

Control of malaria vectors with the insect growth regulator pyriproxyfen in a gem-mining area in Sri Lanka

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Abstract

The study was conducted in eight adjacent villages in central Sri Lanka where there are many shallow pits dug by gem miners that fill with water. These become breeding places of the main malaria vector *Anopheles culicifacies*, and of the second most important vector *Anopheles subpictus*, but not of *Anopheles varuna*, the third most important vector. With the help of local volunteers, data on the adult populations of these three species was collected by various standard methods, and data on the incidence of malaria cases was collected by two clinics set up for the project and through the existing hospitals. Prevalence of malaria infection in symptom-less people was investigated by mass blood surveys. On the basis of a year's pre-intervention data the villages were stratified into four with high levels of malaria transmission and four with lower transmission. Within each stratum two villages were randomly assigned for mosquito control by treating all the gem pits, as well as river bed pools, with a granular formulation of the insect growth regulator pyriproxyfen at a target dose of 0.01 mg a.i./litre. The intervention caused significant reductions in the adult populations of *An. culicifacies* and *An. subpictus*. Similarly, incidence of malaria was reduced in the intervention villages to about 24% (95% c.i. 20–29%) of that in the controls. Prevalence of parasitaemia also declined significantly. It is concluded that in this situation where, with active community participation, the breeding sites of the main vectors could be located; vector control by a highly active and persistent insect growth regulator can be a very effective means of malaria control. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

In Sri Lanka there are four main gem-mining areas, in one of which there are deep mines but in

the others there are many hand dug, shallow pits. When these are left by the gem miners, the pits fill with water in the rainy season and form one of the major types of *Anopheles* breeding site in the country. The hand digging of shallow pits is permitted after obtaining a license from the State Gem Corporation. A deposit of money is ob-

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tained by the Corporation that is intended to ensure closure of gem pits after mining ceases. However, many licensed gem pits have been found left unfilled (Punchiappuhamy, 1976) and many pits are dug illicitly.

Gem mining in Elahera, situated in Matale and Polonnaruwa Districts, has been carried out since the 1960s over an area of 6000–7500 ha (Wickramasinghe, 1991). The density of shallow gem pits that could form potential breeding habitats for mosquitoes was estimated to be 247–370 per ha. At the peak of gem-mining activities in the 1970s a nomadic population of 25 000–50 000 people was estimated to have been engaged in mining operations (Wickramasinghe, 1981).

The slide positivity rate for *Plasmodium vivax* and *Plasmodium falciparum* from passive detection of malaria cases among fever patients in Elahera gem-mining area reaches a maximum of about 40%, which is one of the highest rates in Sri Lanka (Anti-Malaria Campaign, 1965, 1968 and 1976). After the near eradication of malaria from Sri Lanka by DDT residual spraying in the 1960s there was resurgence which was thought to have originated in the Elahera gem-mining area due to mass migration of people from all parts of the island in and out of this area.

Anopheles culicifacies Giles is regarded as the principal vector of malaria in Sri Lanka with *Anopheles subpictus* Grassi as a secondary vector (Amerasinghe et al., 1991, 1992, 1999). From ELISA tests for circumsporozoite proteins of *P. vivax* and *P. falciparum*, these two mosquito species, as well as *Anopheles varuna*, were incriminated as vectors in the Elahera gem-mining area (Yapabandara, 1997). Breeding of *An. culicifacies* is almost entirely in gem pits but some breeding of *An. subpictus* and most of that of *An. varuna* is in other sites such as river bed pools and slow moving river margins (Yapabandara, 1997).

The huts occupied by the gem miners are mostly made out of woven palm leaves and polythene that give no protection from mosquitoes and are unsuitable surfaces for insecticidal spraying. Residual insecticide operations have failed to give adequate protection against malaria vectors in this area. Therefore, although worldwide emphasis for malaria vector control in the last 50

years has been on adulticides in bedrooms, the important Elahera area appeared to be a case where larviciding should be investigated. However the efficiency of such programmes depends on searching out all possible larval habitats for treatment within the flight range of vector mosquitoes from the community, which it is desired to protect (Gratz and Pal, 1988).

Various methods such as chemical larviciding, use of oil, larvivorous fish and refilling with soil have been considered to control the mosquito larvae in the gem pits (Anti-Malaria Campaign, 1976). However there are practical difficulties with these methods, e.g. the need for repeated application of larvicides or re-introduction of fish when the pits refill after a dry spell. The limited access to the area makes filling of pits by bulldozers difficult. Oil and temephos have been used in Sri Lanka to control *An. culicifacies* larvae and pupae since 1911 and 1977, respectively, and the cost effectiveness of these in gem pits have been calculated (Wickramasinghe, 1981). Other methods, which have been reported effective for larval control in other countries, include the use of expanded polystyrene beads (Maxwell et al., 1990) and insect growth regulators (IGRs) (Schaefer and Mulligan, 1991). Kanda et al. (1995) reported that the application of granules containing the IGR pyriproxyfen at 0.005 mg/l to slow moving rivers in an area of low malaria endemicity in Thailand, reduced the transmission of malaria and the numbers of *Anopheles dirus*, *Anopheles maculatus* and *Anopheles minimus* collected from cattle-baited net traps and human-baited double nets. However, the intervention was not effective in controlling malaria transmission in an area highly endemic for malaria. In the first phase of the present work a small-scale field trial was carried out to assess two concentrations of pyriproxyfen (0.5% sand granule formulation at the rate of 0.01 mg and 0.1 mg a.i./l), used engine oil, temephos, expanded polystyrene beads and filling the pits with soil. Two annual applications of pyriproxyfen at the rate of 0.01 mg a.i./l were found to be the method of choice (Yapabandara, 1997; Yapabandara and Curtis, in preparation).

The main objective of the trial described in this paper was to determine the effect in the Elahera

area of applications of pyriproxyfen to the gem pits and river bed pools on the adult vector populations and the malaria incidence, and parasite prevalence. Four treated villages were compared with four controls to test the impact and determine the practicability of using this method.

2. Methods and methods

2.1. Study area

This study was carried out in Kaluganga, which is part of Elahera gem-mining area situated in Matale District (7°40'N, 80°50'E) in the dry zone of Sri Lanka (Fig. 1). A cluster of eight villages with a total area of 23 km² was selected for this study. The eight villages are named Dasgiriya, Dewaladeniya, Kaluganga, Kapuyaya, Maoya, Morathanna, Thorapitiya and Wallewela. The numbers of gem pits per village ranged from 311 to 3622. The villages are surrounded by thick jungle. The area is a settlement scheme, which was established about 30 years ago around the rivers, Aban ganga and Kalu ganga.

The study area had been under three monthly indoor residual spraying of 5% malathion (50% WDP at 2g/m²) and larviciding of river and stream bed pools with temephos. These control methods were withdrawn during the trial period.

2.2. Geographical reconnaissance

The eight villages in the gem-mining area with a 1.5 km wide surrounding zone were mapped to show the geographical distribution of houses and potential breeding places, before the commencement of collection of baseline data. The location of every house, path and major water body was surveyed by counting paces along compass bearings. Each house encountered during the geographical reconnaissance (GR) was given a number and the GR number was marked on the door. Within the 1.5 km zone around each village all the gem pits were numbered. Actual or potential water bodies were classified as gem pits, river bed pools, river margins, rain water pools, paddy fields and wells. Two surveys were carried out in

the dry and wet seasons to identify the potential breeding places and their approximate water surface area.

2.3. Census

After the GR, information on the name, age, sex, occupation and time of residence in the area were recorded for each inhabitant, by visiting each house. At that time household cards marked with this information were issued. Four of the eight villages had populations of less than 500 while the other four had populations of 600–1100.

2.4. Methods of entomological monitoring

Anopheline populations in the study area were estimated by seven sampling methods: window exit trap collection; pyrethrum spray sheet collection; indoor human landing collections; all night or for the first part of the night up to midnight; cattle-baited hut collection and cattle-baited net trap collection (WHO standard methodology given in WHO, 1975); and light trap collection (Lines et al., 1991). The locations chosen for applying these methods were near the centres of each village to try to avoid interference by immigration of mosquitoes from neighbouring villages.

2.5. Parasitological parameters

2.5.1. Passive case detection

Malaria cases were monitored by passive case detection by two field clinics set up for this trial and at two clinics at the existing government health facilities. One malaria field clinic was established in the field laboratory at Dewaladeniya, and was open day and night. The other malaria field clinic was set up in Dasgiriya temple and was open every alternate day from 09:00 to 17:00 h. Records of malaria cases were collected once a week from outpatient departments (OPD) of the nearest medical facilities at Laggala Pallegama government hospital and Haththotaamuna dispensary.

The people in the area were requested to bring their household cards which were issued during

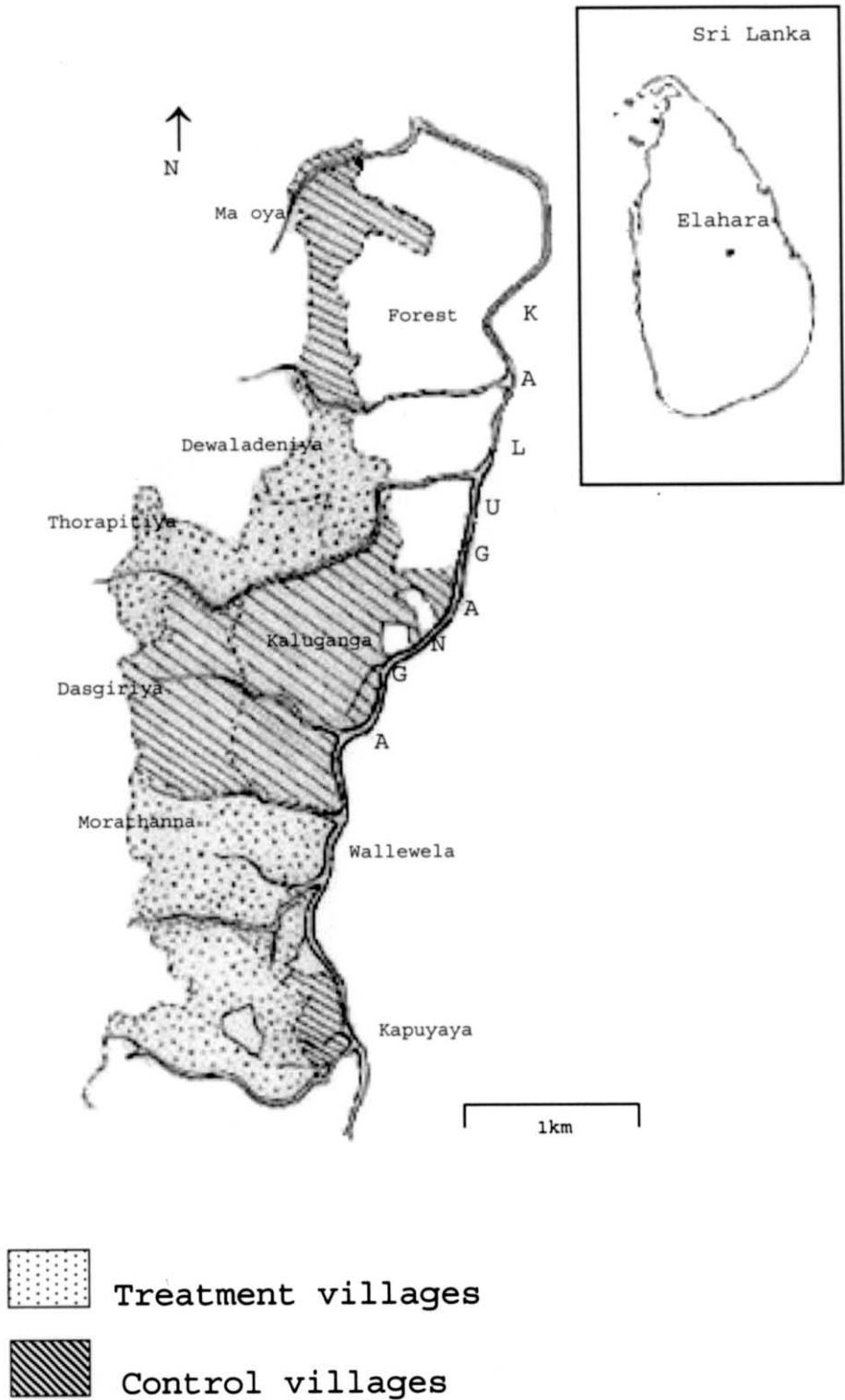


Fig. 1. Sketch map of the eight villages (inset: their position in Sri Lanka).

the census survey when they came to the clinics. These cards allowed patients who were permanent residents in the area to be quickly assigned to their village of residence, and hence to whether they were from a treatment or control area. If a patient had forgotten to bring the card then the patient's name was traced from the records maintained at the four clinics.

From all patients reporting fever or with fever detectable by an axillary thermometer, thick and thin blood films were taken, stained with Giemsa and examined for malaria parasites using oil immersion microscopy. The thick films were used for screening and the thin films for species diagnosis. Patients positive for *P. vivax* or *P. falciparum* were treated with chloroquine and primaquine, according to the National Malaria Control Programme drug policy (Anti-Malaria Campaign, circular no. 406). The *P. falciparum*-positive patients were asked to attend the clinic on the eighth day after the treatment to check whether their infections were resistant to chloroquine. The chloroquine-resistant *P. falciparum* patients, except pregnant mothers and infants under one year of age, were treated with sulfadoxine, pyrimethamine and primaquine. Pregnant mothers and infants with chloroquine-resistant infections were referred to the nearest hospital for quinine treatment.

All the blood films positive for *P. falciparum* or *P. vivax* parasites and 10% of negative blood films were cross checked monthly by a senior microscopist (technician) at the AMC Regional Laboratory, Matale.

2.5.2. Mass blood surveys

Two mass blood surveys were carried out in July and December during the pre- and post-intervention years. Blood films were taken, regardless of the presence/absence of fever, from all the residents of the eight villages by visiting each house. All the blood films were checked for malaria parasites and positive patients were treated.

2.5.3. Community participation

Meetings were held for the residents (one group meeting per 20 houses) in the eight villages and

they were informed of the potential benefits of the project and the importance of community participation for its success. A volunteer was selected from each group of 20 houses at the beginning of the project. They helped the field staff to hold group discussions with the villagers and to find new gem pits, breeding places and the application of pyriproxyfen. The villagers were requested to inform the staff in the field station or the volunteers if new gem pits were excavated in their areas so that the new pit could be rapidly treated with pyriproxyfen granules.

2.5.4. Stratification of villages and randomisation for treatment or control

On the basis of the collections of the three vector species and the malaria data in the pre-intervention year, the eight villages were arranged into two strata with apparently lower and higher malaria risk. Within each stratum two villages were randomly assigned for treatment and the others to be controls. The result of this procedure was that Dewaladeniya, Morathanna, Thorapitiya and Wallewela were assigned for treatment and the other four to be controls (Fig. 1).

2.5.5. Application of pyriproxyfen

Pyriproxyfen, S-31183 (Adeal 0.5% G), was applied at the rate of 0.01 mg a.i./l (2 g of granules per cubic metre) to the gem pits and pools of the four treatment villages. Treatment commenced in December 1994. The depth of the water was measured using a rope attached to a stone and marked in centimetres. This measurement together with the diameter was used to calculate the weight of pyriproxyfen granules to be applied. A few polystyrene beads were put into the breeding places to mark those that had been treated. The gem pits and pools up to 1.5 km from the villages were treated, as the vectors could be expected to fly over that range (Rawlings et al., 1981).

The second application of pyriproxyfen was conducted between June and July 1995 in the post-monsoon season when river bed pools were formed. The requirement of pyriproxyfen in these pools was calculated on the basis of an assumed depth of 10 cm together with the measured diameter of the pools. The third application of

pyriproxyfen was carried out at the end of November 1995 to the gem pits and water collections.

2.5.6. Efficacy of pyriproxyfen

The schedule of reapplication of pyriproxyfen to the gem pits and river bed pools was decided on the basis of field bioassays. Five gem pits were randomly selected from each of the four treatment villages. In these pits the efficacy of pyriproxyfen was recorded monthly using 5 l capacity buckets with two 7 × 5 cm holes covered with nylon netting mesh. A hole was cut in the middle of a 25 × 25 × 3 cm expanded polystyrene board and the bucket was inserted and floated in the water of the gem pits. Ten laboratory reared 3rd–4th instar larvae of *An. culicifacies*, *An. subpictus* or *An. varuna* were put into the bucket and fed with Farex baby food. The bucket was covered with a net and observations were made until all larvae died or developed into adults. Earlier work had shown that in untreated pits almost all the larvae developed successfully to adulthood (Yapabandara et al., in preparation). This was not so in freshly treated pits but when, several months after treatment, adults emerged in the bioassays of the treated pits, re-application of pyriproxyfen to gem pits in all the treatment villages was started. Bucket bioassays were also carried out in river bed pools to decide when to re-apply pyriproxyfen to that habitat.

2.5.7. Statistical analysis of entomological data

To assess the statistical significance of the effect of intervention, data on the three vector species from the three most successful mosquito collection methods (cattle-baited huts and partial and all night landing catches) were used. For each of the treatment and control villages, the differences between the catches (transformed to $\log(x+1)$) were calculated for corresponding quarters of the pre- and post-intervention years. The means and standard deviations of these differences for the villages designated as controls and for those designated for treatment were calculated. The antilogs of these mean differences, minus one, gave estimates of the percent change after the introduction of the intervention and, with the standard devia-

tions of the differences, paired *t* tests of the differences and 95% confidence limits of the percent change were calculated.

2.5.8. Statistical analysis of parasitological data

Incidence rates of fever with malaria parasitaemia for pre- and post-intervention years were calculated, using as denominator the person years of follow up in each village. To test the statistical significance of the differences in these rates in the pre-intervention and in the post-intervention years, between the villages designated for treatment and for control, Mantel–Haenszel chi-squared tests were used, stratified by sex and age. The rate ratios in each year for the treatment and control villages, with their 95% confidence limits, were also calculated.

To test the association between intervention and reduction in malaria prevalence during the mass blood surveys, odds ratios were calculated to compare the treatment with control villages using Mantel–Haenszel chi-squared tests stratified by the season of collection and age group.

3. Results and discussion

3.1. Entomology

A total of 13 591 anopheline mosquitoes were caught, representing 14 species, from cattle-baited huts and net traps, partial and all night human landing catches, light traps, pyrethrum spray sheet and window exit traps from August 1993 to December 1994 during the baseline data collection year. The most abundant endophagic and endophilic mosquitoes collected from cattle-baited huts, partial and all night human landing catches, light traps, pyrethrum spray sheet and window exit traps were the three best known vector species *An. culicifacies*, *An. subpictus* and *An. varuna*. The most productive methods for catching these species were cattle-baited huts and all night or partial night human landing collections. The other anopheline species were mainly caught in the cattle-baited net traps, which accords with their known mainly zoophilic nature.

There was a seasonal peak of *An. culicifacies* in the monsoon season of October to December (Fig. 2). Similar results were obtained in the pre-intervention year for the villages later assigned for controls (Fig. 2(a)) and treatment (Fig. 2(b)). There were indications that in the post-intervention year the October–December peak were largely prevented in the intervention villages (mean catches in this quarter 0–16 in the four intervention villages compared with 17–81 in the controls). On the basis of this and equivalent data for the other quarters and all three vector species and the three most productive monitoring methods, data on the percent change between the pre- and post-intervention year and its statistical significance were calculated (by the methods indicated in the previous section) and the results are shown in Table 1.

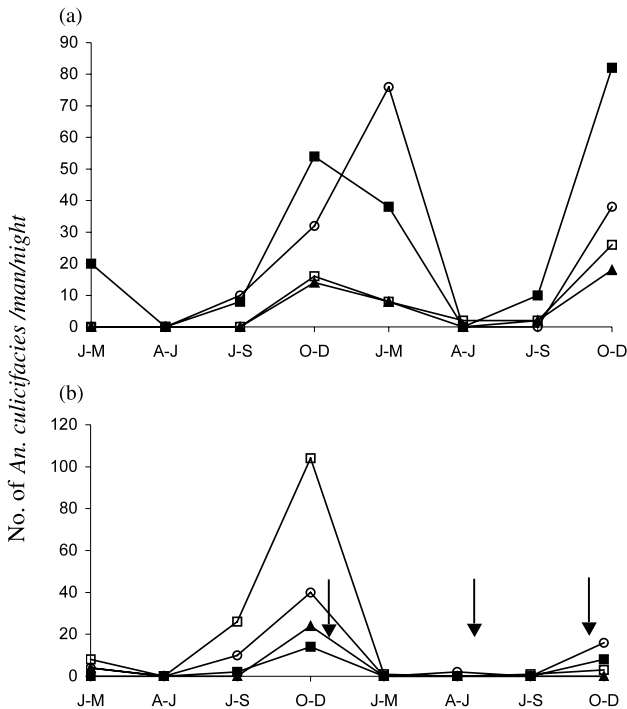


Fig. 2. Number of *An. culicifacies* collected per human per night from partial night (18:00–24:00 h) human landing catches in each quarter of the pre- and post-intervention years in the eight villages: a—control villages, □ Dasgiriya, ■ Kaluganga, ▲ Kapuyaya, ○ Maoya; b—treatment villages, □ Dewaladeniya, ■ Morathanna, ▲ Thorapitiya, ○ Wallewela. NB: black arrows indicate the application of pyriproxyfen to treatment villages.

There was an apparent upward trend of *An. culicifacies* and *An. subpictus* numbers between the pre- and post-intervention years in the control villages, and a downward trend in the treated villages. These apparent trends were statistically significant in four out of the six comparisons for each species. However, there was no such evidence that the treatment had any impact on the number of adults of *An. varuna*. The apparent difference in the response of these species is explicable by the fact that *An. culicifacies* breeds almost wholly in the gem pits which were the main target of treatment, and much of the *An. subpictus* breeding is also in these sites, whereas *An. varuna* breeds in many other sites such as slow moving river margins, which could not be treated.

3.2. Parasitology

3.2.1. Passive case detection

The resident population in the area in the middle of the pre- and post-intervention periods was 4566 and 4659 but it was not stable, being affected by considerable emigration and immigration. Malaria fever incidence rates (*P. falciparum* and *P. vivax* and both sexes and all age groups of human subjects combined) for each village in each three-month period in the pre- and post-intervention periods are shown in Fig. 3. This strongly suggests that the rates in the two groups of villages were similar in the pre-intervention years but that they diverged after intervention. Table 2 shows the data for both *Plasmodium* species stratified by age and sex of human subjects. The data indicate little difference in incidence between these strata except that older females seem to be somewhat under-represented, presumably because they are disinclined to seek treatment.

The incidence rates of malaria fever in the pre-intervention year did not differ significantly between the villages assigned for later treatment or to be controls, as shown by the non-significant Mantel–Haenszel χ^2 stratified by age and sex, and the fact that the confidence limits of the rate ratio between treatment and control overlapped with 1.0 for all age–sex strata. It is concluded that the randomisation of villages to the treatment and

Table 1

Summary of percent change (with 95% confidence limits) between pre- and post-intervention years for *An. culicifacies*, *An. subpictus* and *An. varuna* collected from cattle-baited huts and human landing catches (partial night and all night) in the treatment and control villages

| Species | Treatment/control | Cattle-baited huts | | Partial night landing catches | | All night landing catches | |
|-------------------------|-------------------|----------------------------------|-----------------|----------------------------------|-----------------|----------------------------------|-----------------|
| | | % Change ^a (95% c.l.) | <i>t</i> (d.f.) | % Change ^a (95% c.l.) | <i>t</i> (d.f.) | % Change ^a (95% c.l.) | <i>t</i> (d.f.) |
| <i>An. Culicifacies</i> | | | | | | | |
| | Treatment | −84.1% (−67 to −92%) | 5.5 (11) *** | −69.6% (−47 to −83%) | 4.7 (11) *** | −58% (−84 to +5%) | 2.44 (5) n.s. |
| | Control | +303.8% (+101 to +711%) | 4.4 (11) *** | +64.6% (−30 to +83%) | 1.3 (11) n.s. | +98.2% (+2 to +287%) | 2.63 (5) * |
| <i>An. subpictus</i> | | | | | | | |
| | Treatment | −52.0% (−80 to +21%) | 1.75 (11) n.s. | −59.1 (−3 to −83%) | 2.27 (11) * | −35.5% (−16 to +147%) | 2.63 (5) n.s. |
| | Control | +237.9% (+39 to +720%) | 3.02 (11) * | +511% (+158 to +1346%) | 4.62 (11) * | +387% (+71 to +1288%) | 3.88 (5) * |
| <i>An. Varuna</i> | | | | | | | |
| | Treatment | +65.6% (−29 to +259%) | 1.27 (15) n.s. | −5.1% (−57 to +110%) | 0.14 (15) n.s. | +19.2% (−82 to +685%) | 0.22 (7) n.s. |
| | Control | +60.6% (−34 to +313%) | 1.20 (15) n.s. | +24.1% (−17 to +99%) | 0.98 (15) n.s. | +64.6 (−27 to +172%) | 1.44 (7) n.s. |

Significance of the observed percent change values is indicated by results of paired *t*-tests based on data for each village in corresponding quarters of each year. n.s. = not significant.

^a Negative differences indicate reduction during the course of the trial; positive differences indicate increase.

*** *P* < 0.001.

* *P* < 0.05.

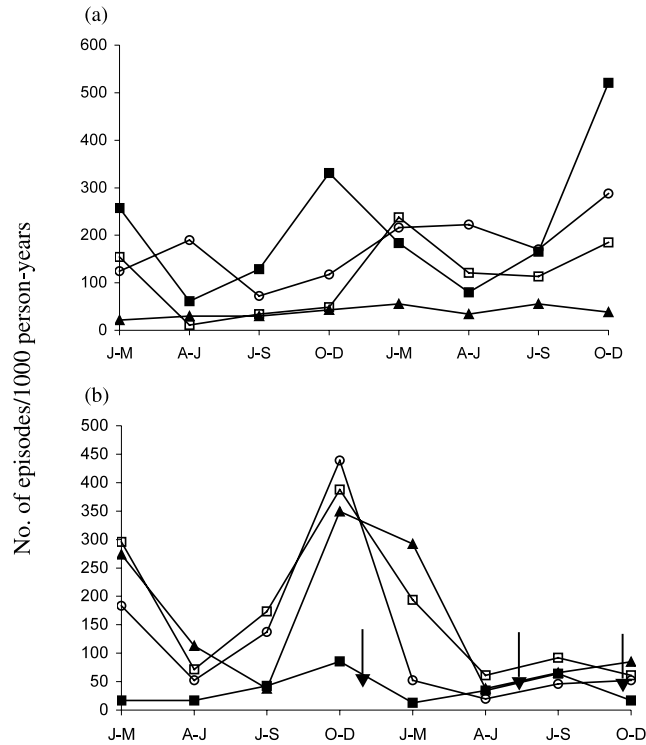


Fig. 3. Incidence of malaria fever due to either parasite species per thousand person-years. Symbols as in Fig. 2.

Table 2

Comparison of malaria fever incidence rates stratified by age and sex in the pre-intervention year of the villages later assigned for treatment and control and in the post-intervention year in the treatment and control villages

Incidence/1000 person years (no. episodes/no. person years)

| Age (Years) | Pre-intervention | | | Post-intervention | | |
|---------------|--------------------------------|---------------|---------------------|---------------------------------|---------------|---------------------|
| | Treatment | Control | Rate ratio (95% CI) | Treatment | Control | Rate ratio (95% CI) |
| <i>Male</i> | | | | | | |
| 0–4 | 153 (29/190) | 233 (32/137) | 0.65 (0.42–1.03) | 64 (13/203) | 322 (48/149) | 0.20 (0.11–0.35) |
| 5–14 | 147 (53/361) | 202 (47/233) | 0.73 (0.51–1.04) | 37 (13/352) | 155 (37/238) | 0.24 (0.13–0.44) |
| >15 | 55 (114/735) | 189 (138/702) | 0.79 (0.63–0.99) | 66 (49/741) | 307 (220/716) | 0.22 (0.16–0.29) |
| <i>Female</i> | | | | | | |
| 0–4 | 174 (29/166) | 153 (22/144) | 1.14 (0.69–1.90) | 87 (15/173) | 200 (30/150) | 0.43 (0.24–0.77) |
| 5–14 | 144 (47/326) | 156 (38/243) | 0.92 (0.62–1.37) | 42 (14/337) | 249 (61/245) | 0.17 (0.10–0.29) |
| >15 | 132 (77/584) | 103 (77/745) | 1.28 (0.95–1.72) | 51 (30/592) | 157 (118/753) | 0.32 (0.22–0.48) |
| | M–H $\chi^2 = 2.55, P = 0.110$ | | 0.95 (0.80–1.04) | M–H $\chi^2 = 293.4, P < 0.001$ | | 0.24 (0.20–0.29) |

control categories had the desired effect of producing reasonably well-balanced samples with regard to an overall malaria risk. However, Table 3 shows that when the data are stratified by *Plasmodium* species there was significantly more *P. falciparum* in the villages assigned to be controls. Tables 2 and 3 show that in the post-intervention year, for all age–sex strata and for both *Plasmodium* species, there were significantly lower incidence rates of malaria fever in the treated villages.

3.2.2. Mass blood survey

The results stratified by age and survey month of the mass blood surveys for malaria infections (regardless of fever symptoms), which were carried out in June and December in the pre- and post-intervention years, are shown in Table 4. In the pre-intervention year there was little indication of differences in prevalence of infection between seasons of survey or age group. Mantel–Haenszel χ^2 and the confidence limits of the rate ratios showed no significant differences

Table 3

Comparison of *P. falciparum* and *P. vivax* incidence rates in the pre-intervention year of the villages later assigned for treatment or control and in the post-intervention year of the treatment and control villages

| Incidence/1000 person years (no. episodes/no. person years) | | | | | | |
|---|--|------------------|---------------------|---|------------------|---------------------|
| | Pre-intervention | | | Post-intervention | | |
| | Treatment | Control | Rate ratio (95% CI) | Treatment | Control | Rate ratio (95% CI) |
| <i>P. falciparum</i> | 20.3 (48/2362) $\chi^2 = 6.45$, DF = 1, $P = 0.011$; | 32.2 (71/2204) | 0.63 (0.44–0.91) | 2.92 (7/2398) $\chi^2 = 39.2$, DF = 1, $P < 0.001$; | 24.4 (55/2251) | 0.12 (0.08–0.26) |
| <i>P. vivax</i> | 123.6 (292/2362) $\chi^2 = 0.01$, DF = 1, $P = 0.91$; | 122.0 (269/2204) | 1.01 (0.87–1.18) | 53.0 (127/2398) $\chi^2 = 239.8$, DF = 1, $P < 0.001$; | 203.9 (459/2251) | 0.26 (0.21–0.31) |

Table 4

Slide positivity rates stratified by age of subjects from mass blood surveys conducted in December and June in the pre- and post-intervention years

| Percent of slides positive (no positive/no slides) | | | | | | | |
|--|-------------|----------------------------------|--------------|----------------------------|------------------------------------|--------------|----------------------------|
| Month | Age (years) | Pre-intervention year | | | Post-intervention year | | |
| | | Treatment | Control | Rate ratio (95% CI) | Treatment | Control | Rate ratio (95% CI) |
| December | 0–4 | 1.4 (4/281) | 2.9 (4/139) | 0.49 (0.13–1.95) | 0.53 (1/190) | 6.0 (9/149) | 0.09 (0.01–0.68) |
| December | 5–14 | 2.9 (18/612) | 2.1 (8/385) | 1.42 (0.62–3.22) | 0.70 (4/567) | 6.9 (26/377) | 0.10 (0.04–0.29) |
| December | > 14 | 2.7 (31/1158) | 2.7 (21/776) | 0.99 (0.57–1.71) | 0.32 (3/925) | 2.9 (22/755) | 0.11 (0.03–0.37) |
| June | 0–4 | 1.3 (2/151) | 2.1 (3/140) | 0.62 (0.10–3.64) | 0.0 (0/185) | 3.8 (5/133) | 0.00 |
| June | 5–14 | 0.25 (1/396) | 2.0 (5/250) | 0.13 (0.01–1.07) | 0.21 (1/483) | 4.8 (20/418) | 0.04 (0.01–0.32) |
| June | > 14 | 0.48 (3/627) | 1.1 (6/566) | 0.45 (0.11–1.80) | 0.10 (1/1000) | 2.6 (19/715) | 0.04 (0.01–0.28) |
| | | M–H $\chi^2 = 0.63$, $P = 0.43$ | | Mean = 0.84 (0.57–1.23) | M–H $\chi^2 = 104.5$, $P < 0.001$ | | Mean = 0.07 (0.04–0.14) |

Data from pre-intervention year divided into those from villages later assigned for treatment and control and from post-intervention year into those from treatment and control villages. Mantel–Haenszel χ^2 tests stratified by age of subjects and month of survey.

between the villages assigned for later treatment or to be controls. By contrast, in the post-intervention year there were significantly greater prevalence of infection in the control, compared with the treated, villages.

4. Conclusions

The entomological monitoring showed the effect on the adult anopheline populations of three annual applications of a low dose of granular pyriproxyfen to gem pits and to river bed pools at seasons when water suitable for anopheline breeding existed in these sites. Treatment in all the sites that could be found in four of the eight villages in the area clearly suppressed the populations of two of the three vector species, relative to the populations in the control villages. Further, monitoring the incidence of malaria fever and prevalence of malaria infection clearly showed that this form of vector control was sufficient to lead to the suppression of malaria in the treated villages compared with the controls. Because of the closeness of the treated and control villages, there was concern that, in view of the considerable flight range of *An. culicifacies* and *An. subpictus* as shown by mark–release–recapture experiments (Curtis and Rawlings, 1980; Rawlings et al., 1981), mosquito movement from village to village would obscure any effect on malaria. However, impact on malaria was clearly evident and it can be emphasised that where anopheline breeding sites can be readily identified with the help of active community participation, larval control by a highly efficient and economical insect growth regulator can be very successful in suppressing malaria.

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