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Impact of human blood groups on reproductive fitness and offspring morphometrics of dengue vector *Aedes aegypti* (Diptera: Culicidae): a laboratory-based study

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Abstract

Objective The reproductive success of *Aedes aegypti*, a primary vector of dengue and other arboviruses, depends on host blood nutrition. Variations in blood group composition may significantly influence mosquito fecundity, egg viability, and offspring fitness, potentially impacting disease transmission dynamics. This study investigates the effect of human blood groups on the egg hatching rate and offspring morphometric development of *Aedes aegypti*.

Results Blood groups B^- and O^- demonstrated the highest fecundity rates, with significantly larger egg sizes and greater egg viability compared to other groups ($P < 0.05$). Offspring from these groups exhibited superior morphometric traits, including longer larval lengths and larger head capsule widths. In contrast, blood groups B^+ and AB^+ resulted in the lowest reproductive success, with smaller egg sizes and reduced larval fitness. The observed differences suggest blood group-specific variations in nutritional quality influencing mosquito reproductive potential and offspring development. The findings reveal that host blood group significantly impacts *Aedes aegypti* reproduction and larval fitness, with B^- and O^- blood groups providing the most favorable outcomes.

Keywords *Aedes aegypti*, Blood groups, Fecundity, Egg viability, Larval morphometrics, Vector control

Background

The dengue vector mosquito, *Aedes aegypti*, is a highly domesticated mosquito considered one of the most significant threats to human health. Female adult mosquitoes target humans as a preferred host for their blood meal to obtain nutrition for egg development and maturation. This ultimately makes them vulnerable to disease transmission as vectors [1–3]. Further, blood meal constituents may play a vital role, indicating the importance of isoleucine and amino acids in the blood, influencing the rate of egg production in some species of mosquitoes [4]. Blood meal volume and quality can play an important

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role in egg production and subsequently influence potential population dynamics and vector competence [5].

Previous research studies conducted using *Ae. aegypti* have indicated that mosquitoes could produce fewer eggs when feeding with animal blood than when feeding with human blood [6]. Evidence shows the average number of eggs laid by females of *Ae. aegypti* that were fed on human blood was 50.4 eggs. In contrast, the average number of eggs/females which were fed on blood meals from rabbits and pigeons were 33.2 and 46.3 eggs, respectively, re-iterating human blood meal increase an average number of egg production/female by rate 34.1% [7]. Therefore, ascertaining the attraction of many vector mosquitoes to human hosts could assist in determining the role of individual mosquito species in outbreaks of mosquito-borne diseases and provide critical information to inform mosquito control and surveillance programs [8].

Female mosquitoes rely on various cues, including physical, visual, and chemical signals, to locate their hosts [9]. Among these, organic molecules emitted by the host are considered the most critical for mosquito attraction. Human sweat, containing a diverse array of components, plays a significant role in this process, as even minor differences in the composition of organic chemicals can influence mosquito behavior [10]. Interestingly, variations in human attractiveness to mosquitoes have been a topic of considerable research. Hematophagous female mosquitoes exhibit differing levels of attraction to individual humans, which is thought to be closely linked to body odor [11]. For instance, *Aedes* and *Anopheles* mosquitoes are often more drawn to pregnant women, potentially due to higher levels of estrogen excreted in their urine [12]. The widespread belief that certain individuals are more prone to mosquito bites than others has fueled studies focusing on differences in human odor profiles and mosquito responses [13, 14]. Although a person's odor profile is complex and influenced by multiple factors, blood type is frequently suggested as a key determinant of mosquito attraction [15].

Different studies have identified the different blood type preferences. A study conducted by Khan et al., 2022 [16] indicated that *Ae. aegypti* fed on blood group B had the highest average feeding rate, number of females with eggs, and fecundity level [16]. However, a laboratory study conducted in Sri Lanka has stated blood group O negative has the highest preference by *Ae. aegypti*. This study also indicated no significant influence of human blood groups or rhesus factor in the life-history parameters of *Ae. agypti* [17]. Therefore, this study was designed to evaluate the effect of human blood groups on the egg hatching rate and morphometry-based developmental parameters of *Ae. aegypti* mosquitoes.

Method

Initiating a mosquito colony and mosquito-rearing

Aedes aegypti mosquito embryos were obtained from the mosquito colony housed at the Entomology Training School at the Medical Research Institute, Colombo 8, Sri Lanka and reared for one generation at the inspector facility available at the Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya, Sri Lanka. For hatching, eggs were put into a closed, one-liter jar filled with deionized water. Emerged larvae were fed with the recommended diet composition specified by the International Atomic Energy Agency (IAEA) comprised of 50% tuna meal (12.5 g), 36% bovine liver powder (9.0 g), and 14% brewer's yeast (3.5 g) in 100 ml of distilled water [18, 19]. Insect rearing and all experiments were conducted under standard laboratory conditions (27 ± 2 °C, 75 ± 5% relative humidity [RH], and a photoperiod of 12: 12 [L: D] h).

Feeding *Aedes aegypti* females with different human blood types

Eight different types of fresh human blood (i.e. A^+, A^-, B^+, B^-, AB^+, AB^-, O^+, O^-) were obtained from the National Blood Transfusion Service Narahenpita, Sri Lanka, with the approval of the Director of the National Blood Transfusion Service. EDTA (10%) was used as the anticoagulant. The blood samples were stored at 4°C until further use in this experiment. Adult mosquitoes were maintained in adult mosquito-rearing cages (24 × 24 × 24 cm^3) with nylon mesh. They were given 10% sugar with vitamin B complex and water. Blood feeding of adult females was facilitated with the artificial membrane-feeding metal plate method as described previously [20]. The mosquitoes were starved of sucrose solution for 24 h before blood feeding. In this experiment setup 15 male and female mosquitoes each were housed in separate mosquito cage and facilitated artificial feeding of a human blood group. Similarly, 8 experimental setups were conducted for separate blood groups. The experiment setup was repeated 3 times for each blood group, separately. Females in each cage were allowed to lay eggs on white filter papers in plastic cylindrical containers (diameter 9.5 cm, height 5 cm) filled with de-chlorinated water.h.

Hatching rate and egg viability of *Aedes aegypti* fed withh different blood groups

The filter papers with eggs were dried under standard conditions as batches for 4, 10, 25, 40, 55, 70, 85 and 100 days of desiccation, separately. The egg batches with different desiccation periods were facilitated to hatch according to the previously described procedure. The number of eggs hatched in each experiment was enumerated. The egg viability was measured as the mean of

hatches, calculated as the ratio to the number of total eggs laid [21].

$$\text{Egg viability} = \frac{\text{Hatched eggs}}{\text{Total number of eggs on each sheet}}$$

Differences in the morphometry of the mosquitoes fed with different blood groups

All morphometric measurements of the eggs, larvae, pupae and adult stages were measured by a stereo microscope (BOECO BST-606, Germany) fixed with a microscopy digital USB camera (Optika 4083.B6) and OPTIKA version 2.12 image processing software. The following developmental parameters were monitored.

Egg volume

A total of 10 eggs each from each experimental setup was obtained randomly to estimate the egg volume. The variation in the egg volume of the *Ae. aegypti* with the blood group they received was estimated according to the method described by Armbruster et al., 2001 [22].

$$V = \frac{\pi}{6} \times \text{length} \times \text{width}^2$$

V = Volume of the egg.
π = 3.14.
6 = Numerical value 6.

Larval, pupal and adult morphometry

From each hatching experiment (fed with different blood groups), 4th instar larvae were picked randomly and killed by a heat shock. Morphometric parameters, namely head length, head width, thoracic length, thoracic width, abdominal length, abdominal width, and total length of larvae, were measured. For pupal morphometry, 5 male and female pupae, each fed with different blood groups, were selected randomly based on their size. Length and width of the cephalothorax of pupae were considered. Five adult males and females that emerged from each blood group were collected randomly into separate collection tubes using mouth aspirators. They were killed using ethyl acetate. Thoracic length and abdominal length were considered as morphometric analysis.

Statistical analysis

The data obtained during the experiment was analyzed using the MINITAB 17 software. All the data were first tested for homogeneity of variance by the Levene's Test and normal distribution of data by the Anderson-Darling test. Analysis of Covariance (ANCOVA) followed by Tukey's test was carried out to determine the effect of ABO and Rh blood groups on the egg viability of *Ae. aegypti*. One-way analysis of variance (One-way ANOVA) followed by the Tukey's test was carried out to determine the effect of ABO and Rh blood groups on the morphometry of *Ae. aegypti*.

Results

Egg hatching rates

The highest egg hatching rate was observed in the B^- blood group, followed by O^+, AB^-, A^+, O^-, AB^+, and A^-, respectively. The lowest egg-hatching rate was observed in the B^+ blood group (Fig. 1). The results indicate a significant difference in the egg-hatching rate of *Ae. aegypti* among the eight blood groups ($F_{7,183} = 7.76$; $P < 0.05$).

Morphometric changes in *Aedes aegypti* fed with different blood types

Egg volume

The egg volume of *Ae. aegypti* was highest among the eggs laid by the females fed with B^- blood group, followed by O^+, A^+, AB^+, and AB^-. The lowest egg volume was observed in the A^-, B^+, and O^- blood groups (Fig. 2). A significant difference in the egg volume of *Ae. aegypti* was observed with different blood group types ($F_{7,72} = 2.84$; $P < 0.05$).

Larval morphometry

No significant difference was observed in the mean head length of larvae (Fig. 3a) ($F_{7,72} = 1.69$; $P > 0.05$). The highest head width (Fig. 3b) and thoracic length (Fig. 3c) were recorded in larvae fed with B^- and O^- blood groups, respectively. The thoracic width (Fig. 3d) of fourth instar larvae was highest in those fed with the B^+ blood group. The abdominal length (Fig. 3e) was greatest in females whose eggs were fed with the AB^+ blood group, while no significant difference was observed in the abdominal width of larvae (Fig. 3f) ($F_{7,72} = 1.88$; $P > 0.05$) across blood groups. The total length (Fig. 3g) was also highest in females whose eggs were fed with the AB^+ blood group.

Significant differences were observed in head width ($F_{7,72} = 4.54$; $P < 0.05$), thoracic length ($F_{7,72} = 5.49$; $P < 0.05$), thoracic width ($F_{7,72} = 2.77$; $P < 0.05$), abdominal length ($F_{7,72} = 18.20$; $P < 0.05$), and total length ($F_{7,72} = 18.04$; $P < 0.05$).

Pupal morphometry

The cephalothorax length of male pupae varied significantly among the eight blood groups ($F_{7,32} = 3.42$; $P < 0.05$). The highest cephalothorax length (Fig. 4a and b) and width (Fig. 4c and d) of both male and female were observed in the B^+ blood group. Significant differences were observed in cephalothorax length and cephalothorax width for both male ($F_{7,32} = 3.42$; $P < 0.05$; $F_{7,32} = 3.70$; $P < 0.05$)} and female ($F_{7,32} = 11.99$; $P < 0.05$; $F_{7,32} = 9.75$; $P < 0.05$) pupae.

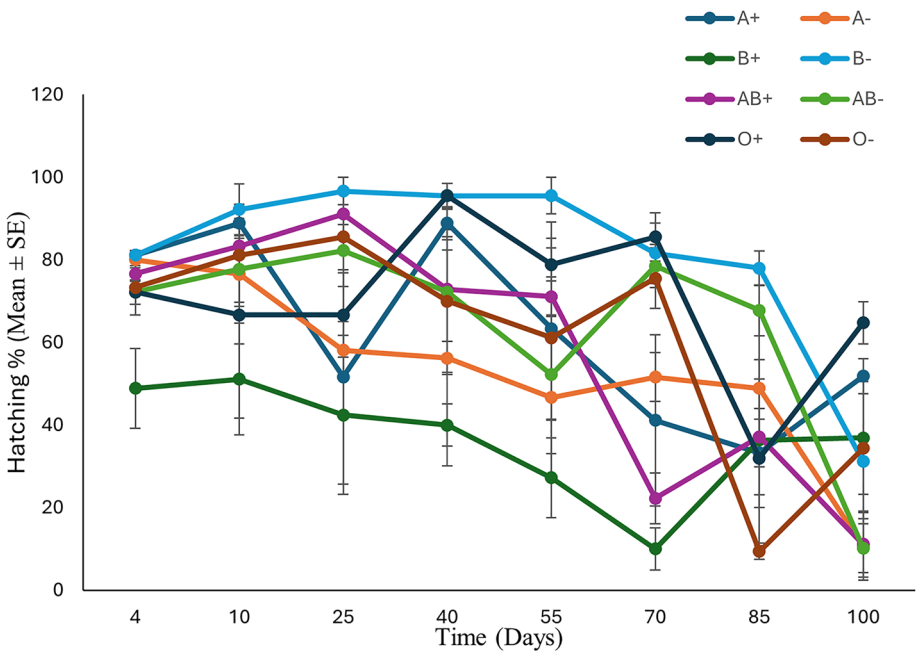

Fig. 1 Mean hatching percentage ± SE of *Ae. aegypti* eggs from ABO and Rh blood groups, plotted against time (days). Error bars represent SE of the mean. Hatching rates with different lower-case letters are significantly different ($P < 0.05$, Tukey's pairwise comparison test after ANCOVA)

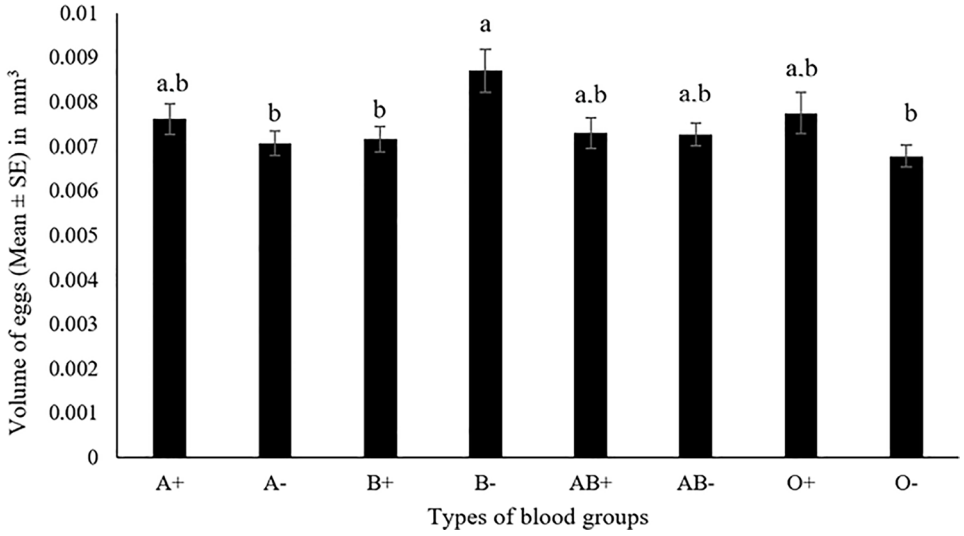

Fig. 2 Mean egg volume ± SE of *Ae. aegypti* in mm^3 versus ABO and Rh blood groups. Error bars represent SE of the mean. Means with different lower-case letters are significantly different ($P < 0.05$, Tukey's pairwise comparison test after one-way ANOVA)

Adult morphometry

No significant differences were observed in the thoracic length (Fig. 5a) and abdominal length (Fig. 5b) of adult males of *Ae. aegypti* ($F_{7,32} = 0.38$; $P > 0.05$; $F_{7,32} = 2.31$; $P > 0.05$). Similarly, no significant differences were found in the thoracic length (Fig. 5c) and abdominal length (Fig. 5d) of adult female *Ae. aegypti* among the blood groups ($F_{7,32} = 0.38$; $P > 0.05$); $F_{7,32} = 1.59$; $P > 0.05$).

Discussion

This study investigated the impact of ABO and Rh blood groups on the reproductive biology and morphometric parameters of *Ae. aegypti*. The findings revealed significant differences in egg hatching rates, viability, and morphometric traits associated with different blood groups, underlining the importance of host blood type in shaping mosquito reproductive success and development.

Understanding how host blood type affects mosquito reproduction is essential for predicting population

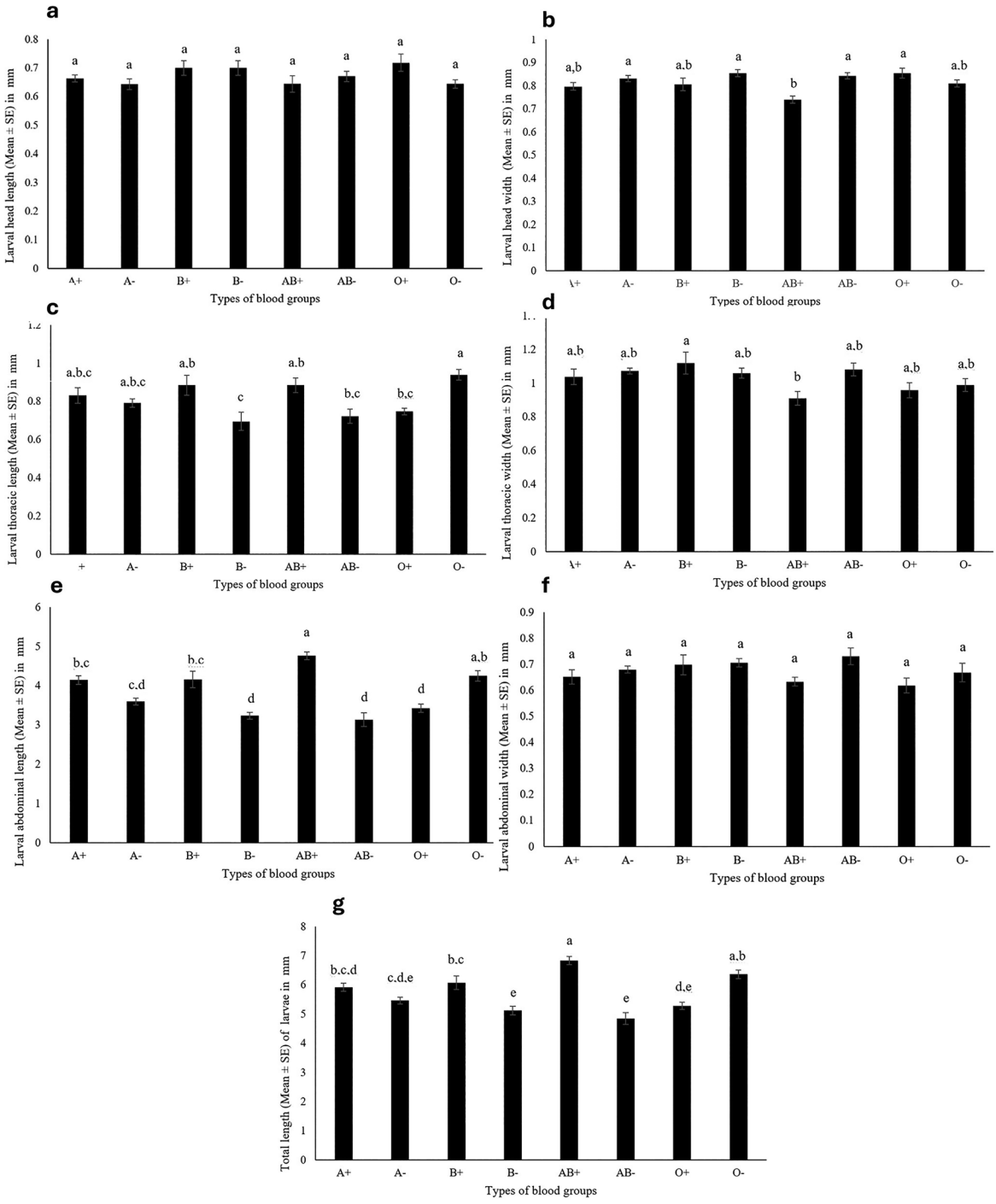

Fig. 3 Larval morphometry; (**a**) Head length (**b**) Head width (**c**) Thoracic length (**d**) Thoracic width (**e**) Abdominal length (**f**) Abdominal width and (**g**) Total length of *Ae. aegypti* larvae in mm versus ABO and Rh blood groups. Error bars represent SE of the mean. Means with different lower-case letters are significantly different ($P < 0.05$, Tukey's pairwise comparison test after one-way ANOVA)

dynamics in areas with differing blood group distributions. *Ae. aegypti* is an anautogenous mosquito which means it needs a blood meal to complete its gonotrophic cycle [23]. A study carried out previously found that eight amino acids are essential for the egg production of *Ae. aegypti* [24]. In the absence of any of these amino acids, egg production does not occur. The other four amino acids (histidine, glutamate, cystine, and methionine) reduce egg production, although not essential [25].

A previous study has highlighted higher levels of lipids, mainly triglycerides, present inside the diapausing insect eggs [26]. Diapausing eggs have higher resistance to

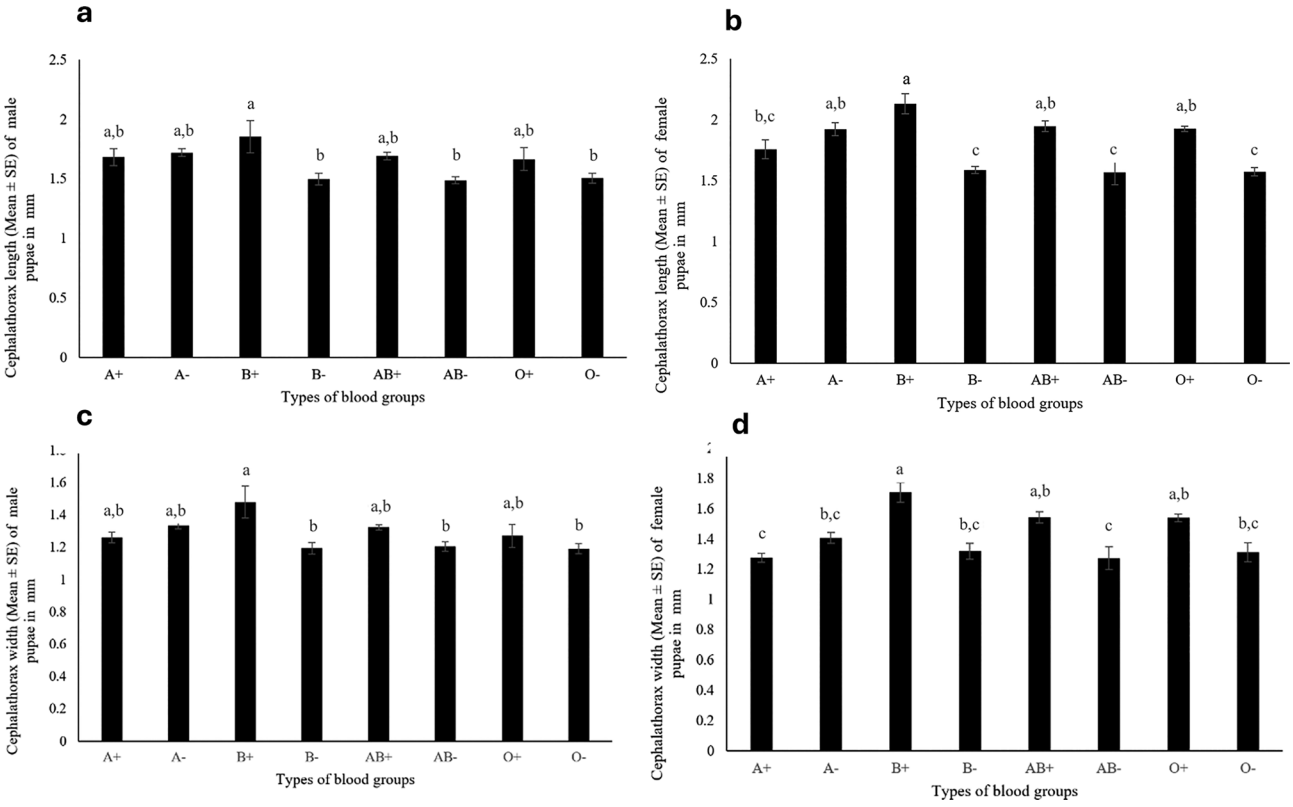

Fig. 4 (**a**) Mean cephalothorax length ± SE of *Ae. aegypti* male pupae (**b**) Mean cephalothorax width ± SE of *Ae. aegypti* male pupae (**c**) Mean cephalothorax length ± SE of *Ae. aegypti* female pupae (**d**) Mean cephalothorax width ± SE of *Ae. aegypti* female pupae in mm versus ABO and Rh blood groups. Error bars represent SE of the mean. Means with different lower-case letters are significantly different ($P < 0.05$, Tukey's pairwise comparison test after one-way ANOVA)

desiccation and their egg viability is also high. In the current study the differences of egg viability among different blood groups also may be due to the amount of lipids, mainly the triglycerides deposited in the egg. According to Contiero et al. [27], it has been found that there is a relationship between the different types of blood groups and the triglyceride levels in the relevant blood group. Triglyceride levels are higher in individuals with the B antigen in their blood which means B and AB blood groups. However, AB blood groups have both A and B antigens, while B blood groups contain only B antigens. Therefore, the highest egg viability in B- the blood group in the present study also re-iterates the fact that triglycerides might be the driving factor for egg viability.

The study also demonstrated significant effects of blood group on egg volume, larval morphometry, and pupal cephalothorax dimensions. Mosquitoes fed with B^- and O^- blood produced larger eggs and exhibited increased larval and pupal body dimensions, suggesting that these blood groups provide superior nutritional support for early developmental stages. Many factors can affect the egg volume of *Ae. aegypti* such as female adult size, genetic background, conditions during the immature stage and blood volume intake during feeding [28]. Larger eggs have been associated with increased offspring fitness, potentially enhancing survival rates and contributing to population growth [29]. Interestingly, the thoracic and abdominal lengths of adult mosquitoes did not show significant variation among blood groups, suggesting that the influence of blood type on morphometry is more pronounced during the early developmental stages.

The observed variations in reproductive and developmental traits may be associated with the physiological roles of specific amino acids in mosquitoes. Amino acids such as isoleucine and phenylalanine are known to be vital for egg protein synthesis and lipid storage, while leucine plays a key role in regulating growth pathways [23]. Although the present study did not analyze amino acid profiles of different blood groups, it is possible that differences in the availability and assimilation of these amino acids contributed to the observed patterns in fecundity and morphometry. Additionally, varying excretion rates of non-essential amino acids such as arginine and histidine may influence nutrient utilization efficiency and could partially explain the trends noted in this study [25].

These findings have significant implications for vector control and surveillance programs. The preference of

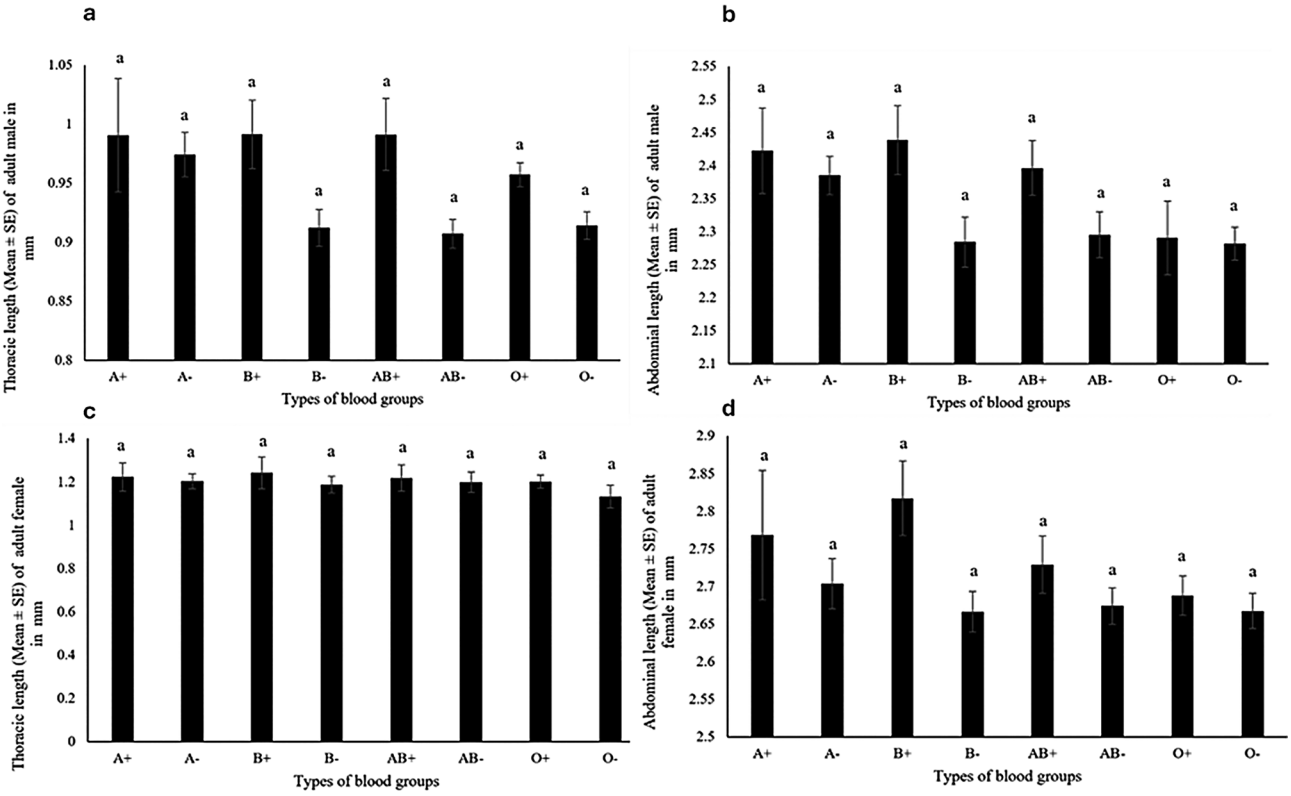

Fig. 5 Adult morphometry; (**a**) Thoracic length of adult male (**b**) Abdominal length of adult male (**c**) Thoracic length of adult female (**d**) Abdominal length of adult female of *Ae. aegypti* adults in mm versus ABO and Rh blood groups. Error bars represent SE of the mean. Means with similar lower-case letters are not significantly different ($P > 0.05$, Tukey's pairwise comparison test after one-way ANOVA)

Ae. aegypti for blood groups associated with enhanced fecundity, particularly B^- and O^-, may influence local mosquito populations and disease transmission dynamics in regions where these blood groups are prevalent. This knowledge could inform interventions such as synthetic attractants mimicking preferred blood types to enhance mosquito traps or optimizing blood-feeding protocols in sterile insect technique programs.

Furthermore, understanding how host population characteristics influence mosquito reproduction could guide targeted vector control strategies. For example, areas with a high prevalence of blood groups associated with increased mosquito reproductive success may require intensified control measures to reduce vector populations effectively.

Despite its valuable insights, this study has limitations. The use of artificial membrane feeding may not fully replicate natural blood-feeding behavior, as factors such as host skin microbiota, metabolic byproducts, and feeding dynamics were not accounted for. Additionally, the study focused on laboratory conditions, which may not capture environmental influences on mosquito development and reproduction.

Future research should explore the molecular mechanisms driving the observed differences, focusing on nutrient assimilation pathways and amino acid utilization. Field studies investigating natural host-mosquito interactions could validate the findings and provide insights into regional variations in mosquito behavior and reproduction. Furthermore, studies examining blood group preferences among different mosquito species and their implications for disease transmission could enhance the generalizability of these results.

This knowledge could guide the development of innovative control strategies, such as synthetic attractants or repellents that mimic or counteract the effects of specific blood groups, enhancing the efficacy of vector management tools. Furthermore, the results emphasize the need for further research to explore the biochemical and molecular mechanisms driving these blood group effects and to validate these findings in field settings. By unraveling these relationships, the study contributes to advancing targeted approaches for mitigating the public health burden of mosquito-borne diseases.

Limitations

- Only artificial membrane feeding was used, which may not fully represent how mosquitoes feed on humans in natural settings.

- The number of samples used for morphometric measurements was relatively small, which may limit the strength of the conclusions.

Conclusion

The study findings highlight the significant influence of host blood group on the reproductive success and viability of *Aedes aegypti*. Mosquitoes fed on blood groups B^- and O^- demonstrated markedly higher fecundity, egg viability, and larger egg sizes, indicating these blood groups provide superior nutritional support for mosquito reproduction. Additionally, larvae hatched from these eggs exhibited enhanced morphometric traits during early development, emphasizing the role of blood group-specific nutrient compositions in shaping offspring fitness. Conversely, blood groups such as B^+ were associated with reduced reproductive success, underscoring the variability in mosquito fitness based on the host's blood group. These findings have critical implications for vector management, as regions with a higher prevalence of favorable blood groups may face elevated risks of mosquito proliferation and disease transmission.

Abbreviations

EDTA	Ethylenediaminetetraacetic Acid
USB	Universal Serial Bus

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Author contributions

DJ and MG were responsible for conceptualization of the study. DJ conducted the experiment, analyzed the data, and drafted the manuscript. MG contributed to the experimental design, supervised the execution of the experiment, and reviewed and corrected the manuscript. NG performed a critical review of the manuscript and made necessary revisions, including rearranging sections. All authors reviewed and approved the final manuscript.

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Data availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was a laboratory-based investigation and did not involve human or animal participants. Human blood samples used in the study were obtained from the existing storage of the National Blood Transfusion Service of Sri Lanka, with formal permission granted by the Director of the institution. As the samples were anonymized, non-identifiable, and sourced from previously stored units, ethics approval was not required for this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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