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Research Article

Entomological Surveillance and Alternate Methods for Species Identification of *Aedes* in a Dengue-endemic District of Punjab, India

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I N F O

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A B S T R A C T

Background: Entomological surveillance is a vital component of vector control programmes as it helps to determine the geographical distribution, density, and species of vector populations in areas at risk of arboviral diseases. Moreover, calculating *Stegomyia* indices is an integral component of *Aedes* surveillance in areas which are at high risk of dengue.

Methodology: In the year 2018, entomological surveillance was carried out in a high dengue endemic district of Punjab to identify the different species of *Aedes* through morphological identification on the basis of hypopygium. For this, all artificial and natural containers holding stagnant water were inspected for the presence of immature larvae. The larval indices were also recorded.

Results: Aedes breeding was found to be high in urban areas as compared to rural areas. Based on the differences in their morphological characteristics as well as hypopygium structure, three different species of *Aedes* were identified: *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus*. Among the three species, the most common species was *Ae. aegypti*. A positive correlation was recorded between dengue cases and *Stegomyia* indices.

Conclusion: This is the first study which has recorded the presence of species *Ae. vittatus* in a district of Punjab, India. The present results indicate that for the identification of damaged specimens, other morphological characteristics must be explored.

Keywords: Surveillance, Identification, Hypopygium, *Aedes*

Introduction

Dengue is an arboviral vector-borne disease and as per a WHO report, the global incidence of this disease in the last five decades has increased up to 30-fold and it is the most rapidly growing arbovirus infection in tropical and subtropical regions.[1,2] Entomological surveillance is a vital component for monitoring vector-borne diseases and forming baseline data by studying the *Stegomyia* indices throughout the year which helps in forming a strategic plan of action in dengue risk areas.[3] Moreover, the other most important objective of entomological surveillance is to identify the species of *Aedes*, its geographic distribution and seasonal prevalence in areas at risk of dengue and this is a prerequisite step for accessing its vectorial capacity in arboviral disease transmission.[4] The most common method for the identification of species of mosquitoes is following the standard morphological keys developed on the basis of external morphological characteristics and distinguishing features of different species but it can become difficult in case of damaged specimens and following the developed molecular technique is not possible for vector control programmes as it is not cost-effective and requires well-equipped laboratories.[5] Thus, the most reliable and economically feasible method for morphological identification is to study and identify the species-specific structural features of the genitalia of mosquitoes. The genital features of male and female mosquitoes are a modified structure beyond the eighth abdominal segment which differs considerably among the different genera.[6,7] Therefore, it facilitates the correct identification of the sibling, new species, and damaged specimens of mosquitoes.[6] Keeping in mind the above points, the present study was planned in a dengue-endemic district of Punjab state, India to develop an easy, accurate and economic method for rapid identification of *Aedes* mosquitoes.

Materials and Methods

Study Area

The selected district, S.A.S. Nagar, is situated next to Chandigarh, a union territory. It has a subtropical climate with hot summers, cold winters and mild to average monsoons. This district is divided into three tehsils: Dera Bassi, Kharar, and S.A.S. Nagar.

Entomological Surveillance

In the year 2018, house-to-house entomological surveillance (257 houses in the urban area and 277 houses in the rural area) was carried out for larval collections in different areas of district S.A.S. Nagar, Punjab, India. For immature collections, all kinds of breeding habitats (indoor and outdoor) were examined. They were reared in standard conditions in the laboratory at NIMR Field Site, S.A.S. Nagar, Punjab till the emergence of adults. All the adults of *Aedes* were identified up to the species level by following standard morphological keys.[8,9]

Hypopygium Preparation

On the basis of morphological characteristics, male mosquitoes were separated from females. To separate the hypopygium, both male and female mosquitoes were dissected under a stereomicroscope, the last few segments of the abdomen were snipped off and the specimens were kept for 15 minutes in 90% alcohol. After that, the hypopygium was transferred to a test tube containing 10% potassium hydroxide (KOH) and was slowly heated over a Bunsen burner up to nearly the boiling point. Then, the hypopygium was washed for 2-5 minutes with a few drops of distilled water and was mounted in Hoyer's medium. The slides were examined under a compound microscope with 40 X magnification.[6]

Data Analysis

The different larval indices *viz.* House index (HI), Container index (CI), and Bretaeu Index (BI) of district S.A.S. Nagar were recorded month-wise as per standard WHO guidelines.[10]

Results

From the entomological surveillance, a total of 257 and 277 houses were searched for *Aedes* breeding from urban and rural areas, respectively. From the total houses surveyed, 78 houses from urban and 53 houses from rural areas were found positive for *Aedes* breeding. The container positivity was found to be high in urban areas i.e., 16.2% as compared to 11% in rural areas. Moreover, the analysis of *Stegomyia* indices *viz.* HI, CI and BI showed that during the monsoon period i.e., July to September, the breeding of *Aedes* was significantly high (Table 1) which positively correlated with the dengue cases in the same period (Figure 1).

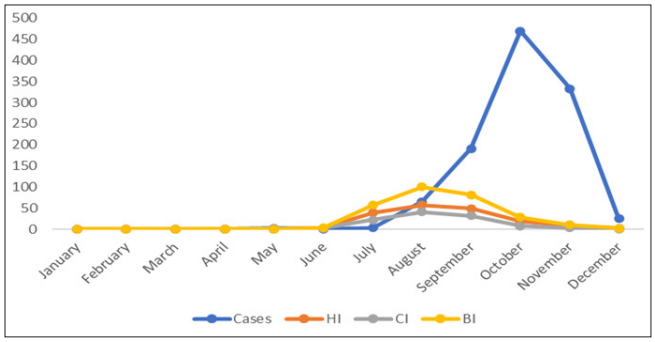

Figure 1.Trend of Dengue Cases and Larval Indices Month-wise in District S.A.S. Nagar, 2018

HI: House Index, CI: Container Index, BI: Bretaeu Index

From the surveillance, three species of *Aedes* were recorded *viz. Ae. aegypti, Ae. albopictus,* and *Ae. vittatus* (Figure 2). All three species were identified on the basis of their distinct morphological features as *Ae. aegypti* has a white lyre shape

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while *Ae. albopictus* has a median white line and *Ae. vittatus* has three pairs of small white distinct spots on the dorsal side of the thorax (Figure 2). *Ae. aegypti* was recorded as the most prevalent species with 25.9% prevalence in comparison to *Ae. albopictus* with 4.5% and *Ae. vittatus* with 2.1%. Thereafter, for their correct identification and confirmation of species, along with morphological characteristics, the slides of hypopygium were also prepared for all three species. All the morphologically identified species on the basis of taxonomic keys were further identified on the basis of differences in their hypopygium structure. It was observed that in male *Ae. aegypti,* paraproct of hypopygium (Figure 3a) has a well-developed ventral arm whereas, in male *Ae. albopictus* (Figure 3b), the hypopygium consists of a spoon-shaped style with an appendage arising near the tip, and the crown of the paraproct also has a series of hairs arising from the single plane. On the other hand, the hypopygium in male *Ae. vittatus* (Figure 3c) is distinct from all other *Aedes* species as the style carries preapical curved appendages and is much enlarged at the extremity.

In the female genitalia, no such conspicuous difference was observed. In all three species, a postgenital lobe and a pair of cerci were present with short and long setae (Figure 4).

In the present study, to implement these findings in the field, in entomological training conducted by NCVBDC, Punjab, the entomologists were tested to identify the male *Aedes* mosquitoes of these three species on the basis of their external morphological characteristics as well as from their distinctive features of hypopygium.

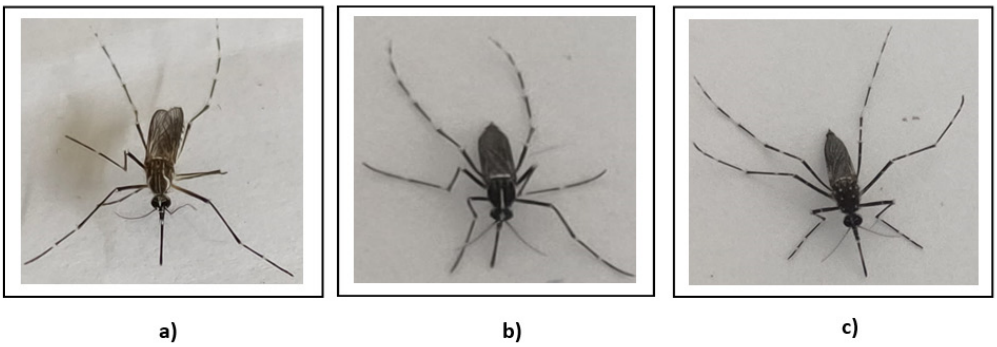

Figure 2.Different Species of *Aedes* collected from District S.A.S. Nagar. a) *Ae. aegypti* b) *Ae. albopictus* and c) *Ae. vittatus*.

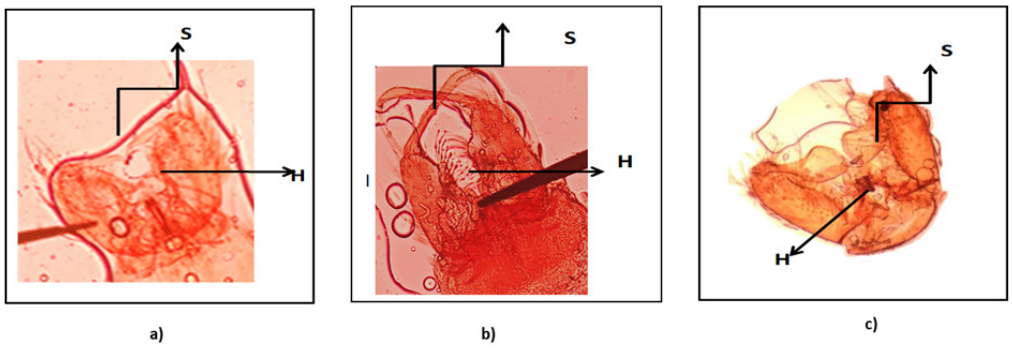

Figure 3.Diagrammatic Representation of Male Hypopygium of Different Species of *Aedes* (a).*Ae. aegypti* (b).*Ae. albopictus* (c).*Ae. vittatus* (S: Style, H: Hypopygium)

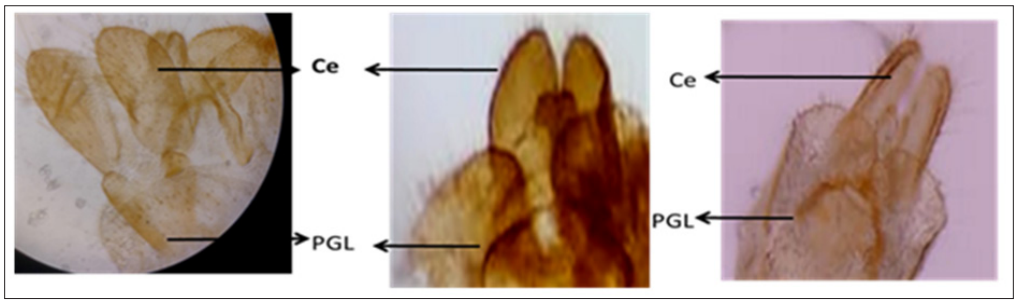

Figure 4.Diagrammatic Representation of Female Genitalia of Different Species of *Aedes* (a).*Ae. aegypti* (b).*Ae. albopictus* (c).*Ae. vittatus* (Ce: Cerci, PGL: Postgenital Lobe)

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Table 1.Month-wise Larval Indices of District S.A.S. Nagar, Punjab, 2018

Larval Indices	January	February	March	April	May	June	July	August	September	October	November	December
HI	0.00	0.06	0.14	0.21	0.7	2.27	38.75	57.36	49.06	18.49	7.75	2.09
CI	0.00	0.02	0.09	0.16	0.41	1.85	21.55	41.25	31.65	7.76	3.39	0.85
BI	0.00	0.06	0.14	0.3	0.77	2.99	56.58	100.56	81.23	28.16	10.22	2.43

HI: House Index, CI: Container Index, BI: Bretaeu Index.

Discussion

Aedes mosquitoes have immense medical importance as they act as prominent vectors for arboviral diseases like dengue, chikungunya and Zika. The control of these arboviral diseases mainly relies on vector control interventions. The key step for the vector control programme is the accurate identification of species of mosquitoes prevalent in any area.[6] Many morphological characteristics are included in the identification of species of mosquitoes such as colour of thorax, scales on legs, banding pattern on legs and wings etc. though there are some other distinguishable characteristics also which can be used for the identification as they are not lost in transportation and storage.[5,11]

In India, very few studies have been conducted on the comprehensive analysis of male and female genitalia of *Aedes* species. In this study, an effort has been made to understand the anatomical differences in male and female genitalia of *Aedes* species detected in the study areas. The specific structure of male hypopygium connected to the last three abdominal segments differs greatly among distinct species of *Aedes* i.e., *Ae. aegypti, Ae. albopictus* and *Ae. vittatus* because anatomical differences of mosquito species are more prominent in male hypopygium. The anatomical feature "male genitalia" remained the most reliable characteristic for identification up to the species level as it is less exposed to harm/ destruction. Among several species of mosquitoes, anatomical differences are very distinct in male hypopygium as compared to larval and female characteristics.[12] Various studies are in agreement with the present observation where male and female genitalia characteristics were reported. This is the first study in Northern India where the male and female genitalia of *Ae. vittatus* was also studied.[6,13] In this study, entomological surveillance was planned in a dengue-endemic district of Punjab. The container positivity was recorded to be more in urban areas as compared to rural areas. Moreover, three species of *Aedes* were recorded, though *Ae. aegypti* was the most prevalent in comparison to the other two species *viz. Ae. albopictus* and *Ae. vittatus*. The present findings are in concordance with a previous study of Punjab where *Ae. aegypti* was recorded as the most prevalent species.[14] Similarly, another study also recorded three species and *Ae. aegypti* was again the most prevalent species as compared to *Ae. albopictus* and *Ae. vittatus*.[15] Moreover, *Aedes* breeding was found to be higher in those areas where dengue cases were reported to be high in the last five years on the basis of the calculation of all *Stegomyia* indices. In our findings too, the *Stegomyia* indices have shown a positive correlation with dengue cases. Thus, more such studies should be carried out to study the correlation of *Stegomyia* indices with dengue cases so that an early warning system can be developed to reduce the havoc created by these arboviral infections.

To validate the use of genitalia in the identification of different species of mosquitoes, in future, more studies should be carried out in different locations and comparisons must be made among other species which will help to generate confidence and accuracy in this taxonomic tool.

Conclusion

This study highlighted the importance of identifying the species of mosquitoes in areas where a continuously high number of dengue cases are being reported as identification of vector species is the prime thing in any vector control programme. More similar studies should be planned to explore the morphological characteristics for the identification of species so that broken specimens can be easily identified.

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Conflict of Interest Statement

The authors declare that they have no known competing interests.

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