Spatiotemporal distribution of insecticide resistance in *Anopheles culicifacies* and *Anopheles subpictus* in Sri Lanka


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**Summary**

The malaria situation in Sri Lanka worsened during the 1990s with the emergence and spread of resistance to the drugs and insecticides used for control. Chloroquine resistance has increased rapidly over this period, but adverse changes in malaria transmission are more closely associated with insecticide use rather than drug resistance. Insecticide susceptibility tests were routinely carried out in key anopheline vectors across the country for more than a decade. These sentinel data were combined with data collected by other research programmes and used to map the spatial and temporal trends of insecticide resistance in the main vectors, *Anopheles culicifacies* and *A. subpictus*, and to examine the relationship between insecticide resistance, changes in national spraying regimens and malaria prevalence. Both species had widespread resistance to malathion, the insecticide of choice in the early 1990s. Both species were initially susceptible to the organophosphate and pyrethroid insecticides used operationally from 1993, but some resistance has now been selected. The levels of malathion and fenitrothion resistance in *A. subpictus* were higher in some ecological regions than others, which may be related to the distribution of sibling species, agricultural pesticide exposure and/or environmental factors. The study highlights that the emergence and spread of insecticide resistance is a constant threat and that active surveillance systems are vital in identifying key vectors and evidence of resistance.

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1. Introduction

Malaria is a major public health problem in Sri Lanka. Annual cases range from 300,000 to 400,000, with malaria-endemic regions in the North-East and North-Central Provinces. The disease is caused by the Plasmodium falciparum and Plasmodium vivax parasites, which are transmitted by the Anopheles mosquito species. The Anopheles subpictus mosquito is the main vector of malaria, and the Anopheles culicifacies mosquito is a significant secondary vector in Sri Lanka.

Malaria control in Sri Lanka has been largely based on chemotherapy and indoor residual insecticide spraying. The use of DDT was replaced by malathion in 1976, and later by permethrin and deltamethrin. Resistance to these insecticides has spread throughout the country. Chloroquine, the first-line treatment, has been affected by resistance, and its effectiveness has decreased over the years. The introduction of permethrin-impregnated bed nets has become more common, particularly in the northern regions where malaria-endemic areas have been identified.

2. Methods

2.1. Mapping trends of insecticide resistance

Annual insecticide susceptibility testing data were collected for malaria-endemic regions of the country. Susceptibility testing was carried out using a range of insecticides, including DDT, malathion, permethrin, deltamethrin, and fenitrothion. The data were mapped using geographical information system (GIS) software (ArcGIS 8.2; Environmental Systems Research Institute, Redlands, CA, USA). Levels of insecticide resistance were calculated based on the recommended discriminating dosages of insecticides used during the monitoring period.

The mapping of resistance trends was achieved through the National Imagery and Mapping Agency database (http://earth-info.nga.mil/gns/html/). Mosquito collection sites were georeferenced using longitude and latitude coordinates obtained from the National Imagery and Mapping Agency database (http://earth-info.nga.mil/gns/html/). The GIS software was used to create a map showing the spatial distribution of insecticide resistance in Sri Lanka.

The monitoring of insecticide resistance is crucial for the effective control of malaria. The mapping of resistance trends helps in identifying regions where insecticide spraying is needed to reduce the vector population and prevent malaria transmission. The use of resistant insecticides can lead to the development of resistance in the mosquito population, which can reduce the effectiveness of malaria control strategies.
3. Results

3.1. Mapping trends of insecticide resistance

Insecticide resistance in malaria vectors in Sri Lanka were imported from the Sri Lankan Department. Maps were digitised on screen in ArcGIS 8.2, and each variable was divided into two distinct regions based on the zones provided. For each mosquito species, mortality rates in each region were pooled, and the overall means were compared using the Mann–Whitney U test with Bonferroni correction for multiple comparison. Resistance data were examined only for the insecticides that were commonly used between 1991 and 2003. These included malathion, fenitrothion, cyfluthrin (2003) were evident in some localities (Figure 1).

In addition, mortality rates of other Anopheles spp. at each location were compared with those of A. culicifacies or A. subpictus to determine whether the vector species had similar patterns of resistance to the non-vectors and whether the latter could be used as surrogates for testing the vectors when their abundance was low.

Three examples are presented as population case studies, which examined the relationship between insecticide resistance, insecticide treatment regimens, malaria incidence and chloroquine resistance over time. Case study locations involved data sets with more than 5 years of mortality data between 1991 and 2003 for one insecticide, tested against either A. culicifacies or A. subpictus. Insecticide resistance data were compared with information on insecticide treatment regimens and the number of mosquitoes caught at each location. Until 1999, AMC entomological teams recorded and tested all Anopheles mosquitoes caught at each collection site, thus providing basic information on vector abundance. Malaria incidence data from passive surveillance carried out between 1991 and 2002 were available from the AMC offices and based on blood slide positivity (%). Information on the level of chloroquine resistance by district was obtained from Galappaththy (2002).

In addition, mortality rates of other Anopheles spp. at each location were compared with those of A. culicifacies or A. subpictus to determine whether the vector species had similar patterns of resistance to the non-vectors and whether the latter could be used as surrogates for testing the vectors when their abundance was low.

Malathion and fenitrothion were the most frequently tested insecticides for both mosquito species. Resistance levels varied between locations and over time (Figure 1). Overall, levels of malathion resistance were high in all regions (mortality ≤50%), except for A. subpictus in most coastal areas (mortality ≤75%). High levels of resistance to DDT (mortality ≤50%) occurred in both species. From 1995, low levels of resistance (mortality >75%) to lambdacyhalothrin, permethrin, deltamethrin, cyfluthrin, cypermethrin, propoxur and etofenprox were detected. However, higher levels of resistance in A. culicifacies to fenitrothion (in 2000 and 2001) and permethrin (2001), and in A. subpictus to fenitrothion (1997), permethrin (1995 and 2001), lambdacyhalothrin (2001) and cyfluthrin (2003) were evident in some localities (Figure 1). Collection sites and regional mortality rates for A. culicifacies and A. subpictus are shown in Table 1. For A. culicifacies, minimal differences in resistance were found between regions, however tests with permethrin were limited due to the small number of collection sites. For A. subpictus, higher levels of resistance to malathion (P<0.001) and fenitrothion occurred in inland regions (compared with coastal), to malathion where annual average temperatures were less than 30 °C, and to fenitrothion in areas 100 m a.s.l. (compared with <100 m). Some resistance was also evident in high paddy field density and sparsely populated areas (compared with <105 000 ha paddy fields and >100 inhabitants/km²).
Figure 1  Locations and levels of resistance in Anopheles culicifacies and A. subpictus between 1991 and 2003.
Insecticide resistance in malaria vectors in Sri Lanka

3.2. Mapping trends of insecticide resistance

3.2.1. Population case study 1
The Rambukkana Health Area in the northern region of the Kegalle District is close (4–6 km) to the borders of the Kurunegala and Kandy Districts (Figure 2). Malathion susceptibility tests were carried out in *A. culicifacies* in 1991–1994, 1996–1997 and 2001. There was a significant decrease in mosquito mortality between 1993 and 1996 (Figure 2). The numbers of *A. culicifacies* collected for testing during this period were 4703 in 1992, 40 in 1993, 29 in 1994, 52 in 1995, 1031 in 1996 and 34 in 1997.

*Anopheles aconitus*, *A. annularis*, *A. barbirostris*, *A. jamesi*, *A. pallidus*, *A. tessellatus*, *A. vagus* and *A. varuna* showed high mortalities (≥80%) with malathion. For malaria control, malathion was replaced in the Kegalle District with deltamethrin in 2002, in Kandy with fenitrothion in 2002, and in Kurunegala with lambdacyhalothrin in 1994 (used until 2000), etofenprox in 1999 (until 2001) and deltamethrin in 2002 (Figure 2). Malaria data from two health areas in this region were examined together owing to their close proximity and similar prevalence patterns. The average proportion of blood slides testing positive for *P. vivax* was 10.7% and for *P. falciparum* was 1.9% in 1991, which decreased to 2.0% and 0.3%, respectively, by 2002 (Figure 2). Overall, *P. falciparum* prevalence remained low and constant (<2%), whilst *P. vivax* fluctuated inter-annually, with peaks recorded in 1993 (23.2%) and 1996 (9.3%). The greatest decrease in *P. vivax* prevalence occurred between 1993 and 1995. In 1999, 42.0% of *P. falciparum*-positive patients (RI late 52.8%, RI early 22.2%, RII 2.8%, RIII 22.2%) had parasites that were resistant to chloroquine in Kurunegala (n = 100). No data were available for Kegalle.

![Figure 1](Continued)

3.2.2. Population case study 2
The Galewela Health Area lies in the western region of the Matale District, close (5–9 km) to the borders of the Kurunegala and Anuradhapura Districts (Figure 2). Malathion susceptibility tests were carried out in *A. culicifacies* in 1991–1994 and 1996–1997, with no significant difference in mortality found between years. The numbers of *A. culicifacies* collected for testing during this period were 4703 in 1992, 40 in 1993, 29 in 1994, 52 in 1995, 1031 in 1996 and 34 in 1997.

*Anopheles aconitus*, *A. annularis*, *A. subpictus* and *A. varuna* had high mortalities (≥80%) with malathion, whilst *A. vagus* was resistant. Malathion antimalarial spraying in the Matale District ceased in early 1997 and was replaced in 1998 with deltamethrin (until 2003) and lambdacyhalothrin (until 1999), whilst in Anuradhapura, fenitrothion (1996–1998), lambdacyhalothrin (1999–2001) and cyfluthrin (2000) were used as alternatives (for Kurunegala see previous case study). In this region, the proportion of blood slides testing positive for *P. vivax* was 23.8% and for *P. falciparum* was 12.1% in 1991, which gradually decreased to 0.8% and 0.04%, respectively, by 2002 (Figure 2). The greatest decrease for *P. vivax* occurred between 1994 and 1996. In 1999, 36% of *P. falciparum*-positive patients (RI late 52.8%, RI early 22.2%, RII 2.8%, RIII 22.2%) had parasites that were resistant to chloroquine in Matale (n = 100).

3.2.3. Population case study 3
The Buttala Health Area lies in the central western region of the Monaragala District, 10–15 km from the border of the Badulla District (Figure 2).
Figure 2  Population case studies. Shown in each location are: (top) percent mortality of *Anopheles culicifacies* after exposure to malathion (white bars) or fenitrothion (shaded bars) (including 95% binomial CIs), with a number of small sample sizes included for general trend analysis in Rambukkana 1994 (n = 13), 1995 (n = 30) and 1997 (n = 11), in Galewela 1997 (n = 32) and in Buttala 1992 (n = 31); (middle) annual *Plasmodium vivax* and *P. falciparum* malaria positivity (and 95% binomial CIs) based on an average of 22 087 blood slides in Rambukkana, 17 965 in Galewela and 21 570 in Buttala; and (bottom) year in which a particular insecticide was used in the region surrounding the health area, with the vertical arrows indicating the introduction of a new insecticide. Mal: malathion; Lam: lambdacyhalothrin; Del: deltamethrin; Fen: fenitrothion; Eto: etofenprox; Cyf: cyfluthrin.

*Anopheles annularis*, *A. jenasi*, *A. tessellates*, *A. vagus* and *A. varuna* had high mortalities with malathion and fenitrothion (≥80%), in contrast to *A. nigerrimus*, *A. subpictus* and *A. peditaenius*. Malathion was replaced in the Monaragala District with fenitrothion in 1999 (until 2001), lambdacyhalothrin in 2000 (until 2001) and cyfluthrin in 2001. The proportion of blood slides testing positive for *P. vivax* was 17.8% and for *P. falciparum* was 2.0% in 1995, which decreased to 1.0% and 0.2%, respectively, by 2002 (Figure 2). Overall, *P. falciparum* prevalence remained relatively low (<4%), whilst *P. vivax* increased dramatically in 1998 (28.6%) and 1999 (26%), and then decreased to 1% by 2002.

In 1999, 62.0% of *P. falciparum*-positive patients (RI late 46.8%, RI early 25.8%, RII 17.7%, RIII 9.7%) had parasites that were resistant to chloroquine in Monaragala (*n* = 100).

### 4. Discussion

Mapping the spatial and temporal trends of insecticide resistance in *A. culicifacies* and *A. subpictus* in Sri Lanka over a decade has illustrated the heterogeneity of resistance present on the island.

Malathion was primarily used for indoor residual spraying until the mid-1990s, when increases in malaria and malathion resistance led to its gradual replacement.

The high levels of malathion resistance in many locations for both Sri Lankan vectors are probably a direct result of malathion used for malaria control for 20 years, as the major resistance mechanism in *A. culicifacies* is malathion-specific and this insecticide has never been used for agricultural treatments on the island. There is now evidence of emerging resistance to the newly introduced insecticides after only a few years of usage. In contrast to malathion, this may be the result of extensive agricultural use of the same insecticides over many years. Cross-resistance, whereby a mechanism responsible for resistance to one insecticide confers resistance to other chemically or functionally related insecticides (Brogdon and McAllister, 1998; Hemingway et al., 2002, 2004; Karunaratne, 1999) may also be playing a role. Major mechanisms
of resistance involve either an alteration within the target site of the insecticide, which prevents it from binding to its target, or an alteration in the rate of insecticide metabolism, i.e. detoxification or sequestration, which occurs when increased levels or modified activities of esterases, mono-oxygenases or glutathione S-transferases (GST) prevent it from reaching its site of action. Selection by insecticides with shared target sites or detoxification pathways may therefore produce cross-resistance to insecticides such as fenitrothion, even though this insecticide has had very restricted agricultural use in Sri Lanka.

The main mechanisms associated with DDT and malathion resistance in Sri Lankan A. culicifacies and A. subpictus are primarily metabolic and involve carboxylesterases (malathion) or mono-oxygenases and GSTs (DDT) (Hemingway et al., 1991; Herath et al., 1987, 1988; Karunaratne and Hemingway, 2001). More recently, an altered acetylcholinesterase conferring organophosphate resistance has been detected in both species (Karunaratne, 1999). These mechanisms can confer broad-spectrum cross-resistance and may now be maintained by selection with the new insecticides used for malaria control.

The selection of malathion resistance was almost certainly a direct result of antimalarial activities, because malathion use was restricted to public health treatments from the early 1980s and the resistance selected in A. culicifacies was malathion-specific (Karunaratne and Hemingway, 2001). However, identifying sources of selection pressure with the insecticides currently employed in vector control is more complex, as most are used both in agriculture and public health (Department of Agriculture, 2004). Anopheles subpictus is subjected to more agricultural insecticide pressure than A. culicifacies, as the former breeds in paddy fields whereas the latter mainly breeds in riverbed pools (Hemingway et al., 1987; Herath and Joshi, 1989). However, as both species rest and feed indoors, adult female mosquitoes will continue to experience pressure from permethrin-impregnated bed nets and AMC residual spray activities.

The alternation of chemically unrelated insecticides was introduced in the mid-1990s to reduce malaria transmission and to delay the development of insecticide resistance. Our analyses, despite their limited sentinel nature, suggest that the changes in spray regimens have stopped resistance to the new insecticides from becoming widespread, and potentially reduced malaria transmission. This is in contrast to the earlier protracted use of DDT, which eventually selected higher levels of resistance in both vectors which subsequently remained in the populations despite the withdrawal of DDT in 1977, and the malathion use that rapidly selected for resistance which also subsequently stayed at a high level despite the reduced use of malathion since 1994. The challenge now is to maintain or improve the efficacy of control without exacerbating the insecticide resistance problems. In Mexico, studies have shown that annual planned rotations or a fine-scale mosaic system of two unrelated insecticides are effective in slowing the rate of selection for pyrethroid resistance compared with long-term use of a pyrethroid. The baseline studies for this work are published (Hemingway et al., 1997; Penilla et al., 1996, 1998) and the full results are detailed in a series of 10 papers that have been accepted for publication by 'Medical and Veterinary Entomology'.
and reflects similar organophosphate resistance trends as the Japanese encephalitis vector, *Culex tritaeniorhynchus*, which is a paddy field breeder in Sri Lanka (Karunaratne and Hemingway, 2000). *Anopheles subpictus* species A is also more endophilic and seasonally abundant than species B (Abhayawardana et al., 1996), and thus should be more exposed to indoor residual insecticide treatments.

Our three population case studies suggest that regular residual spraying of a single insecticide increases resistance, thereby sustaining or increasing the risk of malaria if effective alternatives are not introduced. For instance, in the Rambukkana Health Area, malathion was used until 2002 and resistance patterns changed during this time, with the highest levels found in 1996 and 1997. Interestingly, the additional use of lambdacyhalothrin in 1994 and etofenprox in 1999 in this region corresponded with a reduction in malaria. Similarly, in the Galewela Health Area, the introduction of lambdacyhalothrin in 1994 and cidofothin in 1996 and etofenprox in 1999 probably played a key role in the decline of malarial disease. In contrast, in the Buttala Health Area, malathion resistance (+50%) increased between 1991 and 2001, although not significantly. There was a rapid increase in malaria in 1998 and 1999, which prompted a switch from malathion to fenitrothion. This delay in changing to a more effective insecticide may account for the significantly higher malaria prevalence found in Buttala compared with Rambukkana and Galewela, where alternatives to malathion were introduced 5 years earlier.

It appears that alternating different insecticides for malaria control in Sri Lanka has the potential to reduce the incidence of disease and to extend the effective lifespan of insecticides. In the three population case studies, there was a 2–6-fold reduction in malaria prevalence within 2 years of introducing additional or alternative insecticides to malathion. Whilst *P. vivax*, which remains fully chloroquine susceptible, may have been affected by increased drug treatment, these reductions, particularly in *P. falciparum prevalence*, are not likely to be a result of better drug treatment, as all districts had moderate to high levels (36–62%) of chloroquine resistance and no new antimalarials were introduced as first-line drugs during the study period (Galappaththy, 2002). In a similar situation in southern Africa, sulfadoxine–pyrimethamine drug resistance and pyrethroid insecticide resistance were selected coincidently in the same region (Hargreaves et al., 2000; Roper et al., 2003). In this instance, as both drug and insecticide treatments were changed simultaneously, it was not possible to attribute accurately the contribution of each change to the resultant reduction in malaria transmission.

The success of malaria control programmes is dependent on knowledge of the local epidemiology and associated risk factors, as these vary between populations. In Sri Lanka, human migration, housing type, age, gender, socio-economic status, proximity to water sources, rainfall, vegetation and cultivation type are all risk factors for malaria (Gamage-Mendis et al., 1991; Gunawardena et al., 1998; Klinkenberg et al., 2004; Konradsen et al., 2003; Mendis et al., 1990; van der Hoek et al., 1998, 2003). The populations presented in our case studies vary environmentally, socio-economically and demographically, and therefore will have different prevailing risk factors that will impact on control programmes. For example, Rambukkana and Galewela are more urbanised than Buttala, which has a large rural population, with many living in poorly constructed houses, which is an important risk factor. This region also receives an annual influx of visitors and religious pilgrims to the Kataragama Shrine (Hargigan, 1998), which may contribute to the region’s high malaria incidence. *Anopheles peskonius*, *A. nigerrimus* and *A. vagus* had varying degrees of insecticide resistance. These species are primarily exophagic paddy field breeders ( Yapabandara, 1997), hence their resistance patterns should reflect selection by agricultural insecticides. Many other non-vectors remained fully insecticide susceptible. Hence, non-vectors cannot be used as surrogates to extrapolate to the resistance status of the vectors.

Although the malaria situation in Sri Lanka initially worsened over the past two decades, more recently there has been a dramatic drop in malaria case numbers (Briet et al., 2005). This is presumably predominantly because of renewed access by control personnel to the northern districts of the country following the ceasefire in the civil war. From our data, we consider that in the districts less affected by war, the switching of spraying from malathion to alternatives has also contributed importantly to the decline in national totals of malaria cases. The emergence and spread of insecticide resistance is a constant threat to these control programmes as the vector populations respond to increasing insecticide selection pressures, and active surveillance systems are vital in identifying key vectors and evidence of resistance.

Conflicts of interest statement
The authors have no conflicts of interest concerning the work reported in this paper.
References


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