EVALUATION OF DIFFERENT METHODS OF CATCHING ANOPHELINE MOSQUITOES IN WESTERN VENEZUELA

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ABSTRACT. During a longitudinal study of vector biology and malaria transmission in western Venezuela, adult mosquitoes were collected by different methods and their efficiency was compared with human landing catches. CDC light traps, a double-net, a calf-baited trap and collection of resting mosquitoes on vegetation were tested. These methods did not prove to be effective substitutes for human landing catches.

INTRODUCTION

Collection of mosquitoes landing on human "baits" is considered the most representative method for monitoring the man-biting mosquito population which is what is relevant to the transmission and control of malaria (World Health Organization 1975).

This method has recently been subject to several ethical and practical objections. The use of humans as baits to catch mosquitoes landing on them may increase their chances of contracting malaria, and the procedure is labor intensive, tedious, uncomfortable and expensive. Also, unless the catching team is well motivated and supervised, their results may be unreliable.

In search for a satisfactory method of sampling anophelines which would allow reduced use of human landing catches for routine evaluation of control programs, especially in areas such as southern Venezuela where *Plasmodium falciparum* resistant to chloroquine is the main parasite (Dirección de Endemias Rurales, 1989[3]), other methods were evaluated and their efficiency in relation to human landing catches was determined. The following devices and methods were evaluated between February 1988 and October 1989: CDC miniature light traps (Sudia and Chamberlain 1962), a double-net modified from Gater (1935), a calf-baited trap (World Health Organization 1975, Service 1976) and collection of resting mosquitoes on vegetation with an aspirator (World Health Organization 1975, Service 1976).

MATERIALS AND METHODS

Study area: The study area is located on the southern slopes of the Andes near the Venezuelan border with Colombia (approximately 7°31′N, 71°41′W). The area and the 3 villages selected for intensive study have been described by Rubio-Palis and Curtis (1992). Malaria transmission occurs in these villages throughout the year and they have a range of ecological conditions representative of the area. In each village an experimental hut was built and kept free of insecticide.

Human landing catches: Human landing catches were standardized as follows: 12 h (1900–0700 h) indoor and outdoor catches were carried out 2 nights per week per village over 15 months (August 1988-October 1989). Collections were made by a team of 6 catchers with 2 supervisors. The catchers worked in pairs and in shifts of 4 h, being rotated each day (indoors/outdoors and between shifts).

Mosquitoes were collected with mouth aspirators and placed in paper cups. Mosquitoes were limited to 20 per cup to avoid damage which would make difficult their identification.

In the laboratory the mosquitoes were killed, identified, counted and an aliquot of 20 were dissected and examined for parity by the Polovodova technique (Detinova 1962), i.e., presence or absence of dilatations on the ovariole stalks, but without attempting to count the dilatations.

Light trap catches: The method was standardized as follows: CDC light traps were operated for 12 h a night by a 6 volt rechargeable battery and were run simultaneously in the 3 huts with 2 human baits per hut sleeping under nets. This procedure was carried out for 2 nights per week, three weeks per month for 15 months. In the morning, mosquitoes were killed, identified and an aliquot of 20 mosquitoes dissected for parity as described above.

Double-net catches: Twelve h (1900–0700 h) collections were carried out in a double-net in the experimental huts using a person in a hammock as "bait." The bottom of the outer net was raised about 15 cm from the ground to allow entry of mosquitoes. The outer net was held out laterally from the inner net with 2 sticks about 1 m long. Searches for mosquitoes trapped between the 2 nets were made hourly with a flashlight and mouth aspirator by a collector other

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[3] Dirección de Endemias Rurales. (1989). Informe del Programa de Erradicación de la Malaria Año 1986. Maracay. 25 p.

than the "bait." The bait and catcher changed position every 6 hours.

Calf-baited trap: Between September and October 1989 a calf-baited trap was used in Jabillos between 1900 and 0600 hours. The wooden pen (180 × 120 cm) was covered by a netting "roof" attached to canvas "walls" which terminated 20 cm above the floor to allow entry of mosquitoes. In the morning, the trapped mosquitoes were collected with a large battery operated aspirator modified from that described by D. Natal (personal communication), which consisted of a tube of Polyvinyl chloride (PVC) (14 cm diam × 125 cm long), and a small fan operated by two 6 volt rechargeable batteries connected in series. Mosquitoes were accumulated in the aspirator in removable plastic containers. After removal from the aspirator the containers were placed in polystyrene boxes, covered with wet towels and taken to the field laboratory. Mosquitoes were killed and identified.

Resting mosquitoes: Collections of resting mosquitoes were standardized as follows: mosquitoes were collected out of doors with the large aspirator described above by sweeping vegetation within a radius of about 1 km of the experimental huts between 0610 and 0800 h on 4 days per month at each village over a 14 month period. Mosquitoes were taken to the laboratory and killed either by freezing or with chloroform. Female mosquitoes were identified, counted and kept dry over silica gel for future bloodmeal identification by ELISA. The Statistical Package for Social Scientists (SPSS/PC+ 1989) was used for data analysis.

RESULTS

Human-landing catches: A total of 21,748 mosquitoes representing 11 anopheline species were collected in all night catches indoors in the 3 villages. The 4 commonest species were *Anopheles* (*Nyssorhynchus*) *nuneztovari* Gabaldón, *An.* (*Nys.*) *triannulatus* (Neiva and Pinto), *An.* (*Nys.*) *albitarsis s.l.* Arribálzaga and *An.* (*Nys.*) *oswaldoi* (Peryassú) (Table 1). The most abundant species was *An. nuneztovari*, comprising over 70% of the anophelines collected in the 3 sites. Less than 2% of anophelines could not be identified due to the loss of identifying characters.

Light trap catches: Light traps operated 6 nights per month in each village collected far fewer anophelines (7,636) than indoor human landing catches (21,748) on 2 nights per month in each village during the same period (Table 1). As in human landing catches, the 4 commonest anopheline species collected in light traps were *An. nuneztovari*, *An. albitarsis s.l.*, *An. triannulatus* and *An. oswaldoi*. More than 20% of the anophelines collected were unidentifiable.

The regression lines and correlation coefficients of the log-transformed monthly mean catches in each village in light traps and human landing catches were calculated (Fig. 1). There were significant correlations between the 2 methods for the 4 commonest species (r values between 0.58 and 0.81, $P < 0.01$). For *An. triannulatus* the correlation was weakest, but it was still significant (Fig. 1B).

In order to quantify the efficiency of the light trap catches compared with the indoor landing catch on humans, the ratios were calculated by dividing the mean of light trap catches by the mean of indoor human landing catches for each of the 5 most abundant species. The confidence limits were calculated, based on the variances of the ratios over different months (Fig. 2). Light traps were particularly inefficient for catching the human landing population of *An. nuneztovari* (the traps only caught 10% as many as the indoor human landing catches), but they appeared somewhat more efficient for the human landing population of *An. albitarsis*, *An. triannulatus* and *An. oswaldoi*, and even more efficient for *An. neomaculipalpus*.

From the data available, it cannot be determined whether the variation observed between species in Fig. 2 was due to variation between the members of different species which had entered houses in their tendency to enter the traps or to land on humans.

Parous rate: A total of 1,497 anophelines collected landing on humans at the 3 villages and 964 collected in light traps were dissected and parity determined. The parous rates for *An. nuneztovari*, *An. albitarsis* and *An. triannulatus* are shown in Table 2. For each species the significance of the differences observed between the data for human landing samples and corresponding light trap samples were tested by the Mantel-Haenszel chi-square test (Kirkwood 1988). This statistical method is appropriate since there may well be heterogeneity in the parity between different samples caught by each method. The data were stratified by season (wet and dry) and village, resulting in 9 separate 2 × 2 contingency tables for each species.

As shown in Table 2, the parous rate was significantly higher in the human landing sample than in the light traps for *An. nuneztovari*, but not for *An. albitarsis* or *An. triannulatus*. Thus, light traps would not give an exact representation of the parous rate in the human landing *An. nuneztovari* in this area.

The correlation coefficient of the parous rates in corresponding catches by the 2 types of catch for *An. nuneztovari*, *An. albitarsis* and *An. trian-*

Table 1. Anophelines collected on human baits (H.B.) and in light traps (L.T.) in Jabillos, Caño Lindo and Guaquitas between August 1988–October 1989. Catches on human baits were carried out on a total of 30 nights (360 h), and the traps were run on 82 nights (984 hours) in each village.

Species	Jabillos H.B.	Jabillos L.T.	Caño Lindo H.B.	Caño Lindo L.T.	Guaquitas H.B.	Guaquitas L.T.	Total H.B.	Total L.T.
nuneztovari	5,653	1,263	4,501	995	8,084	2,105	18,238	4,363
triannulatus	204	252	13	5	686	469	903	726
albitarsis	357	181	139	31	760	274	1,256	486
oswaldoi	164	64	76	20	222	67	462	151
neomaculipalpus	21	49	1	6	17	47	32	102
rangeli	80	52	62	7	113	36	255	95
strodei	65	12	47	3	113	35	225	50
benarrochi	1	0	4	0	10	1	15	1
pseudopunctipennis	0	0	4	1	0	0	4	1
punctimacula	0	0	2	0	1	0	3	0
mediopunctatus	0	0	1	0	1	0	2	0
Unidentifiable*	66	475	95	527	192	659	353	1,661
Total	6,611	2,348	4,945	1,595	10,199	3,693	21,748	7,636

* Unidentifiable due to loss of legs, wings and scales showing characters of taxonomic importance.

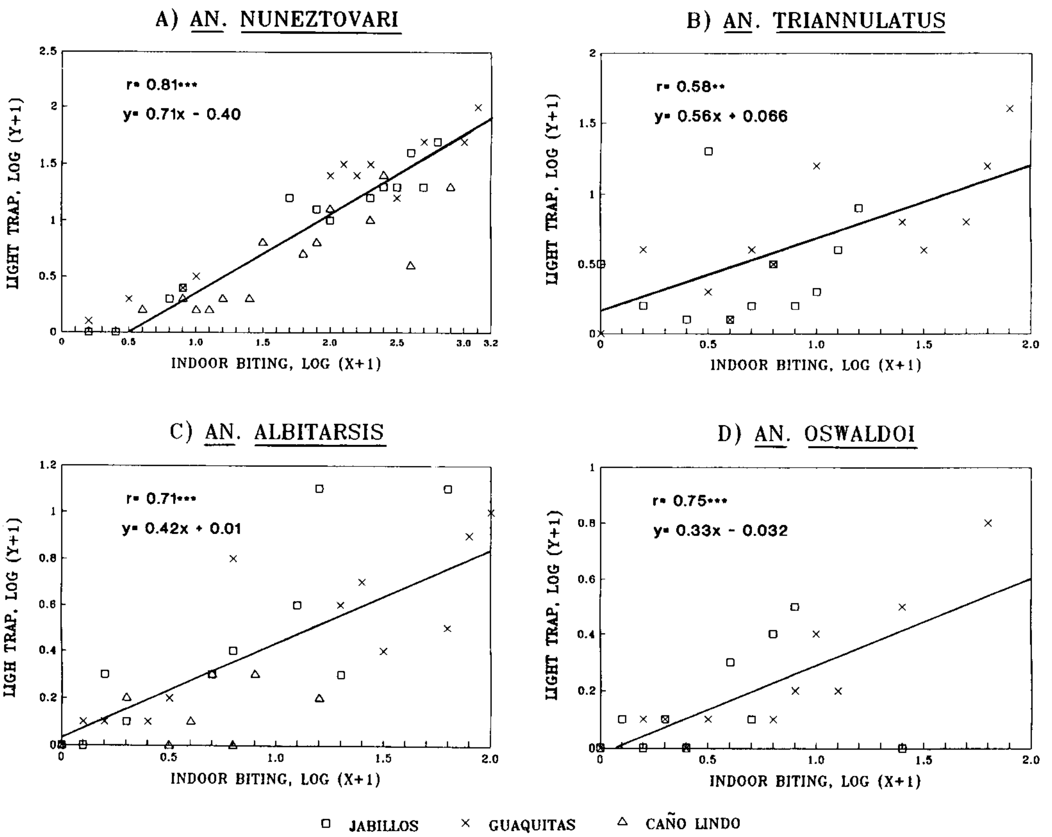

Fig. 1. Numbers of anophelines of the 4 commonest species caught by light traps, plotted against the numbers caught in human landing catches.

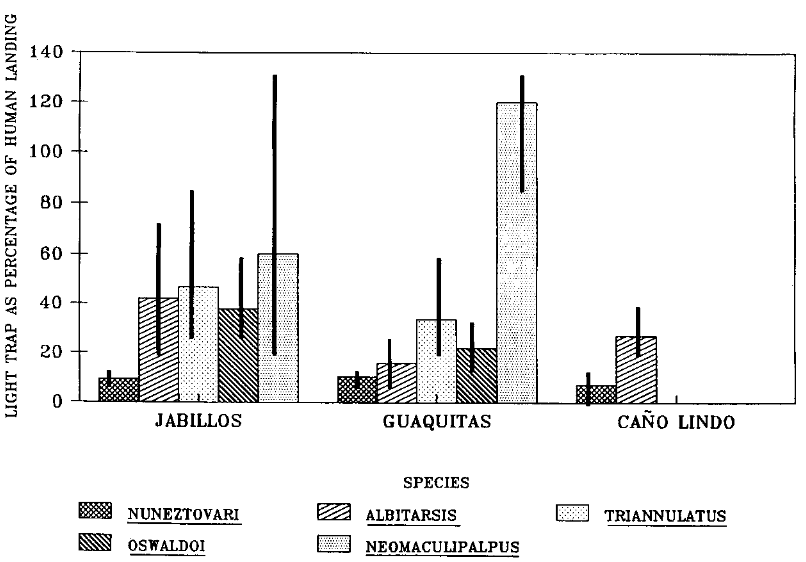

Fig. 2. Numbers of anophelines caught in light traps expressed as percentages of the numbers caught in human landing catches. Bars indicate 95% confidence limits.

Table 2. Parous rate in the 3 commonest species of *Anopheles* caught by light traps and human baits (sample sizes in parentheses). The significance of the differences between the samples caught by the 2 methods was tested by the Mantel-Haenszel chi-square test, stratifying by village and season.

Anopheles	Human	Light trap	$\chi^2_{M\text{-}H}$	*P*
nuneztovari	34.2% (1,149)	28.9% (702)	5.14	0.02
albitarsis	44.3% (133)	31.2% (93)	3.16	0.08
triannulatus	48.1% (106)	45.3% (106)	0.17	0.92

nulatus was calculated. In no case was the correlation coefficient significant. Among the anophelines that were dissected after being caught in light traps, 18% were fully or partially blood-fed.

Double-net catches: A double-net collection conducted outdoors in Jabillos in June 1988 (wet season) for 3 h failed to catch any mosquitoes, while 50 mosquitoes were caught in the same period by a human landing catch. In the following month, collections were made indoors for 12 h in Jabillos and Caño Lindo. During 36 h of collection only 3 anophelines were collected, while 1,237 were collected in the contemporary human landing catches. Since the double-net method was ineffective in the collection of anophelines in western Venezuela, it was abandoned after obtaining these results.

Calf-baited trap: Sixty-nine anophelines were collected from the calf-baited trap during 13 nights at Jabillos. The trap caught all 4 of the main human-biting species, but relatively fewer *An. nuneztovari* (20) than *An. triannulatus* (29). During the 4 human landing catches in September and October 1989, 1,423 anophelines were collected, i.e., a yield per night which was 67× greater than in the calf baited trap.

Collection of outdoor resting mosquitoes: Between August 1988 and September 1989, 2,470 anophelines of 8 species were collected with a large electric aspirator at the 3 sites (Rubio-Palis and Curtis 1992). About 13% of the anophelines collected were unidentifiable.

The log-transformed monthly mean outdoor resting catches were plotted against the log-transformed indoor human landing catches for the 4 most abundant species (Fig. 3). There were significant correlations between the 2 sampling methods for the 4 commonest species (r values between 0.45 and 0.74, $P < 0.003$).

In order to determine the relative sampling efficiency of the resting catch using the aspirator in relation to the indoor human landing catch, the ratios were calculated by dividing the mean of the resting catch by the mean of the human landing catch and 95% confidence limits were determined (Fig. 4). The aspirator out of doors was relatively very efficient in collecting *An. triannulatus* in Jabillos and Guaquitas. This method was less efficient for *An. albitarsis* and

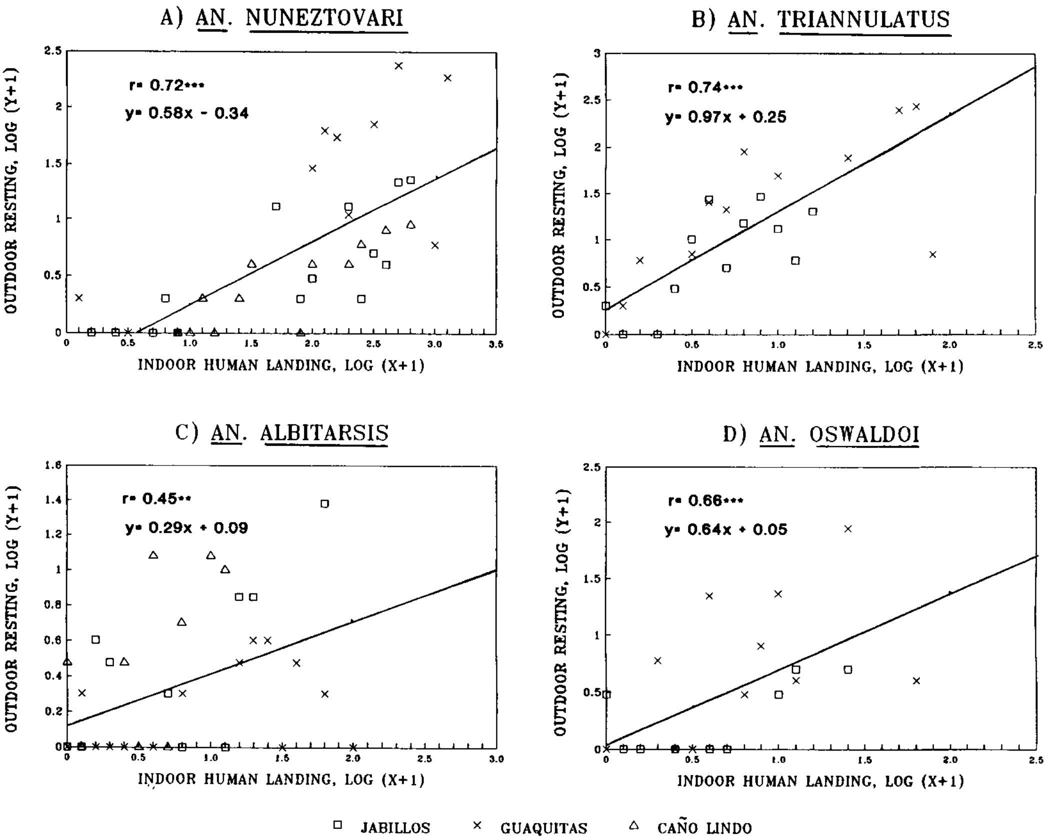

Fig. 3. Relationship of catches of outdoor-resting anophelines using the aspirator and human landing catches indoors.

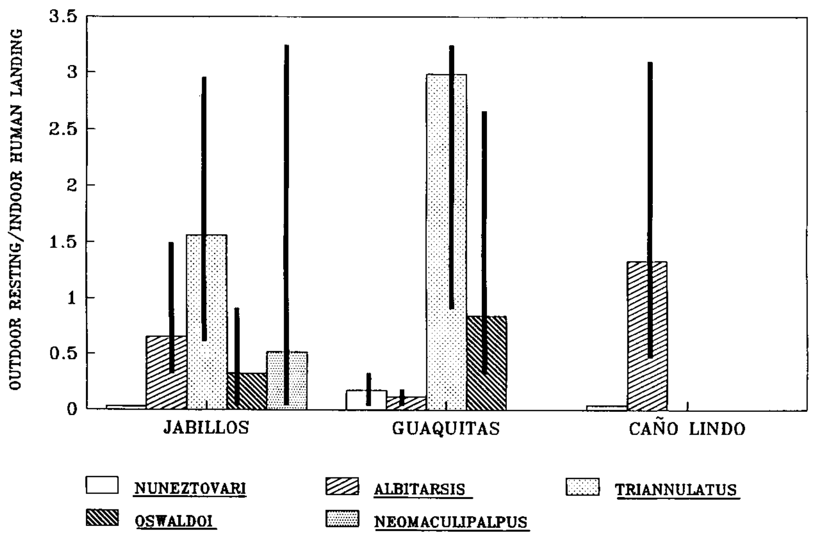

Fig. 4. Outdoor resting/indoor human landing ratios. Bars indicate 95% confidence limits except for *Anopheles nuneztovari* in Jabillos and Caño Lindo where the limits were 0.015–0.06 and 0.021–0.088, respectively.

An. oswaldoi in Jabillos and Guaquitas, while in Caño Lindo more *An. albitarsis* were collected resting on vegetation than in indoor human landing catches. Relatively few *An. nuneztovari* were collected resting around houses compared with their dominant position among the human landing catches.

DISCUSSION

Anopheles nuneztovari is the most abundant anopheline species in western Venezuela and a well established malaria vector (Gabaldón and Guerrero 1959, Pintos et al. 1968). Any method used to sample its population must produce results comparable to the human biting population in which we are interested.

Light traps have been shown in several studies to be a useful method for entomological evaluation of malaria control programs. In the present study, significant correlations were found in the numbers of anophelines caught by the 2 methods, suggesting that light traps are adequate to monitor general seasonal trends. However, the numbers caught in light traps were significantly ($P < 0.01$) smaller than in human landing catches, which might be critical to short-term decision making for control operations.

The light traps were inefficient for sampling *An. nuneztovari* in that the parous rate were significantly lower than in human landing catches. Similar results were reported for *An. gambiae* Giles by Carnevale and Le Pont (1973) and *An. nili* (Theobald) (Carnevale 1974). However, Lines et al. (1991) found a good correlation of numbers caught and the age structure of *An. gambiae* for light trap compared with human landing catches.

In our study where morphologically similar anophelines had to be distinguished, a disadvantage of light traps was that a considerable percentage of mosquitoes were damaged by the trap fan and were therefore unidentifiable.

Despite the present results, mechanical traps should be further evaluated and ultraviolet light should be compared with the light from the small incandescent bulbs used in the present study. Recent studies have shown that the ultraviolet light trap was a very effective method for collecting *An. albimanus* Wied., being superior to human bait catches and CDC light traps for determination of vector densities (Sexton et al. 1986, Mekuria et al. 1990). However, parous rates were not determined and compared in their studies.

The double-net method was found to be almost completely ineffective in catching anophelines. Similar results were reported by Hamon (1964) and Charlwood et al. (1986). However, double-nets using as bait a man, a calf or a goat were successfully used in Malaya by Reid (1961) to compare the attraction of different species of mosquitoes vectors of malaria, filariasis, dengue and Japanese encephalitis to these baits. This method was also used routinely in Japan to monitor the populations of culicine vectors of Japanese encephalitis (Wada et al. 1970).

The aspirator method of sampling the resting population was in general efficient in collecting *An. triannulatus*, *An. albitarsis*, *An. oswaldoi* and *An. neomaculipalpus* but not *An. nuneztovari*. This indicates that, except for *An. nuneztovari*, the anophelines which bite in or near houses tend to rest on the low vegetation nearby.

Collections from the calf-baited trap seemed to indicate that either the anophelines in western Venezuela are less attracted to bovines than to humans, or that the trap, as operated, allowed many mosquitoes to escape. In any further evaluation of this method to sample anophelines, the mosquitoes should be collected at 2 or 4 h intervals to reduce the chances of the mosquitoes escaping.

It is concluded that for monitoring anopheline populations and evaluating their control in western Venezuela, the only currently available, reliable method is by the human landing method. However, efforts should continue to seek satisfactory alternative collection methods.

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