THE EFFICACY OF DIFFERENT MOSQUITO TRAPPING METHODS IN A FOREST-FRINGE VILLAGE, YUNNAN PROVINCE, SOUTHERN CHINA

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Abstract. Despite a control program, malaria incidence in Yunnan has increased and knowledge of vector bionomics is needed for efficient control. Multi-drug resistant *Plasmodium falciparum* necessitates alternatives to human landing catches as a means of studying vectors. Therefore CDC light traps with UV or ordinary incandescent bulbs were tested for 57 trap nights. 2,703 mosquitos were caught, including the vector species *An. minimus* and *An. sinensis* and the suspected vector *An. maculatus*. Larval *An. dirus* were found around the village but no adults were trapped. UV light traps caught more mosquitos than the traps with incandescent bulbs, but caught many insects other than mosquitos requiring time-consuming separation, and were unpopular with villagers. Traps placed in living areas of houses caught more mosquitos than those placed beside bednets and the catch mainly comprised species that were active in the early evening. Encephalitis Vector Surveillance (EVS) traps hung outdoors and baited with CO_2 caught few mosquitos. CDC traps in the same position baited with CO_2 or lactic acid caught large numbers of *Culex tritaeniorhynchus*. Indoor spray catches recovered human fed *An. vagus* and *An. minimus*. This work confirmed that CDC light traps could be used to trap local vectors, and the abundance of early active mosquitos in the living area suggests that personal protection measures may be required in the evening, to supplement bed net use.

INTRODUCTION

Yunnan Province had 2,988 reported malaria cases in 1998, but due to underreporting the actual number of cases is estimated to be ten times this figure (China Advisory Committee on Malaria MOH, 1999). Despite a malaria control program, malaria incidence has increased slightly over the last decade, and *Plasmodium falciparum* remains endemic (China Advisory Committee on Malaria MOH, 1999). Malaria control in Yunnan involves passive surveillance, case finding and treatment of malaria cases (Xu and Liu, 1997). Spraying of houses with DDT is performed in endemic villages but DDT may not be applied effectively, and non-malarious villages are also sprayed (Xu, 1999).

Yunnan Province is situated in the South-west of China and has over 4,060 km of frontier with Myanmar, Lao PDR and Vietnam. Climatic, topographical and cultural diversity throughout the region result in much variation in malaria incidence. The major factors affecting malaria transmission are vector species and abundance, population movement and forest contact. One third of the population of the province are members of minority populations (Yunnan Institute of Malaria Control, 2000). The minority people have close links with the neighboring countries, and travel between them is frequent. Other population movements are due to migrant farmers and workers involved in mining, construction and the lumber industry; as well as refugees, particularly from Myanmar. This results in a high proportion of imported malaria cases through movements of non-immune populations into areas of high transmission (Xu and

Liu, 1997; Tin and Tun, 1991). Population movements associated with agriculture are most frequent during the wet season, which coincides with peak vector densities (Xu, 1999). Although malaria incidence in the Southeast Asian region is closely related to forest activity, it has been shown that transmission in forest-fringe villages is also important (Somboon *et al*, 1998; Wahab Rahman *et al*, 1995; Rosenberg *et al*, 1990).

Collecting basic entomological information before devising control strategies is particularly important in the Southeast Asian region as *An. minimus*, *An. maculatus* and *An. dirus* exist as species complexes. The range of behavioral differences exhibited by these species, along with human population factors influencing transmission requires control programs to be tailored for different areas. The limited health budget of the region requires effective use of resources; therefore control programs need to be carefully monitored. Mosquitos are currently sampled using human bait, and some use has been made of UV light traps. *Plasmodium falciparum* is over 90% resistant to chloroquine, piperaquine and amodiaquine in Southern Yunnan (Yunnan Institute of Malaria Control, 2000). There is no resistance to mefloquine, but the cost and possible side effects of this drug are prohibitive. The study was designed to evaluate some simple methods of monitoring mosquito populations, which are both less dangerous and time consuming than man baited collections, by comparing the efficacy of different light traps and aspirator catches.

MATERIALS AND METHODS

The study site

The study was carried out between July 9th and 26th at the beginning of the rainy season. The study village, Manlu, is located in the far south of Yunnan, in Mengla County, close to the Laotian border. It comprises 250 people of Dai nationality, in 41 families. The only occupation of the village is farming. The village is situated in a valley at 750 m altitude, surrounded by mountains. These mountains are covered in primary tropical rain forest, although the slopes closest to the village have been cleared and planted with maize, tea, rubber trees and small areas of other food crops. A large river flows beside the north side of the village and rice fields are located within 300 m northeast of the village. There is forest located on the other three sides. Within the village small rice fields, puddles, a fish pond and small streams provide breeding sites for several mosquito species including *An. minimus*, *An. maculatus*, *An. sinensis* and *An. vagus*.

The village is typical of Dai villages in the region. Each family lives within a large wooden house, with a tiled roof, which is elevated on stilts. Domestic animals including pigs, cows and water buffalo are kept beneath the houses. Each house comprises one large living space. The space is utilized as a kitchen area at the far end where a cooking fire is located, a living room and bedrooms, which are partitioned for privacy. Each house has at least one incomplete wall. The use of bed nets in Mengla County is not common (Xu and Liu, 1997) although all the members of Manlu village used them. This was largely due to the extra privacy that the bednets afforded sleepers. The village had not been sprayed with insecticide since 1997.

Light traps

Village houses were selected from the village on the grounds of their proximity to vector breeding sites, identified by larval dipping. They were matched by number of adults, children and water buffalo living within them. Houses 1 and 5 were situated at the forest fringe, while houses 2, 3 and 4 were situated near the river, closest to the rice fields. House 5 was also located 10 m away from a small rice paddy, measuring 50 m^2. Both houses 1 and 5 had small streams flowing past them.

The trial was run over 16 consecutive nights, using two miniature CDC traps fitted with normal incandescent bulbs (Bioquip 2836) and two updraught UV traps with a spectral range of 320 - 420 nm. The traps were used beside bed nets, at a distance of 0.3 m, or placed in the living room of the house, 2 m from the bedroom door. In all cases the traps were 1.5 m above the floor. The trial was run with a Latin square design to reduce sampling bias. Each house had both types of traps placed in each location for four nights over the course of the trial. The traps were set at 19.00 hr each night and collected and taken to the field station at 07.00 hr.

The CDC catch was killed using chloroform and the UV catch was killed by freezing, as the collecting bag was large. Mosquitos were immediately morphologically identified. The ovaries of approximately thirty anopheline mosquitos were dissected for parity each day by examining the tracheoles around the ovaries (Detinova, 1962).

On the ninth day of the experiment, house 3 was withdrawn from the study due to objections from the occupants regarding the use of UV traps. House 4 was chosen as a replacement. It was located next to house 3 and had a similar number of occupants.

A single UV trap and one CDC trap was used in the forest for 12 hours, for one night baited with CO_2. A UV trap and CDC trap were placed in the forest, both without CO_2, also for one night. The forest site used was the same as for the EVS forest collections described below.

Two Encephalitis Vector Surveillance (EVS) traps (Bioquip 2801A), were used for eight nights baited with CO_2, and baited with 10 ml of lactic acid for five nights. CO_2 was introduced into the tin of one of the traps from a locally bought tractor tyre inner tube via a rubber hose. The flow of the gas was regulated via the tire valve and by constricting the rubber hose. The other trap had gas introduced at an equivalent rate from a cylinder of CO_2 via a rubber hose. One trap was hung from a tree 1.5 m above the ground within the forest fringe, 20 m from the edge of the village. Larval dipping revealed *An. maculatus* and *An. minimus* breeding in the vicinity. The other EVS trap was located in the center of the village. The traps were set at 19.00 hr and collected at 07.00 hr. Each morning the flow of gas into the traps was checked to ensure it was still constant. The catch was then collected and identified after killing with chloroform.

Collections of resting mosquitos in animal houses

Collections were made in 10 pig sheds located beneath the study houses on three occasions. The structures were examined between 07.30 hr and 08.30 hr using a torch and a mouth aspirator for 15 minutes per shed. Captured mosquitos were killed using chloroform. Blood engorged mosquitos were prepared for ELISA blood meal analysis (Service *et al*, 1986). Three collections were also made in the same way from the lower surface of the houses as cattle shelter beneath the houses.

Spray catches in houses

A local fly spray was used to kill indoor resting mosquitos. The active ingredient was tetramethrin (0.1%) and cis trans cypermethrin (>0.05%). These mosquitos were then identified and the blood fed mosquitos were tested for human and bovine antigens using ELISA.

The data for *Aedes* spp, *An. barbirostris*, *An. culicifacies*, *An. jayporensis An. kochi* and *An. philippinensis* were not analysed because of the low numbers caught. Data for the other species were analysed using the STATA 6 statistical package. Data were normalized by a (Ln +1) transformation. ANOVA was used to investigate the effect of position and type of trap whilst correcting for any species differences which were present. Day and house were also included in the ANOVA equation, although the design of the experiment should have minimized these effects.

RESULTS

Light trap catches inside houses

More mosquitos were caught in the UV

traps than in the CDC light traps (p=0.01). The catch difference being significantly greater in the UV trap for *An. minimus* (p=0.008), *An. sinensis* (p≥0.001), and *An. vagus* (p≥0.001). More *Culex* mosquitos were caught using the CDC light trap but this difference was not significant.

Comparing numbers caught when traps were placed in the living area of the house (labelled "away" in Fig 1) with the numbers caught in traps placed beside bednets (labelled "bednet" in Fig 1) there was a significant difference between the two trapping sites (p≤0.01). When the data were analysed for each species more *An. sinensis* (p≥0.001) and *An. vagus* (p≥0.001) were caught in the living area than in the bedroom. A Kruksall Wallace test showed that more *Aedes* spp (h=4.26, p=0.039) and *An. kochi* (h=6.93 p=0.008) were also caught in the living area. However, this test did not consider the effects of day or house.

A Mantel Haenszel test performed using Epi Info 6 on parity data for the most common species showed that there was no significant difference in the proportion of parous

Table 1
Total numbers of mosquitos caught.

Species	Total trapped	%
An. barbirostris	2	0.12
An. culicifacies	4	0.23
An. jayporensis	31	1.81
An. kochi	137	7.98
An. maculatus	221	12.89
An. minimus	386	22.49
An. philippinensis	1	0.06
An. sinensis	405	23.60
An. vagus	529	30.83
Ae. albolaterus	85	92.39
Ae. harveyi	1	1.08
Ae. annandalei	6	6.52
Cx. tritaeniorhynchus	409	72.64
Cx. bitaeniorhynchus	51	9.05
Cx. eumelomyia	8	1.42
Cx. fuscocephala	49	8.70
Cx. pseudovishnui	30	5.33
Cx. [Uranotaenia][a]	12	2.13
Cx. [Heizmania][a]	4	0.71

[a]Subgenus requires dissection of genitalia for species identification.

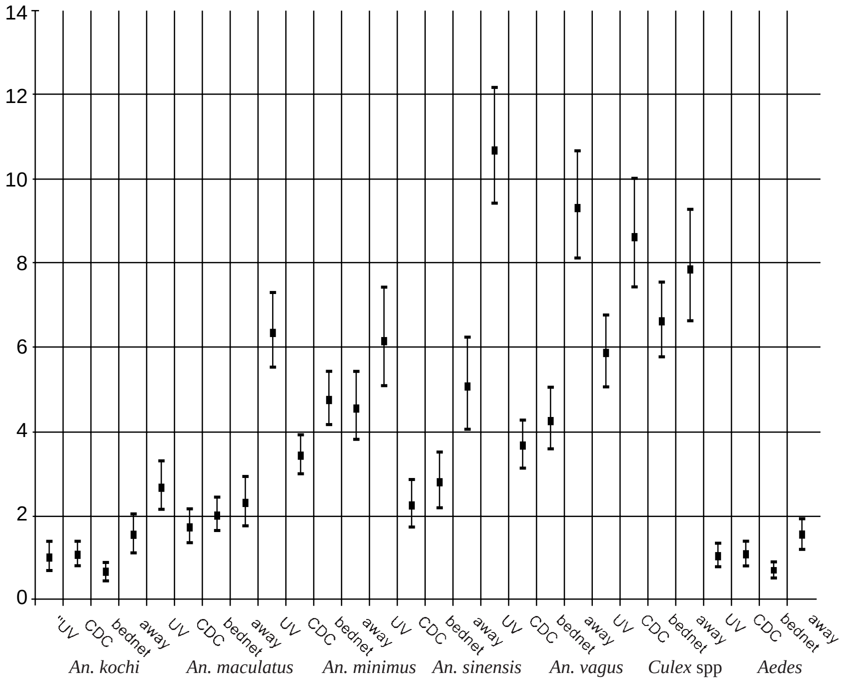

Fig 1–Summary of catch. Figures are geometric means of daily catches with 95%CI.

females caught in the two light traps.

The daily total indoor catch of mosquitos was compared to the daily precipitation data obtained from Mengla County Meteorological Station by linear regression using Microsoft EXCEL 2000. The two sets of data showed a significant relationship ($p = 0.0482$). The total indoor catch of mosquitos was positively related to the amount of precipitation each day, indicating that mosquitos were more likely to be found indoors when rainfall was heavy.

CDC and UV light trap catches outdoors

Twenty-four *Cx. tritaeniorhynchus* and one *An. sinensis* were captured in the CO_2 baited CDC trap. The CDC without CO_2 caught no mosquitos. The UV trap, used the same night as the CDC without bait captured 19 mosquitos comprising 3 *An. maculatus*, 2 *An. sinensis*, 9 *Cx. fuscocephala*, 3 *Cx. tritaeniorhynchus*, 1 *Cx. bitaeniorhynchus*, and 1 *Cx. pseudovishnui*. Two thousand insects other than mosquitos were trapped. The night that the test took place had 85 mm of rain, which began to fall at 22.00 hr. The mosquitos caught are likely to have been trapped in the two hours prior to the onset of rain. The UV trap caught 107 mosquitos of *Culex* spp when baited with CO_2. These consisted of 80 *Cx. tritaeniorhynchus*; 18 *Cx. bitaeniorhynchus*; 9 *Cx. fuscocephala*; 2 *An. sinensis*; 2 *An. jayporensis* and 2 *An. kochi*. Four thousand other insects were also trapped.

In sixteen trap nights the EVS traps baited with CO_2 caught only 28 mosquitos at the forest site and 12 in the village. The trap at the forest site caught 5 different species of mosquito - 5 *Ae. albolaterus*, 2 *Cx. bitaeniorhynchus*, 3 *Cx. fuscocephala*, 17 *Cx. tritaeniorhynchus* and 1 *An. kochi*. The trap set in the village caught only 2 *An. minimus*, 2 *An. maculatus* and 8 *An. vagus*. No mosquitos at all were caught using an EVS trap baited with lactic acid in ten trap nights.

Collections of resting mosquitos from animal housing

Only two mosquitos were captured from aspirator catches in ten pig sheds - one *An. jayporensis* and one *An. minimus*. More success was obtained with the collections made under the house, where the cattle are kept. 3 *An. barbirostris* were collected in the two catches plus 10 *An. minimus*, 2 *An. kochi*, 3 *An. sinensis* and 59 *An. vagus*. All the mosquitos were newly blood-fed, gravid or semi-gravid. All but one of the mosquitos collected from the cow housing had fed on bovine blood.

Spray catches in houses

From the two indoor spray catches, two *An. minimus*, one *An. culicifacies* and fifteen *An. vagus* were collected. All of these mosquitos were blood fed. Both of the *An. minimus* contained human blood, along with six of the *An. vagus* collected.

DISCUSSION

The UV light traps caught more mosquitos than the CDC light traps. The species composition in both the traps was similar. However, the UV traps posed several operational difficulties. Firstly the UV traps caught an average of 398 insects other than mosquitos per night whereas the CDC traps caught only 58 of these. When the UV trap was used outdoors it captured 4,000 insects other than mosquitos. Sorting the mosquitos from unwanted insects was time consuming. The UV trap is an updraught trap, which is cumbersome, and the rapidly oscillating fan damaged some mosquitos. The UV tube requires a large twelve-volt battery, which needed recharging after each use. In a remote setting the small size and lower energy requirement of the incandescent CDC trap is desirable. In addition, the Dai people found the UV traps disturbing. The UV light caused items of clothing and bednets to fluoresce and the noisy fan disturbed their sleep when hung close to the bednet. In this setting the CDC trap is more acceptable. During sixty-one trap nights CDC traps caught eight species of mosquito that have been incriminated as malaria vectors, either in Yunnan, or elsewhere in the Southeast Asian region. However, the ratio of CDC catch to man landing catch needs to be determined to discern the number of CDC traps required to provide similar information to that obtained by man landing catches.

All the mosquito species trapped are active in the first half of the night (Yunnan Institute of Malaria Control, 1997; Wahab Rahman *et al*, 1995). The villagers stay up until around midnight as the village has electricity and satellite television. The televisions are switched off at midnight by the head of the village. This may explain the higher numbers of mosquitos trapped in the living areas, compared to the bedrooms, particularly among the species whose activity peaks in the early evening. The villagers do not employ personal protection measures before going to bed. However the cooking fire often becomes extremely smoky and this may drive mosquitos towards the end of the room by the door where the traps were located, as this is the least smoky point. Smoke from the fire may provide the villagers with some protection as has been shown in Papua New Guinea (Vernede *et al*, 1994). Nevertheless, the activity of vector mosquitos before midnight means that people are still exposed to the risk of receiving an infected bite before they go to bed. This has implications for malaria control in the area. Impregnated bednets have been advocated as the most suitable intervention due to the exophilic habits of the two major vectors, *An. dirus* and *An. minimus* (Li *et al*, 1987), but they cannot provide complete protection. Indoor residual spraying is impractical due to the exophilic vectors and remote, mountainous terrain. The presence of large numbers of transient breeding sites makes larviciding and source reduction impossible. Therefore prevention of man-vector contact is needed for the early evening. A potential intervention would be some form of mosquito repellent. The Dai people traditionally rely on herbal preparations for medicinal and cosmetic purposes. A locally grown herbal mosquito repellent would be desirable, as it would be cheap and available in remote villages.

There was *An. dirus* breeding around the village. The breeding sites were similar to those described by Rosenberg (1982). The road, which passed along the side of the village, had many shallow puddles containing *An. dirus* larvae. In addition, small puddles containing *An. dirus* and *An. vagus* larvae were found beneath a house. Even so, no adults were trapped, although they have been trapped indoors in Myanmar (Tun-Lin *et al*, 1995), and caught in Thailand using light traps with incandescent bulbs (Rattanarithikul *et al*, 1996). It is possible that no adult *An. dirus* were trapped during this study as it was limited to a few weeks and had to be terminated before the population of *An. dirus* in Mengla reaches high levels, which is usually in September (Yunnan Institute of Malaria Control, 1997).

The low numbers of mosquitos caught using the EVS trap suggests that for collecting *Culex* sp outdoors it would be more effective to use a CDC trap baited with CO_2. A serological survey in Southern Yunnan found 77.4% of samples positive for flavivirus antibodies, of which 27.9% had antibodies to Japanese encephalitis (Zhang, 1989). Four species that have been incriminated as vectors of JE in Yunnan were trapped using CDC traps baited with CO_2: *Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, *Cx. fuscocephala* and *An. sinensis*. *Cx. tritaeniorhynchus* comprised 72.64% of the catch, and this species is the main JE vector in Yunnan (Zang *et al*, 1998).

Although *An. minimus* is considered to have become exophilic in parts of Yunnan due to extensive indoor residual spraying (Li *et al*, 1987), it was found resting indoors in this location. This highlights the need for localized surveys of vector bionomics to optimise control programs. Interestingly, the indoor spray catches also showed that *An. vagus*, which is not considered a vector mosquito in Yunnan due to its zoophily, could be found indoors, fed on human blood. *An. vagus* comprised 30.8% of the total anopheline mosquitos collected in indoor light traps during this study. The population comprised many older individuals. Sixty percent and 59.57% of the *An. vagus* were parous of those caught in CDC and UV traps respectively. This species is active early in the evening, with peak biting densities occurring after dusk (Wahab Raman *et al*, 1995). Wild individuals have been found to be sporozoite positive for both *Plasmodium vivax* (1.9%) and *P. falciparum* (1%) in Thailand (Baker *et al*, 1987). However, these figures may be misleading due to false positive results caused by cross-reaction between the ELISA and factors in cow blood (Somboon *et al*, 1993a).

In the present study a majority fed with bovine blood was found among resting mosquitos caught using mouth aspirators. This may be due to the fact that the collections were made the morning after six cows had been kept beneath the house. A CDC light trap inside the house caught only sixteen mosquitos that night, whereas the previous night when cattle were not kept beneath the house ninety-six mosquitos were caught in a CDC trap. If this result can be replicated it suggests the applicability of zooprophylaxis in houses of this type. Tun-Lin *et al* (1995) found evidence that cattle may reduce human exposure to infective bites by attracting sporozoite positive *An. dirus* in Myanmar. However, Somboon (1993b) carried out man landing catches in Thailand and found that men in huts with buffalo underneath caught significantly greater numbers of *An. maculatus* than men in huts without buffalo beneath. In Yunnan all the local vectors also bite animals (Yunnan Institute of Malaria Control, 2000), if cattle deflect infective bites from humans then the design of Dai village houses could be protective, provided the cattle are kept beneath the houses.

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