Physiological state of *Aedes (Stegomyia) aegypti* mosquitoes captured with MosquiTRAPs™ in Mirassol, São Paulo, Brazil

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ABSTRACT: We examined the best location in a house to install MosquiTRAPs, sticky ovitraps that attract and capture *Aedes aegypti* females and investigated the physiological state of captured female mosquitoes. The study was performed in a twenty-block area in Mirassol, São Paulo State, Brazil, in which five blocks were randomly chosen for MosquiTRAP installation. In each block, four houses were selected for the installation of eight traps: four indoors (bedroom, living room, bathroom, and kitchen) and four outdoors in the shade (two at the front of the house and two in the backyard). These houses were visited over an eight-week period. The outdoor MosquiTRAPs captured five times more females than indoor traps and appeared to be the best places to install MosquiTRAPs. There were no significant differences among indoor sites or among outdoor sites with respect to the number of females captured. The capacity of the MosquiTRAP to capture a large number of gravid *Ae. aegypti* females reinforces its potential as an entomological surveillance tool in dengue control programs. ***Journal of Vector Ecology* 31 (2): 285-291. 2006.**

Keyword Index: Aedes aegypti, sticky ovitraps, mosquito surveillance, physiological state.

INTRODUCTION

In the state of São Paulo, Brazil, the number of registered cases of dengue increased during the years 1995 to 2001 when the greatest outbreak was recorded (51,248 cases with an incidence of 136.2 per 100,000 inhabitants). From 2002 onward, the number of cases significantly decreased, with an incidence in 2005 of 12.4 cases per 100,000. The levels of *Aedes (Stegomyia) aegypti* are still high, however, and the risk of outbreaks continues. Thus, entomological surveillance is of great importance for the early detection of transmission risk and in directing vector control measures.

The main entomological surveillance methods for *Ae. aegypti* in the State of São Paulo are based on the numbers of larvae in containers. The Breteau Index (number of containers with *Ae. aegypti* larvae per one hundred houses inspected) is the most frequently used method (SUCEN 2002, Breteau 1954). However, these fail to monitor the adult vector population (Focks 2003). Another method, although not routinely used, is the ovitrap. This consists of a black container partially filled with water holding a vertical wooden paddle with a rough side where females lay their eggs (Fay and Eliason 1966). A study performed by Braga et al. (2000) in Salvador, Brazil, included a comparison between larval census and ovitraps in the detection and monitoring of *Ae. aegypti* and demonstrated that the trap is more sensitive in detecting this mosquito, as well as being cheaper and operationally more viable in vector surveillance. Rawlins et al. (1998) found similar results in Trinidad.

The capture of adult mosquitoes in buildings with aspirators (Nasci 1981) can provide information regarding the number of *Ae. aegypti* females per person in a determined area. The use of this technique is more useful in the development of research projects than in the routine activities of dengue control programs.

Various types of sticky traps have been used as an alternative method of collecting adult mosquitoes, in particular *Ae. aegypti* (Muir and Kay 1998, Ordóñez-Gonzales et al. 2001, Russell et al. 2005). Kay et al. (2000) and Montgomery et al. (2004) used sticky ovitraps to collect mosquitoes in subterranean habitats, whereas McCall et al. (1996) tested sticky traps baited with mouse odor. Ritchie et al. (2003, 2004) tested the efficacy of an adulticidal sticky ovitrap and demonstrated that its use may be important in ecological and epidemiological studies. Russell and Ritchie (2004) showed that sticky ovitraps are important instruments in the investigation of the dispersion oviposition behavior of mosquitoes such as *Ae. aegypti*.

A new sticky ovitrap that uses a synthetic oviposition attractant (Eiras and Santanna 2001) to capture female adults of *Ae. aegypti,* known as the MosquiTRAP™, was developed by Eiras (2002). The MosquiTRAP allows *Ae. aegypti* females to be identified and counted in the field, reducing the necessity for human and operational resources, as well as providing rapid information on the adult vector population. Once the trap has been shown to be effective in the laboratory (Eiras 2002) and in the field, it is important to perform studies to characterize the best installation sites for the trap and to know the physiological state of the females that are captured by it.

The aims of this study were to detect the best location to install MosquiTRAPs in houses for capturing *Ae. aegypti* females and to investigate the physiological state of the captured females, including ovarian follicle development, parity and the contents of the midgut.

MATERIALS AND METHODS

The municipality of Mirassol is located in the northwestern region of the state of São Paulo about 460 km from the capital city of São Paulo. The estimated population was 51,993 in 2004 (DATASUS 2005) and comprises an area of 243.8 km^2. It was infested by *Ae. aegypti* in 1987. Recently, two larval infestation surveys (outdoors and indoors) showed high Breteau Indices: in January 2001, 14.3 containers per 100 houses were positive for *Ae. aegypti* and/or *Ae. (Stg.) albopictus*, whereas in February 2002, the index was 9.9 (Superintendência de Controle de Endemias: unpublished data).

A twenty-block area of 667 residences, mostly one-floor houses without existing concentrations of breeding containers, was chosen to install the traps. The area, located in the suburb of São José, is homogeneous in respect to socioeconomic status and the design of houses.

The MosquiTRAP™ (Version 1.0, Ecovec Ltd) consists of a matte black container (16 cm high x 11 cm diameter) containing approximately 280 ml of water with a removable sticky card inside on which the mosquitoes are captured (Eiras 2002). The trap also contains a synthetic oviposition attractant (AtrAedes™ Ecovec Ltd), developed from the volatile substances of *Panicum maximum* grass infusions (Eiras and Santanna 2001, Eiras et al. 2001). The oviposition trap consists of a black opaque container of approximately one liter. The container is filled with 500 ml of water with a wooden paddle measuring 12 cm x 2 cm placed inside.

Surveys to measure the larval density (Gomes 1998) were carried out on two different occasions in an area comprising 20 blocks in which the traps were installed and in the 27 blocks surrounding the study area: first during the week when the traps were installed (from March 1-5, 2004) and then after their removal (from April 10-14, 2004). The area, which comprises 1,374 houses, was checked by systematically surveying all blocks visiting one in every three houses. During each visit, the containers were checked that had been installed indoors and outside of the houses.

Five blocks were randomly chosen for the installation of traps. One house on each of the four sides of the five blocks was selected, for a total of twenty houses. The criteria for the choice of the houses were the existence of sheltered sites, preferably with plants outdoors, and the residents' agreement to have the traps installed. At each house, eight MosquiTRAPs were placed according to the following distribution: four indoor sites (living room, bedroom, kitchen, and bathroom) and four sheltered outdoor sites (two at the front yard: F1 and F2; two at the backyard: F3 and F4). A total of 160 MosquiTRAPs were installed on March 4-5, 2004, preferably near plants and out of reach of children and animals at a height of from 0 to 1 m above the ground. An ovitrap was also installed in an unshaded outdoor site as an indicator of the presence or absence of the vector.

The MosquiTRAPs were inspected three times weekly during eight weeks (from March 8 to May 1, 2004). On the fourth week of the experiment, the attractants and the sticky cards were replaced with new ones. The captured female mosquitoes were identified *in situ* by trained workers who removed them from the cards with the aid of probes and forceps and placed them in boxes with moist filter paper. They were stored in thermal boxes cooled with ice and taken to the laboratory of Superintendência de Controle de Endemias (SUCEN) for dissection.

The analysis of material was performed using probes under a stereomicroscope, where the presence or absence of blood in the middle intestines was observed. The condition of the blood was also analyzed: digested (brown) or recently ingested (red). The identification of ovarian development was observed according to techniques described by Christophers (1911) and Mer (1936) who classified development in five stages (I to V). Parity was established by analyzing the condition of the tracheoles using the technique of Detinova (1962). Females with coiled tracheolar filaments covering the ovarioles were considered nulliparous. Females with extended tracheolar filaments were considered parous. In stages III-V, it is not possible to identify parity (Forattini 2002). Females in phases IV and V were considered gravid as were those that had laid their eggs on the sticky card after being captured by the MosquiTRAP. The females that had their abdomens damaged during removal from the sticky cards were classified as "without identification."

The ovitrap paddles were changed once a week. The removed paddles were packed in plastic bags and taken to the SUCEN laboratory to check for the presence of eggs. The eggs were not hatched for later identification because Dibo et al. (2005), in the same municipality, found that 99.2% of the eggs on a paddle were *Ae. aegypti*.

The Breteau Indices (BI) for *Ae. aegypti* and *Ae. albopictus* were calculated using the number of positive breeding containers with larvae of each of these species per the number of houses surveyed on each occasion (Breteau 1954). The large number of negative breeding containers did not approximate a normal distribution even after transformation to the square root. Thus, the option was to compare the values obtained at the beginning and at the end of the study using the non-parametric Mann-Whitney test. The results for the ovitraps over the eight-week period is presented together with the respective 95% confidence intervals (Altman 1999).

Ae. aegypti females captured by the MosquiTRAPs during the eight weeks of collection were totalled for each residence according to the installation sites: bedroom, living room, bathroom, and kitchen; front yard (F1+F2) and back yard (F3+F4); indoors and outdoors. Division of these values by the number of residences surveyed produced mean numbers of females per MosquiTRAP for each site analyzed. The numbers of *Ae. aegypti* females were interpreted as rates (number of females over eight weeks) and considered as the result of Poisson processes. Thus, the square root of these values was calculated, providing a good approximation of the normal distribution. The numbers of females using the square root scale at indoor and outdoor sites were compared using the paired Student t-test; at bedroom, living room, bathroom, and kitchen sites, they were compared through variance analysis for repeated measures. At the front yard and back yard sites, they were compared using the paired Student t-test (Altman 1999). The number of collected females per week was paired with average minimum and average maximum temperatures and total rainfall of the same week and the three previous weeks to calculate the respective Pearson correlation (Altman 1999). The rainfall data were obtained from the Department of Agriculture in Mirassol and data on temperature from the Department of Agriculture in São José do Rio Preto (15 km from the study area) were used as these data were unavailable in Mirassol.

The criterion to consider a MosquiTRAP positive was at least one female *Ae. aegypti* mosquito. The positivity of the MosquiTRAPs installed in the bedroom was calculated by dividing the number of times that they were positive by the total number of weekly evaluations (twenty traps multiplied by eight weeks). The same operation was applied to calculate the positivity of the living room, bathroom, and kitchen. The other positivities were calculated for the environment where the traps were located, using a similar procedure. The front yard (F1+F2) was considered positive when an *Ae. aegypti* female was found in at least one of the two traps. An identical criterion of positivity was used for back yard (F3+F4), indoor, and outdoor sites. The positivity of the front yard and back yard of the house were compared through proportion tests. The same test was used to compare indoor and outdoor sites.

The sensitivity of MosquiTRAPs to detect *Ae. aegypti* females outdoors was calculated. To consider a house positive in a certain week, at least one of the eight MosquiTRAPs had to contain *Ae. aegypti* females and/or the oviposition trap contained eggs. These results are presented with their respective confidence intervals.

The proportion of females in relation to ovarian development and the presence and condition of the ingested blood were calculated and the respective 95% confidence intervals are presented. The frequency of females captured in MosquiTRAPs and their physiological states are also presented for *Ae. albopictus*.

An alpha error of 5% and a power of 90% were considered when calculating the sample sizes for the BI. These are also capable of identifying differences in five positive breeding containers for *Ae. aegypti* and *Ae. albopictus* larvae per 100 houses or more, between the initial and final surveys. A sample of 350 houses was obtained and increased to 583 with an expectation of finding 40% of residences inaccessible. The size of the samples to compare the two populations of *Ae. aegypti* females obtained from the MosquiTRAPs placed at distinct sites was calculated from the following parameters: an alpha error of 5%, a power of 71%, considering the measurements of the two populations of 30 and 20 females per house, respectively, and a variance equal to 121 (females per house)^2. The sample size for the MosquiTRAPs was calculated in 20 houses (Altman 1999). The formula used to calculate the sample size for the BI and MosquiTRAP measurements was:

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2)(z_{1-\alpha} + z_{1-\beta})^2}{(\mu_1 - \mu_2)^2}$$

where σ^2 is the variance, α is the alpha error, $1-\beta$ is the power, and $\mu_1 - \mu_2$ is the difference between means (Rosner 2000).

The project was approved by the Ethics Committee of the Medical School in São José do Rio Preto. During the first visits to install the traps, the residents were informed about the schedule of the project (day of collection of adult mosquitoes and changes of paddles) and about the research and its aims. The same procedure was performed for the visits to measure the indices of larval infestation. The participants signed written consent forms.

RESULTS

The values of the initial and final BIs for *Ae. aegypti* (14.1 and 12.9, respectively) were not significantly different (Mann-Whitney test, p=0.5182). For *Ae. albopictus*, the initial and final BIs (1.2 and 0.9, respectively) were also not significantly different (Mann-Whitney test, p=0.2123). The positivity of ovitraps was 87.5% (95% CI: 81.4-92.3) over the eight weeks of the study. All Pearson coefficients calculated with pairing of numbers of females with temperature and rainfall data did not present values significantly different from zero.

During the eight weeks of the study, the MosquiTRAPs captured 488 females of *Ae. aegypti*, 79 (16.2%) indoors and 409 (83.8%) outdoors. The number of *Ae. aegypti* females captured by the MosquiTRAPs outdoors was significantly higher than indoors (Students t-test, p=0.0000). The outdoor MosquiTRAPs captured approximately five times more females than indoor traps. A comparison of the bedroom, living room, bathroom and kitchen did not present significant differences in relation to the number of captured females (F test, p = 0.8214). The same trend occurred at outdoor sites (front yard and back yard) (Students t-test, p = 0.6991) (Table 1).

Indoors, the numbers of females captured per trap and week with the respective 95% confidence intervals were: 0.09 (95% CI: 0.03-0.15) for the bedroom, 0.09 (95% CI: 0.03-0.14) for the living room, 0.16 (95% CI: 0.08-0.24) for the bathroom, and 0.15 (95% CI: 0.08-0.22) for the kitchen. Outdoors, the numbers of females per trap and week were 0.62 (95% CI: 0.47-0.77) for F1, 0.57 (95% CI: 0.42-0.72) for F2, 0.84 (95% CI: 0.63-1.05) for F3, and 0.53

(95% CI: 0.38-0.67) for F4. The positivity of the outdoor MosquiTRAPs was significantly higher than indoor traps (Proportion test, p = 0.0000). The positivity did not present significant differences in relation to the place of installation in indoor sites (Chi-square test, p = 0.2495) or outdoor sites (Proportion test, p=0.9136, Table 1).

Indoor and outdoor environments were similar in relation to the capture or non-capture of *Ae. aegypti* females by MosquiTRAPs in 37.5% of the occasions (95% CI: 30.0-45.5), both positive in 24.4% (95% CI: 17.9-31.8) and both negative in 13.1% (95% CI: 8.3-19.4) of the occasions. They were different in 62.5% of the occasions (95% CI: 54.5-70.0), the great majority (55.6% - 95% CI: 47.6-63.5) related to the positivity of outdoor MosquiTRAPs and negativity of indoor traps. Only 6.9% (95% CI: 3.5-12.0) were related to the inverse situation.

Of a total of 160 evaluations, 156 were considered positive, that is, with at least one female *Ae. aegypti* in the MosquiTRAPs or eggs on the paddle. The sensitivity of outdoor MosquiTRAPs, in relation to the results presented above, was 82.1% (CI 95%: 75.1-87.7). The sensitivity of oviposition traps in relation to this pattern was 89.7% (CI 95%: 83.9-94.0).

The results of the ovarian development analyses in respect to the presence of blood and its condition in *Ae. aegypti* females captured by MosquiTRAPs are presented in Table 2. There was a high proportion of gravid females (87.3%) and of females without blood (86.7%). Among the females in phases III, IV, and V, 15 (3.5%) had ingested blood recently. Of the females captured indoors and outdoors, 90.9% and 86.6% were gravid, respectively (p=0.2987). Of the females captured indoors and outdoors, 80.5% and 86.7% respectively, had no blood (p = 0.1535).

The MosquiTRAPs captured eleven females of *Ae. albopictus* just in outdoor sites. Ten were gravid and one was considered "without identification." None of them had blood.

DISCUSSION

The BI values obtained during the surveys of larval infestation and the high positivity of the ovitraps in the eight-week period demonstrated that the site chosen was appropriate to perform the study because of its significant level of infestation with the main vector of dengue, *Ae. aegypti*.

All results suggest the outdoor sites are the best places to install MosquiTRAPs. They showed a higher number of captured females, higher positivity, as well as the great discordance of finding positive traps in this environment and negative traps at indoor sites. A study performed by Dibo et al. (2005) in the same municipality also identified outdoor sites as the best place to install ovitraps.

The results of this study, with a high proportion of captured gravid females without blood and outdoors, are not in agreement with the study by Barata et al. (2001) in São José do Rio Preto (SP), a town close to Mirassol, in which the females were collected with aspirators (Nasci 1981). In this study, only 12.7% of the captured females were outdoors, 45.3% were pregnant, and 31.8% had no blood. The parallels between the present work and that of Barata et al. (2001) are due to the fact that the MosquiTRAP is similar to an ovitrap as it provides water to mosquitoes and is a synthetic oviposition attractant (Eiras and Santanna 2001, Eiras et al. 2001). Thus, the females are attracted to the traps to lay their eggs. Such characteristics are demonstrated in this study. Collection with manual aspirators not only captures gravid females but also resting and feeding females. The hypothesis of Dibo el al. (2005) that females enter the residences to feed and to rest and leave the houses to lay their eggs seems to be confirmed. Even when MosquiTRAPs were available indoors, the great majority of females were captured outdoors.

The sensitivity of outdoor MosquiTRAPs was similar to oviposition traps and are considered very sensitive for vector detection (Braga et al. 2000). This trend indicates their potential in monitoring the *Ae. aegypti* population. Although it is an important observation, new studies about these traps must be performed, as the comparisons were made between data from one oviposition trap against data from four MosquiTRAPs.

Our studies raise questions about the effectiveness and the utility of each available method of entomological surveillance. Focks (2003) suggested that these methods must provide information about the transmission risk as a function of the quantity of mosquitoes and on the importance of several breeding containers in the environment. The most commonly used methods for *Ae. aegypti* are based on larval samples, do not provide information about the true adult population, and are limited to the evaluation of dengue transmission risk only (Focks 2003). The indicators do not take into account that different types of breeding containers can produce different numbers of adult mosquitoes (Chadee 2004) and do not provide information on the area or people, factors strongly associated with dengue transmission (Focks and Chadee 1997).

The main criticism of the use of ovitraps as an entomological surveillance method, according to Focks (2003), is that in spite of their great sensitivity to detect the presence of the vector, they do not allow estimations of the differences in populations in surrounding areas and also do not evaluate the risk of disease occurrence. The main problem in relation to female collection with manual aspirators is related to the great quantity of human and operational resources involved (Focks 2003).

The capacity of the MosquiTRAP, such as the sticky ovitrap (Ritchie et al. 2003), to capture mostly gravid females of *Ae. aegypti* and to allow them to be counted, reinforces the trap's potential to be used in activities of entomological surveillance in dengue control programs. In contrast to larval research methods, the MosquiTRAP can provide information on the number of females per person or area, considered by Rodrigues-Figueroa et al. (1995) as a good indicator of the risk of the occurrence of dengue transmission. Different from techniques of adult collection using manual aspirators, the MosquiTRAPs present easy

Table 1. Mean numbers of *Ae. aegypti* females captured by the MosquiTRAPs and the positivity over eight weeks, according to sites in Mirassol, 2004.

Site	Number of traps	Number of Females			Positivity	
		Mean	Variance	P-value	%	P-value
Indoors	80	4.0	14.9		31.3	
Outdoors	80	20.6	243.3	0.000[a]	80.0	0.000[c]
Indoors						
Bedroom	20	0.8	1.5		6.9	
Living room	20	0.7	1.5	0.8214[b]	7.5	0.2495[d]
Bathroom	20	1.3	8.7		11.9	
Kitchen	20	1.2	2.7		11.9	
Outdoors						
Front yard (F1+F2)	40	9.6	54.9		57.5	
Back yard (F3+F4)	40	11.0	140.5	0.6991[a]	56.9	0.9136[c]

[a]T-Test; [b]F-Test; [c]Proportion test; [d]Chi-square test.

Table 2. *Ae. aegypti* females captured by MosquiTRAPs according to ovarian development, presence or absence of blood, and the condition of ingested blood, in Mirassol, 2004.

Variable	Number of females	%	CI (95%)
Ovarian development			
gravid	426	87.3	84.0 – 90.1
parous	30	6.2	4.2 – 8.7
nulliparous	1	0.2	0.0 – 1.1
phase III (C&M)	10	2.0	4.1 – 3.7
without identification	21	4.3	2.7 – 6.5
Presence and condition of blood			
no blood	423	86.7	83.3 – 89.6
digested blood	32	6.6	4.5 – 9.1
recently ingested blood	16	3.3	1.9 – 5.3
without identification	17	3.5	2.0 – 5.5
Total	488	100.0	

functionality and economic viability and thus can be used on a large scale. Moreover, they are environmentally safe as they do not utilize insecticides or other toxic agents.

The MosquiTRAPs may be distributed in different neighborhoods of a city and checked periodically to verify the presence and quantity of females on the sticky cards. The quantities of captured *Ae. aegypti* females should be related to the population and the areas of these suburbs. The use of electronic charts for immediate registration of the information and geo-referencing of the sites where the traps are installed would contribute to fast decision making (Ai-leen and Song 2000). The installation of the traps at outdoor sites would eliminate or decrease the rejection of the service by the population, frequently associated with the necessity of entering houses.

Further studies on the MosquiTRAPs are necessary before they can be used as an entomological surveillance method and to direct control actions. The main points include the establishment of the relation between the total number of *Ae. aegypti* females in a determined environment and the proportion of females captured by the trap, the determination of the necessary quantity of traps per area, measurement of its sensitivity to identify the presence of the vector, and, if possible, a correlation of the quantity of captured females with the occurrence of dengue cases.

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REFERENCES CITED

Ai-leen, G.T. and R.J. Song. 2000. The use of GIS in ovitrap monitoring for dengue control in Singapore. Dengue Bull. 24: 110-116.

Altman, D.G. 1999. *Practical Statistics for Medical Research*. Boca Raton, Chapman & Hall/CRC.

Barata, E.A.M.F., A.I.P. Costa, F. Chiaravalloti-Neto, C.M. Glasser, J.M.S. Barata, and D. Natal. 2001. Populações de *Aedes aegypti* (L.) em área endêmica de dengue, sudeste do Brasil. Rev. Saúde Públ 35: 237-242.

Braga, I.A., A.C. Gomes, M. Nelson, R.C.G. Mello, D.P. Bergamaschi, and J.M.P. Souza. 2000. Comparação entre pesquisa larvária e armadilha de oviposição, para detecção de *Aedes aegypti*. Rev. Bras. Med. Trop. 33: 347-353.

Breteau, H. 1954. La fiève jaune en Afrique-Occidenatlle Française. Un aspect de la médecine préventive massive. Bull. WHO 11: 453-481.

Chadee, D.D. 2004. Key premises, a guide to *Aedes aegypti* (Diptera: Culicidae) surveillance and control. Bull. Entomol. Res. 94: 201-207.

Christophers, S.R. 1911. The development of the egg follicle in anophelines. Paludism 2: 73-78.

DATASUS. 2005. Departamento de Informação e Informática do Sistema Único de Saúde, Informações Demográficas. Disponível em: <*http://tabnet.datasus.gov.br/cgi/tabcgi*>. Acesso em 15 ago.

Detinova, T.S. 1962. *Age grouping methods in Diptera of medical importance*. Geneva, World Health Organization. 216 pp.

Dibo, M.R., F. Chiaravalloti-Neto, M. Battigaglia, A. Mondini, E.A. Fávaro, A.A.C. Barbosa, and C.M. Glasser. 2005. Identification of the best ovitrap installation sites for gravid *Aedes (Stegomyia) aegypti* in residences in Mirassol, state of Sao Paulo, Brazil. Mem. Inst. Oswaldo Cruz 4: 339-343.

Eiras, A.E. 2002. Armadilha para captura de mosquitos. Patente: Privilégio e Inovação. n. PI0203907-9, "Armadilha para captura de mosquitos".05 de set de 2002 (Depósito).

Eiras, A.E., A.L. Santanna, and K. Stein. 2001. Identification of volatiles from grass infusions that attract gravid *Aedes aegypti* mosquitoes. In: *3rd International Congress of Vector Ecology*, Barcelona, Spain. Abstract book p. 64.

Eiras A.E. and A.L. Santanna. 2001. Atraentes de Oviposição de Mosquitos. Patente; Privilégio e Inovação. n. PI0106701-0. "Atraentes de Oviposição de Mosquitos". 20 de dez. de 2001.

Fay, R.W. and D.A. Eliason. 1966. A preferred oviposition site as a surveillance method for *Aedes aegypti*. Mosq. News 26: 531-535.

Focks, D.A. 2003. *A review of entomological sampling methods and indicators for dengue vectors*. Gainsville, World Health Organization. 40 pp.

Focks, D.A. and D.D. Chadee. 1997. Pupal survey: an epidemiologically significant surveillance method for *Aedes aegypti*: an example using data from Trinidad. Am. J. Trop. Med. Hyg. 56: 159-167.

Forattini, O.P. 2002. *Culicidologia Médica*, volume 2. São Paulo, Editora da Universidade de São Paulo. 864 pp.

Gomes, A.C. 1998. Medidas dos Níveis de Infestação Urbana para *Aedes* (Stegomyia) *aegypti* e *Aedes* (Stegomyia) *albopictus* em Programa de Vigilância Entomológica. IESUS 7: 49-57.

Kay, B.H., K.A. Sutton, and B.M. Russell. 2000. A sticky entry-exit trap for sampling mosquitoes in subterranean habitats. J. Am. Mosq. Contr. Assoc.16: 262-265.

McCall, P.J., G. Harding, J. Roberts, and B. Auty. 1996. Attraction and trapping of *Aedes aegypti* (Diptera: Culicidae) with host odors in the laboratory. J. Med. Entomol. 33: 177-179.

Mer, G.G. 1936. Experimental study on the development of the ovary in *Anopheles elutus* Edw. (Diptera: Culicidae). Bull. Entomol. Res. 27: 351-359.

Montgomery, B.L., S.A. Ritchie, A.J. Hart, S.A. Long, and I.A. Walsh. 2004. Subsoil drain sumps are a key container for *Aedes aegypti* in Cairns, Australia. J. Am. Mosq. Contr. Assoc. 20: 365-369.

Muir, L.E. and B.H. Kay 1998. *Aedes aegypti* survival and dispersal estimated by mark-release-recapture in Northern Australia. Am. J. Trop. Med. Hyg. 58: 277-282.

Nasci, R.S. 1981. A light weight battery-powered aspirator for collecting mosquitoes in the field. Mosq. News 41: 808-811.

Ordóñez-Gonzalez, J.G., R. Mercado-Hernandez, A.E. Flores-Suarez, and I. Fernández-Salas. 2001. The use of sticky ovitraps to estimate dispersal of *Aedes aegypti* in Northeastern Mexico. J. Am. Mosq. Contr. Assoc. 17: 93-97.

Rawlins, S.C., R. Martinez, S. Wiltshire, and G. Legall. 1998. A comparison of surveillance systems for the dengue vector *Aedes aegypti* in Port of Spain, Trinidad. J. Am. Mosq. Contr. Assoc. 14: 131-136.

Ritchie, S.A., S. Long, G. Smith, A. Pyke, and T.B. Knox. 2004. Entomological investigations in a focus of dengue transmission in Cairns, Queesnland, Autralia, by using the sticky ovitraps. J. Med Entomol. 41: 1-4.

Ritchie, S.A., S. Long, A. Hart, C.E. Webb, and R.C. Russell. 2003. An adulticidal sticky ovitrap for sampling container-breeding mosquitoes. J. Am. Mosq. Contr. Assoc. 19: 235-242.

Rodriguez-Figueroa, L., J.G. Rigau-Perez, E.L. Suarez, and P. Reiter. 1995. Risk factors for dengue infection during an outbreak in Yanes, Puerto Rico in 1991. Am. J. Trop. Med. Hyg. 52: 496-502.

Rosner, B. 2000. *Fundamentals of Biostatistics*. Duxbury Press, Pacific Grove.

Russell, R.C., C.E. Webb, C.R. Williams, and S.A. Ritchie. 2005. Mark-release-recapture study to measure dispersal of the mosquito *Aedes aegypti* in Cairns, Queensland, Australia. Med. Vet. Entomol. 19: 1-7.

Russell, R.C. and S.A. Ritchie. 2004. Surveillance and behavioral investigations of *Aedes aegypti* and *Aedes* *polynesisensis* in Moorea, French Polynesia, using a sticky ovitrap. J. Am. Mosq. Contr. Assoc. 20: 370-375.

SUCEN 2002. Superintendência de Controle de Endemias, Normas e recomendações técnicas para vigilância e controle do *Aedes aegypti* no estado de São Paulo. São Paulo, Secretaria de Saúde do Estado de São Paulo. 70 pp.