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CONTROL OF VECTORS AND INCIDENCE OF MALARIA IN AN IRRIGATED SETTLEMENT SCHEME IN SRI LANKA BY USING THE INSECT GROWTH REGULATOR PYRIPROXYFEN

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ABSTRACT. An evaluation of pyriproxyfen as a larval control agent with the aim of reducing malaria vector populations and incidence of malaria was conducted in 12 villages in an irrigated settlement scheme in the dry zone of central Sri Lanka. In these villages, there are many pools in the beds of rivers, streams, and irrigation ditches during the dry season of the year. These are the major breeding places of the malaria vectors *Anopheles culicifacies* and *An. subpictus*. Collections of adult mosquitoes were carried out by using standard methods and parasitological data were collected by daily malaria clinics set up for the project and through the 2 government hospitals. All villages in the study area were under residual house spraying with lambdacyhalothrin water-dispersible powder. Using the 1st year's baseline data collection, the villages were stratified into 6 villages with high malaria incidence and 6 villages with low incidence. Within each group, 3 villages were randomly assigned for larval control by treating all the pools in the beds of rivers, streams, and irrigation ditches and agricultural wells with a granular formulation of the insect growth regulator pyriproxyfen at the rate of 0.01 mg active ingredient/liter. The field bioassays indicated that a single treatment of pyriproxyfen effectively inhibited the emergence of adult mosquitoes in the riverbed pools for a period of 190 days. The treatment caused significant reduction of the adult populations of *An. culicifacies* (78%) and *An. subpictus* (72%). Similarly, incidence of malaria was reduced in the treatment villages by about 70% (95% confidence interval 58–78%) compared with the controls. The conclusion is made that pyriproxyfen can be a very effective means of malaria control if all possible vector breeding places in the area can be located.

KEY WORDS Malaria, riverbed pools, Sri Lanka, *Anopheles culicifacies, Anopheles subpictus,* pyriproxyfen

INTRODUCTION

Anopheles culicifacies Giles is regarded as the principle vector of malaria in Sri Lanka (James and Gunasekara 1913, Amerasinghe et al. 1999). *Anopheles subpictus* Grassi is considered to be a secondary vector. Recently, several studies using enzyme-linked immunosorbent assays (ELISAs) and the sporozoite dissection method have reported natural *Plasmodium* infections in several other anopheline species (Mendis et al. 1992, Amerasinghe et al. 1992, 1997). Riverbed and streambed pools are among the major breeding sites of *An. culicifacies* and *An. subpictus* (Gill 1936, Rajendram and Jayawickrame 1951, Amerasinghe and Munasinghe 1988, Amerasinghe and Ariyasena 1990). Application of temephos has been widely used to attempt to control anopheline larvae in riverbed and streambed pools in Sri Lanka. Pyriproxyfen (0.5% sand granule formulation) at the rate of 0.01 mg active ingredient (AI)/liter was applied to gem pits as well as riverbed and streambed pools as part of a small-scale trial in a gem-mining area (Yapabandara et al. 2001). The results indicated that pyriproxyfen inhibited emergence of adults for more than 185 days, which included a 4-month dry period. The trial showed that 2 annual treatments with pyriproxyfen at the rate of 0.01 mg AI/liter would be more cost effective than the use of temephos, expanded polystyrene beads, used engine oil, or filling the pits with soil (Yapabandara and Curtis 2002). That trial also showed a 60% reduction in the density in adult *An. culicifacies* and a significant reduction of incidence of malaria fever due to either *Plasmodium falciparum* or *P. vivax* (Yapabandara et al. 2001). Gem pits are localized in only a very few areas of Sri Lanka, but pools in the beds of rivers, streams, and irrigation ditches are common breeding places in most of the malarious areas in the country. Therefore, a study was carried out to check whether the successful results in the original trial were repeatable in a more typical area where pools in the beds of rivers, streams, and irrigation ditches are the predominant breeding sites. The methodology was similar to that used in the original trial.

MATERIALS AND METHODS

Study area, geographical reconnaissance, and census: The study area was Kandalama, which is situated in the Matale District in the dry zone of Sri Lanka. This is one of the highly malarious areas of the country. A cluster of 12 villages with a total area of 9,800 ha was selected for the study. This area is a settlement scheme that was established about 30 years ago around the Kandalama reservoir. Residual house spraying with lambdacyhalothrin water-dispersible powder combined with larviciding with temephos is being used in the 12 villages. The larviciding with temephos was withdrawn during the trial period starting in August 1998 but the house spraying continued with coverage equal in all villages during the months of November and June of each year.

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The 12 villages were mapped to show the geographical distribution of houses and potential breeding places 3 months before the beginning of baseline data collection in November 1998. The location of every house, path, and major water body was surveyed by counting paces of measured length along compass bearings. Each house encountered during the geographical reconnaissance (GR) was given a number, which was marked on the door. Two surveys were carried out in the dry and wet seasons, to investigate the potential breeding places and their approximate total water surface area. After GR mapping, information on the name, age, sex, occupation, and whether a permanent resident or a recent immigrant to the area was recorded for each inhabitant by visiting each house. At that time, a household card marked with this information was issued.

Entomological monitoring: The mosquito population in each village was monitored monthly by several standard methods (WHO 1975, Lines et al. 1991), but the most productive was found to be collections of resting mosquitoes from cattle-baited huts, and the entomological data presented in the present paper are only from the latter collection method. Each of the 12 villages was defined as a circle of 1.5-km radius centered on both sides of a stream or irrigation canal. The mosquito monitoring was conducted in these middle parts of each village to try to minimize interference by immigration of mosquitoes from neighboring villages into the village fringes.

Mosquito larvae were sampled in all types of water collections in the study area once a month from November 1998 to May 2000. All the water collections except wells were sampled by using ladles of 150-ml capacity. Five dips were taken from each 1 m^2 of water surface. The number of dips taken was proportional to the estimated breeding surface area. A single sample was taken from each small ground pool (<50-m^2 area). The data were recorded by date, site, village, number of dips, and number of larvae. The collected larvae (3rd and 4th instars) were identified to species by using the keys of Amerasinghe et al. (1992). Pupae were kept until emergence and adults were identified (Amerasinghe 1990).

Enzyme-linked immunosorbent assay: The anopheline mosquitoes collected from all the methods were brought to the laboratory at the Regional Office, Anti Malaria Campaign, Matale, and stored at 20°C. The protocol described by Wirtz et al. (1985) was used for the ELISA for circumsporozoite proteins (CSPs) of *P. falciparum* and the 2 known variants of *P. vivax.*

Passive case detection: Starting in September 1998, malaria cases were monitored by passive case detection by 2 field clinics and 2 clinics at outpatient departments at Dambulla Base Hospital and Kimbissa dispensary. The patients were requested to bring their household card, which was issued during the census survey when they came to the clinic. The people who were permanently resident in the area were traced by these household cards. A yellow card was given to migrants. Thick and thin films were taken and stained with Giemsa (WHO 1991). If the patients were positive for *P. vivax* or *P. falciparum,* they were treated with chloroquine and primaquine according to the National Malaria Control Programme drug policy. The patient's date of visit, name, address with the GR number, age, sex, presence of fever, and species of parasite were recorded. Subsamples of blood films were cross-checked by a senior technician.

Selection of villages for treatment with pyriproxyfen: On the basis of the collections of the 2 main vector species (*An. culicifacies* and *An. subpictus*) from cattle-baited huts and on the malaria fever incidence recorded in 2 malaria clinics and 2 hospitals, the 12 villages were stratified into 6 villages with high vector prevalence and malaria incidence and 6 with low rates. Three villages from each group were randomly picked for pyriproxyfen treatment, with the other 3 left as controls.

Application of pyriproxyfen: Pyriproxyfen (S-31183 Sumilarv 0.5% granules, Sumitomo Chemical Co., Osaka, Japan) was applied at the target dosage of 0.01-mg AI/liter (2 g of granules/m^3) by using a spoon to the pools in the beds of rivers, streams, and irrigation ditches; quarry pits; and agricultural wells in the 6 treatment villages at the end of July and December 2001. The requirement of pyriproxyfen for the pools was roughly calculated on the basis of an assumed depth of 10 cm, together with the measured area of the pools. For the agricultural wells, the depth of water was measured by using a rope marked in centimeters attached to a stone. This measurement together with the well diameter was used to calculate the amount of pyriproxyfen granules to be applied. Pyriproxyfen was not added to drinking-water wells; instead, the fish *Poecillia reticulata* was introduced into these wells.

Efficacy of pyriproxyfen: Reapplication of pyriproxyfen to the riverbed pools and agricultural wells was decided by field bioassays by the method used by Yapabandara et al. (2001). A total of 3 riverbed pools in each treatment and control village were randomly selected. In these pools, the efficacy of pyriproxyfen was recorded at 2-wk intervals by using 5-liter floating buckets with two 7 × 5-cm holes covered with nylon netting mesh, and into which 10 laboratory-reared 3rd or 4th instars of *An. culicifacies* or *An. subpictus* were placed. The bucket was covered with a lid of nylon netting. When any adults emerged in the bioassay of any of the treated pits or pools, reapplication of pyriproxyfen to the pools and wells in all treatment villages was started. Control bioassays in untreated pools consistently showed normal development of larvae to pupae and adult emergence.

Statistical analysis of entomological and parasitological data: Statistical analysis was carried out

Table 1. Detection of circumsporozoite proteins of *Plasmodium vivax* (Pv) and *P. falciparum* (Pf) by enzyme-linked immunosorbent assay of mosquitoes in the genus *Anopheles* sampled by 6 methods of collection.

Species	No. tested	No. positive			Sporozoite rate (%)		
		Pv	Pf	Total	Pv	Pf	Total
An. aconitus	263	0	0	0	0	0	0
An. annularis	319	0	0	0	0	0	0
An. barbirostris	364	0	0	0	0	0	0
An. culicifacies	242	2	0	2	0.826	0	0.826
An. jamesi	460	0	0	0	0	0	0
An. maculatus	9	0	0	0	0	0	0
An. nigerrimus	5,282	0	1	1	0	0.019	0.019
An. peditaeniatus	9	0	0	0	0	0	0
An. subpictus	1,007	8	3	11	0.794	0.297	1.092
An. tessellatus	1,242	1	0	1	0.081	0	0.081
An. vagus	4,883	2	1	3	0.041	0.020	0.061
An. varuna	191	0	0	0	0	0	0
Total	12,988	13	5	18	0.100	0.038	0.139

with the methods described by Yapabandara et al. (2001). For the entomological data, *t*-tests were carried out between treatment and control villages on the differences between the pre- and posttreatment years in the logs of the catches for each village in each quarter of the year. For the data on malaria case incidence and total numbers of person years in the treated and control villages, χ^2 tests were carried out in the preintervention year to confirm that there was no significant pre-existing difference between these villages, and in the postintervention year to test whether the intervention had a significant impact.

RESULTS AND DISCUSSION

Demographic and geographical features

The study area is about 9,800 ha in extent, with a resident population of 15,415, to which was added a variable number of migrants. A total of 4,480 houses and 659 huts was recorded. About 87% of the males over 15 years of age were employed in rice, onion, vegetable, and tobacco cultivation and most of them sleep near their crops in huts made out of palm (cadjan) leaves. The extent of water in the streams and rivers, irrigation canals, rice paddies, and what in south Asia is called a tank (i.e., a large reservoir) was about 29, 46, 776, and 1,002 ha, respectively. In all treatment and control villages during the trial period, residual spraying of lambdacyhalothrin was carried out twice a year in all permanent houses and huts.

Entomological monitoring

A total of 28,781 anopheline mosquitoes were collected, representing 12 species from the 6 collection methods during the baseline data collection year. The heads and thoraces of 12,988 mosquitoes belonging to 12 species caught by 5 methods were tested for the presence of CSPs of *P. falciparum* and *P. vivax*. Eighteen mosquitoes belonging to 5 anopheline species, namely the well-known vectors in Sri Lanka *An. culicifacies* and *An. subpictus*, and also the much more zoophilic species *An. nigerrimus*, *An. tessellatus*, and *An. vagus*, were found to carry CSPs of either *P. vivax* or *P. falciparum* (Table 1). All the *P. vivax* found were of the standard PV-210 type and none were of the PV-247 variant. When comparing the proportion positive for CSPs of *P. vivax* in *An. culicifacies* and *An. subpictus*, no significant differences were found between the mosquito species ($P = 0.518$). None of the *An. culicifacies* were positive for CSPs of *P. falciparum*. A significant difference was found between the proportions of positive *An. subpictus* and *An. nigerrimus* ($P < 0.001$) and of *An. vagus* ($P < 0.001$) positive for CSPs of *P. falciparum*. The results of the ELISA seem to indicate that *An. subpictus* acts as a major vector in this study area.

Based on mean larval densities and area of water bodies of different types, it was estimated that streambed pools contributed about 80–90% of the production of *An. culicifacies* and *An. subpictus* during the dry season (April–September). Similarly, it was estimated that rice paddies contributed about 60% of the *An. subpictus* breeding in the wet season, whereas in the dry season, paddy fields contributed only about 6% because most of the paddy fields dry up during the dry season. None of the *An. culicifacies* were collected from the paddy fields. Rainwater puddles contributed about 65% and about 25% of *An. culicifacies* and *An. subpictus* breeding, respectively, but these water puddles dried up after a few weeks.

The field bioassays with larvae introduced into buckets with netting bottoms floating in breeding sites indicated that a single treatment of pyriproxyfen at the rate of 0.01 mg/liter effectively inhibited the emergence of adult mosquitoes in the riverbed pools for a period of 190 days. This remarkable result (backed up by controls in untreat-

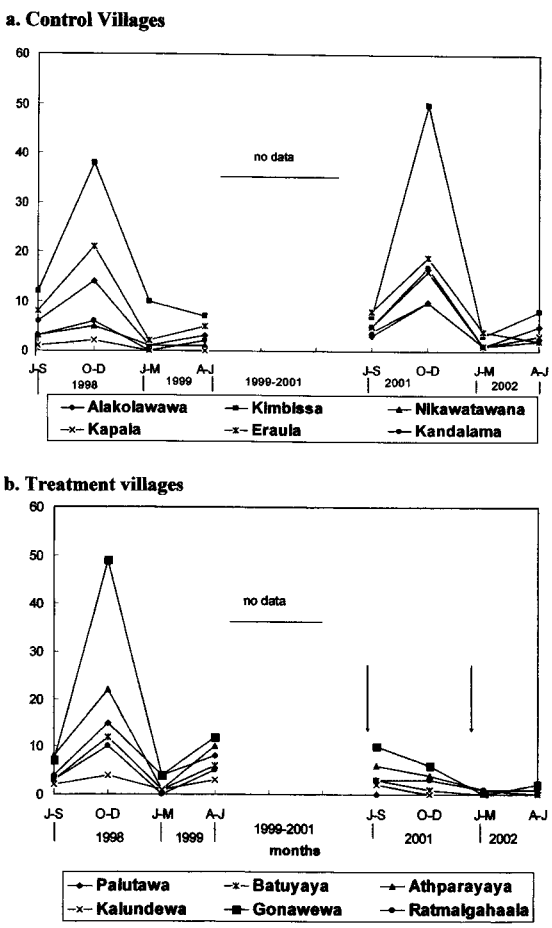

Fig. 1. Number of *Anopheles culicifacies* collected in cattle-baited hut collections in each quarter of pre- and postintervention years in the 12 villages. The arrows indicate the application of pyriproxyfen in the pools in the treatment villages. (a) Control villages. (b) Treatment villages.

ed water bodies where emergence of adults was normal) was fully consistent with published data from our previous trial in gem pits (Yapabandara and Curtis 2002).

Cattle-baited hut collection was the most productive for *An. subpictus* and *An. culicifacies*. Figure 1 shows the number of *An. culicifacies* collected by this method in each quarter of the pre- and postintervention years in the 12 villages. There was a peak of *An. culicifacies* in the monsoon season of October to December (Fig. 1). Similar results were obtained in the preintervention year (1998–99) for the villages later assigned as controls (Fig. 1a) and for treatment (Fig. 1b). Inspection of Fig. 1 indicates that in the postintervention year (2001–02), the October–December peak was largely prevented in the intervention villages.

Paired *t*-tests were used to assess the statistical significance of the effect of the intervention on cattle-baited hut collections of the 2 vector species in corresponding quarters for each village in the preintervention year (July 1998 to May 1999 and in July 2001 to May 2002) in each treatment and control village. Table 2 shows that there were significant reductions of *An. culicifacies* and *An. subpictus* after the pools and wells were treated with pyriproxyfen. Significant upward trends of *An. culicifacies* and *An. subpictus* occurred in the control villages, presumably because of the withdrawal of temephos treatment.

Parasitological monitoring

In 12 preintervention months from June 1999 to May 2000, there were 260 malaria fever episodes with either *P. vivax* or *P. falciparum* from a resident population of 15,415 in the area. The malaria fever incidence rates due to *P. vivax* and *P. falciparum* were 18 and 1 per 1,000 person years, respectively. A seasonal peak occurred in January (Fig. 2). Similar results were obtained in the preintervention year for the villages later assigned as controls (Fig. 2a) and for treatment (Fig. 2b). Comparison of Figs. 2a and 2b indicates that in the post-

Table 2. Summary of paired *t*-tests for comparison of differences between log values of *Anopheles culicifacies* and *An. subpictus* collected from cattle-baited huts in corresponding quarters of the pre- and postintervention years in the treatment and control villages.

Species	Difference between mean of log of posttreatment catches and those of pretreatment catches	Anti-log	% change	95% CI* (%)	*t*-value	df	*P*
An. culicifacies							
Treatment	−0.4832	0.2335	−76.7	−66.8 to −86.5	5.92	23	0.0001
Control	0.1199	1.3179	31.8	29.3 to 34.2	2.06	23	0.03
An. subpictus							
Treatment	−0.2359	1.7214	−72.1	−60.4 to −93.7	2.64	23	0.005
Control	0.0849	1.2159	21	4.22 to 39	3.36	23	0.001

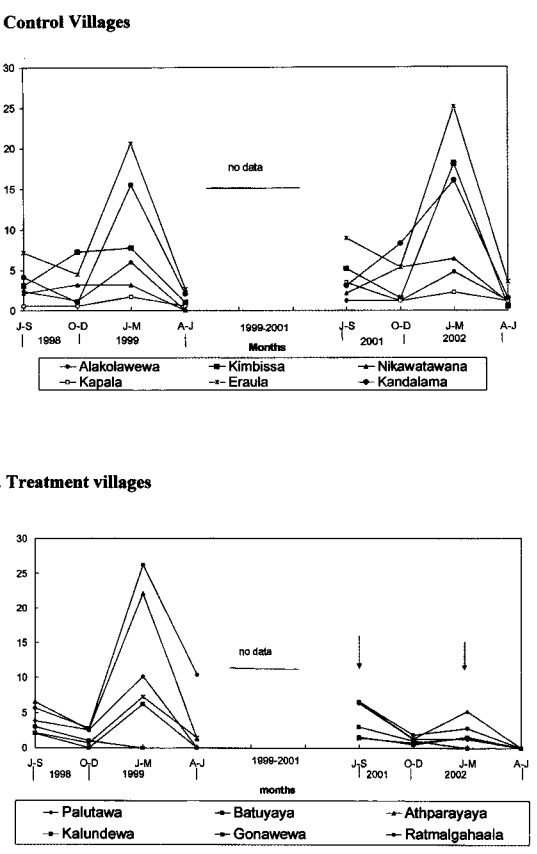

Fig. 2 Incidence of malaria fever due to either parasite species per 1,000 person years. (a) Control villages. (b) Treatment villages.

intervention year, the January–March peak was largely prevented in the intervention villages.

The incidence rate of malaria fever in the preintervention year did not differ significantly between the villages assigned for later treatment or to be controls, as shown by the nonsignificant χ^2 test results and the confidence limits of the rate ratios included 1.0 (Table 3). However, in the postintervention year, the malaria incidence rate in the treatment and control villages was highly significantly different, and the intervention was associated with a 70% (95% confidence interval = 58–88%) reduction in malaria incidence.

The incidence of malaria in males and in people older than 15 years was significantly higher than in females (χ^2 = 32.22, df = 1, $P < 0.001$) or in children (χ^2 = 18.68, df = 1, $P < 0.001$), presumably because of the fact that men slept in huts in the fields to look after the crops and were then exposed to biting mosquitoes.

Conclusion

Even though the houses in the study area were sprayed with lambdacyhalothrin before the pyripro-

Table 3. Comparison of malaria fever incidence rates stratified by sex and age in the preintervention year of the villages later assigned for treatment and control and in the postintervention year in the treatment and control villages.[1]

Effects	Preintervention incidence rate (episodes/1,000 person years)			Postintervention incidence rate (episodes/1,000 person years)		
	Treatment	Control	Rate ratio (95% CI)	Treatment	Control	Rate ratio (95% CI)
Sex						
Male	24.1 (105/4,344)	26.6 (122/4,570)	0.91 (0.70–1.17)	8.9 (39/4,367)	35.1 (161/4,579)	0.25 (0.18–0.36)
Female	4.6 (12/2,583)	5.3 (21/3,918)	0.87 (0.43–1.76)	2.3 (6/2,598)	5.6 (22/3,921)	0.41 (0.17–1.02)
Age (years)						
<1	0 (0/485)	0 (0/559)	—	0 (0/490)	0 (0/562)	
2–14	11.8 (24/2,029)	14.2 (33/2,320)	0.83 (0.49–1.40)	2.4 (5/2,037)	12.8 (30/2,334)	0.20 (0.08–0.50)
>15	21.1 (93/4,413)	19.6 (110/5,609)	1.07 (0.82–1.41)	9.0 (40/4,438)	27.2 (153/5,614)	0.33 (0.23–0.47)
Overall	16.9 (117/6,927)	16.8 (143/8,488)	1.00 (0.79–1.28)	6.0 (45/6,965)	21.5 (183/8,510)	0.30 (0.22–0.42)

[1] χ^2 = 0.00, df = 1, P = 0.98 for preintervention; χ^2 = 58.83, df = 1, $P < 0.001$ for postintervention.

xyfen intervention, there were considerable populations of the vector species (Fig. 1) and serious levels of malaria, especially in men (Table 3). This can be attributed to the fact that most of farmers sleep in the fields in temporary huts consisting of a roof covered by woven palm leaves and polyethylene sheeting, with the sides being partially covered or often left open. Hence, residual insecticide spraying fails to give the men protection against malaria vectors. Thus, larval control is very important and the malaria clinic data indicated significant (70%) reduction of incidence of malaria cases in the pyriproxyfen-treated villages compared to the controls. The pyriproxyfen treatment led to significant reduction of adult densities of *An. culicifacies* and *An. subpictus. Anopheles subpictus* breeds to a considerable extent in paddy fields but pyriproxyfen was not applied to these paddy fields because of the shortage of funds. It is speculated that even more impact on this species and on malaria could have been achieved if it had been possible to treat the paddy fields. Overall, it is concluded that pyroproxyfen is a highly appropriate means of malaria vector control in Sri Lanka.

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