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The implications for vector control of the feeding preference of female *Aedes aegypti* mosquitoes for different types of human blood and how it affects their fertility

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Abstract
Aim: The aim of the present was to identify the preferential feeding of *Ae. aegypti* on human ABO blood groups and their impacts on fecundity.
Methods: Laboratory reared female *Ae. aegypti* mosquitoes were exposed to all four blood groups at once in separate membrane feeders. After feeding, DNA of blood in mosquitoes was extracted and identified using ABO genotyping PCR. Fecundity was determined by the mean number of eggs in an egg batch.
Results: Among 410 mosquitoes allowed for feeding, 200 individual females were identified as blood fed mosquitoes by amplifying the 334bp fragment in the human DNA detection PCR. In the ABO genotyping PCR, blood groups A (n= 36), B (n=38), AB (n=50) and O (n=61) were detected. Additionally, 15 samples were detected having consumed multiple blood meals. The mean numbers of eggs laid per females were analyzed using one-way ANOVA test with the 0.05 significance level. There was no significant difference in fecundity for different blood groups.
Conclusion: The discovery of the underlying mechanism responsible for the preference of *Ae. aegypti* mosquitoes for blood group O has resulted in the development of a novel trap that attracts adult mosquitoes. This trap shows promise in combating the growing problem of pesticide resistance. Recent research has shown that persons with blood type O are more susceptible to contracting vector-borne illnesses transmitted by the *Ae. aegypti* mosquito.

Keywords: *Aedes aegypti*, Dengue, ABO blood groups, feeding behavior

Introduction
Mosquitoes are well recognized as significant carriers of disease-causing agents that have severe implications for human well-being. Tropical and subtropical climates are the most geographically favourable environments for mosquitoes to thrive and reproduce, making them a significant threat for mosquito-borne illnesses in these regions. Nevertheless, the occurrence of climate change, together with the surge in human travel and migration seen in recent times, has led to the dissemination of these illnesses to regions where they were previously absent [1-4]. *Aedes aegypti* poses a significant public health threat since it is very effective in transmitting diseases such as malaria, dengue, Chikungunya, Zika, and other arboviruses [5, 6].
Female mosquitoes are categorized as pernicious, ferocious, and hazardous ectoparasites that consume the blood of many vertebrate creatures, including mammals. The phylum Chordata includes several classes, such as Mammalia, Aves (birds), Reptilia (crawlers and creepers), Batrachomorpha (amphibians), and Pisces (fishes). In mosquitoes, blood meals are essential for providing the necessary dietary proteins and amino acids that are required for the formation and maturation of eggs in females [7, 8].
Furthermore, the reaction of mosquitoes to their hosts involves a series of three crucial steps: activation, orientation, and alighting. These stages dictate the process and technique by which mosquitoes are attracted to their particular hosts [9, 10].

It is important to note that there is a new trend in Europe where agricultural practices are changing and adopting new methods. The goal is to improve the living conditions of rural communities by providing separate animal shelters, such as pens and barns, away from human living areas. This has resulted in the shift of mosquito vectors, which prefer feeding on human blood, to exclusively feed on domesticated animals as the primary carriers of the malaria-causing pathogens [11].

Implementing personal protective measures to avoid mosquito bites is a crucial aspect in controlling the transmission of dengue by limiting the interaction between the dengue virus and the human host [12]. This is of utmost importance since the transmission of dengue via asymptomatic carriers who do not display any symptoms is a significant factor in the development of the disease [13]. Understanding the correlation between blood group selection and its impact on fertility is crucial for implementing efficient personal protective measures in vector control strategies as part of national dengue preventive and control plans.

The objective of this study was to determine the selective feeding behaviour of *Ae. aegypti* mosquitoes on different human ABO blood types and assess its effects on their reproductive capacity.

Materials and Methods

Female *Ae. aegypti* mosquitoes that were bred in a laboratory were simultaneously exposed to all four blood groups using individual membrane feeders. Following the feeding process, the DNA present in the blood of mosquitoes was collected and then detected using ABO genotyping PCR. The measure of fecundity was derived by calculating the average number of eggs in each batch of eggs. *Aedes aegypti* mosquito colony was developed in the Department of Zoology from a single engorged wild mosquito. The mosquitoes in the colony were kept under a 12:12 (light: dark) cycle, 28 °C constant temperature and 80% relative humidity. The mosquitoes were bred under standardized conditions [14] in order to produce individuals of equal size. The adult mosquitoes were kept in mosquito cages of 15 × 15 × 30 cm, which had mesh screening on top. They were given a 20% sugar solution and unlimited access to water. The eggs deposited by the female mosquitoes from the 12th generation of the colony were used in the present investigation.

Mosquito Rearing and Maintenance

Mosquito breeding was conducted in the insectary. The water was heated to its boiling point in order to remove oxygen and then transferred into glass bottles with a capacity of 250 ml. Subsequently, the bottles were left to reach ambient temperature while their lids were partially closed. The eggs of female *Ae. aegypti* collected from the colony were immersed in deoxygenated water to stimulate their hatching [15]. Newly hatched larvae were moved to the enamel trays filled with water. The larvae were fed with commercially available fish feed at a dosage of 0.32 mg per larva until they reached the 4th instar stage. Each day, the trays were thoroughly examined for pupae. The pupae were then carefully placed into plastic cups and moved to the adult cages to let the emerging adults to emerge.

Following the maturation of the adult mosquitoes, 200 individuals consisting of 100 males and 100 females were relocated to six new cages measuring 15 x 15 x 30 cm. The cages were covered on top with a net and the transfer was conducted using a mouth aspirator. A consistent 1:1 sex ratio was maintained in each cage to assure the mating of all females [16]. Cotton pads soaked in a 10% sucrose solution were placed on top of the cage to serve as the food source for adult insects [17]. The insectary maintained a constant temperature of 28 °C and a relative humidity of 80%.

Mosquitoes' Hematophagy

A group of 50 female mosquitoes, aged five days, were placed in a new cage measuring 20 x 20 x 20 cm. The cage had a mesh on the top and the mosquitoes were deprived of food and water for a period of 16 hours [15]. Feeding using blood supplies was conducted using an artificial membrane feeder. The membrane feeding system consisted of water jacketed glass feeders and a water circulation mechanism. The blood samples were analysed using a commercially available technology that used murine monoclonal anti sera to identify their group. Parafilm membranes from Marrifield, USA were used to cover the bottoms of four glass feeders. The temperature of the circulating water in the system was consistently maintained at 37 °C. Four glass feeders containing 3 ml of human blood were used for feeding, with each feeder representing blood types A, B, AB, and O. The blood-filled feeders were attached to the water circulating system and allowed to remain for 30 minutes in order to reach the same temperature as the circulating water. The mosquitoes were let to feed for a duration of one hour. Following the feeding procedure, fully fed mosquitoes were promptly refrigerated and then frozen until DNA extraction. The feeding process was conducted in six individual cages to guarantee that all 410 female mosquitoes were well fed, facilitating their ability to mate.

DNA extraction from human blood ingested by mosquitoes

The cryogenically preserved mosquitoes were subjected to ambient temperature for about 30 minutes to facilitate the thawing process. The abdomens of the mosquitoes were dissected. The samples were mixed well in 100 µl of extraction buffer [18]. Following a one-hour incubation at a temperature of 65ºC, the samples were subjected to treatment with 8 M cold potassium acetate and then incubated on ice for a duration of 45 minutes. The DNA was precipitated by adding 100% ethyl alcohol. The DNA was kept at a temperature of -20 ºC until it was needed for future use. The same protocol was executed to extract DNA from four different blood groups, serving as positive controls to guarantee precise amplification for each blood type.

The assessment of the collected DNA's relative quality was conducted by identifying the presence of human DNA using PCR amplification, utilizing human-specific primers, namely Human 741F and UNREV 1025 [19]. The predicted PCR fragment for the identification of human blood was 334 base pairs in length. The PCR mixture included 1X PCR buffer, 1.25 units of Taq Polymerase, 2.5 millimolar $MgCl_2$, 0.8 millimolar dNTP, 50 picomoles of primers, and 10 nanograms of template DNA in a volume of 25 microliters. The experimental protocol included subjecting the samples to a series of temperature cycles. Specifically, the samples were first exposed to a temperature of 95 °C for a duration of 5 minutes. This was followed by a repetitive cycle consisting of 35 iterations, each lasting 1 minute at 95 °C, 1 minute at 58 °C, and 1 minute at 72 °C. Finally, the samples underwent a

final extension phase lasting 7 minutes at 72 °C.

Mosquito blood meal ABO genotyping

Each DNA sample extracted from blood in the mosquito stomach underwent four consecutive PCR reactions to identify the blood group. The primers used for amplification were prepared by Lee *et al*. [20] The identification of six alleles of the ABO gene involves the use of four distinct primer combinations: A101, A102, B101, O01, O02, and cis-AB. The PCR reaction consisted of 1X PCR buffer, 1.25 units of Taq Polymerase, 4.5 millimolar (mM) $MgCl_2$, 0.2 mM dNTP, 0.5 micromolar (µM) of each allele specific primer, and 10 nanograms (ng) of template DNA in a total volume of 25 microliters (µl). The experimental protocol consisted of subjecting the samples to a series of temperature cycles. Specifically, the samples were first exposed to a temperature of 95 °C for a duration of 5 minutes. This was followed by 35 cycles, each lasting 40 seconds, with alternating temperatures of 95 °C, 60 °C, and 72 °C. Finally, a final extension step was performed at 72 °C for 5 minutes.

The reproductive capacity of *Aedes aegypti* Females who are swollen or enlarged with four different blood groups.

The experiment included male and female mosquitoes from the F32 generation. Recently hatched adult mosquitoes were moved into four adult enclosures measuring 15 x 15 x 30 cm. The subjects were given cotton pads soaked in a solution containing 20% sucrose as their food source. They were then given a period of five days to engage in mating. After a period of five days, the females were segregated into fresh adult enclosures, with each cage housing a total of 50 females. Subsequently, the female mosquitoes in each of the four enclosures were deprived of sustenance for a period of 24 hours, during which they were not given any nourishment or access to water. The circulating water system of the artificial membrane feeder was linked to four glass feeders, with each feeder corresponding to one cage. These feeders were filled with four distinct varieties of blood. The mosquitoes were let to feed for a duration of one hour.

The fully engorged females were separately placed in egg-laying cages, which had a diameter of 4 cm and a height of 10 cm. A cotton pad with a solution with a sugar concentration of 20% was delivered as the nourishment. Following a period of 7 days, the females were extracted and the eggs were enumerated using a tally counter and a hand lens. The whole operation was repeated four times. The data was statistically compared using One-way analysis of variance (ANOVA) in SPSS software version 22.

Results

Among 410 mosquitoes allowed for feeding, 200 individual females were identified as blood fed mosquitoes by amplifying the 334bp fragment in the human DNA detection PCR. In the ABO genotyping PCR, blood groups A (n= 36), B (n=38), AB (n=50) and O (n=61) were detected. Additionally, 15 samples were detected having consumed multiple blood meals.

The mean numbers of eggs laid per females were analyzed using one way ANOVA test with the 0.05 significance level. There was no significant difference in fecundity for different blood groups.

Table 1: Number of blood fed mosquitoes detected from the PCR analysis

Blood group	Number of blood fed mosquitoes	Mean percentage (%) ±Standard deviation
A	36	18.62±2.48
B	38	17.33±3.77
AB	50	23.97±2.17
O	61	31.79±3.00
Multiple meals	15	7.33±3.67
Total	200	

Table 2: *Aedes aegypti* egg production in response to the different blood groups

Blood type	Blood fed females out of 410 total mosquitoes	No. of females -Egg laid	Mean no. of eggs/female (± SD)
A	355	350	45.065±7.823
B	350	338	42.188±6.354
AB	344	340	44.456±4.034
O	360	355	45.225±7.633

Discussions

Aedes aegypti, a primary carrier of Dengue, Yellow fever, and Chikungunaya, is now found in tropical areas, including Sri Lanka, as well as subtropical regions such as the South-Eastern United States, the Middle East, Southeast Asia, the Pacific and Indian Islands, and Northern Australia [21]. The transmission of the dengue virus occurs via the bites of infecting *Ae. aegypti* mosquitoes. Following copulation, the female *Ae. aegypti* mosquito need a blood meal from a vertebrate in order to get the necessary nutrients for their reproductive capacity [22]. Therefore, the choice of a high-quality blood supply is crucial for their reproductive success. Hence, the blood feeding habits of mosquitoes provide crucial insights into disease transmission via mosquito bites and may be valuable for implementing successful vector control measures.

Out of the 410 insects used for feeding, 200 individual females were recognized as mosquitoes that had consumed blood by amplifying the 334bp fragment in the PCR test for detecting human DNA. The ABO genotyping PCR identified the presence of blood types A (n=36), B (n=38), AB (n=50), and O (n=61). In addition, 15 samples were identified as having ingested several blood meals. The fecundity experiment demonstrates that there is no statistically significant variation in the success of blood feeding and subsequent oviposition across four blood groups, when they were given various kinds of blood supplies individually. This demonstrates that when individuals are limited to a single option, the rate of feeding is greater compared to situations when several options are available. This may be attributed to the need for a longer length to pick from a range of sources in order to get a enough quantity of the most ideal blood meal for their reproductive success. This research suggests that the choice of blood meal changes depending on the availability of different sources and the time of day. Similarly, Culex species exhibit varying preferences for blood supplies based on factors such as availability and mosquito population [23]. The ongoing research maintained a consistent mosquito population

density during the whole duration of the study.

The average number of eggs deposited per female was analysed using a one-way ANOVA test with a significance level of 0.05. There was no discernible disparity in fertility across various blood kinds. Although this mosquito exhibits a preference for individuals with blood type O, no significant impact on reproductive capacity was observed. The selection and blood feeding on human blood mostly rely on the availability, accessibility, and ease of feeding. Furthermore, the findings indicated that although blood group type O was preferred to a greater extent, significant consumption of other blood types was also seen. Von Willebrand factors (VWFs) are glycoproteins that play a role in blood clotting. Elevated levels of VWF contribute to increased thrombotic activity, leading to the formation of blood clots [24]. ABO blood types that exhibit varying quantities of VWF in the blood plasma. Individuals with blood group type O have VWF levels that are 20% to 30% lower than those without blood group type O [25]. The changes in oligosaccharides found on the erythrocyte membrane are responsible for the disparities seen across blood types. Antigen A has an extra N-acetylgalactosamine terminal that is not present in the other two antigens. Antigen B has a surplus of glycan molecules in comparison to antigens O and A. While there are variations in the oligosaccharides found in erythrocyte membranes, the discrepancies in plasma composition across different blood types remain inadequately elucidated. There is a scarcity of research that have been conducted to elucidate the function of carbohydrates in the process of egg formation. Nevertheless, *Ae. aegypti* mosquitoes do not have any reproductive success other from their extended longevity [26].

Conclusion

The discovery of the underlying mechanism responsible for the preference of *Ae. aegypti* mosquitoes for blood group O has resulted in the development of a novel trap that attracts adult mosquitoes. This trap shows promise in combating the growing problem of pesticide resistance. Recent research has shown that persons with blood type O are more susceptible to contracting vector-borne illnesses transmitted by the *Ae. aegypti* mosquito. This may be a worry over the efficacy of personal protective measures in preventing mosquito bites. Moreover, it is crucial to analyses the correlation between dengue cases and the prevalence of ABO blood groups in the human population in order to infer the connection between symptomatic and asymptomatic dengue infections with certain blood types.

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