The Use of Ovitraps Baited with Hay Infusion as a Surveillance Tool for *Aedes aegypti* Mosquitoes in Cambodia

by

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Abstract

This study was conducted to test (a) if a modified version of the CDC-enhanced ovitrap would attract more gravid female *Aedes aegypti* mosquitoes than standard ovitraps for more frequent monitoring of oviposition activity, and (b) the placement of ovitraps indoors or outdoors affected their performance. Paired ovitraps were placed in 25 strategically selected houses in Toul Kouk, a village on the outskirts of Phnom Penh, Cambodia's capital city. Each pair consisted of one ovitrap with 10% hay infusion and the other with plain tap water, one pair placed inside each house and the other outside the same house. Collections were made every other day for four weeks. The number of positive ovitraps was recorded and egg counts made.

Thirteen collections made over a 4-week period yielded a total of 7758 eggs, of which 5396 were collected in ovitraps with hay infusion. Ovitraps with hay infusion had a higher positivity (weekly range 15.56 – 54.55%) than ovitraps with plain water (weekly range 6.67 – 34.88%) ($t = 4.92$; df 12; $p < 0.01$) and the mean number of eggs collected was significantly more in the enhanced ovitraps (415.07) than in ovitraps with plain water (181.69) ($t = 7.33$; df 12; $p < 0.001$). Indoor and outdoor placement of ovitraps showed no significant differences in positivity or mean number of eggs collected either for infusion-baited traps ($t= 0.25$; df 12; $p > 0.5$ and $t = 0.06$; df 12; $p > 0.5$, respectively) or for plain water traps ($t= 1.97$; df 12; $0.05 < p < 0.1$ and $t = 1.03$; df 12; $0.2 < p < 0.5$, respectively).

Overall results indicate that, in the study site (a) hay infusion-baited ovitraps are a more sensitive indicator of the presence and numbers of *Aedes aegypti* than those with plain water and are suitable for frequent monitoring of *Aedes aegypti* oviposition activity, and (b) the location of ovitraps, indoors or outdoors, does not influence the performance of the traps.

Keywords: *Aedes aegypti*, enhanced ovitraps, surveillance, Cambodia.

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Introduction

Most dengue control programmes rely on the use of *Aedes aegypti* larval indices as indicators of *Aedes aegypti* population densities for targeting control operations; however, they do not reflect adult mosquito population which has a great significance in the epidemiology of dengue infection.

A standardized CDC oviposition trap, developed by Fay & Eliason[1], for use in *Aedes aegypti* surveillance, which indirectly determines the presence of adult gravid females, was improved by Reiter et al.[2] by the use of hay infusion rather than plain tap water as the medium. Paired ovitraps, one containing 100% hay infusion and the other 10% dilution of the same infusion, were found to greatly enhance the collection of *Aedes aegypti* eggs. Subsequent field studies in the Americas have also demonstrated the effectiveness of these CDC-enhanced ovitraps as sensitive surveillance tools[3,4]. A field evaluation of ovitraps with hay infusion was undertaken in Cambodia to determine their effectiveness as a surveillance tool.

Materials and methods

A village, consisting of 50 houses, in Toul Kork, close to Phnom Penh, the capital city of Cambodia, was chosen as the study site. Twenty-five houses (every second house) were selected for the study.

Ovitraps (350 ml plastic cups, 91 mm in height and 75mm in diameter, painted black on the outside) were placed in pairs at each of the 25 houses - one pair outside and the other inside each house. Each pair comprised of one ovitrap with the 10% diluted hay infusion and the other with tap water. Ovitraps were lined with strips of rough, absorbent paper (#76 seed germination paper, Extra Heavy Weight, Anchor Paper Co., Minneapolis, Minnesota) cut to a size that completely covered the inside surface of the ovitrap. Traps were prepared with 175 ml hay infusion or water and lined with appropriately labelled papers before transportation to the field. Hay infusion was made by steeping 125g of dried rice grass (*Oryza sativa*) in 15 litres of tap water in a tightly closed plastic garbage container for seven days. A new batch of hay infusion was started seven days in advance of each collection day. A 10% dilution of hay infusion was compared with tap water.

A total of 50 paired ovitraps were set out and exchanged between 0900-1200 hours, the time of lowest oviposition activity[5] on Mondays, Wednesdays and Fridays each week for four weeks. Ovitraps that had been emptied, removed or interfered with in any way were excluded from the final results. On collection, the hay infusion or water was discarded in the field and papers taken back to the laboratory in their respective cups. Papers were then removed from the cups and left to dry with the egg-bearing side face-up. Egg counts were made using a magnifying glass. Test results were statistically analysed by t-tests.

Results

From 13 ovitrap collections over the four-week period, each using approximately 50 infusion-baited ovitraps and 50 plain water ovitraps, a total of 7758 *Aedes aegypti* eggs were collected (Table). The average number of eggs per infusion-baited ovitrap was 12.13 and for plain water ovitrap was 4.76.

Table. *Total Number of Eggs Collected in Oviposition Traps*

Location	Medium		Total
	Hay infusion	Plain water	
Inside house	2659	1292	3951
Outside house	2737	1070	3807
Total	5396	2362	7758

For ovitrap pairs placed inside houses, the difference in the percentage of positive ovitraps with and without hay infusion was significant (paired t test after arcsine transformation gave $t = 3.26$; df 12; $0.005 < p < 0.01$) (Figure 1). Ovitraps with hay infusion inside houses yielded more eggs per trap than those with plain water (Figure 2). This difference was also significant ($t = 5.86$; df 12; $p < 0.001$).

Similarly, for ovitrap pairs placed outside houses, the proportion of positive traps with hay infusion was higher than with plain water, and again, there was a significant difference ($t = 4.41$; df 12; $p < 0.05$) (Figure 3). *Aedes aegypti* egg yields were higher in traps with hay infusion than in traps with plain water ($t = 5.70$; df 12; $p < 0.001$) (Figure 4).

A comparison of the sensitivity of ovitraps with hay infusion only, both indoors and outdoors, showed no significant difference in positivity ($t = 0.25$; df 12; $p > 0.5$) or mean number of eggs collected ($t = 0.06$; df 12; $p > 0.5$). The same was observed in ovitraps with plain water for – positivity ($t = 1.97$; df 12; $0.05 < p < 0.1$) and mean numbers of eggs ($t = 1.03$; df 12; $0.2 < p < 0.5$). Thus, the placement of ovitraps, indoors or outdoors, did not significantly affect the results.

Figure 1. ***Percentage positive ovitraps with hay infusion or plain water inside houses***

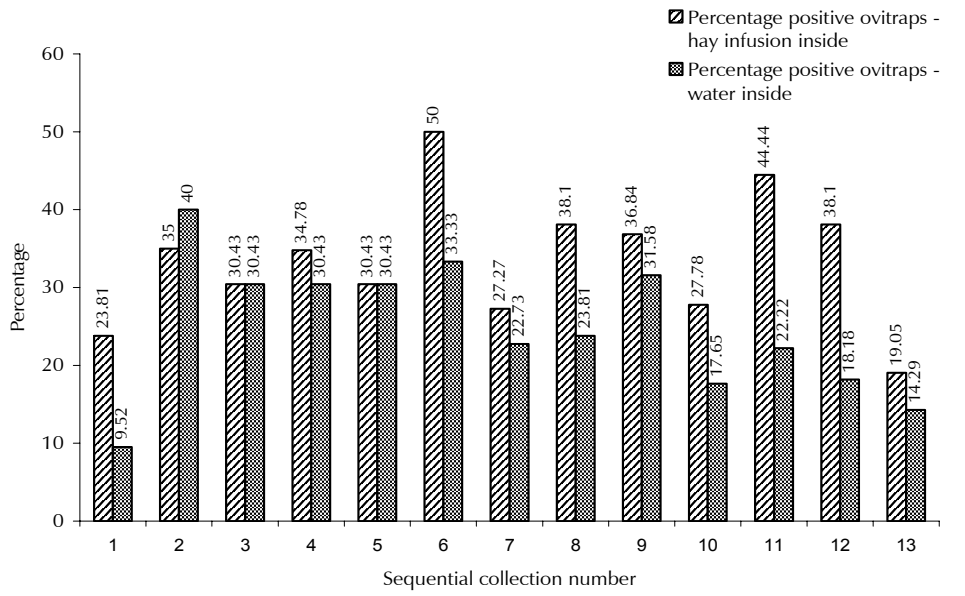


Figure 2. ***Mean numbers of eggs collected in ovitraps with hay infusion or plain water inside houses***

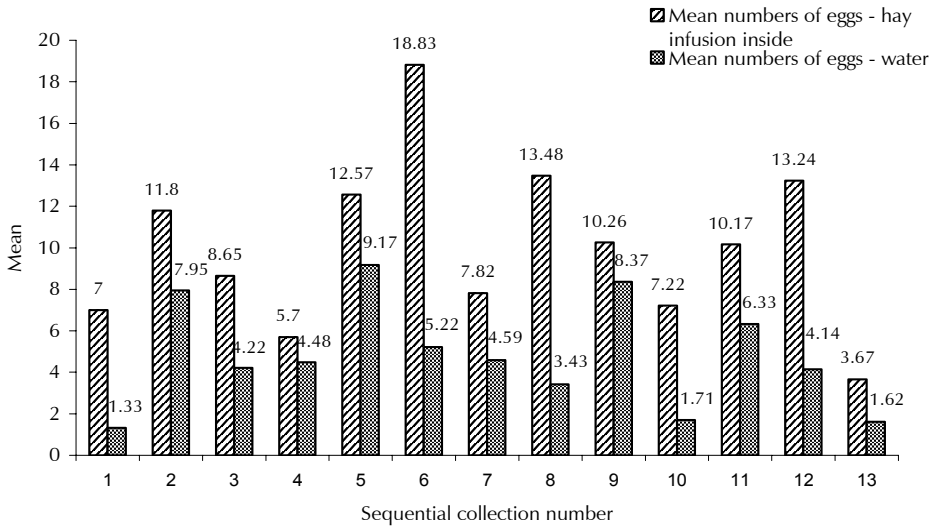


Figure 3. ***Percentage positive ovitraps with hay infusion or plain water outside houses***

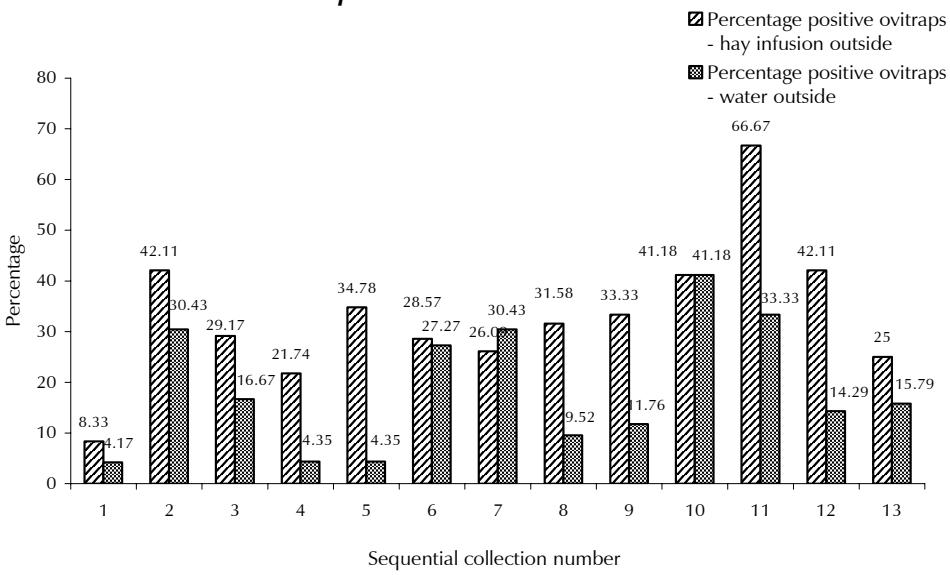
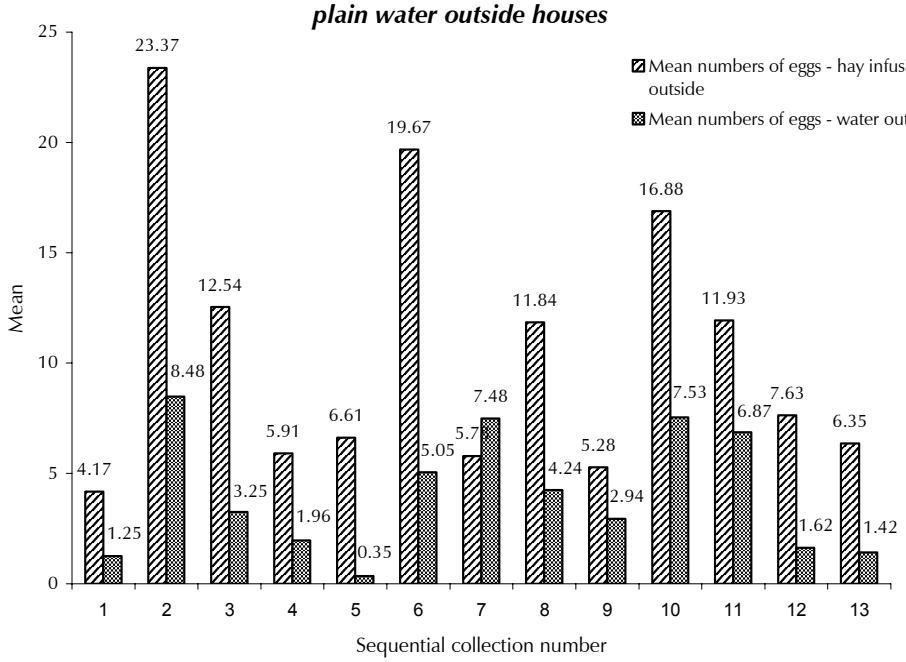


Figure 4. ***Mean numbers of eggs collected in ovitraps with hay infusion or plain water outside houses***



Discussion

It is generally accepted that *Aedes aegypti* mosquitoes prefer to breed in clean water but the results of this study clearly corroborate that of other investigators, who demonstrated that *Aedes aegypti* mosquitoes deposited more eggs more frequently in ovitraps with hay infusion than in those with plain water[2,3]. Reiter et al.[2] investigated the oviposition response of *Aedes aegypti* to various dilutions of hay infusion in groups of ovitraps and found that ovitraps containing a 10% dilution collected the largest number of eggs. In their studies they also investigated several different paired ovitrap combinations of undiluted infusion (100%), 10% infusion and tap water. Their results showed that the 100%/10% pair gave the highest egg yield per collection. In the present study in Cambodia, the 10% infusion/plain water pair was used, which is one of the pairs included in the study of Reiter et al.[2]. The results of the present study are consistent with theirs in that within the 10% infusion/water pair the infusion received twice as many eggs as plain water. Although Reiter and colleagues recommend the use of the 100%/10% infusion paired combination, we suggest that for reasons of practicality, single ovitraps with 10% hay infusion are sufficient to enhance Cambodia's surveillance programme.

For our study we had ready access to a vehicle and the chosen study site was easily accessible from the laboratory so we were

able to transport 50 pre-prepared ovitrap pairs to 25 houses at the field site with two operators servicing them within one hour. Reiter et al.[2] suggest that two operators can service 80 ovitraps in a morning in an urban area without difficulty, but in an urban area in Cambodia where resources are limited and some areas are inaccessible by car, this may not be feasible. Often operators go to the field on motorbikes or on foot.

The operational implication is that the transportation of prepared ovitraps to the field would not be possible and, instead, operators would have to take bottles of infusion with them. If only one ovitrap, which requires hay infusion, is used, then that would lessen the load which operators would have to carry. Only 125g of grass is needed to make 15 litres of concentrated infusion which, when diluted to 10%, can service hundreds of ovitraps as, from our study, we found that eight litres of 10% hay infusion are required to service 50 ovitraps.

Results showed that there was no significant difference in the positivity and mean numbers of eggs between pairs of ovitraps placed indoors and outdoors. In both cases, ovitraps with hay infusion were more productive than those with plain water. For practical purposes it is easier for operators to place ovitraps outside rather than inside houses as this avoids having to deal with the problem of closed houses or householders not wanting field workers entering their homes. In the conduct of our study we were never able to recover all our traps on any of the collection days and this was in part due to householders not being at home to allow us access.

The other reason was that some ovitraps were either disturbed or overturned. Chadee et al.[6] suggested that although ovitraps placed at ground level do collect significant numbers of eggs, they found that in Trinidad, *Aedes aegypti* mosquitoes preferred oviposition sites up to 1.2 metres above the ground. If the same is found to be true in Cambodia, then placement of ovitraps off the ground would reduce the problem of ovitraps being disturbed by domestic animals.

The surveillance of adult female *Aedes aegypti* populations is the most useful indicator for assessing the impact of control programmes on disease transmission. In Cambodia, ovitraps are more practical surveillance tools as they are inexpensive and do not require any special skills on the part of the operator, and as we have shown, they also have the added advantage of being non-intrusive.

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