

## A novel mosquito feeding system for routine blood-feeding of *Aedes aegypti* and *Aedes albopictus*

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**Abstract.** A novel mosquito feeding system for routine blood-feeding of *Aedes aegypti* and *Aedes albopictus* was developed and evaluated. The system consisted of a collagen membrane casing filled with specific pathogen free (SPF) mini-pig blood, which is warmed by a simple in-house designed heating device. Blood feeding rate, fecundity, survival rate and hatchability of *Ae. aegypti* and *Ae. albopictus* colonies maintained by the feeding system were compared with those raised by conventional guinea pig feeding method. *Aedes aegypti*, displayed a significant difference in the feeding rate when offered blood meal using the membrane feeding (85.3%) and the guinea pig feeding (96.2%) methods ( $P=0.012$ ). Though the feeding rate was reduced, the level was acceptable for maintenance of laboratory colonies. There was no significant difference in the fecundity ( $P=0.556$ ), survival rate ( $P=0.715$ ), and hatchability ( $P=0.932$ ) between the two methods. For *Ae. albopictus*, the two feeding methods yielded no significant difference for the three parameters (fecundity, survival rate and hatchability=0.887, 0.580 and 0.564, respectively). Hence, we conclude that this simple collagen based membrane blood feeding system can be used for routine colonization of laboratory strains of *Ae. aegypti* and *Ae. albopictus*.

### INTRODUCTION

Blood feeding of female mosquitoes is an essential activity for colonization and maintenance of mosquitoes, which are often required for research on vector-borne diseases. Most of the earlier studies on mosquito rearing relied on human hosts or live animal hosts (Boyd *et al.*, 1935; Rozeboom 1936; Crowell 1940). Currently, live animals such as guinea pigs and rodents are more commonly used.

The increasing awareness on animal welfare and stringency in regulations governing the scientific use of animals for research, coupled with the inconvenience of using live animals as blood hosts, have led to the impetus for the development of a cheap and user friendly artificial membrane blood

feeding system for mosquitoes. Cosgrove *et al.* (1994) reported on a natural or artificial membrane using glass feeder developed for rearing many blood-feeding insects. Such membrane feeding method has been employed for several species of blood-feeding insects, largely for virus transmission and infection work. However, there was no substantial study on the long-term effects of such artificial systems on the fecundity, survival rate and hatchability of the mosquitoes. Limited studies have shown large reduction, up to 42%, in fecundity and low feeding rate (Cosgrove *et al.*, 1994).

Three predominant species of mosquitoes, namely, *Ae. aegypti*, *Ae. albopictus* and *Culex quinquefasciatus* are being colonized and maintained at the Environmental Health Institute (EHI), a public

health laboratory of Singapore. Live guinea pigs have been used as blood host for colonizing the mosquitoes. In order to replace, reduce and refine the use of animals, EHI developed and evaluated a novel membrane feeding system, with the aim of eliminating or reducing the use of guinea pigs.

During a preliminary trial, the mosquitoes were offered specific pathogen-free mini-pig blood for an hour, using the following methods: (a) Glass feeder-parafilm with blood warmed by circulating water bath; (b) Glass feeder-collagen membrane with blood warmed by circulating water bath; (c) Collagen membrane sausage warmed with an in-house designed heating device. Results showed that the collagen membrane sausage method gave the highest feeding rate of 89%, followed by glass feeder with collagen membrane and glass feeder with parafilm, which gave 64% and 45% respectively.

To determine the feasibility of using this collagen membrane sausage feeding method for maintaining mosquito colonies, four parameters namely, blood feeding rate, fecundity, hatchability and survival rate were studied.

## MATERIALS AND METHODS

### Mosquitoes

*Aedes aegypti* (F167) and *Ae. albopictus* (F182) were hatched, reared, and maintained in the Environmental Health Institute insectary maintained at  $25\pm 2^{\circ}\text{C}$ ,  $75\pm 5\%$  relative humidity and 12:12 h (light:dark) photoperiod. Female and male adults were housed together for 5 to 7 days in acrylic cage (30cm x 30cm x 30cm) for mating. 10% sugar solution was placed in the cage as food source. The females were then separated from males 24 hours before feeding experiments. For the entire study, data on feeding rate, fecundity, survival rate and hatchability was collected from eight consecutive generations of *Ae. aegypti* lab strain and three generations of *Ae. albopictus* lab strain. Using the same colony of mosquitoes to initiate the study, two parallel

lines were established for each species: one was solely blood fed using a guinea pig, and the other using the in-house designed membrane system.

### Guinea pig

Male guinea pigs, *Cavia porcellus* were used as control feed. A guinea pig of body weight 1kg and above was chosen, and its lumbar region was shaved to facilitate the blood feeding. The guinea pig was coaxed into a metal cage lined with a plastic sheet and paper towel and was then transferred into a mosquito cage for blood feeding. The use of animals was approved by Institutional Animal Care and Use Committee.

### Membrane feeding system

Cattle collagen membrane casing (Golden Bridge Pte Ltd) was used for this study. Pieces cut to 15 cm were soaked in boiling water for an hour and rinsed twice with tap water to remove any grease coated on it. About 20 ml of mini-pig blood was collected in EDTA coated tube an hour before feeding. The blood was pre-warmed in hot water ( $40^{\circ}\text{C}$ ) for 5 minutes. A knot was tied at one end of the membrane casing and about 7 ml of mini pig blood was transferred into the casing. Another knot was then tied at the other end. This blood – filled collagen membrane casing is named “blood sausage”. The blood sausage was placed on top of the in-house designed heating device (Fig. 1), which was pre-heated for 3 – 5 minutes prior feeding. The heating device consisted of a ceramic heating element, an aluminum block and a petri dish. It was connected to a transformer and was adjusted to  $40^{\circ}\text{C}$  by adjusting the output voltage of the transformer. The whole heating device was covered with aluminum foil and only the aluminum block was exposed.

### Blood feeding

For each feeding experiment, one hundred female mosquitoes were placed in each cage. Following a deprivation of sugar solution for 24 h, the cage of females was offered a blood meal. While one line of mosquitoes was offered mini-pig blood at  $37\text{--}39^{\circ}\text{C}$ , another was offered guinea pig feeding. The number



Figure 1. Blood sausage with heating device

of fully blood-fed mosquitoes was counted after the 1 hour feeding. Each set of experiments was conducted in triplicates.

Blood feeding rate = (number of blood fed mosquitoes /number of mosquitoes tested) X 100%.

#### **Fecundity, survival rate and hatchability**

Seventy five blood-fed mosquitoes were randomly picked after the blood meal. Each individual mosquito was transferred into a plastic vial (30 ml) for egg laying. A small wet cotton wool and a piece of filter paper (3.5 cm in diameter) were placed in the screw cap of the plastic vial which was inverted for the females to deposit their eggs on the wet filter paper. Each vial of blood fed mosquito was maintained by a cotton wool fully soaked with 10% sucrose solution and placed on top of the inverted plastic vial. After one week, the eggs laid by the blood fed mosquitoes were air-dried for a few days and counted under a stereo-microscope to determine the number of eggs laid per female.

The same seventy-five mosquitoes randomly picked for determining fecundity rate were studied for their survival rate. They were transferred into a large cage and continued to be fed with 10% sucrose solution. Survival rate is the percentage of female mosquitoes that survived 30 days after the first blood meal. Meanwhile, the 75 filter

papers, each holding eggs laid by an individual mosquito, were stored for 60 days before being used for hatchability studies. Firstly, the numbers of eggs on 10 randomly picked filter papers were counted using a stereo-microscope. Three hundred eggs, randomly obtained from the 10 filter papers, were distributed in four trays of aged water for hatching. The number of larvae hatched was counted daily until day four. Hatchability is the total number of larvae hatched per number of eggs tested (n=300).

#### **Statistical analysis**

All data were collated and analyzed by using SPSS version 17. Paired –Sample T test was performed to determine the differences between two feeding methods.

## RESULTS AND DISCUSSION

#### **Blood feeding**

Table 1 and 2, respectively, shows the feeding rates of each generation of *Ae. aegypti* and *Ae. albopictus* offered guinea pig and collagen membrane blood sausage. Feeding rate of *Ae. aegypti* fed on blood sausage achieved an average of 87.5% in the first four generations and was not significantly different from the guinea pig fed ones ( $P>0.05$ ). However, *Ae. aegypti* fed through

Table 1. Comparison of mean percentage blood feeding rate, median fecundity, mean survival rate and mean hatchability of *Aedes aegypti* lab strain fed on guinea pig and fed through collagen membrane for 8 generations

		<i>Ae. aegypti</i>							
Parameters	Method	GENERATION							
		1	2	3	4	5	6	7	8
Blood feeding rate (%)	G	91.13±0.03	94.27±0.72	94.00±1.01	99.54±0.34	98.83±0.35*	96.43±0.69*	97.65±0.58*	97.60±0.41
	M	88.17±3.29	89.27±1.25	85.10±2.76	94.64±1.66	78.82±4.70*	74.38±4.84*	77.53±2.29*	93.65±1.42
Fecundity (No. of eggs)	G	78.00±1.91	70.00±1.76	102.34±1.91	95.00±2.18	106.00±2.13	111.33±2.58	122.30±2.54	134.50±1.75
	M	71.17±1.68	66.67±2.01	93.00±1.91	87.84±2.05	94.00±2.03	106.00±3.11	103.30±2.61	115.00±2.02
Survival rate (%)	G	92.00±5.29	95.00±3.79	94.00±1.86	85.00±12.86	91.00±9.00	82.00±2.85	88.00±2.33	88.00±6.56
	M	94.00±2.85	98.00±2.33	99.00±6.06	90.00±16.83	92.00±18.67	90.00±1.20	87.00±2.33	83.00±7.00
Hatchability (%)	G	98.90±0.49	86.60±0.88	95.51 <sup>#</sup>	92.96 <sup>#</sup>	98.91 <sup>#</sup>	83.10 <sup>#</sup>	77.08 <sup>#</sup>	81.00 <sup>#</sup>
	M	97.40±0.53	86.52±3.79	96.84 <sup>#</sup>	97.56 <sup>#</sup>	96.43 <sup>#</sup>	89.06 <sup>#</sup>	77.80 <sup>#</sup>	75.00 <sup>#</sup>

G: Guinea pig feeding. M: Membrane feeding

\*Significant difference was found between two methods within same generation

<sup>#</sup>Only one replicate was performed for hatchability test from generation four to generation eight

Table 2. Comparison of mean percentage blood feeding rate, median fecundity, mean survival rate and mean hatchability of *Aedes albopictus* lab strain fed on guinea pig and fed through collagen membrane for 3 generations

<i>Ae. albopictus</i>				
Parameters	Method	GENERATION		
		1	2	3
Blood feeding rate (%)	G	97.33±0.63*	95.88±0.39*	97.82±0.30
	M	79.91±3.78*	74.94±5.08*	90.74±6.74
Fecundity (No. of eggs)	G	102.16±3.36	143.33±3.80	131.67±2.70
	M	132.66±2.99	132.67±3.32	118.67±3.03
Survival rate (%)	G	89.00±8.41	86.00±11.3	83.00±1.67
	M	90.00±5.90	87.00±5.90	85.00±7.00
Hatchability (%)	G	69.49 <sup>#</sup>	80.05 <sup>#</sup>	N.A.
	M	70.02 <sup>#</sup>	75.27 <sup>#</sup>	N.A.

G: Guinea pig feeding; M: Membrane feeding; N.A.: No data available

\*Significant difference was found between two methods within same generation

<sup>#</sup>Only one replicate was performed for hatchability test from generation one to generation two

blood sausage showed significant difference in feeding rate after four generations. The decrease in the membrane-feeding rate from generation 5 to 7 could be due to the new batch of collagen membrane used. The feeding rate returned to initial levels at generation 8, when the old batch of collagen membrane was used. Nevertheless, the feeding rates achieved by the old and new batches of membrane were sufficient to maintain colonies of laboratory strains. This result demonstrated the importance of verifying the quality of each batch of membrane.

The first two generations of *Ae. albopictus* that had been offered guinea pig and collagen membrane sausage, showed significant differences in feeding rates. However, there was no significant difference in the third generation ( $P=0.068$ ). This suggests possible adaptation of *Ae. albopictus* to blood sausage feeding.

#### Fecundity

Tables 1 and 2, respectively, shows the fecundity of *Ae. aegypti* and *Ae. albopictus* for both blood feeding methods. There was

no significant difference ( $P > 0.05$ ) in fecundity between the two feeding methods. However, membrane feeding showed a consistently small reduction in fecundity when compared to guinea pig feeding. This could be due to mosquitoes imbibing serum only at the later part of the feeding process, as separation of serum and blood cells occurred about 20 minutes after blood sausage exposure. As serum contains less protein than whole blood, the lower fecundity could be due to the lower protein intake.

#### Survival rate

On average, more than 80% of *Ae. aegypti* and *Ae. albopictus* supported by both feeding methods, survived 30 days throughout the generations. The mean survival rate of *Ae. aegypti* and *Ae. albopictus* fed on guinea pig and fed through collagen membrane showed no significant differences ( $P = 0.177$  and  $P=0.580$ ).

#### Hatchability

There was no significant difference ( $P>0.05$ ) in hatchability between the two feeding methods. The hatchability for *Ae. aegypti* and

*Ae. albopictus* fed through both methods ranged from 80% to 98% and 63% to 80% respectively.

Our study showed that laboratory strains of *Ae. aegypti* and *Ae. albopictus* fed through collagen membrane blood sausage yielded good results. The average feeding rate, fecundity and hatchability for *Ae. aegypti* were 83.98%, 90 eggs/mosquito and 94.95% respectively. The average feeding rate, fecundity and hatchability of the third generation of *Ae. albopictus* were 81.86%, 128 eggs/mosquito and 72.65% respectively. Although the mosquitoes feeding rate were affected by the batch of membrane, the lowered feeding rate did not impact the colonies. In conclusion, membrane blood feeding is a good alternative to reduce usage of guinea pigs for colonizing laboratory strain *Ae. aegypti* and *Ae. albopictus*.

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