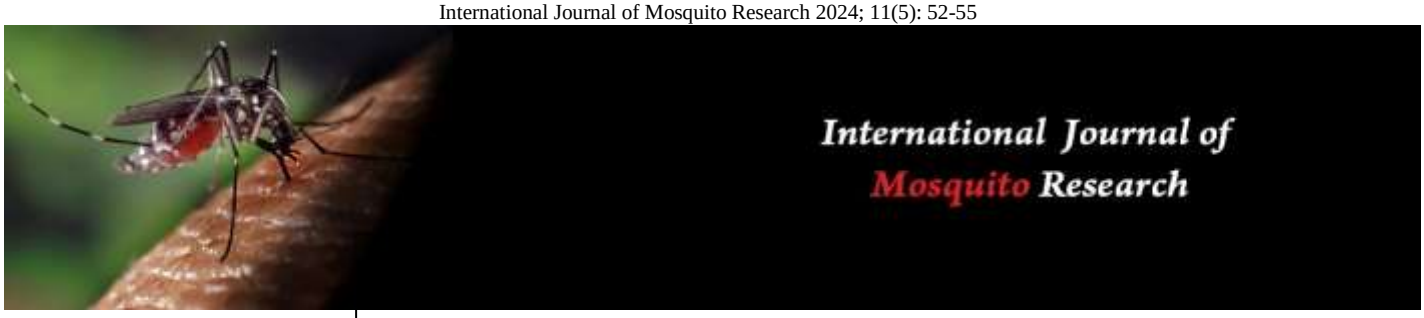

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Dr. Bindiya Ghedia
Assistant Professor, Department of Microbiology, Smt. B. K. Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth deemed to be University, Piparia, Vadodara, Gujarat, India

Dr. Manish R Shah
Professor, Department of Orthopaedics, Dhiraj Hospital, Smt. B. K. Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth Deemed to be University, Waghodia, Vadodara, Gujarat, India

Corresponding Author:
Dr. Manish R Shah
Professor, Department of Orthopaedics, Dhiraj Hospital, Smt. B. K. Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth Deemed to be University, Waghodia, Vadodara, Gujarat, India

A study to identify the preferential feeding of *Ae. aegypti* on human ABO blood groups and their impacts on fecundity

Dr. Bindiya Ghedia and Dr. Manish R Shah

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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: Separate membrane feeders exposed laboratory-reared female *Ae. aegypti* mosquitoes to all four blood types. After feeding, mosquito blood DNA was collected and identified using ABO genotyping PCR. Fecundity was measured by egg batch mean egg count.
Results: Amplification of the 334bp fragment in the human DNA detection PCR revealed 100 female blood-fed mosquitoes among 205 permitted to feed. The ABO genotyping PCR found blood classes A (18), B (19), AB (25), and O (30). Eight samples also had numerous blood meals. The mean number of eggs deposited per female was evaluated using one-way ANOVA with 0.05 significance. No blood group had substantial fecundity differences.
Conclusion: Understanding *Ae. aegypti*'s affinity for blood type O led to the development of a novel adult mosquito attractant trap that can combat pesticide resistance. Blood type O persons have an increased risk of contracting vector-borne illnesses transmitted by *Ae. aegypti* due to more vector bites.

Keywords: *Aedes aegypti*, dengue, ABO blood groups, feeding behaviour

Introduction
Female mosquitoes are recognized as infamous, violent, and lethal external parasites that feed on the blood of many vertebrate creatures, including mammals. Phylum: Chordata; Class: Mammalia; Class: Aves (Birds); Class: Reptilia (Reptiles, including crawlers and creepers); Clade: Batrachomorpha (Amphibians); Phylum: Chordata, Subphylum: Vertebrata, Class: Pisces (fishes). Blood meals are essential for supplying female mosquitoes with the requisite dietary proteins and amino acids necessary for egg formation and maturation [1, 2].
The reaction of mosquitoes to their hosts involves three critical stages: the activation phase, the orientation phase, and the alighting phase [3, 4]. It is significant to note that the emerging trend in Europe involves the adoption of new agricultural practices aimed at enhancing rural housing conditions for humans and establishing distinct animal enclosures, such as pens and barns, to shelter livestock and pigs away from human residences. This has resulted in the shift of mosquito vectors, which exclusively feed on human blood and are responsible for transmitting malaria pathogens, to feeding on domesticated animals [5].
The cultivation of mosquitoes is significant for both theoretical and practical studies. A substantial body of research has been undertaken on mosquitoes and mosquito-borne illnesses, necessitating a continuous supply of laboratory-reared insects. Certain research, such the assessment of pesticide resistance, the cultivation of mosquito diseases for vaccine development, or the enhancement of insect population control techniques, need a rapid approach to growing laboratory populations [6-10]. Maintaining mosquitoes in laboratory settings is a tough endeavor, with the primary responsibility being the provision of blood meals, which is essential for two critical processes in female mosquitoes: oogenesis and egg formation. *Ae. aegypti*i have a strong preference for humans among many host species, including dogs, cats, rodents, bovines, porcines, and avians, in both rural and urban environments, where humans are typically the most prevalent and reliably accessible hosts [11].

The objective of this study was to determine the preferred feeding habits of *Ae. aegypti* on human ABO blood types and their effects on fertility.

Materials and Methods

Laboratory-reared female *Ae. aegypti* mosquitoes were simultaneously exposed to all four blood groups using distinct membrane feeders. Following feeding, the DNA from the blood of mosquitoes was collected and analyzed by ABO genotyping PCR. Fecundity was assessed by the average quantity of eggs in a single egg batch. A colony of *Aedes aegypti* mosquitoes was developed at the Department of Zoology from a single engorged wild specimen.

The mosquitoes in the colony were kept under a 12:12 (light: dark) cycle, at a constant temperature of 28 °C and 80% relative humidity.

Mosquitoes were cultivated under standardized circumstances to produce uniformly sized individuals.[12] Adult mosquitoes were contained in mosquito cages of 15 × 15 × 30 cm, equipped with mesh screening on the top, and supplied with a 20% sugar solution and water ad libitum. The eggs produced by the female mosquitoes of the twelfth generation of the colony were used in the present investigation.

Mosquito Rearing and Maintenance

The mosquito breeding was conducted in the insectary. Water was boiled to remove oxygen and subsequently transferred into 250 ml glass bottles. The bottles were let to reach room temperature with loosely secured lids. The eggs of female *Ae. aegypti* taken from the colony were immersed in deoxygenated water to facilitate hatching [13].

One-day-old larvae were transferred to the water-filled enamel trays. Larvae were fed with commercially available fish feed at a dosage of 0.32 mg per larva until the fourth instar stage. Trays were examined daily for pupae, which were then segregated into plastic cups and moved to adult cages for the emergence of adults.

Following the emergence of adults, 100 mosquitoes (50 males and 50 females) were moved to six new cages (15 x 15 x 30 cm), which were covered with a net, using a mouth aspirator. A constant sex ratio of 1: 1 was preserved in each cage to facilitate the mating of all females [14]. A 10% sucrose solution-soaked cotton pads were placed above the cage as a feeding source for the adults [15]. The insectary maintained a constant temperature of 28 °C and relative humidity of 80%.

Blood Feeding of Mosquitoes

50 female mosquitoes, five days old, were placed in a mesh-topped 20 x 20 x 20 cm cage and starved for 16 hours without food or water [15].

Artificial membrane feeders fed blood. A water circulation system and water-jacketed glass feeders comprised the membrane feeding system. The blood sample groups were determined using commercial murine monoclonal anti sera. Four glass feeders had parafilm (Marrifield, USA) membrane bottoms. System flowing water was 37 °C. Feeding employed four glass feeders with 3 cc of human blood types A, B, AB, and O. Blood feeders were attached to the water circulation system for 30 minutes to equalize blood and water temperatures. Mosquitoes fed for one hour. Engorged mosquitoes were frozen immediately after feeding to harvest DNA. So all 205 female mosquitoes could mate, they were fed individually in six cages.

Fecundity of *Aedes aegypti* Engorged Females with Four Blood Groups

Male and female mosquitoes of generation F32 were employed in the experiment. Newly emerging adult mosquitoes were put into four adult cages (15 x 15 x 30 cm). Mate for five days with 20% sucrose-soaked cotton pads as food. Females were placed in 50-female adult cages after five days. Then the female mosquitoes in all four cages were fasted for 24h without providing any food or water. Circulating water system of artificial membrane feeder was attached to four glass feeders (One feeder for one cage) and filled with four distinct kinds of blood. One hour was allowed for mosquitoes to feed.

Statistical analysis

Statistical comparison of the data was carried out using One-way analysis of variance (ANOVA) in SPSS version 22. P-value was set at 0.05.

Results

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

Blood group	Number of blood fed Mosquitoes	Mean percentage (%) ±Standard deviation
A	18	19.51±2.38
B	19	18.32±3.67
AB	25	24.96±2.28
O	30	32.78±3.00
Multiple meals	8	7.43±3.57
Total	100	

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

Blood type	Blood fed females out of 205 total mosquitoes	No. of females -Egg laid	Mean no. of eggs/female (± SD)
A	178	175	46.054±7.833
B	170	168	43.187±6.344
AB	172	170	45.465±4.046
O	180	170	47.233±7.643

Amplification of the 334bp fragment in the human DNA detection PCR revealed 100 female blood-fed mosquitoes among 205 permitted to feed. The ABO genotyping PCR found blood classes A (18), B (19), AB (25), and O (30). Eight samples also had numerous blood meals.

The mean number of eggs deposited per female was evaluated using one-way ANOVA with 0.05 significance. No blood group had substantial fecundity differences.

Discussion

Aedes aegypti, primary vector of Dengue, Yellow fever and Chikungunaya is presently spread across the tropics and a variety of subtropical areas such as South-Eastern United States, the Middle East, Southeast Asia, the Pacific and Indian

Islands and Northern Australia [19]. Dengue virus is spread via the bites of pathogenic *Ae. aegypti* mosquitoes. Following copulation, female *Ae. aegypti* requires a vertebrate blood meal to obtain the necessary nutrients for their reproductive success [20]. Therefore, the selection of an optimal blood source is crucial for their reproductive success. Therefore, blood feeding habits of the mosquitoes give crucial information on disease transmission by biting of mosquitoes and might be valuable in efficient vector control measures.

Of the 205 mosquitoes permitted for feeding, 100 individual females were identified as blood-fed mosquitoes through the amplification of the 334bp fragment in the human DNA detection PCR. In the ABO genotyping PCR, blood types A (n= 18), B (n=19), AB (n=25) and O (n=30) were found. Furthermore, eight samples were identified as having ingested multiple blood meals. The fecundity experiment demonstrates no significant difference in blood feeding success and subsequent oviposition success among four blood groups, when they were offered various kinds of blood supplies independently. This indicates that when presented with a single option, feeding rates were superior to those with multiple options. This might be attributed to the demand of additional time duration for selection over diversity of sources to collect the appropriate quantity of most suited blood meal for their reproductive success. This indicates that the selection of blood meals fluctuates based on the availability of sources and temporal factors. Culex species exhibit variable selection of blood sources based on availability and mosquito density [21]. The current study sustained a consistent mosquito density throughout the duration of the investigation.

The average number of eggs laid per female was analyzed using a one-way ANOVA test at a significance level of 0.05. There was no substantial variation in fecundity for various blood types. Even though this mosquito favors O blood type, no affect demonstrated on fertility. Selection and blood feeding on human blood may be mainly based on the availability, simple access and easy to feed. Moreover, results showed that although the blood group type O was more favoured than others, considerable ingestion of other blood groups were also observed. The von Willerbrand factors (VWFs) are glycoproteins that are involved in blood haemostasis. The high VWF amounts are responsible to high thrombotic activities which result in blood coagulation.[22] ABO blood types possess varied VWF levels in the blood plasma. Blood group type O individuals are known to possess 20% to 30% lower VWF levels compared to non-blood group type O individuals [23].

The differences among the blood groups are related with variations in oligosaccharides in structures present on erythrocyte membrane. Antigen A has an additional N-acetylgalactosamine terminal which is absent in other two antigens. Antigen B is composed with an excess glycan molecule compare to antigen O and A. Although there are differences in terms of oligosaccharides in erythrocyte membranes, the plasma composition differences among the blood groups is under explained. Few studies have been carried out to explain the role of carbohydrate in egg production. However there is no reproductive success other than the long lifespan in *Ae. aegypti* mosquitoes [24].

Conclusion

Understanding *Ae. aegypti*'s affinity for blood type O led to the development of a novel adult mosquito attractant trap that can combat pesticide resistance. Blood type O persons have an increased risk of contracting vector-borne illnesses transmitted by *Ae. aegypti* due to more vector bites. Personal protection measures may not prevent mosquito bites. If the preferred blood type and mosquito physiology or behavior are linked, national vector control efforts may modify the vector population to reduce dengue's severe impact. Additionally, the association between dengue incidence and ABO brood group distribution in humans is necessary to extrapolate symptomatic and asymptomatic dengue infections to specific blood types.

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