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# Use of a cost-effective artificial feeding system to evaluate the effect of blood meal source and its role in feeding success, reproductive parameters and larval growth of laboratory-reared *Aedes Aegypti* (L.)

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**Abstract**
Five different blood sources, human, cattle, rabbit, sheep and dog were delivered to laboratory-reared *Aedes Aegypti* to determine the effect of blood meal source on reproductive performance and larval growth characteristics of the mosquitoes. The blood meal was delivered with the use of a cost-effective artificial membrane feeding system. The mosquitoes displayed a significant feeding success for human blood over the other given blood meal sources. Thus, significantly a higher fecundity for human blood followed by cattle blood over other given blood meal sources was observed. The mean percentage of egg hatching for human blood and cattle blood was more or less the same, while the values were significantly different from that of other given blood meal sources rabbit, sheep and dog. Furthermore, larval growth parameters did not show any significant relationship with the blood meal source. These findings agree with established literature and facilitate the rearing and maintaining of *Ae. Aegypti* mosquitoes in a cost-effective manner. Hence the findings of the study aid in empowering laboratory research on *Ae. Aegypti* cost-effectively while enabling studies on its biology in *in vitro* conditions which are crucial in implementing disease control strategies.

**Keywords:** *Aedes Aegypti*, artificial- membrane feeding, blood meal sources, hematophagy, host preference

## 1. Introduction
*Aedes Aegypti* is a small dark mosquito with silver scales distributed throughout the thorax, abdomen and legs, which makes the legs appear banded. Being native to Africa *Aedes Aegypti* is currently common in many tropical and sub-tropical regions and throughout the Americas, India and Southeast Asia [1]. Also, it is one of the main vectors of the dengue virus which has caused severe disease outbreaks in Sri Lanka over the past two decades [2].
Among numerous vector control strategies implemented to curb the spread of dengue, vector control has proven to be the most effective [3]. *Ae. Aegypti* mosquitoes are mass-reared in laboratories to study their biology, behaviour, interactions with the virus, hosts, insecticides etc. Hence, it is crucial to raise and maintain a healthy colony of mosquitoes to achieve successful and reliable research outputs. An ideal laboratory-reared colony should not deviate from the original gene pool of the mosquitoes caught in the wild.
Furthermore, an anautogenous, hematophagy (blood-feeding behaviour) is an essential physiological process which provides nutrients required to proceed through the developmental stages until the oviposition in female *Ae. Aegypti* [4, 5, 6]. Hence, a blood meal should be arranged during the rearing period. Many of the previous studies relied on human volunteers or live animal hosts such as guinea pigs and rodents to nourish the mosquitoes [7, 8, 9, 10]. At present, the use of live animals is highly discouraged due to the increasing bioethical and animal welfare concerns, stringency in rules and regulations on using animals for scientific purposes together with the inconvenience of handling and housing live animals. Later, the use of artificial blood feeders that allow mosquitoes to feed on a liquid diet was provided.

Several different artificial feeders have been developed to feed the mosquitoes. Some are simple while others are more complex [11, 12].

The current study used a cost-effective and user-friendly alternative artificial feeding system to expose female *Ae. Aegypti* mosquitoes to five different blood meal sources to evaluate its effect on blood-feeding success, fecundity and larval growth. *Aedes Aegypti* prefer certain host species over the others [13]. Host preference influences the amount and the quality of blood ingested and therefore directly affect the fecundity in mosquitoes [14]. According to the findings of Prasad (1987), this variation in reproductive success across different blood meal sources is attributed to the variations in the amino acid composition in different blood sources [5]. *Aedes Aegypti* shows a significant preference for human blood meals as they receive a prolific nutrient composition and the energy benefits of ingesting human blood [15, 16].

## 2. Materials and methods

### Maintaining the mosquito colony

Eggs of laboratory-reared *Ae. Aegypti* were brought from the Medical Research Institute (MRI), Sri Lanka and mosquito colonies were raised and maintained in the insectary at the University of Kelaniya, Sri Lanka (6º58'20.91" N; 79º54'52.83" E; Gramin etrex©). The laboratory conditions were maintained at a temperature of 29 ± $2^0$C, humidity 80 ± 5% and Photoperiod 12:12 L:D. Eggs were submerged by adding 500 mL of aerated tap water to plastic trays (30 x 35 x 5 cm) and allowed to hatch. Once the eggs hatched, larvae were fed with dried krill powder (0.1g for 1st and 2nd larval instars, 0.5 g for 3rd and 4th larval instars) twice a day and the water was changed every day. Throughout the experimental procedure, a larval density of 250 larvae/ 500 mL was maintained in every tray. Two hundred pupae were transferred into aerated tap water trays and were placed inside the mosquito rearing cages (40 x 50 x 50 cm). Once the adults emerged, they were fed with a 10% sucrose solution soaked in cotton pads. Two days old female and male mosquitoes (1:3) were put in five separate experimental cages and were starved for 24 hours. After starvation, females were fed with blood samples from five different vertebrate hosts separately in an artificial membrane feeding system.

### Provision of blood meals to adult mosquitoes

The five different blood types used in the experiment were; human (*Homo sapiens sapiens*), rabbit (*Oryctolagus cuniculus*), dog (*Canis lupus familiaris*), sheep (*Ovis aries*), and cattle (*Bovis taurus*). The selected blood types were obtained from the Medical Research Institute (MRI) in Colombo and Zonal Veterinary Offices at Mahara and Welisara. Sheep and rabbit blood was obtained from MRI while dog and cattle blood was obtained from Mahara and Welisara veterinary offices. The researcher's own blood was used as human blood. The blood was taken by the registered medical officer in Nawaloka Medical laboratory at Dalugama, Kelaniya. Immediately after blood was drawn, 0.1 mL of EDTA was added to each sample and was transported to the laboratory and maintained at 4 ºC to avoid clotting [10].

Metal container lids (12 cm diameter) having a 0.5 mm uniform depth were used to prepare the apparatus to serve the blood meals to the mosquitoes. The surface of the lid was scarred using a metal nail to make the surface rough. The opening of the lid was covered with a Parafilm membrane and blood (5.0 mL) was introduced into the space created between the metal container lid and the membrane (Figure 1c) [12]. Blood-filled metal lids were placed on the top of the rearing cages in a way that the blood-filled membrane is facing the cage. A stoppered rubber hot-water bag (figure 1b), having hot water (60-80 ºC) was placed on the metal lid facilitating the heat energy to flow towards blood to keep it warm (Figure 1a).

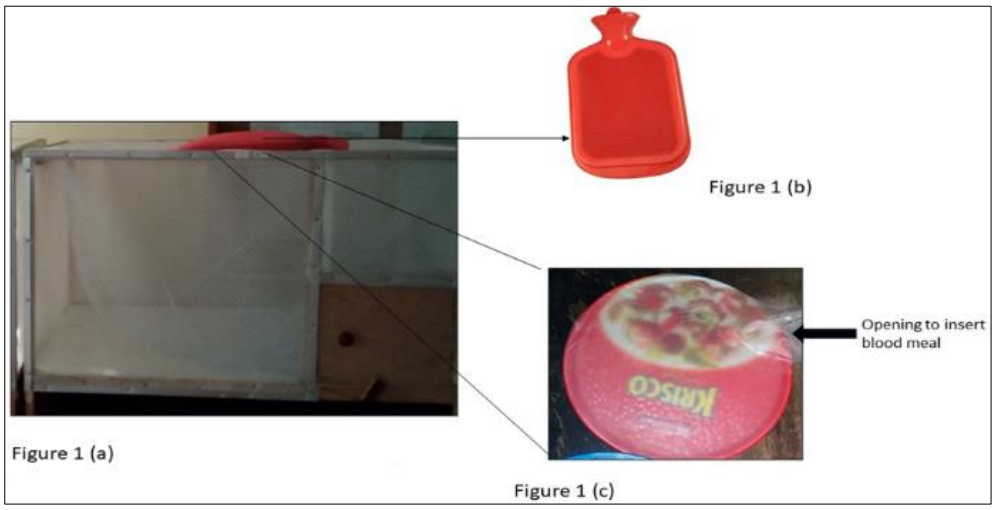


**Fig 1:** (a) Setup of the artificial membrane feeding system on a mosquito rearing cage (b) Stoppered hot water bag (c) Metal lid covered with a para film membrane

### Assessing the effect of blood meal source on landing preference and blood-feeding success of *Aedes Aegypti*

Five insect rearing cages with twenty starved mated females were arranged and the five blood meals were offered separately to each cage using the membrane feeding apparatus. The blood meal was served from 8.30 am to 10.30 am. The number of mosquitoes that landed the blood sample within the first 15 minutes was counted for each type of blood meal.

After two hours the number of fully engorged females was counted and the successfully blood-fed percentage was calculated using the following formula[17].

$$\text{Successfully blood-fed \%} = \frac{\text{Number of successfully blood-fed mosquitoes}}{\text{Total number of female mosquitoes in one cage}} \times 100$$

**Assessing the effect of blood meal source on fecundity and fertility of *Aedes Aegypti***

All the fully engorged females were transferred to mosquito rearing cages for oviposition. Black colour cylindrical plastic cups (200 mL) with a diameter of 7.0 cm and a depth of 6.0 cm were used to prepare the oviposition substrates. The cups were soaked in a water bath for one week to avoid the bad plastic odour. A filter paper stripe of length 22.0 cm and width of 5.0 cm was placed along the plastic cup's interior circumference, which acted as the substrate for egg-laying. One-third of the cup was filled with aged tap water. The number of eggs laid in each oviposition substrate for each blood source was counted to determine the fecundity.

Subsequently, an egg sheet with 100 eggs was placed in plastic trays having 500 mL of aerated water and allowed to hatch. Twenty-four hours later the number of 1st instar larvae that hatched out from the eggs was carefully recorded to determine the fertility.

**Estimating the effect of blood meal source on the duration of embryonic stage and total immature stages of *Aedes Aegypti***

An egg sheet with 100 eggs was placed in a plastic tray filled with 500 mL of aerated water. The time taken for the 1st instar larvae to hatch out from the eggs for each blood source was recorded as the duration of the embryonic stage[17]. Consequently, the hatched larvae were fed with dried shrimp powder and the time taken for the 1st instar larvae to develop into pupae and emerge as adults was recorded as the duration of the total immature stages of *Ae. Aegypti.*

All the experiments were replicated four times following the same laboratory conditions throughout the entire study period.

**Statistical Analysis**

Statistical analysis was carried out using the statistical software MINITAB 17. All the data were subjected to the Anderson-Darling normality test to check if they were normally distributed. Since the data followed a normal distribution one-way analysis of variance (ANOVA) was used to determine the effect of different blood meals on landing preference, blood-feeding success, reproductive performance and larval development of *Ae. Aegypti*. When the resulted means were significant ($p< 0.05$), Tukey's test was used to determine the differences among sample means for significance.

## 3. Results

The mean number of female mosquitoes that landed on different blood meals within the first fifteen minutes varied significantly (one-way ANOVA, $F_{4, 35} = 4.71$, $P = 0.004$). *Aedes Aegypti* showed the highest landing preference for human blood over the other blood sources, cattle, rabbit, sheep and dog. The landing preference for cattle blood was significantly lower than human blood and significantly higher than the other three blood meal sources. Meanwhile, the landing preference for rabbit, sheep and dog blood sources was not significantly different ($p> 0.05$) (Table 1).

Furthermore, the successfully fed percentage varied significantly across the five blood meal sources (one-way ANOVA, $F_{4, 35} = 12.93$, $P = 0.000$). Thus, the highest number of blood-fed mosquitoes were observed in human blood source. However, the number of blood-fed mosquitoes in all other blood sources remained more or less the same (Table 1).

The fecundity of females varied significantly with different blood meal sources (ANOVA, $F_{4, 35} = 38.68$, $P = 0.000$). The highest fecundity was observed in mosquitoes fed with human blood while a significantly lower fecundity was observed in mosquitoes fed on sheep and dog blood meals (Table 1).

The mean percentage of eggs hatched had a significant effect on mosquitoes fed with different blood meals (one-way ANOVA, $F_{4, 35} = 13.48$, $P = 0.000$). The highest egg-hatch percentage was observed in the human blood source. However, the hatchability of eggs did not show any significant difference between human and cattle blood sources while the other blood sources were significantly different (Table 01).

The embryonic period of *Ae. Aegypti* did not vary significantly in mosquitoes fed with different blood meal sources (one-way ANOVA, $F_{4, 35} = 0.38$, $P = 0.818$). However, the lengthiest embryonic period was observed corresponding to the dog blood meal (2.13 ± 0.41 days) while the shortest embryonic period resulted corresponding to human blood (1.25 ± 0.24 days) (Table 01).

The duration of the total immature stages of *Ae. Aegypti* did not vary significantly with different blood meal sources (one-way ANOVA, $F_{4, 35} = 0.092$, $P = 0.646$). However, a relatively shorter duration of the immature stages was observed in mosquitoes that were fed with human (9.38 ± 0.04 days) and dog (9.00 ± 0.50 days) blood sources (Table 1).

**Table 1:** Estimates of the tested parameters of *Aedes Aegypti* for different blood meal sources

| Source of blood meal | Number of mosquitoes landed (1st 15 minutes) | Successfully blood-fed mosquito percentage | Fecundity (Mean number of eggs laid per female) | Fertility (Mean percentage egg hatch) | Embryonic Period (days) | Duration of the total immature stages (days) |
|---|---|---|---|---|---|---|
| Human | 5±0.1[a] | 44.38±0.31[a] | 104±4.8[a] | 83.6±0.3[a] | 1.25±0.24[a] | 9.38±0.04[a] |
| Cattle | 3±0.1 [b] | 11.88±0.06[b] | 74±2.0[b] | 81.8±0.2[a] | 1.75±0.41[a] | 11.25±0.03[a] |
| Rabbit | 2±0.3 [c] | 10±0.08[b] | 87±8.0[c] | 74.8±0.2[b] | 1.5±0.37[a] | 10.38±0.03[a] |
| Sheep | 2±0.2[c] | 14.38±0.44[b] | 33±5.3[d] | 51.7±1.3[c] | 1.75±0.37[a] | 10.25±0.40[a] |
| Dog | 2±0.2[c] | 11.25±0.33[b] | 24±5.5[d] | 27.8±1.2[d] | 2.13±0.41[a] | 9.0±0.50[a] |

## Discussion

Blood feeding preference was evaluated using the data of landed mosquitoes and successfully blood-fed mosquitoes. However, the two parameters were not interrelated. Although many mosquitoes had landed, the feeding success may not be high. This is because the mosquitoes detect preferred hosts with the help of odorants and other chemical and thermal signals emitted by the skin and its microfauna. But using a Parafilm-M© membrane to feed the mosquitoes will prevent all the qualities given by the skin [13].

Subsequently, the current study revealed the fecundity of *Ae. Aegypti* is significantly high when they feed on human blood. Similar findings have been discovered by Dimond *et al*. (1956) [15], Bennett (1970) [14], Harrington *et al.* (2001) [16] and Phasomkusolsil (2013) [18] which attribute the higher fecundity to the nutrient composition and the energy benefits the female mosquito obtain by ingesting human blood [14, 15, 16, 17, 18]. Considering the amino acid requirement of mosquitoes, the presence of isoleucine in the diet can increase the fecundity significantly [15]. Hence isoleucine is considered a limiting factor in determining the extent of blood meal utilization for vitellogenesis during oogenesis [19]. Human blood possesses a unique low titer of isoleucine which is believed to be associated with differences in feeding preference [16]. However, it was observed that *Ae. Aegypti* benefit from an accumulation of more energy reserves and a fitness advantage when fed on isoleucine-poor human blood compared to isoleucine-rich vertebrate blood [16].

Although the percentage of eggs hatched, varied significantly between the blood of rabbit, sheep and dog, the absence of a significant difference between human and cattle blood thwarted our hypotheses, that the hatching of eggs depends solely on the source of blood meal ingested. The findings of the current study align with the studies of Olayemi *et al*. (2011) [17], which state that the hatching of eggs is mainly influenced by the hatching conditions while the quality or the viability of eggs also aids in efficient egg hatching [17]. The quality of the eggs is determined by the nutritional benefits gained from a blood meal. Furthermore, environmental conditions such as water quality of the rearing medium (pH, temperature), room temperature and egg density affect the hatching.

Larval growth indicators such as the embryonic period and duration of immature stages did not vary with different blood meal sources. This indicates that there is a predominant influence of rearing conditions over blood meal source on larval development. Hence, this could be a piece of conclusive evidence to prove the limitation of blood sources on the development of eggs. Moreover, the larvae are not supported by the egg nutrients after hatching hence they feed by themselves to obtain the required nutrition. Consequently, larval development cannot be solely attributed to the benefits of the source of blood meal.

## Conclusion

The consequences of the current study are on par with the previously conducted studies which indicate the efficacy of the implied artificial blood feeding method. Hence, it can be safely concluded that the use of the cost-effective artificial blood feeding method does not deviate significantly from the observations gathered by direct feeding on animals or artificial feeding using costly and complex devices. These findings enable better and cost-effective rearing techniques of *Ae. Aegypti*, which empowers studies on vector control strategies in dengue prevention and disease management.

## Conflicts of interests

The authors declare that they have no competing interests.

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## References

1. Costanzo KS, Schelble S, Jerzy K, Keenan M. The effect of photoperiod on life history and blood-feeding activity in *Aedes albopictus* and *Aedes Aegypti* (Diptera: Culicidae). Journal of Vector Ecology. 2015;40(1):164-171.
2. Noordeen F, Raza M, Pitchai F, Saranga WKC, Sandeepani LKHB, Sadamali L, *et al*. Distribution of dengue vectors, *Aedes Aegypti* and *Aedes albopictus*, in a few selected semi-urban areas of the Central Province of Sri Lanka. Sri Lankan J Infect Dis. 2018;8(1):36-39.
3. Imam H, Sofi G, Aziz S. The basic rules and methods of mosquito rearing (*Aedes Aegypti*). Indian Academy of Tropical Parasitology. 2014;4(1):53-55.
4. Spielman A. Bionomics of autogenous mosquitoes. Annual review of entomology. 1971;16(1):231-248.
5. Prasad RS. Nutrition and reproduction in haematophagous arthropods. Proceedings*:* Animal Sciences. 1987;96(3):253-273.
6. Sanders HR, Evans AM, Ross LS, Gill SS. Blood meal induces global changes in midgut gene expression in the disease vector, *Aedes Aegypti*. Insect Biochemistry and Molecular Biology. 2003;33(11):1105-1122.
7. Boyd MF, Kitchen SF, Stratman-Thomas WK. On the relative susceptibility of *Anopheles Quadrimaculatus* to *Plasmodium Vivax* and *Plasmodium Falciparum*, The American Journal of Tropical Medicine and Hygiene. 1935;15:485-493.
8. Rozeboom LE. The rearing of *Anopheles Albimanus* wiedemann in the laboratory, The American Journal of Tropical Medicine and Hygiene. 1936;16(4):471-478.
9. Crowell RL. Insectary rearing of *Anopheles quadrimaculatus*: A preliminary report, American Journal of Epidemiology. 1940;32:12-20.
10. Deng L, Koou SY, PNG AB, Ng LC, Lam-Phua SG. A novel mosquito feeding system for routine blood-feeding of *Aedes Aegypti* and *Aedes albopictus*. Tropical. Biomedicine. 2012;29(1):169-174.
11. Gunathilaka PADHN, Ranathunga RMTB, Udayanga NWBAL, Abeyewickreme W. Assessment of artificial blood feeding methods and efficacy of different blood meal sources in mass rearing of *Aedes Aegypti* for innovative vector controlling strategies. International Postgraduate Research Conference, University of Kelaniya, Sri Lanka. 2016;2016:247-247.
12. Gunathilaka N, Ranathunge T, Udayanga L, Abeyewickreme W. Efficacy of Blood Sources and Artificial Blood Feeding Methods in Rearing of *Aedes Aegypti* (Diptera: Culicidae) for Sterile Insect Technique and Incompatible Insect Technique Approaches in Sri Lanka, Bio Med Research International. 2017;2017:1-7.
13. Takken W, Verhulst NO. Host preferences of blood-feeding mosquitoes. Annu Rev Entomol. 2013;58(1):433-453.
14. Bennett GF. The influence of the blood meal type on the fecundity of *Aedes* (Stegomyia) *Aegypti* L. (Diptera: Culicidae). Canadian Journal of Zoology. 1970;48(3):539-543.
15. Dimond JB, Lea AO, Hahnert WF, DeLong DM. The amino acids required for egg production in *Aedes Aegypti*. The Canadian Entomologist. 1956; 88(02):57-62.

16. Harrington LC, Edman JD, Scott TW. Why do female *Aedes Aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? Journal of Medical Entomology. 2001;38(3):411-422.
17. Olayemi IK, Ande AT, Danlami G, Abdullahi U. Influence of blood meal type on reproductive performance of the Malaria vector, *Anopheles gambiae* s.s. (Diptera: Culicidae). Journal of Entomology. 2011;8(5):459-467.
18. Phasomkusolsil S, Tawong J, Monkanna N, Pantuwatana K, Damdangdee N, Khongtak W, *et al*. Maintenance of mosquito vectors: Effects of blood source on feeding, survival, fecundity, and egg hatching rates. Journal of Vector Ecology. 2013;38(1):38-45.
19. Briegel H. Metabolic relationship between female body size, reserves, and fecundity of *Aedes Aegypti*. Journal of Insect Physiology. 1990;36(3):165-172.