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SCIENTIFIC NOTE

USE OF A FUNNEL TRAP FOR COLLECTING IMMATURE *AEDES AEGYPTI* AND COPEPODS FROM DEEP WELLS IN YOGYAKARTA, INDONESIA

YOYO R. GIONAR, SAPTORO RUSMIARTO, DWIKO SUSAPTO AND MICHAEL J. BANGS

Department of Entomology, U.S. Naval Medical Research Unit No. 2, Box 3, APO AP 96520-8132

ABSTRACT. During the course of a "dry" season dengue vector survey, indoor and outdoor household wells were sampled for the possible presence of immature mosquitoes and copepods. With a simple floating funnel trap, *Aedes aegypti* immature stages were captured in over 33% of the sampled wells (n = 93) during a 24-h trapping period per well. Average number of larvae (all instars) per positive well was 8.8 (range 1–63). Positive wells varied in depth from 2.7 to 14.7 m (8.8–48.2 ft), with a mean of 7.9 ±SE 0.5 m in well rim to water surface. Only 4 wells (4.3%) contained *Culex quinquefasciatus* larvae. Only 1 of 31 infested wells contained both species. *Aedes albopictus* was not detected in any of the wells. Cyclopoid copepods were captured in 15% of the wells. No significant difference was found between positive and negative wells with regard to the physical characteristics (inside diameter, distance to water level) or the depth and volume of water held at the time of sampling. A significant association was found between wells positive for larvae and numbers of other positive containers in the vicinity of the wells. In general, wells containing copepods had fewer larvae present in the trap, possibly indicating some level of natural population regulation of *Ae. aegypti* occurring in the well; however, this association was not significant. Preliminary results indicate that wells in Yogyakarta serve as important permanent habitats for *Ae. aegypti*, especially during the dry season.

KEY WORDS *Aedes aegypti*, Copepoda, funnel trap, wells, mosquito surveillance, Indonesia

The transmission of dengue viruses represents one of the greatest vectorborne disease threats and control challenges faced by humans (Gubler 1997). In Indonesia alone, dengue fever and dengue hemorrhagic fever accounts for untold numbers of severe illnesses and deaths each year (Sumarmo 1987). The epidemiology of dengue transmitted by *Aedes aegypti* (L.) is complex and much remains unclear about the natural history of the disease and the vector (Gubler 1988, Halstead 1997). Sampling of immature stages (larvae and pupae) serves as the primary method of surveillance for estimating relative population size and degree of *Aedes* infestation in a community (Chan 1985). Such information has been used to develop models for predicting the likelihood for epidemic transmission in communities and for timing intervention methods to prevent outbreaks. Although *Ae. aegypti* has been recorded from a wide variety of habitats, including ground pools and natural containers, it is most commonly found in large and small artificial containers holding fresh water in and around human habitation (Christophers 1960).

As part of a prospective seroepidemiological dengue study in an urban area of Indonesia, periodic vector surveys from sentinel houses have been conducted during dry and wet seasons. Yogyakarta is a city area of around 1 million people in south-central Java, Indonesia. The study area is in the central part of the city, in the subdistrict Gondokusuman, a well-established residential area of mostly middle to low income families. Wells used for general domestic needs are ubiquitous in the area of study, nearly every dwelling having its own. Two previous surveys that concentrated on all indoor and outside peridomestic containers had excluded sampling the wells because no sampling device was available. This problem was corrected on the 3rd survey in September 1998 by the construction and use of a simple funnel trap. The primary purpose of sampling wells was to establish the presence or absence of *Ae. aegypti* in wells relative to other container types in the community.

Yogyakarta well water levels are commonly many meters below ground level, making efficient sampling difficult. Consequently, a funnel trap design similar to that of Kay et al. (1992) was used to sample wells in the Gondokusuman study area. A floating trap is considered an efficient and sensitive surveillance tool for moderate to large sized artificial containers (Harrison et al. 1982), including wells (Kay et al. 1992). The trap operates as a passive collection device, floating at the water's surface, while active aquatic organisms, randomly moving inside the submerged inverted funnel, are guided into the reservoir. Our trap design was comprised of 4 parts: a 20-cm-diameter white plastic funnel, a 1-liter polystyrene bottle (reservoir) with screw cap, a 420-g metal ring (sinker) at the point of attachment of the reservoir to the neck of the funnel, and a plastic cord attached to the funnel apparatus to lower the trap into and remove it from the well. The length of the cord varied depending on the depth of the well. Construct-

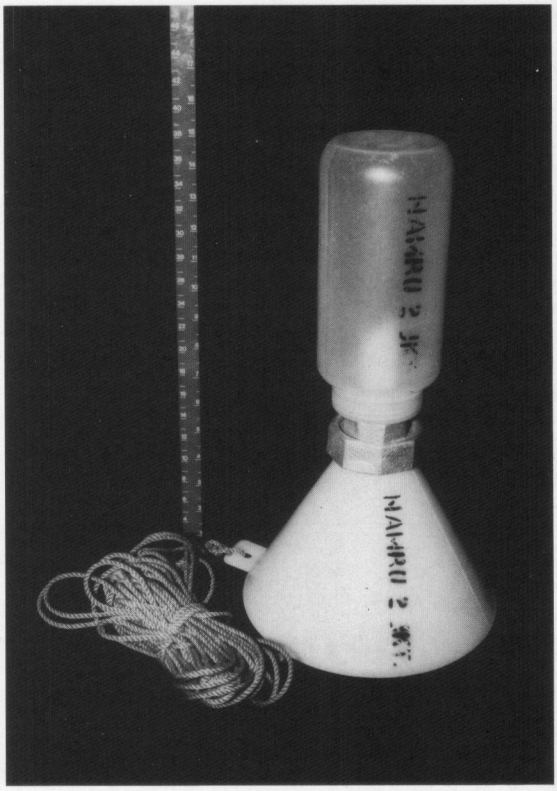

Fig. 1. The funnel trap ready for use with reservoir, funnel, sinker, and plastic cord; left measure in both inches and centimeters.

ed, the entire funnel trap is 37 cm in height and 650 g (Fig. 1).

One funnel trap per well was set in the following manner. The reservoir was filled to half capacity with clean fresh water and attached to the funnel. The trap was then lowered by the cord, reservoir up, into the well. On contact with the water surface, the weight of the sinker naturally inverted the trap into the collecting position. The air pocket in the reservoir allows the funnel to float freely. Each well was sampled for approximately 24 h, beginning at 1000–1200 h, and retrieved the following day. For retrieval, the trap was inverted with the cord and removed from the well. Water in the reservoir was filtered through a 100-mesh screen (W. S. Tyler, Inc., Mentor, OH), and all mosquito larvae and copepods were pipetted into separate plastic bags. Other organisms (fish, other arthropods) were recorded and released back into the wells. Mosquito larvae were enumerated by stage and identified to species by standard keys with the use of dissecting and compound light microscopes. All larvae and copepods were preserved in 80% ethyl alcohol.

Physical characteristics of each well, including inside diameter, depth to water level, and depth of water were measured and compared. A cotton cord with sinker was used for depth measurements. Water from each well was tested for levels (ppt) of salinity with a refractometer (Sper Scientific, Scottsdale, AZ). Standard larval collections from all other containers in and around each house having a sampled well were conducted at the beginning of the trapping period. A container index (percentage of sampled containers positive for *Aedes* immatures) per dwelling was determined, and a small subsample of late larval instars and all pupae from each positive container were examined for species identification. Standard parametric procedures were used in statistical analysis of data, including the Anderson–Darling normality test for all data, independent *t*-tests, proportion analysis, and chi-square statistics with correction for continuity to measure differences and associations of data sets (Freeman 1987, Law 1991). All levels of significance were set at $P < 0.05$ (df = 1).

Tests for normality found all analyzed data sets with normal distributions and homogeneity of variants. All sampled wells were very similar in construction materials, lined with brick or concrete, with an unlined base. Most of the 93 wells sampled were outside the house (86%), and most (outdoor and indoor) had water drawn by electric pump. A smaller percentage (13%) of wells remained completely open and small buckets were used to obtain water. Wells equipped with electric pumps had access openings generally covered with sheet metal or wood planks; however, in many cases, the enclosure was incomplete, allowing access for adult mosquitoes to enter and escape at will. The brick- or cement-lined construction of wells clearly provided a suitable substrate for *Aedes* oviposition. All well water was fresh, with zero to minor traces of salt (<1 ppt). A summary breakdown of well physical characteristics is presented in Table 1.

Two species of immature Culicidae and several cyclopoid copepod species were captured in the wells. Thirty-one (33%) wells contained *Ae. aegypti* larvae, whereas only 4 (4.3%) contained *Culex quinquefasciatus* Say. Only 1 well contained both mosquito species. Average number of *Ae. aegypti* larvae (all instars) per positive well trap was 8.8 (range 1–63) per 24-h period. Positive wells varied in depth from well rim to water surface from 2.7 to 14.7 m ($\bar{x}$ = 7.9 ± 0.5 SE) (8.8–48.2 ft, $\bar{x}$ = 26 ± 8.3 ft). No significant difference was found between mosquito-positive and -negative wells concerning inside diameter, depth to water level, depth of water, or volume of water (*t*-test, $P > 0.05$). *Aedes aegypti* were captured in 8 of 13 (61%) indoor wells and 23 of 80 (28%) outdoor wells. Proportion analysis indicated a significant difference between the 2 locations ($Z = 2.326$, $P = 0.02$), indoor wells being preferred over outdoor. *Aedes albopictus* were not detected in wells by the funnel trap method, and <2% of all positive indoor/outdoor receptacles contained immatures of this species. During a previous dry season collec-

Table 1. Summary of sampled wells for physical characteristics, presence of mosquito larvae and copepods, and container index for houses surveyed (excluding wells).

Parameter	Wells[1]		
	(+) *Aedes*	(+) *Culex*	Negative
Number	31	4	59
Well physical characteristics			
Average inside diameter (m) ± SE	0.81 ± 0.02	0.94 ± 0.10	0.85 ± 0.02
Average depth of water (m) ± SE	1.22 ± 0.06	1.46 ± 0.16	1.26 ± 0.05
Average depth to water surface (m) ± SE	7.89 ± 0.46	5.35 ± 0.71	7.60 ± 0.30
Average volume of water (liters) ± SE	660.38 ± 297.67	1031.20 ± 525.57	716.40 ± 277.98
Copepod presence in well			
Positive copepod	8 wells (25.8%)		5 wells (8.5%)
Negative copepod	23 wells (74.2%)		54 wells (91.5%)
Other containers surveyed			
Numbers of containers inspected	304 containers		668 containers
Numbers of positive Aedes containers	40 containers		53 containers
Container index	13.2%		7.9%

[1] Sample size = 93.

tion, *Ae. albopictus* had been found infrequently from surface-level containers (Bangs et al., unpublished data, 1997).

Copepods were captured in 14 (15%) of the wells surveyed (Table 1). Larval mosquito densities for *Ae. aegypti* captured in the funnel trap were not significantly different (*t*-test, $P > 0.05$) whether the well contained copepods (4.9 larvae/trap) or were negative for copepods (10.2 larvae/trap). Likewise, no significant association was found for the presence of both larvae and copepods in the same well ($\chi^2_{corrected} = 3.64$, df = 1, $P = 0.057$). However, a significant association was found between positive wells and numbers of other positive containers present in and around the same house ($Z = 2.567$, $P = 0.01$). The overall container index (excluding wells) from houses with positive wells was 13.2%, whereas houses having negative wells had an index of 7.9% (Table 1). More importantly, positive wells represented 43.6% of all positive containers from houses possessing positive wells.

Immature *Puntius javanica* (Cyprininae), a small local food fish, were incidentally captured by the funnel trap in 4 (4.3%) wells. Wells containing fish and 1 well containing temephos (Abate® 1% SG) larvicide treatment were negative for mosquito larvae. Other aquatic arthropods (e.g., Ephemeroptera, Hemiptera, Coleoptera spp.) were collected from a small percentage of wells but were not identified to genera/species.

Wells have been described as important breeding sites for *Anopheles stephensi* Liston in India (Batra and Reuben 1979) and *Cx. quinquefasciatus* in Brazil (Kay et al. 1992). *Aedes aegypti* has been reported from wells in India (Panicker et al. 1982), Lagos (Dalziel 1920), French Polynesia (Lardeux 1992), Lao PDR (Jennings et al. 1995), Australia (Russell et al. 1996), Vietnam (Nam et al. 1998), and isolated breeding places in the Sahara (Christophers 1960). However, in general, wells have not been reported as common *Ae. aegypti* larval habitats.

Ground wells are common in Yogyakarta and have been an obstacle in determining an accurate assessment of all potential breeding sites. In many cases, the well water level is found at considerable depth, completely out of range for normal hand-held collection devices. Previous surveys had avoided sampling wells because of the difficulty in doing so and the perceived notion that wells contributed little or nothing to the local vector populations. A variety of trapping methods for immature stages of mosquitoes found in artificial and natural containers have been devised (World Health Organization 1975). Most methods used for sampling wells have had considerable limitations, most notably the disturbance of the water surface during the sampling process, making capture of alarmed organisms more difficult. Devices have included an assortment of dippers, nets, cylinder devices, and buckets, typically used in a series of repeated samples from each well (Service 1993). In contrast, funnel traps have proven effective in overcoming a number of these limitations (Kay et al. 1992).

The 33% positive wells we report here is probably a conservative estimate based on sampling strategy and trapping sensitivity. However, we surmise that wells may likely serve as important permanent larval habitats, especially during times of low precipitation and reduced alternative habitats. This limited survey would also indicate *Ae. aegypti* may prefer to oviposit in wells located indoors versus outdoor locations, possibly a direct consequence of this species' predilection to feed and rest indoors. The funnel diameter in compar-

ison with the average well covered approximately 5.7% of the well water surface area. Because each well was sampled only once in this study, the funnel trap served only as a detection device for *Aedes* larvae. No attempt was made to sample the same well repeatedly to gain more sensitivity or estimate larval population size. Kay et al. (1992) concluded that, on the basis of 1 trap night per well, interpolation of catch size to the size of the natural population would be imprecise. In particular, wells with very low densities of larvae would be especially vulnerable to reduced sensitivity in simple detection of an infestation. This problem would be even greater when attempting to detect or estimate numbers of pupae, which are normally found at much lower densities compared with other immature stages. They estimated that for wells containing very low population numbers (e.g., 10 immatures or less), the success of detection (sensitivity) during a single trapping period would be less than 50%. With consecutive multiple sampling periods (up to 5) from each well, the sensitivity would eventually reach nearly 100%. Unfortunately, the presence of larvae from a percentage of low population density wells would be missed because repeated sampling of negative wells would generally be impractical during large community vector surveillance activities.

The detection of copepods was also noteworthy. We found no statistical association for the presence of both mosquito larvae and copepods in the same wells and no clear indication of a difference in mosquito larval density in relation to presence or absence of copepods. Possible explanations await further surveys, species identification, and analysis to define the natural occurrence of both. Reasons may involve the ecological characteristics of the individual well or the degree of predation (if any) by resident copepods. Because wells rarely run completely dry in this area, the use of entomophagous copepods (e.g., *Mesocyclops* sp.) or fish may be important *Aedes* control options for these habitats in Yogyakarta (Kay 1996, Nam et al. 1998).

This initial study indicates the importance of wells providing acceptable larval habitats for *Ae. aegypti.* The utility of a funnel trap and standardized sampling of wells in Yogyakarta may enable local health authorities to selectively and economically treat infested wells. Further studies are planned to determine if seasonal differences in percentage of positive wells between dry and wet seasons exist and to use larval mark–release studies to assess the level of sensitivity of the funnel traps in the detection of various densities of larvae in wells.

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