/home/vector_control.php#base)

on man [137,138]. These secondary vectors include *An. aconitus, An.annularis* s.l., *An. culicifacies* s.l., *An. sinensis, An. jeyporiensis, An. maculatus* s.l., *An. philippinensis,* and *An. sundaicus* s.l. [136].

2.4.3. Anopheline behavior

Much of the recent work on anopheline bionomics and distribution in Myanmar is attributed to Oo et al. [113, 136] herein. *Anopheles baimaii* is the most common species of the Dirus Complex present in Myanmar, which is also the primary vector species in neighboring Bangladesh [113]. Highest numbers of immature stages were collected during the pre- and post-monsoon periods, while the lowest numbers were seen during the cool-dry and hot-dry months. The larvae were found in rock pools along the banks of thickly shaded streams and in cut bamboo stumps. Adults of this species are plentiful in the monsoon months with a peak densities occurring during September and October. *An. dirus* s.l. was also found daytime resting in the crevices and vegetation around the inner walls of domestic wells and on the underside of banana leaves. Adult behavior indicated this species highly exophilic and it will bite both humans and cattle. A previous study [139] has reported a higher zoophilic tendency despite the breeding sites being found very near human dwellings. Outdoor biting peak has been

shown to occur between 21:00 and 03:00 hr. [139]. The results of the dissection both of midgut and salivary glands together for determination of natural infection rates in different localities ranged from 0.4 to 2.8%. The highest infection rate for midgut dissection was 0.4 % (1/250) and salivary gland dissection 2.4% (6/250).

For *An. minimus* s.l., adult densities vary seasonally, although it is also abundant throughout the entire year in many locations [136]. The highest prevalence of *An. minimus* s.l. occurs during the post-monsoon months of October to December. Adults prefer to rest in houses and cattle sheds during daytime. The preference of *An. minimus* s.l. for human blood is well documented during different periods of the year and various locations. Even when cattle are present, only a small proportion of mosquitoes appear to deviate from biting humans. *Anopheles minimus* s.l. feeds mainly during the early hours of the evening, beginning before 21:00 hr and peaking in activity just before or after midnight. However, when adult densities are high, *An. minimus* s.l. populations will bite throughout the night (both outdoors and indoors) with greater activity during the first quarter of the evening and a gradual decrease in biting till dawn (06:00 hr). The infection rate both in midgut and salivary glands has been reported to vary between 1.1-3.0%. *Anopheles minimus* s.l. is primarily a mosquito of hilly regions, low rolling foothills to narrow river valleys in more mountainous areas; it has not been recorded in locations over 915 m above sea level. When found in lowland plains, it is always in association with irrigation systems.

Anopheles aconitus is a secondary vector in certain localities and is a fairly abundant species from October to February, peaking in November [136]. From March to September it is very seldom seen. *An. aconitus* is more commonly seen in hilly tracts, foothills and also in the plains of central and southern Myanmar closely associated with active rice cultivation. Only a few *An. aconitus* females are found resting in houses or stables during daytime preferring to rest outdoors in scrub and other locations. *An. aconitus* appears to prefer cattle for blood meals, although it will bite humans if cattle are not available or very limited in number. It is active in the early evening, biting as early as 18:00 hr, with very little activity after 01:00 hr. *An. aconitus* had a 0.2% (1/350) infection rate [136].

An. annularis s.l. has been found in few localities with high adult densities. Stagnant water with thick grassy edges in permanent ponds, ground pits, tanks, swamps, stagnant drains and rice fields are common larval habitats of *An. annularis*. Its abundance varies according to rainfall patterns. In coastal areas with heavy rainfall (between 3,800 mm to 5,150 mm annually), *An. annularis* densities typically increase from October to January. This species appears to preferentially feed on cattle with a far greater proportion (80-90%) of biting activity seen during the first half of the night (18:00-24:00 hr). The midgut dissection records on *An. annularis* have seen 0.1-0.2% (350-700 samples) plasmodia infection rates [136].

An. culicifacies s.l. is a suspected malaria vector in central Myanmar, especially in irrigated areas. The larval stage of this species breeds in fresh (unpolluted) water and also in artificial water containers and unused swimming pools. *An. culicifacies* is more abundant in August and September, dropping of in October and virtually none from November to March. Adults prefer to rest in cattle sheds and houses during the day, but it may take shelter in paddy-sheds, stacked fire-wood and piles of straw near the stables and outside houses. *Anopheles culicifa-*

cies is primarily a cattle feeder with generally far fewer numbers attacking humans.This species feeds mostly around midnight with very few biting after 03:00 hr. Midgut infections (with oocysts present) have been recorded at 1.83% (6 infections from 328 examined mosquitoes) [136].

When adult densities are high, *An. sinensis* is a secondary vector along the Myanmar-China border. Larvae are predominately found in stagnant waters and rice fields. This species was found from July to December with a peak in August. It is predominantly zoophilic, preferring cattle over humans. Very few have been caught biting humans at night. The peak biting activity of *An. sinensis* is during the first half of the evening beginning at 18.00 hr. In Shan State, along the Myanmar-China border, 300 specimens of *An. sinensis* were examined of which 2.3% (6/300) were found malaria infected [139]. *Anopheles jeyporiensis* is regarded a secondary vector on the Myanmar-China and Myanmar-Bangladesh borders when adult densities are high. Immature stages are mainly found along margins of slow-moving streams and channels with grassy edges and often sympatric with larvae of *An. aconitus*. Rice fields are also attractive breeding sites for *An. jeyporiensis* when uncultivated or early stages of plant growth but become unfavorable as the plants increase in height. Adults are normally abundant during the pre-monsoon period of March and April. They will feed on both humans and cattle. The peak biting period has been recorded from 23:30 to 03:00. On the Myanmar-Bangladesh border (Rakhine State), 500 specimens of *An. jeyporiensis* were dissected with four having sporozoite-infected salivary glands (0.8% infection rate). On the Myanmar-China border (Shan State), 500 specimens of *An. jeyporiensis* were dissected with a 1.2% (6/500) infection rate.

An. maculatus s.l. has been reported as a primary vector, especially in Tanintharyi Division, and elsewhere as a secondary vector depending on the location. The greatest density of this species in nearly all areas where it occurs is during January (cold dry season). Numbers start to increase at the end of southwest monsoon period in early October and relatively rare during the two annual monsoon seasons.There is only one exception, in Mandalay Division, where *An. maculatus* has been recorded in large numbers during September, at the end of the rainy season. It has not been recorded resting indoors during the day, even though many houses in the foothill areas are semi-enclosed. However, at times of peak densities, *An. maculatus* can be collected in cattle sheds. This species member feeds on both humans and various animals, mainly during the first half of the night beginning at 18:00 hr. The midgut dissections have shown a 0.5% (1/180) infection rate. *Anopheles maculatus* is primarily recorded from forested foothills, around deep forest camps and in mountainous areas at 1, 200m above sea level and typically not found in low lying areas far from foothill environments.

Anopheles philippinensis is a vector of minor importance near the Myanmar-Bangladesh border. This species was not found resting in houses and cattle sheds during daytime and presumably selects natural sites outdoors. *An. philippinensis* has only been found resting in houses during morning collections. *An. philippinensis* is a zoophilic species and feeds mainly on cattle. In areas where cattle are either scarce or absent, this species will readily feed on man. In Innwaing Village (Mawlamyine Township, Mon State) and Patheingyi Township *An. philippinensis* has been reported in large numbers during the post-monsoon months from September to November [136].

An. sundaicus s.l. is a secondary vector restricted to coastal areas where larval habitats are mainly located in sunlit lagoons, natural fresh and brackish water impoundments and back-up streams, often with dense aquatic vegetation (floating algal mats), and brackish water seepage areas. The seasonal abundance of *An. sundaicus* s.l. often increases between May and July and again in October to February. This species was recorded in moderate numbers from houses and cattle sheds from daytime collections. They feed on both human and cattle. In Chaungthar and Seikgyi areas in Ayeyarwady Division, *An. sundaicus* s.l. had a 0.4 % midgut infection rate (1 oocyst positive /220 sampled and 1 positive per 230, respectively). Along the Myanmar-Bangladesh border in Rakhine State, a total of 202 specimens were dissected from which 0.5% had positive salivary gland infections [136].

Myanmar's national malaria control program aims to achieve the WHO Millennium Development Goal of halting the increase in malaria cases by 2015 and significantly reversing the incidence of malaria thereafter. The principle method for malaria vector control in malaria endemic areas of Myanmar relies on the application of ITNs distribution and case management [3]. Biological control using two predacious 'top minnow' fish species, *Poecilia reticulata* and *Aplocheilus panchax* are also effective in certain aquatic habitats and when the correct conditions merit. Inter-sector cooperation, community participation and health education are also part of this integrated approach to reducing disease transmission [131]. Although insecticides are an important component of malaria control operations in Myanmar there is lack information on the status of insecticide resistance in key vector species [12]. Information from the NMCP showed insecticide resistance present in anopheline mosquitoes from Rakhine State. In 2009, both *An. annularis* s.l. and *An. barbirostris* were found resistant to 4% DDT, and *An. barbirostris* was also resistant to 0.25% permethrin, while both species were susceptible to 5% malathion and 0.05% deltamethrin [132]. Although the threat of malaria must be targeted at the local and regional level, especially in the remaining conflict areas of eastern Myanmar, the government does not yet conduct extensive malaria control programmes in many areas in need [140].

2.4.4. Effects of changing environmental conditions on malaria vectors and transmission

Since Nay Pyi Taw, the new administrative capital of Myanmar was opened in November 2005 to include relocation of all government ministries approximately 320 km north of Yangon. This major infrastructural change has had a major impact on the land-use characteristics in the area with new buildings a connecting train network, roads and other projects. [85]. Land-use changes could create ideal new habitats ideal for mosquito propagation, the extension or reduction of a vector's distribution, and modify the composition of the mosquito vectors in an area [141]. *An. dirus* s.l. and *An. minimus* are the major malaria vectors in the hilly regions of Myanmar. There is a profound lack of information about the effects of environmental changes on malaria vectors in Myanmar. Currently there are only a few publications that describe [77, 142, 75] the effects of major infrastructural projects (e.g., dam construction), deforestation, vegetation replacement, increased in human population density and movement, modified topography and hydrological characteristics that can affect the epidemiology of malaria and risk of transmission.

Myanmar is the country where the malaria situation is still poorly understood and well-organized control programs remain lacking in many areas of the country. Current and available information is generally lacking and operational research limited to better assess the epidemiology throughout the country. Both published literature and unpublished departmental reports by the Department of Medical Research (DMR) and Department of Vector Borne Disease Control (VBDC) are regarded as inadequate to address managing an effective malaria control program.

2.5. Thailand

2.5.1. Overview

Thailand is the world's fifty-first largest country in terms of total land area (513,120 km²), and a total population of nearly 67 million people. Thailand shares national boundaries with Myanmar on the west and north, Laos on the north and east, Cambodia on the east, and Malaysia in the south. Gem mining, hunting, logging, agriculture, road construction and other economic activities along Thailand's border areas attract many migrant workers from neighboring countries. The constant movement of workers and the transient, often poorly constructed dwellings they occupy facilitates cross-border transmission of malaria and complicates efforts to control it, making it one of the most serious vector-borne diseases in these areas.

Despite decades of success in reducing the number of cases of malaria in the country, the disease remains a major cause of morbidity and mortality. Approximately 32 million people in Thailand's border areas (50% of the Thailand's population) are at risk of contracting malaria. All four malaria parasites are present with the most common being *P.vivax* with 60% of all reported infections in 2011 [3]. Since 1997, *P. falciparum* and *P. vivax* infections have been recorded at near equal prevalence [70] (Figure 5). The under-developed border areas between Thailand and eastern Myanmar remain the worst affected area for continuing transmission [1, 2]. Non-immune workers who migrate across the international border remain the most susceptible and vulnerable populations. The constant movement of this population involved in gem mining, logging, agriculture, construction and other pursuits, has helped to increase the spread of multi-drug resistant *P. falciparum* malaria in the area and region. Serious outbreaks of malaria have taken place in high risk areas along the Thai-Myanmar border, especially in Kanchanaburi and Tak Provinces [70,143]. In four southern provinces of Thailand, malaria cases have risen to nearly 4,000 per year in the areas bordering Malaysia where social conflict and a local insurgency have greatly complicated control efforts [70]. At the same time, a rapid increase of rubber plantations in northeastern Thailand has become a major concern because of the potential for the reemergence of malaria [144]. Several major malaria vectors, mainly *Anopheles dirus* s.l., *An. maculatus* s.l., and *An. minimus* s.l., can adapt and utilize rubber plantations in place of more typical habitats like hill environments and natural forests [4]. Careful attention and monitoring to land use changes along with climatic and other environmental changes is essential to help prevent or delay the reemergence of malaria in receptive areas.
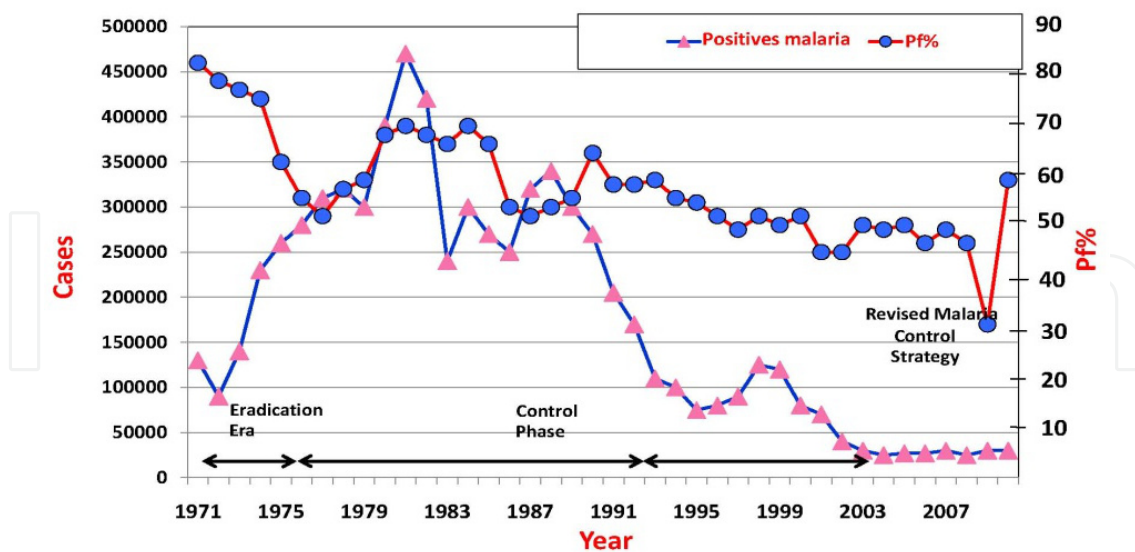
Figure 5. Trends of malaria in Thailand between 1971 and 2010.'Positives' refer to all malaria cases, "Pf" = *P. falciparum* infections only. (http://www.searo.who.int/en/Section10/Section21/Section340_4027.htm).

Based on recorded malaria surveillance activities in Thailand from 1971 to 2011, the peak of malaria cases was seen in 1981 with the total of 473,210 infections, and has since declined thereafter despite another rise in case load seen in 1988 (349,291 infections). In general, from 1988 to 2010, malaria has declined significantly [143, 70]. Despite the significant achievements in malaria control in Thailand over the past five decades, between 25,000 and 35,000 confirmed malaria cases still occur annually [70]. There were 32,502 confirmed cases of malaria in 2010, a decrease of 61.2% compared to 2000. Mortality has also dramatically declined, dropping from 625 in 2000 to 80 in 2010, a decrease of 87.2%. The decline in malaria cases has been attributed to the effective implementation of selective and targeted indoor residual spray of homes and treated netting as vector control measures. Reduction of malaria in Thailand is also the consequence of expanded programs and access to prompt diagnosis and treatment in rural areas as well as an active disease surveillance program.

2.5.2. Malaria vectors and species diversity

Approximately 73 *Anopheles* species are recognized in Thailand. Members within the Leucosphyrus Group, the Maculatus Group and the Minimus Complex are recognized as the most important malaria vectors in the country [145,146,147,148,149]. Molecular techniques based on polymerase chain reaction (PCR) technology have allowed important malaria vectors comprised of sibling species to be correctly identified [33,59]. Within the Dirus Complex, *An. baimaii* and *An. dirus* are considered to be primary malaria vectors in Thailand [149]. Both are forest and forest-fringe inhabiting mosquitoes that are considered highly anthropophilic [150,112,149]. However, a recent study showed a significantly greater number of *An. dirus* and *An. baimaii* collected from cattle-baited traps as compared to human-landing collections, demonstrating that both species could also show strong zoophilic behavior [151].

Among the members of the Maculatus Group, seven known species have been reported in Thailand, including *An. maculatus*, *An. sawadwongporni*, *An. dravidicus*, *An. notanandai*, *An. willmori*, *An. pseudowillmori*, and *An. rampae* [152,153,154,155,147,149,156]. *Anopheles maculatus* and *An. pseudowillmori* has been implicated as important malaria vectors in southern and western Thailand, respectively [145, 147, 149]. *Anopheles sawadwongporni* is a common species often found in high density throughout Thailand, especially along the border provinces with Myanmar and Malaysia [157]. Based on feeding behavior and the natural infection rate detected in this species, *An. sawadwongporni* appears to be a malaria vector in Thailand [16,158,149]. Plasticity in trophic behavior and host preferences over the geographical range of members of this group have been reported [159,153,160]

An. minimus is also an important vector of malaria and is widespread throughout Thailand [161]. Its sibling species, *An. harrisoni* (formerly *An. minimus* C) appears restricted to only two districts of Kanchanaburi Province, western Thailand, where it also occurs in sympatry with *An. minimus* [162]. *Anopheles harrisoni* was previously collected from Mae Sot in Tak Province and Mae Rim in Chiangmai Province, northern Thailand, but no clear confirmation was made at the time [149].

Several other potential secondary or incidental vectors of malaria are also present in Thailand. These mosquitoes can have a close association with humans and include *An. barbirostris* s.l. and *An. epiroticus* (Sundaicus Complex) [163]. Within the *An. barbirostris* Subgroup, *An. campestris* is incriminated as a potential vector of *P. vivax* in Thailand [164]. Additionally, under the correct conditions *An. karwari*, *An. philippinensis* and *An. tessellatus* are also considered to be potential malaria vectors in Thailand. Recently, *An. cracens* (Dirus Complex) and *An. latens* (Leucosphyrus Complex) have been shown natural vectors of *P. knowlesi* in the south of Thailand [165,166,163]. A list of known and potential malaria vector species in Thailand is provided in Table 2.

Anopheles species	Vector in Thailand	Vector in neighboring countries	Vector of *Plasmodium knowlesi* in Thailand
Anopheles dirus	+	+	-
Anopheles baimaii	+	-	-
Anopheles cracens	-	-	+
Anopheles minimus	+	+	-
Anopheles maculatus	+	+	-
Anopheles pseudowillmori	+	-	-
Anopheles sawadwongporni	+	-	-
Anopheles epiroticus	+	+	-
Anopheles campestris	+	-	-
Anopheles latens	-	-	+

+: malaria vector, -: not recorded as vector

Table 2. Known and potential malaria vector species in Thailand [163].

2.5.3. Anopheline behavior

Knowledge of mosquito behavior is of paramount importance to understand the epidemiology of disease transmission and apply effective vector control. Details on mosquito biology, especially blood feeding activity and host preference of a defined species within its particular group or complex is essential to help identify their respective role in disease transmission in specific areas and help vector control operators to design the most appropriate strategy to reduce biting densities. Numerous observations on biting cycles and host preference of the three complexes/group, *An. dirus*, *An. minimus*, and *An. maculatus*, have been conducted in Thailand [167, 168, 169, 170, 171, 172]. However, nearly all previous ecological and behavior studies were based on species populations identified by morphological characters only. Studies on vectors have recognized additional *Anopheles* species within species complexes in Thailand [150, 173, 161, 149]. Infrastructure development and deforestation along the national borders with other countries in the past two decades has led to a significant reduction in malaria incidence, yet many malaria vectors have apparently and successfully adapted to the environmental changes. Using molecular approaches enables investigators to describe the trophic behavior of each species within a complex. For example, the different biting activities of *An. minimus* and *An. harrisoni* were described from two malaria endemic areas of Tak [143] and Kanchanaburi [174] provinces, respectively. Recently, the biting activity and host preference of *An. dirus* and *An. baimaii* have been described from Kanchanaburi [151]. More meaningful investigations on population biology, bionomics and blood feeding activity of sympatric sibling species within medically important complexes can now be conducted with greater accuracy.

2.5.4. Effects of changing environmental conditions on malaria vectors and transmission

Most insect species are generally very sensitive to changes in climatic and environmental conditions, such as ambient temperature, relative humidity, wind speed, and rainfall. The natural environment imposes significant constraints on insect populations [175, 176]. Among the blood-sucking species in the forest-type habitat that transmit diseases to humans, mosquitoes are found to be susceptible to environmental/climatic modifications [144]. Longevity (survival), population density, and ecological distribution of any mosquito can be dramatically influenced by small changes in environmental conditions, and the availability of suitable hosts, larval habitats and adult resting sites. Changes in environmental conditions are directly influenced by modification and increased land use, such as conversion of rice fields to rubber plantations, forested areas to urbanized environments. Human activities are of major concern in changing the patterns of vector-borne diseases. For example, in 1988 a major malaria outbreak along the Thai-Cambodia border was due to transient employment opportunities from gem mining activities with almost 60,000 malaria cases detected in this population [4]. Similarly, between 1998 and 2000 an outbreak of malaria occurred at Suan Ping Village, Ratchaburi Province, western Thailand, in another gem mining area where most of the work force was recruited from Myanmar. This outbreak clearly showed that the man-made activity and population movement could be a significant factor in contributing to disease transmission.

In the past three decades, rubber plantations have expanded in most SEA countries, including Thailand. Although Thailand is known as a significant producer of natural rubber, these plantations were generally restricted to southern Thailand. Recently, rubber trees have been planted in the east and northeastern parts of the country. Rubber plantations placed in once forested hill areas provide potential habitats for several primary malaria vectors such as *An. dirus* and *An. maculatus*, two commonly found vectors in southern Thailand [161]. Recent rubber plantation expansion in the northeast has also opened more job opportunity for migrant workers from neighboring countries. Lacking sufficient labor resources in Thailand, over one million registered migrant workers from neighboring countries have entered the country since 2004 [144]. This has undoubtedly resulted in trans-border movement of malaria into Thailand with the potential of re-introduction of transmission in once malaria-free areas and malaria resurgence and outbreaks in more vulnerable environments.

In summary, efforts are being directed to strengthen malaria control activities along the international borders of Thailand. The problem of border malaria due to inter-country human population movement, both legal and not, is known to greatly complicate the control efforts. In addition, land use modifications have a great influence on vector-borne disease transmission. Careful attention to land use changes along with the climatic and environmental changes is needed to help predict and prevent the reemergence of malaria in all areas of Thailand. Effective collaborative efforts between neighboring countries with trans-border malaria have to be implemented to mitigate continued high malaria transmission in these sensitive areas of the country.

2.6. Vietnam

2.6.1. Overview

Vietnam has a land area of 331,690 km², and 4,550 km long with a total population of approximately 88.2 million [177].) This country shares borders with China in the north, Laos and Cambodia in the west. Malaria is the most important public health burden. A massive epidemic of 1991 resulted in more than one million cases and 4,600 deaths [178]. After this epidemic, the National Malaria Control Program (NMCP) focussed on malaria as its first public health priority and intensive control activities were implemented to help reduce malaria transmission in the country, including mass drug treatment in high endemic areas, indoor residual insecticide spraying and distribution of insecticide-treated bet nets. The successes of the NMCP have been witnessed in many areas, especially in northern Vietnam where no local malaria cases have been reported and malaria entomological inoculated rate has been nil for many years [6, 7, 32]. While malaria control has been successful in northern Vietnam, malaria continues to be a problem further south, particularly in the hilly-forested areas of central and southern Vietnam, and along the international borders with Cambodia and Lao PDR where frequent human population movements occur [92, 43]. Various ethnic minorities are the populations at greatest risk of malaria, suffering five times more malaria paroxysms than the vast majority of the Vietnamese population [179, 180]. From 2010 to 2011, respectively 36% to 18% of the

population were still living in defined high transmission areas, while 54% to 20% were exposed to low transmission and 10% to 63% where in malaria-free, many urbanized, areas [2,3].

All four human malaria parasites and *P. knowlesi* have been reported in Vietnam [181, 182, 183]. Reported malaria cases are mostly due to *P. falciparum* (66%), followed by *P. vivax* (34%), while *P. malariae* and *P. ovale* are seldom recorded [3]. Transmission of zoonotic *Plasmodium knowlesi* has been reported in southern-central Vietnam [184, 185, 186, 187, 188, 189]. *Plasmodium knowlesi* has been found in several *Anopheles* species, especially *An. dirus* considered as the main malaria vector in Vietnam [181, 183].
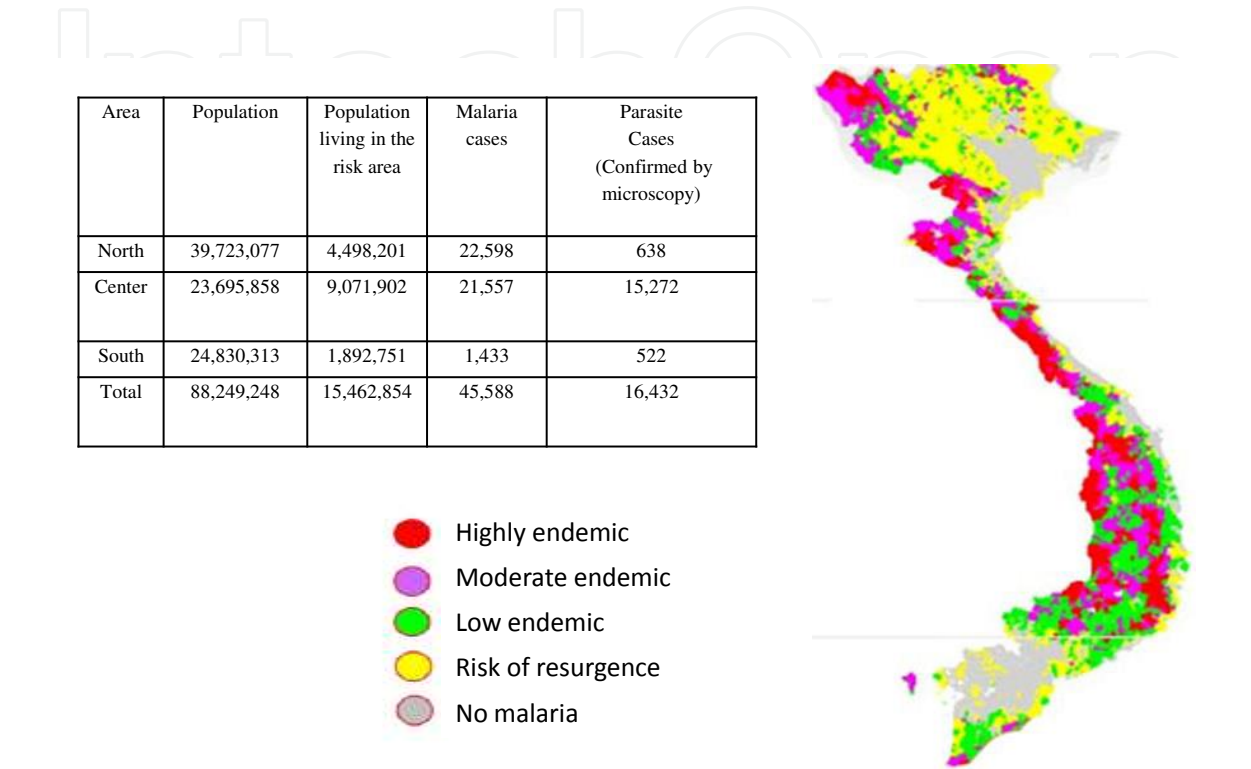
Insecticide use and mass drug treatment were effective measures for controlling vectors and malaria transmission in Vietnam [190]. However, with decades of insecticide and anti-malarial drug use, both resistance of *Anopheles* to insecticides and malaria parasites to malarial drugs has appeared [191, 192, 56, 193, 194]. Moreover, land use modifications caused by deforestation, expansion of agriculture, conversion from rice to shrimp production, have introduced dramatic changes in mosquito habitats and represent new challenges for malaria control strategies in Vietnam. Although considerable effort has been invested applying malaria control activities following the 1991 epidemic, malaria still ranks as an important public health problem. In 2011, 16, 539 malaria cases (6 deaths) were reported in central and highland areas of Vietnam [195]. There are two periods of the year during which malaria transmission is the highest: (1) from the end of the rainy season to the early dry season (September to January) and (2) from the late dry season to the early rainy season (May to August).The dry and rainy seasons may slightly shift from year to year and the intensity of malaria transmission is also dependent on the geographic area and other variables.

The term "forest malaria" is defined within a specific context of transmission epidemiology and involves several sylvatic vectors such as *An. dirus* [7, 196, 43, 183]. The population at greatest risk of infection are the inhabitants of hilly forested areas, particularly ethnic minorities that have the poorest living standards, low educational background, and where their normal life activities include jungle exploitation and subsistence-level slash and burn cultivation practices [196, 71, 180]. Moreover, in both recovered forests and deforested areas, many workers come to live in rudimentary huts and other shelters during harvest time that afford poor protection against mosquitoes. Population movements between different areas, together with generally poor living conditions expose them to high malaria risk. Indeed, the social-ecological factors such as living in remoted areas and the logistical difficulties in implementing and sustaining control efforts against highly efficient forest vectors favour malaria transmission [17, 196, 18, 197, 26].

After the last local malaria cases were reported in northern Vietnam in 1995, malaria transmission has apparently not returned despite reports that malaria vectors remain common [7, 198, 199]. A study on the health information system on malaria surveillance activities in Vietnam [200] called into question the accuracy of data captured and that there was likely a great underestimation for the actual malarial burden reported during the past decade. By applying spatial-temporal analytical tools to determine the association among social aspects, environmental factors and malaria risk in Vietnam, Bui et al., (2011) suspected that malaria transmission is still occurring in some focal areas of northern Vietnam, therefore, emphasizing

that malaria surveillance activities and control capabilities should be sustained to prevent or respond to the reintroduction of malaria in receptive areas.

The prevalence of human malaria and entomological inoculation rates have been reported in several provinces of southern and central Vietnam, such as Binh Thuan, Ninh Thuan, Khanh Hoa, Quang Binh, Binh Phuoc, Dak Nong, Dak Lak, Bac Lieu [7, 181,42, 180, 43, 183, 195].

Area	Population	Population living in the risk area	Malaria cases	Parasite Cases (Confirmed by microscopy)
North	39,723,077	4,498,201	22,598	638
Center	23,695,858	9,071,902	21,557	15,272
South	24,830,313	1,892,751	1,433	522
Total	88,249,248	15,462,854	45,588	16,432

🔴 Highly endemic
🟣 Moderate endemic
🟢 Low endemic
🟡 Risk of resurgence
⚪ No malaria

Source: Meeting on Outdoor Malaria Transmission in the Mekong Countries for 13 countries during 12-13 March 2012, Bangkok, Thailand. [http://www.rbm.who.int/partnership/wg/wg_itn/ppt/ws2/m4VuDucChinh.pdf.]

Figure 6. Total population living in risk area, malaria cases and positive cases (confirmed by microscopy) in Vietnam in 2011.

2.6.2. Biodiversity of Anopheles vectors in Vietnam

In Vietnam, 61 *Anopheles* species have been reported using morphological identification methods [201]. Many species of *Anopheles* from SEA belong to a species complex or group [39]. For species complexes, as often there is either no or unreliable morphological characters to accurately distinguish each sibling species from one another. Therefore, their specific role in malaria transmission remains unclear [202, 203, 40]. The *Anopheles* in Vietnam can be divided into three categories based on their vectorial capacity to transmit malaria: (i) the primary vectors include species in the Dirus (*An. dirus)*, Minimus (*An. minimus*, *An. harrisoni)* and Sundaicus (*An. epiroticus)* Complexes; (ii) secondary or incidental vectors include *An. aconitus*, *An. jeyporiensis*, *An. maculatus*, *An. subpictus*, *An. sinensis*, *An. pampanai*, *An. vagus*, *An. indefinitus*; and (iii) suspected vectors are *An. interruptus*, *An. campestris*, *An. lesteri* and *An. nimpe*. Therefore, 16 (26%) are considered as having some role in malaria transmission in the country.

However, more studies are needed to better define the importance and role of each species, especially secondary and suspected vectors. For example, *An. culicifacies* s.l., an important vector in India, was recently found in Vietnam. However, the species identified was *An. culicifacies* species B of the Culicifacies Complex which is primarily zoophilic and thus regarded as not involved in malaria transmission in the country [54]. In addition, extensive environmental changes have occurred since the 90's, which have modified the *Anopheles* habitats and the presence and prevalence of some species.

2.6.3. Distribution of Anopheles vectors in Vietnam

According to Phan (2008), the anopheline fauna in Vietnam has been sorted based on two criteria [204]:

• **Geographically**, clustered into 4 zones: Northern, South Central-Highlands, Southern and Lam Dong (Province in south-central Vietnam within a temperate zone climate).

• **Physio-geographically** by combining the epidemiology of foci and clustered into 7 different zones: (1) Plains with standing water, (2) Low hills with streams, (3) Low mountains-hills and woodlands with streams, (4) Mountains and forests with streams, (5) Northern plateau, (6) High mountains with streams and waterfalls, and (7) Coastal brackish water habitats.

Vectors such as *An. minimus* and *An. dirus* are present in almost all clusters, whereas *An. epiroticus* and *An. subpictus* are vectors restricted along the coast line with varying degrees of brackish water in natural impoundments (e.g., lagoons, blocked coastal streams and small rivers). The SEA distribution of the dominant vector species has recently been well delineated [59]. Many studies have contributed to new insights on the presence, biology and behavior, and distribution of *Anopheles* in Vietnam. The majority of investigations have focused in the central and southern regions where malaria transmission is most endemic. In Ma Noi and Phuoc Binh Communes, a forested area of Binh Thuan Province, central Vietnam, 24 *Anopheles* species were collected between 2004 and 2006. The predominant malaria vectors were *An. dirus* and *An. minimus* s.l. and also included *An. maculatus* s.l., *An. pampanai, An. aconitus, An. annularis* s.l., *An. nigerrimus, An. philippinensis, An. sinensis, An. annandalei, An. argyropus, An. barbumbrosus, An. crawfordi, An. jamesii, An. jeyporiensis, An. monstrosus, An. tessellatus, An. vagus, An. varuna, An. barbirostris, An. kochi, An. nivipes, An. peditaeniatus,* and *An. splendidus* [43].

A nation-wide study to evaluate the status and the distribution of *Anopheles* malaria vectors in four forested regions in northern Vietnam (northern part of the Hai Van Pass) recorded 30 *Anopheles* species, of which, 20 species were collected in primary forests, 21 in secondary growth forests, 16 in woodland or shrub biomes, and 6 species in tidal mangrove zones. Two main malaria vectors were present, *An. minimus* s.l. and *An. dirus*, as well as potential secondary vectors, including *An. aconitus, An. jeyporiensis, An. maculatus, An. subpictus, An. sinensis* and *An. donaldi*, the latter species representing a new country distribution record for Vietnam [205]. Sympatric sibling species, *An. minimus* and *An. harrisoni*, was confirmed in Hoa Binh Province in north-eastern Vietnam [32] as well as 21 other *Anopheles* species near the Son La hydro-electrical dam (Son La Province), including *An. minimus* [199]. This finding showed that even

though malaria prevalence in this region is very low, malaria risk still remains and vector control capacity in this region should be sustained to prevent or combat possible malaria outbreaks.

Molecular methods have been developed to resolve identification problems due to overlap in morphological characters among sibling species [206, 207, 208, 55, 209, 210, 211]. The distribution of species that were once morphologically identical has been clarified for many localities.

In Vietnam, *An. minimus* has an extensive north-south distribution, while *An. harrisoni* has a much more patchy occurrence [212]. The presence of *An. minimus* and *An. harrisoni* occurs from northern to south-central regions where they often occur in sympatry [213, 32, 212, 42]. In central Vietnam, an increase in density of *An. harrisoni* has been seen compared to *An. minimus* which also coincided with the wide use of permethrin-treated bed nets in the study village [7,213]. The dominance of *An. harrisoni* was also reported in Quang Binh Province, northern central Vietnam [42].

Out of the 8 species that make up the Dirus Complex, only two occur in Vietnam: *Anopheles dirus*, the main vector found in hilly forested areas [32, 41, 18, 42, 43] and the recently described cryptic species, *An.* aff. *takasagoensis* collected in northern Vietnam [40]. Khanh Phu Commune (Khanh Hoa Province in south-central Vietnam) is a hilly-forested area where malaria transmission is endemic. Twelve *Anopheles* species were captured in this area in which *An. dirus* was the dominant (83.2%) species present [183].

Anopheles epiroticus is considered the main malaria vectors in the southern coastal areas below the 11th parallel. Recent studies have shown extremely low infectious rates for this species [46, 58, 7, 214]. *An. epiroticus* is the only member of the Sundaicus Complex present in Vietnam [58, 117, 32, 44].

Anopheles nimpe (Hycarnus Group) is a recently described species which was discovered along the coastal area of southern Vietnam and is suspected as a malaria vector due to its high attraction to humans [45, 215, 32, 42]. To date, very little else is known about this species.

The Maculatus Group has three representatives present in the country, *An. maculatus*, *An. sawadwongporni* and *An. rampae* (Form K), with variable distributions and densities based on geographic area [42, 43]. Only *An. maculatus* is regarded as a vector of minor (secondary) importance [45, 204].

2.6.4. Vector habitats and behavior

Anopheles dirus is primarily a forest malaria vector and the main vector species in many cases. However, in Truong Xuan Commune (Quang Binh Province) and Phuoc Chien Commune (Ninh Thuan Province), locations where malaria transmission is still high, *An. dirus* has not been reported infected [18,42], therefore the role of secondary vectors in malaria transmission may be under estimated [32,42,43].

Species of the Minimus Complex are normally found in forested foothills associated with freshwater streams and canals. *Anopheles minimus* has also been found in sunlit and shaded

ponds, rock pools, and rice paddies. On the outskirts of Hanoi, along the Red River Delta, *An. minimus* was found to oviposit in artificial containers such as rainwater tanks near houses [204,206,216]. *Anopheles epiroticus* is an important malaria vector along the coast of southern Vietnam and has been commonly found in man-made fish and shrimp ponds. This species has been observed to bite humans throughout the night [32].

Species of the *An. maculatus* Group has been found in hilly forested areas, especially in the recovered forest areas. Their larval habitats are closely associated with stream pools and drying river beds. They are generally zoophilic being more attracted to cattle than humans and tend to bite from early evening to the early morning hours [32, 42, 43].

2.6.5. Implication of changing social and environment conditions on vectors and transmission

Extensive environmental changes have occurred in Vietnam since the 1990's [217], which have modified the *Anopheles* habitats and the presence and prevalence of some species. *Anopheles minimus,* known as an endophilic and fairly anthropophilic vector, is abundant mainly during the dry season that generally lasts from November to April in the south and from November to February in northern Vietnam [7]. The use of indoor insecticide residual spraying has been successfully used to reduce malaria transmission as *An. minimus* has a strong behavioral tendency for biting indoors. However, this adaptable vector has shown marked variations in its behavior from endophilic to exophilic and anthropophilic to zoophilic in northern Vietnam where it was more attracted to cattle and other domestic animals kept near the house [32, 34, 42]. In parallel, insecticide use led to the significant increase in density of *An. harrisoni* in Khanh Phu Commune [213].

Human practices are generating important environmental changes throughout the country, such as deforestation, reforestation, plantations, fish and shrimp ponds replacing rice culti‐ vation, road construction, dams, more intensive slash and burn activities, and so on. Such land use changes have an impact on vector habitats, vector diversity and distribution that could either promote or discourage the propagation of some vector species and therefore impact risk of malaria transmission [199,218]. In urban and rural settings, the expansion of electricity to the more mountainous and remote villages encourages people to remain outdoors for longer periods during night time, thereby increasing risk in this unprotected population of being bitten by the *Anopheles* vectors, especially *An. dirus* which is more likely to be exophagic and exophilic [32,43]. Housing construction has implications on malaria transmission. Houses with open construction (e.g., with uncompleted walls, no doors) allow anthropophilic mosquitoes to easily detect human host attractant stimuli and enter the houses to bite [32]. As standard of living and economic development increase in the country, so will the type and quality of houses thus adding additional barriers to host-seeking vectors.

3. Conclusions

Many years of organized malaria control and research have led to some notable successes in reducing the incidence of malaria in countries located on mainland SEA. However, this disease

is still a major health risk in rural and remote communities close to forest and forest fringe areas where socioeconomic conditions remain low, the areas more difficult to -reach, and daily human are closely-related or dependant on the subsistence from forests.

More recent and dramatic changes in the local ecology created by development projects, while aiming to improve the standard of living of the local populations, may have profound and negative effects upon human health and vector-borne diseases. In most countries, deforestation, and reforestation, is one of the most potent factors in relation to emerging and re-emerging infectious diseases. For example, rubber plantations have had the effect of increasing the density of important malaria vectors in Thailand [75]. Southeast Asia has the highest relative rate of deforestation of any major tropical region in the world, and could deplete three quarters of its native forest cover by 2100, effectively removing up to 42% of its fauna and flora biodiversity [19]. Most of the main malaria vectors occurring in mainland SEA are associated with forests, therefore we can anticipate changes in distribution and population densities of malaria vectors, some possibly disappearing while secondary or potential vectors move to exploit the altered habitats to become primary malaria vectors of the future.

Moreover, the expanding exploitation and over utilization of natural resources, together with other forms of economic development can help to improve living conditions, while simultaneously changing the environment in ways that might increase disease transmission risk of malaria or other vector-borne diseases (e.g., dengue). Together with changes in human practices, the adaptation of vector fauna to altered environments, including vector behaviour, might profoundly alter the dynamics of malaria transmission. These are some of the challenges to be raised by all countries in order to reach the goal of malaria elimination by 2015 (Lao PDR), 2020 (Vietnam), 2025 (Cambodia). Clearly there is a need for more studies on *Anopheles* malaria vectors in some countries of SEA, such as Myanmar, where work is now dated. For instance, in order to better control malaria and its vectors, a trans-border network should be organized at the SEA region scale. A better understanding of the mechanisms linking deforestation and development projects with anopheline ecology and malaria epidemiology, and that to contribute to improved health impact assessments in the future, are challenges for further study. Malaria vector control is still predominantly based on the use of insecticides as residual house spraying and bednet impregnation, and still regarded as the most effective way to attack vectors. Yet relatively little work has been done to exploit the behaviour of mosquito vectors as a means of transmission control (e.g., use of spatial repellents to impact outdoor transmission, search of natural substances with insecticide properties respectful of the environment). With expected changes in the distribution and epidemiology of malaria, there will be a critical need to continue to explore and develop new and innovative methods of intervention to complement existing strategies.

Acknowledgements

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