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A STICKY ENTRY–EXIT TRAP FOR SAMPLING MOSQUITOES IN SUBTERRANEAN HABITATS

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ABSTRACT. This paper addresses the problems of sampling adult *Aedes aegypti* and other mosquitoes which utilize subterranean habitats such as wells and service manholes. The sticky pipe trap is a simple device with an adhesive paper insert that can be clipped to the undersides of service manholes to record the entry and exit of adult mosquitoes through the keyhole openings. This trap was 1st used successfully in Townsville, Charters Towers, and Saunders Beach in north Queensland, Australia, in dry seasons of 1996–97 to record usage by 5 species, mainly the *Aedes tremulus* group and *Ae. aegypti,* which together comprised 91% of the 1,140 adults collected. Both males and predominantly nulliparous females were recorded exiting manholes, whereas all fresh-water-breeding species entering manholes were gravid, presumably seeking oviposition sites.

KEY WORDS *Aedes aegypti, Aedes tremulus,* subterranean, manholes, sticky trap, Australia

INTRODUCTION

Townsville, Charters Towers, and Saunders Beach are tropical localities in north Queensland, Australia, where winter conditions become cool and dry. During 1992–93, 1,063 cases of dengue were notified for Townsville and Charters Towers (McBride et al. 1997) and Ross River virus infection is common annually throughout the region. Until recently, surveys for container-breeding mosquitoes have concentrated almost entirely on surface habitats and overlooked subterranean ones that have been shown to be highly productive (Russell et al. 1996, 1997).

Although larval sampling is usually an effective way to establish the importance of most water containers to mosquito breeding, a number of problems are associated with larval sampling of subterranean containers. First, many subterranean habitats have restrictive openings making it impossible or impractical to sample larvae. Second, large amounts of suspended solids in the waters of septic tanks, pit latrines, and polluted wells quickly block sweep nets and may interfere with the operation of funnel traps (Russell and Kay, 1999) and floating emergence traps (Girikumar and Venkateswara Rao 1984). Third, larval sampling may fail to detect mosquito species that utilize subterranean habitats for reasons other than breeding. For example, Hayes (1973) observed large numbers of adult *Culex pipiens* L. overwintering in subterranean habitats. Finally, the differential efficacy of sampling some genera, for example, top-dwelling *Anopheles* and *Culex* compared to side-browsing *Aedes* may lead to bias because of their stratification in large water containers.

Exit and entry traps provide a simple way of sampling subterranean containers that are difficult or impossible to sample using dipping or netting techniques. Unlike many other habitats (e.g., ponds and salt marshes), the openings of subterranean habitats can easily be covered by a trapping device. Such entry and exit traps have been used effectively for collecting *Culex quinquefasciatus* Say and other mosquitoes in septic tanks in central Nigeria (Lyimo and Irving-Bell 1988) and in 1st detecting *Aedes aegypti* (L.) emerging from numerous septic tanks in India (Babu et al. 1983).

A variety of emergence (exit) trap designs have been described; most are similar to the pyramidal traps of Aubin et al. (1973), Lesage and Harrison (1979), and Pajor (1987). However, these configurations are relatively complex and some investigators have employed custom-made adhesives to capture emerging mosquitoes. Slaff et al. (1984) demonstrated that the use of a sticky trap was 2.5 to 3 times more effective than baffled pyramidal traps. Despite the modifications of Slaff et al. (1984) of the trapping mechanism, the conspicuous pyramidal frame of the exit trap still remained. Although pyramidal traps are useful for trapping on private property, they are not suitable for footpaths and public walkways because of public inconvenience and danger. These traps are also are prone to vandalism.

The sticky pipe trap is a simple device designed to capture mosquitoes entering or emerging from manholes. The trap comprises a disposable adhesive trapping surface contained inside a piece of pipe that can be easily fixed beneath the surface of a manhole, therefore eliminating any risk or inconvenience to pedestrians. Our aim was to measure the utility of this device through release of known numbers of adult *Ae. aegypti* and to record ingress and egress of different mosquito species in the field. These were age graded, which then facilitated preliminary understanding of their behavior during the gonotrophic cycle.

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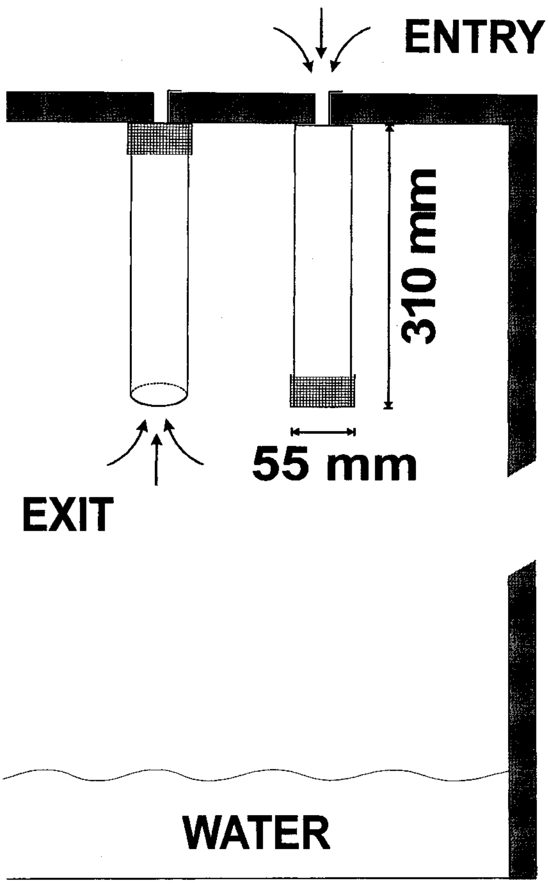

Fig. 1. Diagram of a pipe trap used for monitoring exit and/or entry of adult mosquitoes through keyholes in manhole covers. The adhesive card (not shown) is inserted lengthwise inside the pipe with the adhesive facing inwards.

Table 1. The cumulative percentage of reared then released *Aedes aegypti* entering or exiting a manhole, captured by the sticky pipe trap every 12 h for 48 h.

Mode of action	Total number released	Cumulative percent recapture at time (h)			
		12	24	36	48
Entry					
Male	34	74	79	88	91
Female	36	64	78	86	86
Total	70	69	77	86	87
Exit					
Male	27	28	48	67	67
Female	34	27	35	61	61
Total	61	28	41	63	63

MATERIALS AND METHODS

Design: The trap (Fig. 1) comprises a 310-mm section of 55-mm-outside-diameter polyvinyl chloride (PVC) pipe with a spring steel clip punch-riveted to the top. Red-colored sticky cardboard, with a 310 × 155-mm area of proprietary adhesive on 1 side, was inserted lengthwise to line the inside of the pipe, with the adhesive facing inwards. Although the trap can be used with both ends open, to simultaneously measure entries and exits through the holes in service pits and manholes, the bottom and top of the trap, respectively, can be covered with gauze to selectively collect mosquitoes entering and exiting. The trap is clipped to the underside of the steel plates, thus presenting no hazard to the public who may be walking along sidewalks or across roadways. The sticky cardboard blanks were supplied by Austech International Pty. Ltd., 235A Scarborough Beach Road, Mt. Hawthorne, Western Australia 6016, Australia.

Sticky pipe trap entry trial: Entry and exit trials were done in manholes that were free of mosquitoes. After attaching an entry sticky pipe trap to 1 of the keyholes and blocking the remaining keyholes with tape, the perimeter of the manhole was surrounded by a frame that was covered with mosquito netting. Seventy adult *Ae. aegypti* reared from wild-caught immatures were released into the net enclosure. The sticky insert of the pipe trap was replaced every 12 h for 48 h and cumulative totals of male and female mosquitoes were recorded. The basal end of the trap was covered with gauze.

Sticky pipe trap exit trial: Exit trapping was evaluated in the same manner as the entry trial described above. However, instead of *Ae. aegypti* being released into a net enclosure above the manhole, they were released directly into the manhole. The pipe trap was also fitted with netting on the end nearest the keyhole.

Field collections: In Townsville, Charters Towers, and Saunders Beach, north Queensland, for 1 wk during the dry seasons of July 1996 and September–October 1997, these traps were clipped under the keyholes of 4 steel manhole covers of various shapes and dimensions up to 3,228 × 1,235 × 1,550 mm. Other remaining keyholes were taped off. Precast manholes, fulfilling a variety of service functions, have minimum water capacities of from 75 to 1,700 liters, suitable for mosquito breeding. The physiologic state of females was determined by dissection to ascertain whether they were nulliparous, bloodfed, gravid, or parous empty (Detinova 1962).

RESULTS

Sticky pipe trap trials

After 48 h, the total percentage of mosquitoes (Table 1) caught in the entry trap (87%) was higher than in the exit trap (63%) (χ^2 = 9.73, df = 1, P = 0.002). No significant difference was found between numbers of male and female mosquitoes caught in either the exit (χ^2 = 0.14, df = 1, P > 0.05) or entry traps (χ^2 = 0.07, df = 1, P > 0.05).

Table 2. Species composition of adult mosquitoes and reproductive status of female mosquitoes entering and exiting through keyholes in service manholes in north Queensland.

Species	Trap type and number	Number of males	Number of females	Age grade (%) Nulliparous	Blood-fed	Gravid	Parous empty
Aedes aegypti	Entry	0	29	10.3	0	86.2	3.4
	Exit	124	113	96.5	0	3.5	0
Ae. tremulus	Entry	0	120	4.2	0	93.3	2.5
	Exit	230	426	98.8	0	1.2	0
Ae. notoscriptus	Entry	0	21	0	0	100	0
	Exit	0	1	100	0	0	0
Ae. vigilax	Entry	0	5	100	0	0	0
	Exit	0	0	0	0	0	0
Culex quinquefasciatus	Entry	0	2	0	0	100	0
	Exit	157	52	100	0	0	0
Total	56	371	769	703	0	62	4

Field collections

Five species of adult mosquitoes were collected either exiting and entering through keyholes in service manholes, giving an average of 20.4 mosquitoes/trap night (Table 2). *Aedes tremulus* (Theobald) and *Ae. aegypti* comprised 68% and 23%, respectively, of the 1,140 adults collected. Four species, *Ae. aegypti, Ae. tremulus, Aedes notoscriptus* Skuse, and *Cx. quinquefasciatus* are known container breeders but *Aedes vigilax* (Skuse) breeds in intertidal salt marsh. All of the 177 mosquitoes entering the manholes were female and 93% of the container breeders were gravid. Of the 963 mosquitoes caught exiting the manholes, 62% were female and 98.5% of these were nulliparous.

DISCUSSION

A range of emergence traps have been fitted over cesspits, septic tanks, and pit latrines (see Service 1993) but none of these designs was suitable for manhole covers because of the obstruction caused. The sticky pipe trap can be conveniently clipped to the underside of such covers and will measure ingress and egress of adult mosquitoes, depending on which end of the PVC pipe is covered with gauze. It is important that normal mosquito gauze is used so that the cues used for orientation through the keyholes (i.e., humidity, heat and light) are not blocked off.

The 310 × 55-mm adhesive cards that are inserted lengthwise into the PVC pipe are a lightweight version of sticky lures currently being field-tested (with olfactant added) for *Ae. aegypti* surveillance (Muir and Kay 1994) and they can be easily compressed to fit within the 55-mm-diameter piping. Cost of a total unit is <U.S. $1. Preliminary evaluations (Table 1) indicated that the trap was effective and that the adhesive was able to immobilize both male and female mosquitoes on initial contact. In the trials of reared mosquitoes, more *Ae. aegypti* were recaptured entering the manhole than vice versa, that is, 87% vs. 63%. This difference should not be interpreted with respect to efficacy of the traps themselves but rather as due to differences in mosquito behavior and survival inside and outside the manholes. Mosquitoes have less inducement to leave a warm, humid manhole than vice versa. Because of the size of such manholes, it was not possible to account for all individuals. However, this could be done easily for the entry trials.

In field trials, the majority of mosquitoes entering the manholes were gravid females. Low numbers of nulliparous and parous empty females and the total absence of bloodfed female mosquitoes suggest that the primary reason for entry was oviposition. In contrast, both males and females exited the manholes but 98.5% of females were nulliparous, departing to seek their 1st blood meal. The absence of any bloodfed mosquitoes in our collections indicates that host-seeking and subsequent resting occurs at the surface. The traps not only proved useful in defining cryptic subterranean sites but also in understanding the behavior of such mosquitoes.

Of the species collected in the field, *Ae. aegypti, Ae. tremulus, Ae. notoscriptus,* and *Cx. quinquefasciatus* are known to breed in artificial containers, whereas a few *Ae. vigilax,* an intertidal-breeding species, utilized this habitat as a resting site. *Aedes aegypti* is well recognized as a vector of dengue viruses and is capable of transmitting Ross River virus (Kay et al. 1979), whereas *Ae. notoscriptus* has been linked with transmission of Ross River and Barmah Forest viruses and dog heartworm, *Dirofilaria immitis* Leidy (Russell and Geary 1992; Watson and Kay 1998, 1999). *Culex quinquefasciatus* is generally regarded as a pest species although it has been associated with some arbovirus transmission in Australia (Russell 1995). Nothing is known about the vector competence of *Ae. tremu-*

lus, although this species group has been associated with isolates of Kunjin and Ross River viruses (Mackenzie et al. 1994).

In view of the 1999 West Nile virus outbreak in New York (Centers for Disease Control 2000), it has been pointed out to us that this trap should be useful for collecting *Culex* mosquitoes from the city's storm and sanitary sewer system. Polymerase chain reaction techniques for virus detection from mosquitoes stuck to the adhesive inserts will still be applicable, as this has already been done for dengue (K. Porter, M. Bangs, NAMRU2, Jakarta, and B. H. Kay, unpublished data).

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