A comparison of three *Aedes aegypti* sampling methods in Trinidad, W. I.

Dave D. Chadee [(1)]

Summary

The efficacy of three Ae. aegypti *collection methods, human-bait, larval surveys and ovitrapping were compared before and after insecticide treatment in Flanigan Town, Trinidad, W. I. A comparison between the three collection methods showed ovitrapping as more efficient in collecting* Ae. aegypti *($P < 0.05$) than larval and human-bait collections. However, it was found that the different collection methods suited the collection of different mosquito species ($P < 0.001$). Although ovitrapping monitored the presence of* Ae. aegypti, *it did not identify its larval habitats. Larval surveys were also found inadequate in detecting all the breeding grounds of* Ae. aegypti *suggesting that some larval habitat may be inaccessible to vector control workers. Human-bait captures were useful in determining the number of mosquito vectors present in the study area, but like ovitraps and larval surveys failed to detect their breeding habitats. The effect of residual, focal-perifocal and ULV insecticide treatments on the* Ae. aegypti *population showed a highly significant reduction ($P < 0.001$) in the population size over the first six weeks after treatment. This study emphasizes the difficulty encountered by control programs to detect and destroy low levels of* Ae. aegypti *infestation during the first treatment cycle.*

Key words : *A. aegypti* — Sampling — Human-bait — Larval survey — Ovitrapping — Treatment — Trinidad.

Résumé

Comparaison de trois méthodes d'échantillonnage d'*Aedes aegypti* à la Trinité, Trinidad et Tobago. *L'efficacité de trois méthodes de récolte d'*Aedes agypti, *la capture sur appât humain, la recherche des larves et l'utilisation de pondoirs-pièges, a été évaluée avant et après traitement par les insecticides à Flanigan Town sur l'île de la Trinité. La comparaison entre les trois méthodes a révélé que les pondoirs-pièges étaient plus efficaces ($P < 0,05$) que la recherche des larves et la capture sur homme. Toutefois, il a été montré que les différentes méthodes permettaient la capture d'espèces différentes de moustiques. Mais, si les pondoirs-pièges révèlent la présence d'*Ae. agypti, *ils ne mettent pas en évidence ses gîtes larvaires naturels. La récolte des larves s'est également montrée inadaptée à détecter tous les gîtes larvaires, suggérant ainsi qu'un certain nombre de gîtes reste inaccessible aux équipes de désinsectisation. La capture sur homme permet de déterminer le nombre de moustiques anthropophiles présents dans la zone étudiée, mais de même que les deux autres méthodes elle ne permet pas de trouver leurs gîtes larvaires.*

*Le traitement insecticide des maisons, des gîtes et de leur environnement, ainsi que les pulvérisations en ULV, ont déterminé une réduction hautement significative ($P < 0,001$) des populations d'*Ae. aegypti *dans les premières six semaines qui les ont suivis.*

(1) *Entomologist, Insect Vector Control Division, Ministry of Health and Environment, Trinidad and Tobago, W. I., on study leave at the Department of Biological Sciences, The University, Dundee, DD1 4HN, United Kingdom.*

*Ce travail souligne les difficultés rencontrées par les programmes de lutte quand il s'agit de détecter et de détruire les faibles populations résiduelles d'*Ae. aegypti *au cours du premier cycle de traitement.*

Mots-clés : *Aedes aegypti* — Échantillonnage — Capture sur homme — Récolte de larves — Pondoirs-pièges — Traitement insecticide — La Trinité.

Introduction

Human-bait captures and larval surveys are traditional methods used for monitoring mosquito populations (Smith, 1904). They can be used to monitor the presence, distribution, and density or size of vector populations and to determine the efficacy of treatment procedures (Service, 1976).

Voluminous literature exists on the sampling techniques and equipment suitable for mosquito surveillance. The success of any particular sampling technique depends not only upon the sensitivity of a technique but also upon the most efficient use of time and money. For example, within recent years the use of the commonly used human-bait technique, has sometimes been shown to be inadequate, because of varying degrees of " attractiveveness " among collectors (Slaff *et al.*, 1983). Giglioli (1979) also pointed out that human-bait captures of *Aedes aegypti* (Linn.), together with space-spray collections and house searches for resting adults is labour intensive, low yielding and may be statistically insignificant.

Recent field and laboratory studies have shown that *Ae. aegypti* are readily attracted to artificial oviposition containers (Fay and Perry, 1965 ; Fay and Eliason, 1966) but that the species is seldom collected in light traps. Fay and Eliason (1966) and Tanner (1969) have mathematically calculated the cost/benefit ratio of ovitrapping surveys against larval surveys and concluded that ovitrap surveys make more efficient utilization of personnel. Tanner (1969) pointed out that larval surveys were often unreliable but ovitrapping was more sensitive in determining the presence or absence of *Ae. aegypti*. However, Fay and Eliason (1966) indicated that ovitraps only estimate the extent of potential infestation and cannot detect breeding foci. The present study was undertaken before and after insecticide treatment in Flanigan Town, Trinidad, W. I. to compare the efficiency of the three mosquito collection techniques (human-bait, larval surveys and ovitrapping) traditionally employed to measure *Ae. aegypti* mosquito populations.

Materials and methods

Study Area

This study was conducted from 22 July 1983 to 3 February 1984 in a small agricultural hamlet of Flanigan Town (61°20′ W, 10°26′ N), nestled on the Central Range of Trinidad, W. I. The housing pattern is predominantly linear with cocoa and coffee plantations occupying major stretches of the Brasso-Caparo Road (500 hectares, 500 houses, 1 000 people). The area consists of rolling hills composed of mainly sandstone and limestone formation. The vegetation is typically evergreen seasonal deciduous forest with the *Carapa guianensis* Aubl. (Crappo)/*Eschweilera subglandulosa* (Steud.) Miers (Guatecare) associaton occuring from 360 m and in lower parts of the Central Range. In Trinidad there are two distinct seasons : the wet season which occurs during May to November of each year and the dry season from December to May. The annual rainfall for Flanigan Town is approximately 2 413 mm. A complete description of the study area can be found in Beard (1946).

General

In 1976, the Insect Vector Control Division, Ministry of Health and Environment, Trinidad, embarked on an *Ae. aegypti* eradication program. However, from the time this program was initiated, the *Ae. aegypti* population in Flanigan Town was neither evaluated nor treated with insecticides. Therefore, during weeks 1-11 of this study, the *Ae. aegypti* population in Flanigan Town was evaluated using human-bait captures, larval surveys and ovitrapping. During weeks 12-14 the study area was treated using ultra-low-volume spraying of malathion, focal and perifocal treatment with temephos and fenthion and residual household spraying with fenthion. Post-treatment evaluations were conducted from weeks 15-32 using the same methods employed during weeks 1-11.

Human-bait Captures

During this study, human-bait captures were performed according to the procedures outlined by Haddow (1954). Collections were made on one day of each week between 1600-1900 hours in eight houses, with one man sitting in the porch of each house. This particular time was used for collecting *Ae. aegypti* because their diel biting activity peaked during this period (Corbet and Smith, 1974 ; Chadee, unpublished data). Each catcher was provided with a torch and mosquitoes were caught in hand nets or aspirated from catcher's lower legs and ankles and transferred into plaster-of-paris lined jars. The jars were transported in a Coleman Ice Box, containing a block of ice, to the Insect Vector Control Division laboratory in St. Joseph. In the laboratory, mosquitoes were lightly anaesthetized with chloroform, identified and recorded on standard forms by the author.

Larval Survey

In Flanigan Town, all houses and compounds containing potential *Ae. aegypti* habitats were inspected by Insect Vector Control Division (IVCD) workers using the PAHO (1968) guidelines. All containers including natural habitats like treeholes, which might harbour *Ae. aegypti* and other mosquitoes were inspected. These larval surveys were similar to those conducted in Tobago by Chadee *et al.* (1984). Two days later laboratory technicians revisited the area and re-inspected the houses both to determine the quality of work and to detect any hidden or missed breeding sites of *Ae. aegypti*. Any missed breeding sites detected in the second survey were pooled with the data collected by the IVCD workers.

Ovitrapping

One hundred and twenty conventional ovitraps as described by Fay and Eliason (1966), were used to monitor the *Ae. aegypti* population before and after insecticide treatments in Flanigan Town. Each ovitrap consisted of a cylindrical, black painted glass jar (height 13 cm, diameter 6 cm) containing approximately 375 ml of tap water and a removable thin strip of brown hardboard called a " paddle " (12.5 cm × 2.5 cm) on which mosquitoes laid eggs just above the water level. Two ovitraps were placed under each of 60 houses, all of which were built on stilts. Ovitraps were used according to the criteria proposed by Jakob and Bevier (1969), being placed at ground level and the paddles collected weekly and placed into plastic bags. All eggs were microscopically examined and identified according to their shape and exochorionic pattern (Pratt and Kidwell, 1969). In addition, eggs were hatched and the resultant larvae reared to adults which were checked for identification.

Treatment

During weeks 12-14, both the inside and outside walls of all houses in Flanigan Town were sprayed with a Hudson X-Pert pressure sprayer delivering approximately 1 g/m^2 of emulsion concentrate. All water storage containers found in and around the houses were treated with 1 % temephos (Abate) sand granules to yield a concentration of 1 ppm. The outer surfaces and periphery of the water containers were sprayed with 1.6 g/m^2 of 40 % fenthion (wettable powder). During week 13, the study area was also sprayed twice (Monday and Friday) between 1630-1900 hours by a truck-mounted Leco ULV spraying machine delivering 95 % malathion insecticide at a rate of 130 ml/mn. Droplet size along the pre-established route averaged 16 microns, and weather conditions were within the manufacturer's recommendation for ULV application of malathion. Further details of the ULV treatment procedures can be found in Chadee (1985). The results were analysed using a G-test by transforming the data into contingency tables (Sokal and Rohlf, 1981) to determine whether different collecting methods suited different species and to determine the most efficient method of collecting *Ae. aegypti*.

Results

Table I shows the number of mosquito species collected from July (1983) through February (1984). The " species richness " data are presented by months indicating that during August, September, October, December, January and February, four different mosquito species were encountered during larval collections. Ovitraps on the other hand attracted primarily *Ae. aegypti* but in August eggs of *Haemagogus janthinomys* Dyar were collected. Human-bait captures attracted a total of nine mosquito species but the highest number of mosquito species collected in any month was six, that is during August and September.

The most common mosquito species found in and around domestic and peri-domestic situations in

TABLE I

A comparison of the number of mosquito species collected using three sampling methods in Flanigan Town, Trinidad, W. I. (1983-84)

Sampling methods	No. of species collected							
	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Larval surveys	1	4	4	4	0	4	4	4
Ovitrapping	1	2	1	1	1	1	1	1
Human-bait	5	6	6	3	3	3	5	6

TABLE II

A comparison of the sensitivity of human-baits, larval surveys and ovitrapping in collecting different mosquito species in Flanigan Town, Trinidad, W. I. (1983-1984). $G_{10} = 15.7$, $P < 0.05$. All low values (below 10) were combined for our analysis. The asterisk denotes the number of positive ovitraps and not number of eggs

Mosquito species	No. of mosquitoes collected		
	Human-bait	Larval Survey	Ovitrapping*
Aedes aegypti	194	203	547
Anopheles bellator	8	0	0
Culex quinquefasciatus	63	24	0
Haemagogus janthinomys	4	0	1
Limatus durhamii	20	37	0
Phoniomyia lassalli	1	0	0
Trichoprosopon digitatum	14	10	0
Wyeomyia medioalbipes	26	0	0
Toxorhynchites moctezuma	0	8	0

TABLE III

Mosquitoes collected during larval surveys during the period July (1983) to February (1984) in Flanigan Town, Trinidad, W. I.

Mosquito species	Months								
	1983					1984			
	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Total
Ae.aegypti	15	32	117	25	0	12	23	19	243
Li. durhamii	0	4	19	11	0	2	1	1	37
Tr. digitatum	0	1	1	2	0	1	1	4	10
Cx.quinquefasciatus	0	1	6	1	0	0	9	7	24

Flanigan Town are presented in table II. During this study, the common mosquito species collected at bait were *Ae. aegypti* (58 %), *Culex quinquefasciatus* Say (19 %), *Wyeomyia medioalbipes* Lutz (7.8 %) and *Limatus durhamii* Theobald (6.1 %). These four mosquito species were also collected during larval surveys, but none of them was collected in the ovitraps. In fact 99 % of the mosquito eggs collected in ovitraps were those of *Ae. aegypti* (table II).

Table II also gives the number of mosquitoes captured by the three collecting methods, and shows that the methods differed in their relative efficiencies of collecting the different mosquito species (G-test of 3 × 6 contingency table ; $G_{10} = 18.4$, $P < 0.05$). Although all three methods collected *Ae. aegypti*, ovitrapping was the most suitable method for this species. The differences found between human-bait and larval collections were also significant (G-test of 2 × 6 contingency table ; $G_9 = 39.6$, $P < 0.001$), showing that while human-bait was suitable for collecting many mosquito species, larval surveys were more suitable for collecting *Ae. aegypti*.

Table III shows the effect of focal treatment with temephos on different mosquito species found in artificial containers. Results show that larviciding reduced the larval population of *Ae. aegypti*, and three associated species, with a large reduction over the first six weeks after treatment (weeks 16-21) (fig. 1). These results are consistent with those previously obtained by Chadee (1984). To test the differences between species in respect of the effects of larvicides, pooled data for July-September and November-February were subjected to a G-test as a 2 × 4 contingency table. The results were significant ($G_7 = 15.5$, $P < 0.05$), showing that temephos had a greater proportional effect on *Ae. aegypti* than on other species (the October data were omitted from the analysis as October was a transitional period between pre and post-treatments).

Table IV shows the combined effect of residual, focal-perifocal and ULV treatment on *Ae. aegypti* populations as accessed by human-bait captures. A considerable reduction in the *Ae. aegypti*, and associated mosquitoes, was recorded indicating that with one full treatment cycle, the *Ae. aegypti* biting populations and immature stages were brought under control for six weeks. A similar statistical analysis to that applied to table III gave a highly significant result ($G_7 = 30.2$, $P < 0.001$), combining species with total captures of less than ten, confirming that the *Ae. aegypti* population was reduced by treatment more than other species (eg. *Cx. quinquefasciatus*).

Figure 1 shows the fluctuations in the *Ae. aegypti* population during the pre-treatment and post-treatment periods. The results suggest that although one insecticide treatment cycle, substantially reduced the biting population and immature stages of *Ae. aegypti*, the ovipositing population of *Ae. aegypti* were still maintained, though at a reduced level (fig. 1).

TABLE IV

Monthly totals of mosquitoes collected by human-bait between 1630-1900 hours in Flanigan Town, Trinidad, W. I. (July 1983-February 1984). $G_9 = 30.2$, $P < 0.001$. All values below 10 were combined for the analysis

Mosquito species	Months								
	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Total
Ae. aegypti	47	70	131	0	1	5	0	10	264
An. bellator	2	0	2	0	0	0	0	0	4
Cx. quinquefasciatus	0	6	20	0	5	24	13	10	78
Hg. janthinomys	4	0	0	0	0	0	0	0	4
Tr. digitatum	2	5	6	0	0	0	1	1	15
Lm. durhamii	4	10	6	0	0	0	0	0	20
Ph. lassalli	0	1	0	0	0	0	0	0	1
Wy. medioalbipes	0	10	13	0	1	3	0	1	28
Total	59	102	178	0	7	32	14	22	414

Discussion

Although this evaluation program was conducted during only a single treatment cycle, the efficiency of ovitrapping over human-bait and larval collections in clearly evident during both pre-treatment and post-treatment surveys. These findings confirm results previously obtained by Fay and Eliason (1966) and Tanner (1969).

Fay and Eliason (1966) showed that *Ae. aegypti* ovitrap surveys estimated the extent of potential infestation, but could not identify larval habitats. In the present study, larval surveys failed to detect all larval breeding habitats and consequently, the number of larvae ans pupae collected did not reflect the true population size of *Ae. aegypti* (fig. 1). Failure to find and treat all breeding places was likely responsible for the continuation of oviposition throughout the study, but which was most recognisable during the post-treatment period (fig. 1).

Tanner (1969) pointed out that *Ae. aegypti* larval surveys were often unreliable because of discontinuous sampling and varying skills among collectors. This may be true in most control programmes as well as during this study, despite all houses and potential breeding habitats were re-checked by two independent groups (laboratory staff and the IVCD re-checking unit). Possibly missed in inaccessible areas like tree holes at high elevations which could

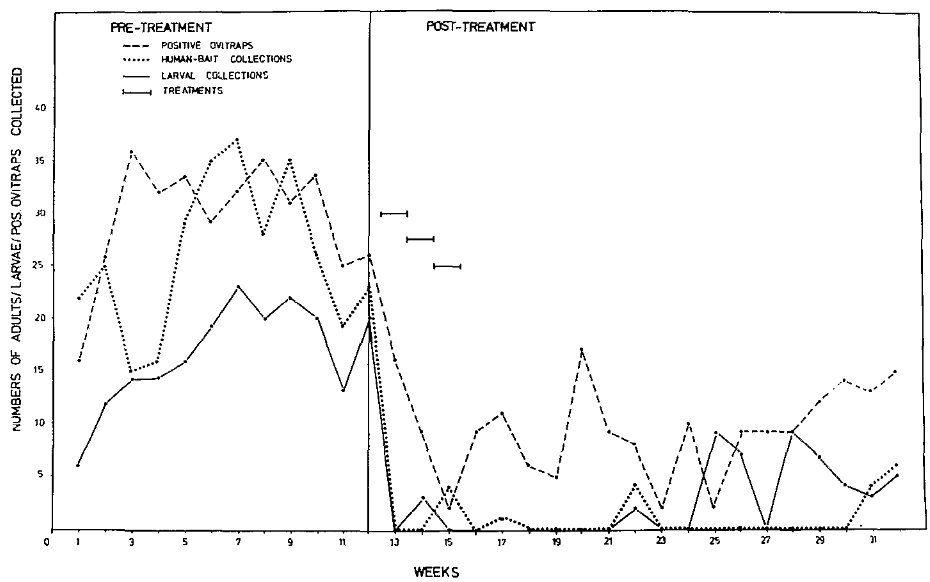

FIG. 1. — Shows the effect of treatment on the *Aedes aegypti* population as accessed by human-bait, larval surveys and ovitrapping in Flanigan Town, Trinidad, W. I. (1983-1984)

not be found by the IVCD workers. The results of this study suggest that in some areas, with a well established population of *Ae. aegypti*, a small proportion of the population may not be detectable using human-bait and larval surveys. Although this level of infestation may be below the transmission threshold of dengue and other arboviruses, it may, if unchecked, serve to re-infest the entire area. This study, therefore, further emphasizes the difficulty encountered by control programs to detect and destroy these low levels of infestation.

Furlow and Young (1970) concluded that ovitrapping alone or in conjunction with larval collections was adequate for the surveillance of *Ae. aegypti* and *Aedes triseriatus* (Say), and the present study seems to support this view. However, failure of these methods to detect all breeding sites of *Ae. aegypti* makes its control very difficult.

Within recent years, human-bait captures have sometimes been found to be inadequate, labour intensive, low yielding and statistically insignificant for determining the population size of *Ae. aegypti* (Giglioli, 1979 ; Slaff *et al.*, 1983). Similar conclusions were reached during the present study, that is only a few adults were collected during the post-treatment period, although numerous ovitraps were positive. Although human-bait captures do not appear to be a very sensitive method of collecting *Ae. aegypti* at low levels, it is the only method presently available for determining man-vector contact. Never theless, human-bait collections were informative in the present study in capturing in addition to *Ae. aegypti*, three other potential disease vectors biting man, namely : *Anopheles bellator* Dyar and Knab the vector of malaria, *Hg. janthinomys* vector of sylvan yellow-fever, and *Cx. quinquefasciatus* vector of bancroftian filariasis.

Chadee (1984) reported that 1 % temephos sand granules placed into water drums gave acceptable results from four-six weeks under field conditions in Trinidad. Data from this study also showed a similar efficacy pattern using temephos (1 ppm), with no *Ae. aegypti* larval collections in artificial containers after weeks 16 to 21 post-treatment.

The efficacy of malathion ULV spraying against both caged and natural populations of *Ae. aegypti* has been found ineffective for use under local conditions in Trinidad (Chadee, 1985). But the combined use, in the present study, of ULV treatment and fenthion residual or intradomiciliary spraying, against adult populations of *Ae. aegypti* was found effective for approximately five weeks. Nevertheless, a small number of positive ovitraps were collected weekly during this period (fig. 1). This may have been due to adults that missed being sprayed or untreated larval habitats.

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