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EFFECTS OF SUGAR AND ANIMAL BLOOD AVAILABILITY ON ATTRACTION OF AEDES (STEGOMYIA) SPP. TO HUMANS

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Abstract *Aedes (Stegomyia)* female mosquitoes feed on sap of herbaceous indoor plants and blood of small mammals. This study investigates whether the female *Ae. aegypti* and *Ae. albopictus* provided with sugar solution and mouse blood are still attracted to human after feeding. Laboratory caged females of *Aedes* were given continuous access to a 10% sugar solution and a white mouse. These female mosquitoes at different feed groups were exposed to a human palm for a period of 5 minutes. Results showed that females *Ae. aegypti* or *Ae. albopictus* at age above 2 days from emergence were significantly ($P < 0.05$) attracted to human palm despite being provided with continuous supply of sugar solution and mouse blood. The attraction varied 20 to 100% between the feed groups. Female *Ae. aegypti* and *Ae. albopictus* mosquitoes were able to survive on the given food while maintaining its attraction to human. Thus the findings suggest the availability of sugar from plants and blood of small mammals in urban area aids the survival of *Aedes* mosquitoes while waiting opportunity of contact with human for blood meal.
Key Words Mosquito, blood host, nutrient, biting behaviour

INTRODUCTION

Aedes (Stegomyia) aegypti and *Aedes (Stegomyia) albopictus* are the important vectors of dengue fevers in the tropical and subtropical regions around the globe (Chan et al., 1971; Hudson et al., 1988; Jensen et al., 1994; Lee and Yap, 1999). After 24 hour from emergence, females of these species would start to display their blood host seeking behaviour. Adults actively search for blood host during the days with the peak activity at dawn and dusk. The active flying activity can be temporarily suspended by two inhibiting mechanisms. The first inhibition mechanism is activated by the dilation of abdomen while the latter is induced by the development of oocyte (Klowden, 1994).

Whenever the total volume of blood stored in the abdomen exceeds 2.5 µl, the stretch receptors at the anterior of abdomen will be activated, which triggers the temporary inhibition. The volume of blood could be from a single feeding or accumulation of several feedings (Madhukar and Jones, 1974; Bowen, 1991). Therefore, the females will continue to exhibit active flying to seek blood host as long as the total volume blood in the abdomen is below the threshold (Klowden, 1994). However, this inhibition mechanism does not have any effect on the sensitivity of lactic acid receptors (Davis, 1984).

The oocyte-induced inhibition mechanism is initiated when the eggs start to develop inside the female mosquito. The development of eggs usually happens around 25 to 30 hours after the digestion of a full blood meal. It has been identified that ovaries that contain oocyte releases ecdisteroid during 6 to 12 hours after a full blood meal (Bowen, 1991). This initial factor will stimulate the fat body to excrete a compound into the hemolymph, which subsequently reduces the sensitivity of the lactic acid receptors. Decrease of the receptors sensitivity will inhibit the active flying associate with searching for host. Once the eggs are fully developed, the oviposition will be completed within 18 hours. The females would require a maximum of 24 hours to recover from this type of inhibition before starting to show their host seeking behaviour (Klowden, 1994).

This study aims to characterize responses of females *Ae. aegypti* and *Ae. albopictus* fed with sugar and mouse blood, to the presence of human palm in proximity.

MATERIALS AND METHODS

Mosquito Colony

Both *Ae. aegypti* and *Ae. albopictus* mosquitoes used in the study were obtained from an established laboratory colony maintained at 25 ± 2°C, 70 ± 10% and photoperiod of 12:12 hours in the insectarium of Vector Control Research Unit, Universiti Sains Malaysia, Penang, Malaysia, a WHOPES regional centre. These mosquitoes were bred using the rearing technique described by Chong, et al. (1998). In brief, male and female mosquitoes were fed with only sugar solution (10% w/w) and caged together to allow mating. Regularly, female mosquitoes were provided mouse blood for eggs production (Jones and Madhukar, 1976; Gerberg, et al., 1994). However, in this study, the 14 groups of mosquitoes were provided with both sugar solution and mouse blood at different feeding periods as shown in Table 1.

Method

Experiments were conducted using transparent polyethylene tubes (inner diameter = 4.3 cm, length = 24.5 cm) with both ends covered with nets. The tubes were exposure tubes that were provided in the WHO test kit for the assessments of mosquito resistance to insecticides (WHO, 1981; WHO, 1998). Each tube consists of two compartments of equal volume, which were separated by a sliding window. The first compartment was named as holding area and the later as exposure area.

The method used in this study was developed based on a classical study by Khan and Maibach (1970). A healthy Malaysian male (age 25 years, weight 55 kg and height 167 cm), who was not hypersensitive to mosquito biting volunteered for the experiment. The right palm of the volunteer was utilized for all the experiments to avoid the effect of human individual variation. Five minutes before the experiment, a total of 10 female mosquitoes from a selected feeding group (Table 1) were transferred into the holding area using manual aspirator, while the sliding window was kept closed. The experiment was initiated by placing the volunteer's palm in proximity (approx. 1 cm) to the netted end of the exposure area for a total period of 8 minutes. On the 3rd minute, the sliding window was opened to permit the females in the holding area to fly into the exposure area towards the volunteer's palm. Concurrently, the highest number of females, which landed and probed (penetration of proboscis through the nets) on the netted end of the exposure area was recorded within five minutes. At the end of the 8th minute, the volunteer's palm was moved away from the tubes. The above experiment was repeated five times using fresh batch of females and clean tubes were used for each group of females (Table 1).

Data Analysis

The highest numbers of females, which landed and probed on the nets within five minutes (NLP) were proportion against the total of ten females per tube (%NLP). The percentage NLP represents the magnitude of mosquito attraction to a human individual. Thus, individual highly attractive to *Aedes* mosquitoes will exhibit high percentage NLP values. These values were transformed using log (x + 1) for normal distribution prior to analysis with one way ANOVA and Student-Newman-Keuls mean comparisons. The statistical analysis was run using the SPSS computer program (Zar, 1974; Kinnear and Gray, 1997).

RESULTS AND DISCUSSION

The mean percentages of attraction (percentage NLP) are shown in Figure 1. Both *Ae. albopictus* and *Ae. aegypti* female mosquitoes of feed group less than 1 day (Al-D0 and Ae-D0) were found to be non-responsive to the volunteer's palm. The non-responsive was also seen for *Ae. albopictus* of feed group Al-D1 (1 to 2 days) but for *Ae. aegypti* females (Ae-D1), it exhibited a sudden peak of mean percentage of attraction to 100%. The non-responsive of both *Aedes* spp. of age less than 24 hours to human palm is probably due to their insensitive sensory organ towards blood host (Bowen, 1991).

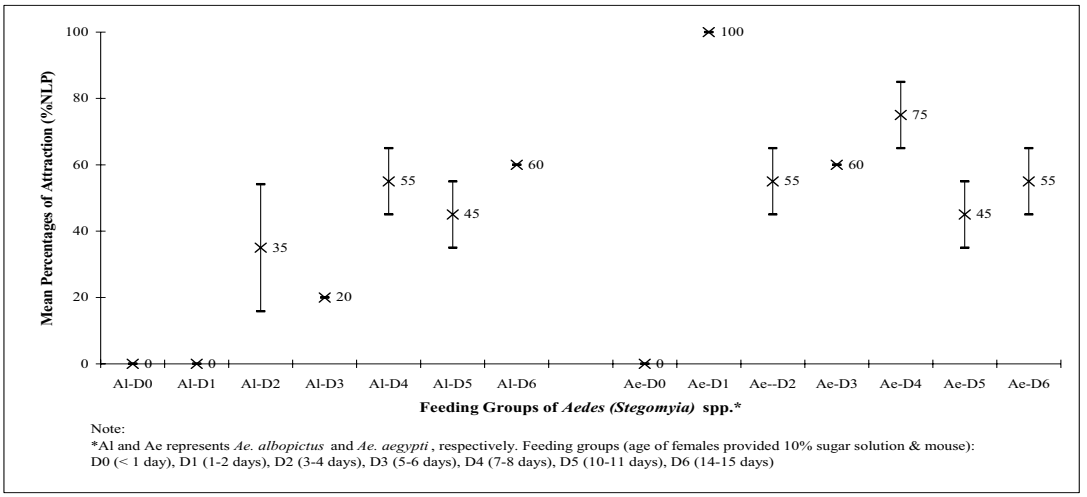

Figure 1. Mean percentage of *Ae. albopictus* and *Ae. aegypti* females provided with sugar solution and mouse attracted to human palm within five minutes

In feed groups from Al-D2, Al-D3 to Al-D6, *Ae. albopictus* mean percentage of attraction to the volunteer's palm fluctuated from 20 to 60%. Feed groups of 3 to 4 days (Al-D2), 5 to 6 days (Al-D3) and 7 to 8 days (Al-D4) exhibited different levels of attraction significantly ($P < 0.05$) at 35, 20, and 55%, respectively. The difference in attractions were insignificant ($P > 0.05$) among feed groups Al-D4, Al-D5 and Al-D6 (within 7 to 15 days feed period), which were at 55, 45 and 60%, respectively. This finding suggests the feeding of sugar solution and mouse blood for 3 to 6 days has greater effect in reducing attraction of females *Ae. albopictus* to human. Beyond the 6 days, the effect of feeding in reducing attraction to human decreases. However for *Ae. aegypti,* there were no significant difference ($P > 0.05$) in attractions to human among feed groups Ae-D2, Ae-D3, Ae-D4, Ae-D5 and Ae-D6 (3 to 15 days) at 55, 60, 75, 45 and 55%, respectively.

More than 45% of both *Ae. albopictus* and *Ae. aegypti* female mosquitoes at age of 7 to 15 days readily display host seeking activity though visual observation showed the females had dark dilated abdomen, an indication of post feeding on the provided sugar solution and mouse blood. As such it is suggested that dilation of abdomen from the feeding of sugar solution and mouse blood does not completely inhibit the host seeking activity in female *Ae. albopictus* and *Ae. aegypti* mosquitoes. Nevertheless, feeding of sugar solution and mouse blood is still found to affect the probing responses of female *Ae. aegypti* as reported by Khan and Maibach (1970).

There is a possibility that mix feeding on both sugar solution and mouse blood were unable to activate the oocyte-induced inhibition mechanism due to insufficient blood meal as reported by Madhukar and Jones (1974). Females *Aedes* sp. were reported to take less blood meals when provided with 10% sucrose compared to those that were only given water. Another study showed that females *Anopheles* sp, which were given corn syrup 10% did not show any inhibition of biting and blood feeding activity (Straif and Beier 1996).

Whilst, the fluctuation among feed groups is possibly due to temporary activation of abdomen dilation inhibition. The results reflect findings by Khan and Maibach (1970), Madhukar and Jones (1974) and Straif and Beier (1996) that *Aedes* sp. given only 10% sucrose displayed a short period of host seeking inhibition due to reduced abdomen size after digestion of sucrose and blood (Bowen, 1991; Klowden, 1994). However, it does not correspond with suggestion by Jones and Madhukar (1976) that the sensitivity of sucrose-fed females towards blood host decreases upon the digestion of the sucrose meal. *Ae. aegypti* and *Ae. albopictus* showed a similar overall trend of host seeking inhibition. Except for feed groups of 1 to 2 days (Al-D1 and Ae-D1) and 5 to 6 days (Al-D3 and Ae-D3), *Ae. aegypti* remain highly attracted to human palm compared to *Ae. albopictus.*

CONCLUSIONS

Based on the findings of this study, it was shown that the abundance of sugar source and alternate blood host could reduce the attraction of both *Aedes* spp. to human but did not completely inhibit them from seeking and biting human. The persistence to bite human whenever opportunity arises signifies the anthropophilic behaviour of both *Aedes* spp. It is obvious, both *Aedes* spp. readily utilizes sugar and animal blood to survive. However, it is uncertain whether *Aedes* spp. are capable of controlling the intake of sugar solution below the threshold level to avoid the distend-induced inhibition. Such capability will allow *Aedes* spp. to always be ready to detect human. The mix feeding of both sugar and animal blood may prolonged period needed by females to accumulate sufficient blood meal for egg production, subsequently activate the oocyte-induced host seeking inhibition. Such condition is seen to encourage multiple biting, allow better opportunity for pathogen incubation and increase the risk of pathogen transmission from animal to human. Overall, feeding of sugar solution and mouse blood simultaneously has similar effects on the attraction of both *Aedes* spp. to human.

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