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RESEARCH ARTICLE

Blood Feeding Preferences of Laboratory-Reared *Aedes albopictus* for Human Blood Groups and Its Effect on Their Fertility

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ABSTRACT

Female mosquitoes require both sugar and blood for feeding. They show distinct host preferences depending on behavioral, ecological, and physiological factors. Knowledge of the feeding behavior of *Aedes albopictus,* one of the primary vectors of Dengue, is critical in disease prevention measures. This study was aimed to determine the preference of *Ae. albopictus* on human blood groups (ABO) and their effects on female fecundity. Laboratory colonies of these mosquitoes were offered O, A, B, and AB blood via artificial membrane feeders, and blood meal preferences were identified using multiplex allele-specific PCR. Fertility was assessed by the mean number of eggs laid. Results showed that *Ae. albopictus* species significantly preferred the O blood group compared to others and blood type choice did not affect the fertility of the mosquitoes. To our knowledge, this is the first study assessing blood feeding choice of *Ae. albopictus* mosquitoes directly using human blood, hereby reducing the effects of factors such as odor when using human participants. The results of this study provide a new perspective on the still partially unknown ABO blood group host selection of mosquitoes, promoting the personal protection of individuals in at-risk populations.

Keywords: ABO Blood Groups, *Aedes albopictus*, Feeding preference, Multiplex allele-specific PCR

INTRODUCTION

Mosquitoes are a significant threat to public health as a result of their inclination to bite people for blood, and their role in the transmission of diseases. *Aedes*, *Anopheles,* and *Culex* genera contain the most important species that vector viral and protozoal pathogens of several human and animal diseases, like Dengue, Yellow fever, Zika, Chikungunya, and Malaria [1-3]. Such widespread infections have been global health problems for years; this is directly associated with upsurges in international trade and travel, the distribution of vector mosquitoes, and ecological changes affecting these organisms [4,5]. *Aedes albopictus* (Asian tiger mosquito), in particular, is found in many sections of the world such as Southeast Asia, Africa the United States, and Europe [6-8], where it transmits Dengue, Chikungunya, and Zika, which are viral infections that affect nearly half a billion people annually [9-11]. In Türkiye *Ae. albopictus* is present in Thrace, Aegean, Central Anatolia, Marmara, and Mediterranean Regions [12-15]. This invasive species has the potential to further spread to other regions of Türkiye; this might be a huge public health problem as Türkiye receives many immigrants from war-torn areas and the spread of *Aedes*-transmitted diseases will significantly affect more unprotected people [15]. Therefore, countries infested by these mosquitoes should initiate vector and pathogen surveillance and control to hinder any potential epidemics [16,17].

Female mosquitos need blood for oogenesis [18], hence understanding blood meal preferences of harmful species is important in assessing disease risk. Quality and quantity of a blood meal can impact oviposition and consequently affect vector competence and possible population dynamics [19]. For instance, more eggs are oviposited by *Ae. albopictus* after feeding from human blood than on animal blood [20]. Elucidating mosquito attraction to human hosts might help in ascertaining the role of individual mosquito species in diseases epidemics and provide information vital to mosquito surveillance and control [21]. Female mosquitoes locate host using physical, visual, and chemical cues [22] hence they have different levels of attraction to different humans [23] which some studies have linked to genetic factors such as odor, ABO blood group system, and onsite host availability [21].

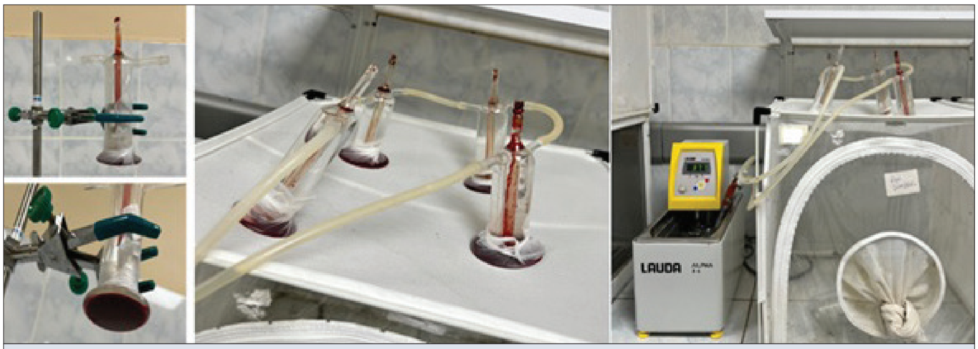
Fig 1. Experimental setup

Studies have been conducted in many parts of the world in regards to mosquito blood preference at feeding time as well as the relationship between different mosquito species and the forms of pathogen transmission [24,25]. Awareness on blood group choice and their effect on fertility is fundamental in developing personal protection and vector control in Türkiye. So, the current study determined which human blood type was preferred by *Ae. albopictus* females and the impact of the blood type on their fertility. Elucidating the effects of different human blood groups on such insects enables better recognition of the behavior of hematophagous arthropods that transmit important vector-borne diseases.

Material and Methods

Ethical Statement

This study was approved by the Aydin Adnan Menderes University Human Ethical Committee (Approval no: 2015-714).

Maintenance of Mosquito Species

Aedes albopictus larvae were collected from Aydın and reared to adult stage in the Vector Control Laboratory, Aydin Adnan Menderes University, under laboratory conditions of 25±2°C temperature and 70±10% relative humidity; adults were obtained from these larvae and reared in the insectarium. Females were fed through a membrane feeding system containing human blood and allowed to lay eggs. Mosquitoes laid eggs in plastic containers filled with water and sides lined with filter papers after about 5 days after blood feeding. The eggs were hatched in plastic trays (size 33-24-8 cm) with distilled water. Groundfish food (Tetramin) was added to each tray as feed for larvae. Pupae were transferred to 50 mL disposable plastic containers filled with distilled water and allow developing to adults. Hundred mosquitoes (50 females: 50 males) were separately removed to six insect cages (50 x 50 x 50 cm) using a mouth aspirator and were given cotton balls drenched with 10% sucrose which were removed from the cages for 12 h prior to blood feeding.

Blood Feeding Choice of Mosquitoes

Fifty newly emerged female mosquitoes (5-7 days old) were transferred to new insect cages (50 x 50 x 50 cm) and starved for 12 h [26]. Commercial human blood in EDTA tubes was obtained from the Blood Bank, Aydin Adnan Menderes University Hospital. Approximately 50 mL of blood was taken under appropriate ethical conditions, transported appropriately, and stored in the refrigerator until use in the experiment and used in the experiment within 2 days.

The females were provided with four blood feeding systems which have blood from different groups in the same time. Feeding experiments using these blood types was conducted with an artificial mosquito membrane feeding system [27,28]. The glass feeding system is made up of an inner chamber which had blood and an outer chamber that had circulating water from a water bath system at 37°C. Parafilm was stretched over the undersides of four feeders and filled with 10 mL of human blood (types O, A, B, and AB) for blood-feeding. The blood in the feeders were warmed up for 30 min before the experiments and mosquitoes were allowed to feed for an hour. All artificial blood feeding procedures were performed in replicates of six on different days (*Fig. 1*). After the feeding, blood-fed female mosquitoes were collected from the cages with an aspirator, labeled, frozen 4°C and kept until molecular analysis.

Determination of Blood Feed Type

Genomic DNA of 245 blood-fed mosquitoes was isolated to determine the ABO blood groups in mosquito abdomen using the Invitrogen PureLink Genomic DNA isolation kit by following manufacturer's instructions. Unfed *Ae. albopictus* female was used as negative control and identified human ABO blood groups served as positive controls. The amount of isolated DNAs (ng/µL) was measured in the Nanodrop (Thermo Scientific™ NanoDrop™ 2000/2000c Spectrophotometers). Total DNAs obtained from the samples were stored at -20°C until PCR was performed. All extracted DNA was checked to verify human DNA by PCR amplification with human-specific

Table 1. *Primers used in this study to detect blood type in mosquito abdomen*

Target Gene	PCR Reaction	Primer Names and Sequences	Fragment Size (bp)	Allele/Genus/Species Specificity
ABO	1	261G: 5´-CAGTAGGAAGGATGTCCTCGTGtTG-3´ int6: 5´-AGACCTCAATGTCCACAGTCACTCG-3´	205	A101, A102, B101, cis-AB01
		467C: 5´-CCACTACTATGTCTTCACCGACCAtCC-3´ 803G: 5´-CACCGACCCCCCGAAGAtCC-3´	385	A101, O01, O02
	2	297A: 5´-CCATTGTCTGGGAGGGCcCA-3´ int6: 5´-AGACCTCAATGTCCACAGTCACTCG-3´	164	A101, A102, O01, cis-AB01
		467C: 5´-CCACTACTATGTCTTCACCGACCAtCC-3´ 803C: 5´-CACCGACCCCCCGAAGAtCG-3´	381	B101
	3	261A: 5´-GCAGTAGGAAGGATGTCCTCGTGtTA-3´ int6: 5´-AGACCTCAATGTCCACAGTCACTCG-3´	205	001, 002
		467T: 5´-CCACTACTATGTCTTCACCGACCAtCT-3´ 803G: 5´-CACCGACCCCCCGAAGAtCC-3´	381	A102
	4	297G: 5´-CCATTGTCTGGGAGGGCcCG-3´ int6: 5´-AGACCTCAATGTCCACAGTCACTCG-3´	164	B101,002
		467T: 5´-CCACTACTATGTCTTCACCGACCAtCT-3´ 803C: 5´-CACCGACCCCCCGAAGAtCG-3´	381	cis- AB01

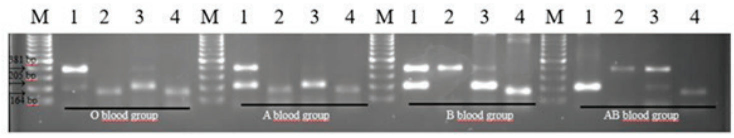

Fig 2. Agarose gel image of selected ABO blood groups amplified by multiplex ASPCR. Lane M, size marker (100-1000 bp ladder); lane 1-4 PCR reaction

primers: Human 741F, UNREV 1025 [29]. A 334 bp PCR fragment indicated human blood. Then the genomic DNA was verified for ABO blood groups by multiplex allele-specific PCR (ASPCR) [30]. Ten different primers were used in a four-reaction multiplex ASPCR genotyping assay to determine the differences in the specific nucleotide sequence between the ABO alleles - A101, A102, B101, 001, 002, and cis- AB01 *(Table 1)*. PCR mixture had 2× Taq DNA Polymerase Master mix, 20 pmol of primers, and 15 ng of template DNA in 25 µL and PCR settings are as follows: denaturation for 5 min at 95°C, 35 cycles of 1 min at 95°C, annealing for 1 min at 58°C, elongation for 1 min at 72°C and a final extension of 7 min at 72°C. The products were run on a 1% agarose gel and visualized under UV light inVilbert lourmat transillumінator.

Effects of Blood Groups on *Aedes albopictus* Female Fecundity

Newly emerged adult mosquitoes were transferred to four cages. Each cage housed 30 males and 30 females. Four artificial membrane feeding systems as described above were used and each was filled with different blood types. Mosquitoes in the cages were allowed to feed on blood for one hour. Afterward, fully engorged females were moved to paper cups (one female/cup) which held water and had filter papers on the sides for oviposition. The total number of eggs laid per individual was collected seven days post-blood-feeding and counted under a stereomicroscope (Leica GZ4 Stereo Zoom Microscope). Feeding success was ascertained by recording the numbers of fully engorged female mosquitoes and calculating the percentage of fed mosquitoes. The study was replicated three times.

Statistical Analysis

Analysis of data was in Minitab 14 and comparison of means of the number of blood fed females and the number of eggs laid by each female mosquitoes was carried out using ANOVA with Tukey's test ($P<0.05$).

Results

Preferences of *Aedes albopictus* from ABO Blood Sources

Out of the 300 mosquitoes used in the experiment, it was determined that 245 females blood-fed according to PCR amplification of human DNA. Agarose gel images of some selected samples are given *(Fig. 2)*.

In the ABO genotyping PCR, the number of mosquitoes that preferred the blood groups are O (n=93), A (n=51), B (n=66), and AB (n=35). The most fed on blood group was O (37.9%). Statistical analysis showed a significant preference in the selection of the blood groups ($F=18.385$; df=3.15 $P<0.01$) *(Table 2)*.

Table 2. *Number of blood-fed mosquitoes detected by PCR analysis and mean number of eggs laid per female*

Blood Group	Number of Blood-fed Mosquitoes	Mean (%) no. of Blood-fed Mosquitoes	Mean no. of Eggs Laid/Female
O	93	37.96	55.72±1.08
A	51	26.94	55.01±0.95
B	66	20.82	55.27±0.95
AB	35	14.29	55.72±0.95
Total	245	F=18.385; df=3.15; P<0.01	F= 0.128; df=3.232; P=0.943

Effect of Blood Sources on *Aedes albopictus* Fecundity

In total 60 mosquitoes were selected from 4 different cages fed from each blood group. On average 55 eggs were laid per female after blood feeding and no statistical difference was observed in the number of eggs laid after blood feeding from different blood groups (F=0.128; df=3,232; P=0.943).

Discussion

This study demonstrated that laboratory reared *Ae. albopictus* mosquitoes preferred O blood group compared to the other blood groups in artificial membrane feeding and that the different blood types did not affect fertility of the mosquito. The ecology and behavior of this and other mosquito species has been studied in the past to better understand disease transmission and control. It has been shown that blood feeding behavior varies and can be quite complex with different species displaying varying degrees of pattern, preference and specificity on a wide range of invertebrate and vertebrate hosts, and even preference variation between individuals and populations of a single host [38,39]. Several studies have assessed the blood feeding and host finding behavior of several important mosquito species [21,24,25,31,40-47] but few have focused on the effects of blood type preference on mosquito choice. Shirai et al.[25] determined the landing preference of *Ae. albopictus* females on forearms of human with different blood types. They showed that *Ae. albopictus* landed more on the forearm of volunteers with O blood group than B, A, and AB blood groups. In another study Prasadini et al.[31] reported that *Ae. aegypti* also displayed a significant preference for O blood when offered different blood groups. The authors suggested that blood meal choice differs with the availability of hosts and the time. Takken & Verhulst [21] hypothesized that blood type influences mosquito host choice. At first these researchers suggested that the disaccharide group (antigen H) on red cell membranes of O group was responsible for mosquito attraction. But data collected after their experiment did not explain the preference for O-type blood hence there may be other unknown influences underlying blood preferences. Based on these studies it seems blood typing has no clear effects on mosquito preference due to the interference of factors such as odor when using human participants instead of blood in in vitro tests. Hence other studies like the present studies and Prasadini et al.[31] are recommended where only the individual blood types are used.

Some common hypotheses have been put forth regarding blood preference of mosquitoes. Blood choice may mostly depend on what is available and accessibility to feed from. *Ae. albopictus* species originated from Asia, where there is a higher prevalence of A-type blood in the population, than O-type blood [21-25]. Tursen et al.[34] suggested that even though mosquito eyesight and color perception, allows them to successfully explore their surroundings, they are unlikely to play a role in host preference and choice.

ABO blood groups contain different von Willerbrand factors (VWFs), which are glycoproteins in the blood plasma associated with blood hemostasis and are responsible for blood coagulation [35]. People with O blood group possess 20-30% less VWF levels than other blood group types [36]. In addition VWF molecules of blood group type O have a short survival rate and cleared faster than other molecules linked with the other three blood groups [37].

Blood feeding behavior of female mosquitos is directly connected to reproductive activities. It has been reported that serum proteins are important blood components linked to oviposition. After taking a blood meal, serum proteins are broken down into amino acids like isoleucine in the digestive tract that have a huge effect on the vitellogenesis process of mosquitoes [26]. Hence, this study reasoned that besides oviposition, the result from this study will help in understanding the behavioral patterns of *Ae. albopictus* and provide additional information to improve vector control programs.

Since blood group O individuals are more likely to be bitten by vectors, they are more likely to be infected with *Ae. albopictus*-transmitted diseases. Similarly, other mosquito species like *Ae. aegypti* [32] and *Anopheles gambiae* [24] show a significant preference for O blood group. This might be a concern about personal protection measures effectively to prevent mosquito bites. Identification of the connection between mosquito physiology or behavior and blood group preference can be useful for national vector control programs conducting vector control to effectively to control *Aedes*-transmitted diseases in Türkiye. Also, the link between dengue incidences and ABO blood group distribution in the humans is vital to infer the relationship of asymptomatic and symptomatic dengue infections in different individuals. On the contrary, Khan et al.[33] showed that *An. stephensi* displayed a strong preference for individuals with B blood group when on the search for hosts. An *in vivo* study Anjomruz et al.[24] demonstrated that *An. stephensi* species however prefers AB blood type. It was then suggested that mosquito behavior is mediated by semiochemicals and that possibly the first females who came into contact with AB-type blood individuals produced an aggregation pheromone that attracted other mosquitoes to that same host.

Individuals with blood groups more preferred by vectors are more prone to be bitten and should be protected more than others if vectors have specific ABO host choice. The most preferred blood type of *Ae. albopictus* is O according to the current analysis. The fecundity of these mosquitoes was not affected by the blood type. The existence of different factors that influence mosquito human blood preferences is a fact, however, this topic still needs more conclusive data that could be used to prevent diseases transmitted by these vectors. Also, if more studies are carried out on the blood meal preference of other mosquito species, personal protection will be easier. Since personal protection is one of the effective control measures to prevent the diseases that this species is vector, it will be very important to choose the blood group of female mosquitoes in personal protection measures against mosquito bites.

Availability of Data and Materials

All data sets collected and analyzed during the current study are available from the corresponding author (F. B.) on reasonable request.

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Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

F. B. and F. M. S. designed and planned the work. F. B. drafted the experiment and analyzed data. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

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