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A study to assess the *Aedes* mosquto diversity and the different mosquito genera in a rural area in Thrissur, Kerala

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ABSTRACT

Background: Mosquito is one of the leading causes of vector borne causing infections leading to morbidity and mortality worldwide. It is the reason for spreading various viral, parasitic infections to humans. India has favorable climatic and environment conditions for mosquito breeding and in addition to these, influence of human activity has been significant in the increased breeding of mosquitoes. The objective of the study was to determine the various artificial containers which led to the *Aedes* mosquito larvae breeding and to understand the various mosquito genera in a rural area in Kerala.

Methods: A cross sectional study was conducted over a period of 4 months September, October, December and January in a ward of Kaiparambu Panchayath, Thrissur. A house-to-house survey was done where every water holding containers outdoor and indoor of each house was inspected, looked for larvae, collected and examined. Adult mosquitoes were also collected from the premises.

Results: Total house examined were 520 over four months and positive containers were 271. The House index, Container index and Breteau index for the 4 months were moderate to high risk level. Maximum breeding was seen in plastic containers and the most common species identified during larval and adult stages from indoor and outdoor were *Aedes albopictus*.

Conclusions: Mosquitoes are the main risk for various vector borne diseases. Influence of manmade habitats has been significant in the increase of mosquito diversity. By educating the people, appropriate waste management and reducing use of plastics can to extent reduce the problems associated.

Keywords: Aedes aegypti, Aedes albopictus, Artificial container, Larvae breeding, Mosquito

INTRODUCTION

Two winged flying mosquitoes under family Culicidae of the order Diptera are considered as the most important vectors for transmission of various protozoan and viral pathogens which effects human health. Consisting of approximately 3500 species, the mosquitoes are considered to transmit serious infections through their bites causing public health problem.¹

The various infections caused by mosquitoes is listed in Figure 1. WHO has listed dengue among ten diseases as

potential threat for 2019 and outbreaks in many countries has confirmed this observation.²

The spatial distribution and density of mosquitoes are influenced by the climate and topographic features in addition to the changes brought about humans to the environment.³ Even though rainfall, humidity and temperature influence the increase in mosquito density, human habitats have got major role where water clogging has increased which led to increased breeding of mosquitoes.

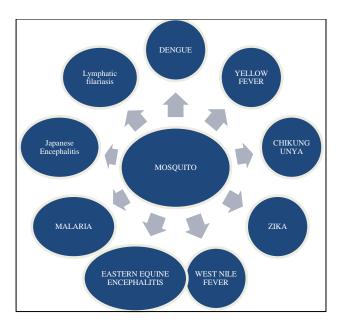


Figure 1: Various mosquito borne infections.

Kerala the southernmost part of India has favourable topographic and climatic condition for the mosquitoes.⁴ First incidence of dengue epidemic in Kerala at 2003 with 3546 cases and 68 deaths.⁵ Since then, dengue cases have been increasing over the years with presence of multiple serotypes.⁵ In 2017, peak of dengue cases reported in Kerala with 9104 cases with 115 deaths.⁶ Chikungunya was also seen in Kerala with epidemic twice in 2006 and 2007. Japanese encephalitis also seen in some districts of Kerala.⁷ Around 118 species of mosquitoes have been recorded from Kerala.

The various mosquito infections reported in Kerala 2019 is summarized in Table 1.8-15 Many studies are there regarding vector indices, but importance of various man causes water collection which have led to mosquito breeding is not stressed into. The primary objective of the study was to determine the various artificial containers which led to the *Aedes* mosquito larvae breeding and secondary objective was to understand the various mosquito genera in a rural area in Kerala.

| Mosquito | Disease | Cases in Kerala | Deaths in Kerala | Cases in Thrissur | Deaths in Thrissur |
|-----------|-----------------------|--------------------|---------------------|----------------------|-----------------------|
| Aedes | Dengue fever | 4651 | 14 | 113 | - |
| | Chikungunya | 109 | - | 2 | - |
| Culex | Japanese encephalitis | 11 | 2 | 3 | 0 |
| | West Nile fever | 11 | 2 | 0 | 0 |
| Anopheles | Malaria | 656 | 1 | 76 | 0 |

METHODS

Cross sectional study design, done in Kaiparambu Panchayath which is a rural area in Thrissur district, Kerala, over four months of September, October, December 2019 and January 2020.

Study procedure

Non probability convenience sampling technique was used. Series of house survey was done as part of field practice area of Amala Rural Community Health (ARCH) programme, Amala Institute of Medical Sciences, Thrissur which was led by a team formed under the department of Community Medicine of Amala Institute of Medical Sciences, Thrissur. The team consisted of undergraduate students, post graduates, health inspector, medical social worker and entomologist. Conducted a house-to-house survey in two wards (ward 9 and 10) out of the 18 wards of Kaiparambu Panchayath and covered a house approximately 120 houses every four months.

Standard entomological techniques have been used for the study. Our team were split to cover the houses in two days per month in the respective wards between 7:00 to

10:00 am. The tools used included a survey form, pipettes, plastic bottles, test tubes, cotton and a flashlight. After getting the consent from the households of the house, the premises and inside of the house were meticulously searched for: 1) mosquito larvae breeding areas both natural and man cause habitat collections and also for any other potential containers, 2) mosquitoes inside and outside house and vicinity.



Figure 2: Barraud cage.

Larvae collected into cylindrical vessel or half broken plastic container using dipping and pipetting method and the larval collections were recorded in predesigned survey forms. Adult mosquitoes were captured at rest by using test tubes. Larvae and adult forms collected brought to entomological lab of our department and species identified. Larvae species identified using taxonomic key. The species larvae in doubt are then transferred into Barraud cage where they are reared for 7-10 days and the adult ones formed are dissected for species identification (Figure 2).

Larval indices analysed by calculating House index, Container index and Breteau index over four months and the trend was analysed.

With the help of entomologist of our department the mosquito genera and species were identified.

Data analysis

Descriptive analysis was done manually to calculate mosquito larval indices and the proportion of different types of containers.

Ethical approval

Institution ethics were not taken as the data collected from respected households were part of the field practice area of the ARCH programme of Amala Institute of Medical Sciences, Thrissur.

RESULTS

A total of 515 houses were surveyed over a period of four months; a minimum of 121 houses were covered each month (Table 2). Of these houses, 170 houses were found to be infested. A total of potential containers (both wet and dry) were identified, of which 2136 contained water. Of these, 271 were total positives for larval breeding. Table 3 shows the different potential containers outdoor and indoor respectively. Number of wet containers during the months September 2019, October 2019, December 2019 and January 2020 were 807, 680, 200 and 449 respectively and of these 93 (12.5%), 120 (17.6%), 24 (12%) and 26 (6%) of the respective months were found to have larval breeding. The containers were emptied and placed upside down.

Table 2: List of dry, wet and positive containers.

| | September 2019 | October 2019 | December 2019 | January 2020 |
|---------------------------------|----------------|--------------|---------------|--------------|
| Total number of houses surveyed | 126 | 136 | 126 | 132 |
| Number of infested houses | 61 | 72 | 17 | 20 |
| Number of dry containers | 219 | 510 | 240 | 576 |
| Number of wet containers | 807 | 680 | 200 | 449 |
| Number of positive containers | 93 | 120 | 24 | 26 |

Table 3: Outdoor containers-man-made and natural.

| | | September 2019 | October 2019 | December 2019 | January 2020 | |
|------------------|----------|----------------|--------------|---------------|--------------|--|
| | Wet | 191 | 25 | 8 | 62 | |
| Coconut shell | Dry | 54 | 33 | 26 | 160 | |
| | Positive | 7 | 10 | 1 | 5 | |
| | Wet | 72 | 109 | 0 | 13 | |
| Coconut husk | Dry | 31 | 115 | 0 | 18 | |
| | Positive | 8 | 4 | 0 | 0 | |
| | Wet | 23 | 65 | 5 | 32 | |
| Egg shell | Dry | 6 | 63 | 3 | 59 | |
| | Positive | 2 | 12 | 0 | 0 | |
| | Wet | 21 | 34 | 12 | 4 | |
| Tyre | Dry | 8 | 25 | 30 | 22 | |
| | Positive | 2 | 6 | 0 | 0 | |
| | Wet | 12 | 1 | 0 | 16 | |
| Can | Dry | 7 | 0 | 3 | 4 | |
| | Positive | 2 | 1 | 0 | 7 | |
| | Wet | 3 | 0 | 0 | 0 | |
| Pipe | Dry | 0 | 0 | 0 | 0 | |
| | Positive | 0 | 0 | 0 | 0 | |
| | Wet | 2 | 0 | 0 | 0 | |
| Tank | Dry | 0 | 0 | 0 | 0 | |
| | Positive | 0 | 0 | 0 | 0 | |
| | Wet | 1 | 2 | 0 | 0 | |
| Unused fish tank | Dry | 0 | 1 | 0 | 0 | |
| | Positive | 0 | 1 | 0 | 0 | |

Continued.

| | | September 2019 | October 2019 | December 2019 | January 2020 |
|----------------------------|-----------------|----------------|--------------|---------------|--------------|
| | Wet | 95 | 19 | 3 | 15 |
| Flower pot | Dry | 19 | 21 | 6 | 58 |
| • | Positive | 10 | 2 | 0 | 0 |
| | Wet | 37 | 16 | 14 | 20 |
| Earthen pot | Dry | 22 | 17 | 14 | 6 |
| • | Positive | 3 | 3 | 3 | 3 |
| | Wet | 0 | 6 | 0 | 0 |
| Organic bag | Dry | 0 | 0 | 0 | 0 |
| | Positive | 0 | 0 | 0 | 0 |
| Utensils (steel/aluminium/ | Wet | 45 | 0 | 26 | 32 |
| copper/iron) | Dry | 15 | 0 | 14 | 14 |
| соррегинопу | Positive | 7 | 0 | 3 | 1 |
| Glass jar/ plate broken/ | Wet | 12 | 57 | 0 | 3 |
| bulb/tube/medicine bottle | Dry | 2 | 29 | 0 | 28 |
| buis/tuse/medicine socie | Positive | 3 | 3 | 0 | 1 |
| | Wet | 5 | 6 | 5 | 15 |
| Drum | Dry | 0 | 0 | 2 | 6 |
| | Positive | 2 | <u> </u> | 4 | 2 |
| Tile piece | Wet | 41 | 9 | 0 | 0 |
| | Dry | 9 | 11 | 0 | 3 |
| | Positive | 3 | 0 | 0 | 0 |
| C . 1. | Wet | 1 | 2 | 0 | 0 |
| Grinding stone | Dry | 0 | 0 | 0 | 0 |
| | Positive | 0 | 0 15 | 0 | 0 |
| 7D 1: 1 | Wet | 19 | | 4 | 3 |
| Tarpolich | Dry | 2 | 20 | 14 | 4 |
| | Positive | 5 | 3 | 0 | 1 |
| Asbestos sheet | Wet | 0 | 2 2 | 0 | 0 |
| Aspestos sneet | Dry Positive | | 1 | 0 | 0 |
| | Wet | 6 | 1 | 0 | 0 |
| Broken wood piece/chair | Dry | 0 | 0 | 0 | 0 |
| broken | Positive | 1 | 0 | 0 | 0 |
| | Wet | 6 | 0 | 0 | 0 |
| Shoes | Dry | 1 | 0 | 0 | 0 |
| Silves | Positive | 1 | 0 | 0 | 0 |
| | Wet | 20 | 70 | 2 | 31 |
| Plastic bottles | Dry | 8 | 52 | 12 | 57 |
| Tastic bottles | Positive | 1 | 9 | 0 | 0 |
| | Wet | 9 | 27 | 0 | 2 |
| Plastic cover | Dry | 0 | 3 | 0 | 4 |
| | Positive | 1 | 6 | 0 | 0 |
| | Wet | 139 | 81 | 41 | 107 |
| Plastic bucket | Dry | 24 | 28 | 49 | 84 |
| | Positive | 22 | 18 | 5 | 3 |
| | Wet | 19 | 116 | 45 | 47 |
| Plastic can/paint can/ | Dry | 4 | 85 | 65 | 42 |
| container/mug/cup/ vessel | Positive | 4 | 38 | 6 | 3 |
| | Wet | 0 | 10 | 0 | 0 |
| Toys | Dry | 7 | 14 | 0 | 0 |
| | Positive | 0 | 1 | 0 | 0 |
| | Wet | 0 | 1 | 0 | 0 |
| Mud plate | Dry | 0 | 0 | 0 | 0 |
| | Positive | 0 | 1 | 0 | 0 |
| Drainage water pit | Wet | 14 | 0 | 0 | 0 |
| | Dry | 0 | 0 | 0 | 0 |
| | Positive | 5 | 0 | 0 | 0 |
| | Wet | 2 | 0 | 0 | 0 |
| Pit | Dry | 0 | 0 | 0 | 0 |
| | Positive | 1 | 0 | 0 | 0 |
| | Wet | 3 | 3 | 0 | 2 |
| Leaf axis | Dry | 0 | 1 | 0 | 3 |
| | Positive | 0 | 0 | 0 | 0 |
| | | | | | |

Common positive containers included plastic containers, followed by coconut shells, egg shell, water tanks, tyres, earthen pot and tarpaulin sheets (Figures 3 and 4).

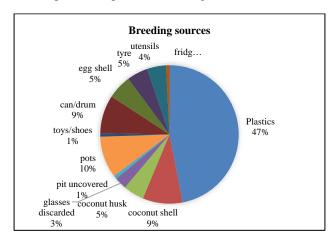


Figure 3: Different breeding sources.

For any locality, a House index >5 and/or a Breteau index >20 is an indication that the locality is dengue sensitive

and therefore adequate preventive measures should be taken. ¹⁵ House index calculated was high risk for all four months (48.41%, 52.95%, 13.49% and 15.15%). Breteau index calculated found high risk for months of September and October; moderate risk for December and January (Table 4, Figure 5).

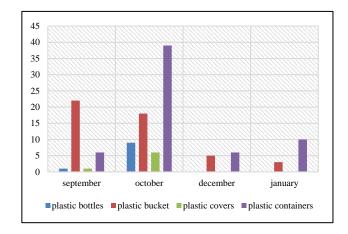


Figure 4: Mosquito breeding sites in plastics.

| | September 2019 | October 2019 | December 2019 | January 2020 |
|---|-------------------|-----------------|------------------|-----------------|
| Total number of houses surveyed | 126 | 136 | 126 | 132 |
| Number of infested houses | 61 | 72 | 17 | 20 |
| Number of dry containers | 219 | 510 | 240 | 576 |
| Number of wet containers | 807 | 680 | 200 | 449 |
| Number of positive containers | 93 | 120 | 24 | 26 |
| House index = $\frac{Total\ houses\ infested\ ^{\times}100}{Total\ no.of\ houses\ inspected}$ | 48.41% | 52.95% | 13.49% | 15.15% |
| Breteau index = Total positive containers 100 Total no.of houses inspected | 74 | 88 | 19 | 20 |
| Container index = Total positive containers * 100 Total no. of Containers inspected | 11.5% | 17.6% | 12% | 5.7% |

Table 4: List of dry, wet and positive containers.

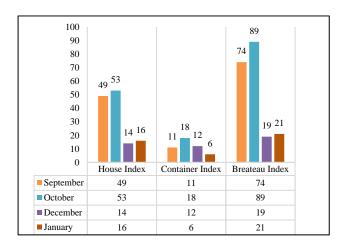


Figure 5: Trend in larvae indices over the four months.

Table 5 show the mosquitoes identified over the four months. The adult mosquitoes collected where *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, Anopheles and Armigeres, of which *Aedes albopictus* and *Culex quinquefasciatus* were seen more.

The larvae species identified during larval and adult stages collected from indoor and outdoor were *Aedes albopictus*, *Aedes aegypti* and *Culex quinquefasciatus*. *Culex quinquefasciatus* larvae and *Aedes albopictus* larvae were seen predominantly. It was interesting to note that mixed breeding such as *Aedes albopictus*, *Toxorhynchites* larvae and *Culex* larvae were seen in certain containers over the months of September and October. Adult *Toxorhynchites* mosquito also known as elephant mosquito, acts as a bio control agent against mosquito vectors. ¹⁶

| Genera of mosquitoes identified | | | | | | | | | | | | |
|---------------------------------|------|-----------|-------|-----|---------|-------|----------|----|---------|---|----|-------|
| Genera | Sept | September | | Oct | October | | December | | January | | | |
| | M | F | Total | M | F | Total | M | F | Total | M | F | Total |
| Aedes albopictus | 5 | 22 | 27 | 2 | 10 | 12 | 0 | 10 | 10 | 0 | 7 | 7 |
| Aedes aegypti | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 |
| Culex quinquefasciatus | 0 | 12 | 12 | 3 | 18 | 21 | 3 | 19 | 22 | 3 | 18 | 21 |
| Armigeres | 0 | 3 | 3 | 0 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anophelesstephensi | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 5: Different types of mosquitoes that were identified over the four months.

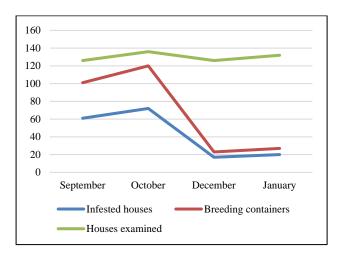


Figure 6: Trend in breeding over the four months.

DISCUSSION

Kerala is experiencing mosquito borne infection epidemic since 2003, the favourable climatic conditions and collection of artificial containers has led to the increase breeding in mosquitoes.

In this study, 520 houses were examined over the four months, more than 60% of the houses had positive breeding containers during September and October while 17-20% of the houses had positive containers during December and January showing a trend (Figure 5). Rainfall in Kerala was more in October 2019 than in September 2019 while less in December 2019 and least rainfall during January 2020.16 This shows the increased breeding after rainfall owing to the increased water collections in the discarded containers. This finding is similar to Vaz et al where high larvae indices was seen during monsoon season compared to post monsoon season.¹⁰ Heath et al also supports the fact.¹² Study done in 2018, Kerala showed high larvae indices during monsoon June and July 2018 and following floods which was in August 2018 with low larvae indices in September. 9,10 In our study dry containers were more in the month of January when there was no or scanty rainfall but these can later be a potential source for the breeding to occur if these containers remain as such till next rainy season.

Most common positive containers were plastics followed by coconut shell which is similar to studies done by Krishnadas et al and Jerome et al.^{3,11} Again study done in Trivandrum showed commonest breeding in plastics followed by metal container and mud pot.⁵

Vector density was high with *Aedes albopictus* in this study, it is supporting with the study done by Vijayakumar et al, a study done in 5 districts of Kerala.⁷

Aedes aegypti is associated closely with human habitation where artificial habitats are seen and predominantly in urban areas. It is usually, a day time biter. It breeds in clean water collections in tanks, containers, disposables, junk materials in domestic and peri domestic situations. Just one teaspoon of water is required for the Aedes mosquito to lay its eggs and for the larvae to develop into an adult mosquito. Female mosquito lay eggs in clean artificial collection of water and takes 7-8 days to complete life cycle. This is important as transovarial transmission of chikungunya and dengue viruses occurs.⁷ Reservoir responsible for dengue, chikungunya, vellow fever and Zika virus. Mostly infected female mosquitoes transmit the disease. Aedes albopictus equally distributed in urban and rural areas also plays a major role in mosquito disease transmission. It is highly adaptive and flexible in its behaviour transmission and hence has a secondary role in disease transmission of dengue, chikungunya and Zika virus.^{5,7}

Till recently dengue and chikungunya were the only reported cases in Kerala caused by *Aedes*, but the emergence of Zika virus infection on July-August 2021 in Trivandrum, Kerala with 65 reported cases shows the impending risk our state facing.¹³

Culex quinquefasciatus mosquito feeds on human hosts, mammalian or avian, is responsible for transmission of diseases like Japanese encephalitis, Bancroftian filariasis and West Nile fever. Their larvae are normally found in the eutrophic water. Open ponds, ditches and drains containing human sewage (septic tanks) or animal sewage are the common areas seen. ¹⁴ There is predominant increase of this species in the similar sites of study area but some of their larvae were seen in the artificial

containers also. High predominance of the larval species may be due to dirty stagnant water collections in the area.

Anopheles mosquitos normally breed in natural water collections. They usually start biting by late evening around 5 pm with peak of biting at midnight and early hours of morning. Hence, by keeping the windows and doors closed during this period and wearing garments that cover the whole body at bedtime in addition to using long lasting insecticide treated nets can prevent infection especially areas where malaria disease is reported. ¹⁴ In our study area, we only got one adult Anopheles mosquito in the month of October may be due to the timing of our collection.

Bottles, tins, tender coconut shells, buckets, tyres, plastics discarded utensils etc. are thrown out in the open space and thereby providing ample breeding ground for the mosquitoes. Other breeding sources include open wells, ponds, water tanks, paddy fields etc. including construction sites where water on the concrete slabs (used for curing), water collected in tanks, water collected in and around the construction site (manmade impoundments) owing to blockage of water drains promotes breeding. Hence it is very important to destroy these water collections or to keep them properly covered to prevent breeding. 9-11,14

By just emptying once a week the vessels or containers which are used for storing water, does not solve the problem. The egg especially of *Aedes* may not get washed off on draining the water, the containers sides should be scrubbed and washed off otherwise high chance for the mosquito egg to cling on to the sides of the container. This mosquito egg can live for six months to one year in dry container hence these dry containers can be a potential source during rainy season where the eggs can grow to larvae and to adult mosquito in a week.¹⁷

For convenience purpose, study was done in only two wards of the panchayat area, this gives limited evidence of the specific area only which cannot be compared to the whole of rural area.

CONCLUSION

The results of the study imply that the increase in artificial containers and man-made impoundments are the reasons for increasing mosquito diversity thereby abounding risk for vector borne diseases and increase susceptibility of an epidemic outbreak especially dengue and chikungunya.

Source reduction activities should be stressed into. Starting from educating households on segregation of home waste required, rather than disposing the coconut shells, husks, discarded/broken different containers, utensils, tyres etc. into the peri domestic area, segregate them separately into different bags/cartons available. The government should provide arrangements to collect the

waste at least once a month in each area thereby promoting the households to segregate the waste. Secondly, educating the individuals on problems of stagnant water collections, dry containers and the associated breeding and risk of transmission of diseases through mosquito bites and also improving the awareness by constantly observing "dry day" per week. Thirdly, implementing focused active surveillance and to implement aggressive source reduction measures especially the use of plastics. Fourthly, personal protective measures-mosquito repellants, bed nets, full sleeve dress, biological measures, anti-larvicidal and anti-adult mosquito measures to be continued.

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