ORIGINAL ARTICLE

# Distribution and Abundance of *Aedes* Mosquito Breeding Sites at Schools in Bukit Tinggi, Klang

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**ABSTRACT**

**Introduction:** In tropical nations including Malaysia, dengue fever is one of the most widely recognized viral ailments spread to people by the *Aedes* mosquito. The state of Selangor, Malaysia has become a dengue outbreak hotspot since several years ago and understanding the potential of breeding sites within the outbreak hotspot is vital. **Methods:** This study was conducted in Bukit Tinggi, Klang. This town in Selangor is one of the dengue hotspots. This study assessed the distribution profile of *Aedes* mosquitoes and then determined the differences in the population and distribution between schools with hostels and schools without hostels. The number of positive ovitraps and egg counts were analyzed using descriptive analysis and inferential statistics. **Results:** Out of 210 ovitraps deployed in each type of school, the schools with hostels had the most positive ovitraps, whereby 89 positive ovitraps were successfully collected out of 207 (42.99%). In schools without hostels, 61 positive ovitraps were gathered out of 209 (26.19%). Additionally, 471 eggs with a mean egg per trap (MET) value of 16.02 were identified from schools with hostels, while schools without hostels, recorded 185 eggs with an MET value of 9.17. **Conclusion:** Schools with hostels are more likely to have a higher number of *Aedes* mosquito population and breeding rather than schools without hostels. This could be due to increased human activity in these schools. Local authorities and the school's management must take accountability to devise a mitigation plan to reduce mosquito breeding at the school and hostel compounds.

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## INTRODUCTION

Dengue fever (DF) is one of the major health problems in Malaysia that influence human wellbeing. The rising number of DF cases is a national and developing medical concern, that may eventually become an outbreak (1, 2, 3). Dengue is viewed as the most significantly severe and deadly mosquito-transmitted viral infection. Two types of *Aedes* mosquitoes transmit dengue fever. They are *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse). Numerous examinations have presumed that *Aedes* mosquitoes are consistently found inside and outside the human settlement. *Aedes* mosquitoes live among humans to feed themself, rest within the vicinity of humans and lay their eggs in man-made water bodies (4). *Aedes* mosquitoes are consistently found in water compartments, culverts, plastic drums, metal drums, flower vases, disposed containers, canals, and open spaces (4, 5, 6).

Environmental factors such as food availability, human activity, and climate change are significant factors that affect the transmission of DF. Controlling the disease is difficult due to these factors that influence the disease pathophysiology and transmission (7). However, minimizing vulnerable breeding sites in the surrounding is one of the safest ways to prevent the disease from spreading, by breaking the transmission chain (8, 9).

The majority of DF outbreaks in people are between the ages of 10 and 29 (10, 11). Due to ongoing human-vector-human transmission in the enclosed environments of schools, school-aged children are more prone to the infection. Upon getting infected, children are typically asymptomatic or show mild symptoms, while adults more commonly develop DF. However, when children are exposed to the same viral load as adults, the rate of DF in children were five folds higher than adults (4). This data shows that school-aged children are at a vulnerable age to be exposed to dengue.

As students spend a significant amount of time at their schools, the school and its surroundings must be clean and free from potential breeding sites. Controlling the vector with a thorough and effective vector management program helps to prevent disease transmission, particularly in schools. Therefore, the study of dengue vector distribution and breeding population in schools is essential. This information will help in controlling the virus vector's transmission and breaking the mosquito's life cycle, ultimately preventing a significant number of human infections (12). Data on the profiles of the *Aedes* population can help improve the existing vector control methodologies at schools. The only approach to prevent the infections is by breaking the transmission chain. This can be done by limiting the potential breeding location in the area (9, 13).

The purpose of this study was to assess the profile distribution of *Aedes* mosquitoes in selected schools at Bukit Tinggi, Klang. The profile distribution included the changes of population abundance and distribution in the selected schools with and without hostels. The presence of *Aedes* mosquitoes in the schools was measured using the positive ovitrap index (POI) and mean eggs per trap (MET). The POI value represents mosquito dispersion, whereas MET value represents vector population. The hypothesis assumes that schools with hostels at Bukit Tinggi, Klang will have a higher *Aedes* population than schools without a hostel.

## MATERIALS AND METHODS

### Location

A total of 3,893 dengue cases in Selangor were reported until 27 March 2021, with the Klang District having recorded a total of 583 cases (15% of the total cases in Selangor) (14). There are 22 active outbreak localities reported until March 2021 in the Klang District. Bukit Tinggi, Klang is located within the active localities. A total of four primary schools and two secondary schools (three with hostels and three without hostels) were selected for the search for potential *Aedes* breeding sites. The schools were located within a 400-meter radius of the hotspot area in Bukit Tinggi, Klang. The description of the schools are summarized in Table 1.

**Table 1 Description of the schools selected for profile distribution**

| Category | Code | Types of school | Total number of students | Total number of staff | Hostel capacity |
|---|---|---|---|---|---|
| **Schools with hostels** | A | Primary School | 1444 | 110 | 440 |
| | B | Primary School | 1895 | 130 | 580 |
| | C | Primary School | 1768 | 129 | 500 |
| **Schools without hostels** | D | Primary School | 1907 | 95 | - |
| | E | Secondary School | 1338 | 81 | - |
| | F | Secondary School | 750 | 45 | - |

Source: Pejabat Pendidikan Daerah Klang or Klang District Education Office (2021)

### Procedure

Figure 1 shows the entire procedure in assessing the profile distribution of the *Aedes* mosquito in the schools. The schools with hostels have dormitories for students, while schools without hostels do not have dormitories. The field experiment was conducted in all selected schools to identify the number mosquito resting and breeding sites in the area.

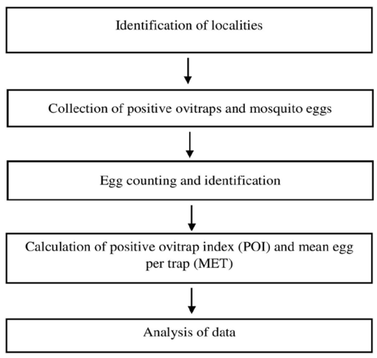


**Figure 1: The methodology flow in assessing profile distribution of *Aedes* mosquitoes.**

An ovitrap is a device which consists of a dark container containing water and a substrate where mosquitoes can lay their eggs. The ovitraps were placed in a shaded location to avoid direct sunlight exposure and egg loss during rainfall. The ovitraps were collected weekly for 7 weeks during the morning hours. Each school were positioned with indoor and outdoor ovitraps. The indoor ovitraps were placed in the bathroom, toilets and canteen, whereby the outdoor ovitraps were placed near drains, flowerpots, abandoned dumpsters, and food and plastic waste litter. A total of 60 ovitraps were placed in the selected schools each week, with ten ovitraps per school. Results were documented based on the number of ovitraps that contain the mosquito eggs. The mosquito eggs were observed under the microscope and counted using hand tally counter.

Data analysis was divided into two parts. The first part focused on Aedes breeding distribution using positive ovitrap index (POI), and egg population abundance with mean egg per trap (MET) to acquire the population of *Aedes* mosquitoes. The second part was to compare the difference in the population of *Aedes* mosquitoes between the schools with hostels and schools without hostels. The data were analyzed using both descriptive analysis and inferential statistics. For inferential statistics, the Statistical Package for Social Sciences (SPSS) for t-test analysis was used to determine a difference between the variables' means.

The purpose of the descriptive analysis was to further understand the distribution and abundance of *Aedes* mosquitoes in both dengue risk zones. The Ministry of Health (15) has recommended entomological measures, such as the POI and MET to assess the population abundance of *Aedes* mosquito eggs. The data obtained were analyzed as;

$$\text{Positive Ovitrap Index (POI)} = \frac{\text{Number of positive traps}}{\text{Number of inspected traps}} \times 100 \quad \text{(Equation 1)}$$

$$\text{Mean eggs per trap (MET)} = \frac{\text{Number of eggs}}{\text{Number of positive traps}} \times 100 \quad \text{(Equation 2)}$$

The value of POI represents mosquito dispersion, whereas the value of MET represents vector population abundance.

## RESULTS

### *Aedes* mosquito distribution and abundance in schools with hostels

A total of three schools with hostels were chosen to place 210 of the ovitraps for seven weeks. Table II presents the total number of recorded data for schools with hostels. From the total, 207 were successfully retrieved and taken into consideration. A total of 89 (42.99%) of the 207 ovitraps had *Aedes* mosquito eggs. The distribution of *Aedes* mosquitoes was estimated from the positive ovitraps, and ranged from 30% to 60%.

The population density of *Aedes* mosquitoes was determined by the number of eggs laid at the sites. A total of 471 eggs were collected from the schools with hostels during the seven-week study. Eggs were collected from the 89 recovered positive ovitraps. Week 5 had the highest number of *Aedes* mosquito eggs at 124 eggs. The second highest number of eggs was recorded in Week 3 (90 eggs) and the third highest in Week 2 (64 eggs). The lowest number of eggs was recorded in Week 1, with only 22 eggs. These numbers show the significant abundance of *Aedes* mosquitoes breeding in the selected schools with hostels.

**Table II Distribution of *Aedes* mosquito population in ovitraps deployed in schools with hostels**

| School | Week | No of total ovitraps | No of chec-ked ovit-raps | No of positive ovitraps | Ovi-trap index (%) | No of eggs | Mean eggs per trap (MET) |
|---|---|---|---|---|---|---|---|
| Schools with hostels | W1 | 30 | 30 | 9 | 30.00 | 22 | 2.44 |
| | W2 | 30 | 30 | 10 | 33.33 | 64 | 6.40 |
| | W3 | 30 | 29 | 17 | 58.62 | 90 | 5.29 |
| | W4 | 30 | 30 | 12 | 40.00 | 83 | 6.92 |
| | W5 | 30 | 30 | 17 | 56.67 | 124 | 7.29 |
| | W6 | 30 | 28 | 14 | 50.00 | 56 | 4.00 |
| | W7 | 30 | 30 | 10 | 33.33 | 32 | 3.20 |
| | Total | 210 | 207 | 89 | - | 471 | - |

Figure 2 shows the distribution and abundance of *Aedes* mosquitoes in schools with hostels. Week 3 had the highest percentage of POI (58.62%) with 17 positive ovitraps out of the 29 checked ovitraps. Week 5 had the second highest POI (56.67%) with 17 positive ovitraps, which is only less than 2% compared to Week 3. The third highest POI was in Week 6 at 50% (14 positive

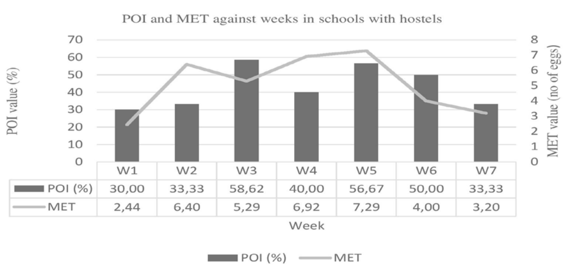


**Figure 2: POI and MET against eweeks in schools with hostels**

ovitraps). Lastly, Week 1 showed the lowest POI (30%) with nine positive ovitraps. In overall, the average POI value for schools with hostels was 43.13%, with 12.71 positive ovitraps. The distribution of the *Aedes* mosquitoes was not drastically scattered for the seven weeks of study.

Data on the number of eggs was collected to calculate MET, which alluded to the abundance of *Aedes* mosquitoes breeding in the area. The MET values were calculated by the number of eggs divided by the number of positive ovitraps. Week 1 recorded the lowest value of MET, at 2.44, with only 22 eggs over nine positive ovitraps. The average MET in the seven-week of study at the schools with hostels, was 5.08.

**_Aedes_ mosquito distribution and abundance in schools without hostels**

Table III shows the data of schools without hostels for seven weeks of observation. During this time, a total of 209 ovitraps were deployed from the 210 ovitraps that were placed. Out of these numbers, only 61 ovitraps (26.19%) were positive with eggs. The POI from schools without hostels ranged from 20% to 37%.

**Table III Distribution of *Aedes* mosquito population in ovitraps deployed in schools without hostels**

| School | Week | No of total ovitraps | No of checked ovitraps | No of positive ovitraps | Ovitrap index (%) | No of eggs | Mean eggs per trap (MET) |
|---|---|---|---|---|---|---|---|
| Schools without hostel | W1 | 30 | 30 | 6 | 20.00 | 14 | 2.33 |
| | W2 | 30 | 29 | 9 | 31.03 | 28 | 3.11 |
| | W3 | 30 | 30 | 7 | 23.33 | 21 | 3.00 |
| | W4 | 30 | 30 | 11 | 36.67 | 31 | 2.82 |
| | W5 | 30 | 30 | 11 | 36.67 | 44 | 4.00 |
| | W6 | 30 | 30 | 10 | 33.33 | 34 | 3.40 |
| | W7 | 30 | 30 | 7 | 23.33 | 13 | 1.86 |
| | Total | 210 | 209 | 61 | - | 185 | - |

A total of 185 eggs were collected from schools without hostels. The number of eggs was used to calculate MET, which value was then used to determine breeding density. The three highest number of eggs collected was in Week 5 (44 eggs), followed by Week 6 (34 eggs) and Week 4 (31 eggs). The lowest number of eggs collected from the positive ovitraps was in Week 7, which only contained 13 eggs. This shows that schools without hostels have a low percentage of POI and number of eggs throughout the study.

Figure 3 shows the percentage of MET and POI for schools without hostels. The proportion of POI did not noticeably vary across weeks, recording values between 20% and 37%. The highest value of POI was 36.67 % in Weeks 4 and 5. Week 6 recorded a slightly lower percentage, at 33.33%, compared to Week 4 and Week 5. The lowest percentage of POI was in Week 1, at only 20%.

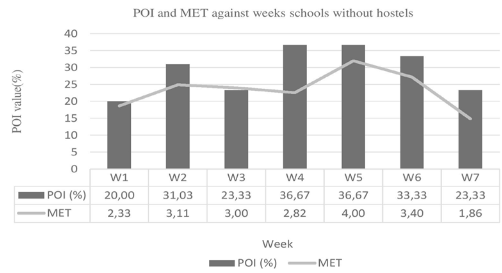


**Figure 3: POI and MET against weeks in schools without hostel**

The number of positive ovitraps in the schools without hostels was 61. The highest MET recorded was in Week 5 (4.00) with 44 eggs. Week 6 had the second highest MET (3.40), and Week 2 (3.11) was the third. The lowest MET for schools without hostels was in Week 7, at only 1.86 (13 eggs).

**Mean difference of _Aedes_ mosquito distribution and abundance between schools with hostels and schools without hostels**

T-test analysis was conducted to comprehend the difference between means recorded for schools with hostels and schools without hostels, and the abundance and distribution of *Aedes* mosquitoes. The test was done for two purposes; i) determine the difference between means of schools with hostels and schools without hostels using POI, and ii) determine the difference between means of schools with hostels and schools without hostels using MET values. A normality test was performed before the t-test to assume a normally distributed population (16).

**Positive ovitrap index (POI)**

The t-test data indicates that there is a significant difference between schools with hostels and schools without hostels. The mean of schools with hostels ($M = 43.00$, $SD = 7.15$) was higher than schools without hostels ($M = 29.18$, $SD = 3.19$), ($t(4) = 3.06$, $p < .05$). The difference between the mosquitoes' distribution and type of schools was significant.

**Mean eggs per trap (MET)**

T-test analysis comparing the mean of schools with hostels and schools without hostels on the MET values were performed. The mean of schools with hostels ($M = 5.34$, $SD = 0.47$) was higher than schools without hostels (M = 3.06, SD = 0.43), (t (4) = 4.28, p < .05). The difference between the mosquito density and type of schools was significant.

## DISCUSSION

### *Aedes* mosquito distribution and abundance in schools with hostels

The current data coincides with several studies that have also revealed that schools have a larger number of *Aedes* mosquito breeding sites (17, 18, 19). It is therefore evident that transmission of dengue can occur in schools and affect students, if the situation is not adequately monitored and abated. Thus, awareness on the abundance of *Aedes* mosquitoes in schools is key, as abundance relates to dengue transmission.

The distribution of *Aedes* mosquitoes in schools with hostels was higher than schools without hostels, at 58.62% and 36.67% respectively. The facilities and buildings available at the hostels tend to accommodate the vector, as female *Aedes* are airborne, and students living in the hostels become human hosts (20). Vector-host interaction has a direct impact on the likelihood of the infection. For reproduction, the female mosquito needs to perform a gonotrophic cycle (21). In this cycle, females will host-seek, conduct ingestion, and digest the blood meal that matures their eggs. This is followed by looking for appropriate oviposition sites. As mosquitoes have a flying distance of lesser than 100 meters (22), the host-seeking task will appear within the school's surroundings. This influences the spread of the dengue virus vectors in the host's environment (23).

A female mosquito in the reproductive period needs more blood meals during the day. The blood meals must be consumed before production of the eggs, to trigger the brain to release hormones that activate the process of maturing the eggs (24). During the active biting periods of the mosquitoes, students are highly exposed to receive the dengue virus from the infected mosquito. Infection occurs when an infected mosquito sucks human blood for egg maturation and nutritional intake (25). When their eggs have matured, female mosquitoes will begin looking for breeding sites. Thus, the dispersion of mosquitoes for oviposition is associated with disease propagation, and frequent feeding in a single gonotrophic cycle may intensify dengue transmission.

### *Aedes* mosquito distribution and abundance in schools without hostels

Based on the results, schools without hostels have a lower population and abundance of mosquitoes compared to schools with hostels. This can be explained by lesser host presence during blood meals, as students are not required to stay within the school's area. Consequently, the female mosquitoes may not have enough blood meals to mature their eggs (25). In this case, the mosquitoes disperse to other breeding sites. Furthermore, the schools without hostels have fewer facilities and buildings that accommodate water containers, which can be oviposition sites for the *Aedes* mosquitoes. However, the breeding sites can be indoors like in the classrooms, toilets, and canteen, or outdoors, such as the potted plant with stagnant water, food and plastic waste litter, and abandoned bins (17, 22).

### Comparison of *Aedes* mosquito distribution and abundance between schools with hostels and schools without hostels

A total of 420 ovitraps were distributed in the selected schools, of which 416 were recovered. Of the 416, 150 ovitraps (36.1%) were positive with the presence of *Aedes* mosquito eggs. The 89 (59.3%) positive ovitraps in schools with hostels were higher than schools without a hostel, at 61 (40.1%) ovitraps. The distribution of *Aedes* mosquitoes can be estimated from the POI. It is thus evident that schools with hostels have a higher *Aedes* mosquito abundance compared to schools without hostels.

Additionally, the number of *Aedes* mosquito eggs collected was higher in schools with hostels (471 eggs (71.8%)) compared to schools without hostels (185 eggs (28.2%)). The highest total number of eggs collected from schools with hostels was 124 eggs, on Week 3 of the study. The distribution of egg count corresponded to the 59.3 and 40.1 percent of positive ovitraps for schools with and without hostels, respectively.

By comparison, the schools with hostels had a higher POI in Week 3 (58.62%), followed by Week 5 (56.67%) and Week 6 (50.00%). The highest POI in schools without hostels was only at 36.67% in Week 4 and Week 5. The highest value of MET was from schools with hostels at 7.29 in Week 6, while schools without hostels only reached 4.00 in Week 4. Hence, it can be inferred that schools with hostels have a higher *Aedes* mosquito distribution and abundance breeding, than schools without hostels. As the students need to stay in the hostels for many hours a day, they are prone to the vectors during their progressive biting times.

The presence of students during the active biting periods of the vector enhances the breeding of their eggs (22, 25). Although the vector can only bite during the day, students in the dorms are more likely to be exposed to the vector, since mosquitoes bite more frequently after dawn (early morning) and before sunset (late evening) (22). During these times, students head out of their rooms to get their breakfast and dinner, while the vectors are present indoors and outdoors. This poses a risk as female *Aedes* mosquitoes feed several times a day. Students who stay outside their rooms during dusk and dawn attract the mosquitoes to disperse in the surrounding area.

Difference in the lifestyle of students that live in hostels compared to students in schools without hostels had also affected mosquito population and distribution. Students in schools with hostels were required to stay in the school compounds many hours a day and may only exit with permission. This situation suggests that students are active within the school, either to play, relax and complete their homework. Students who exercise or play sports during the morning or evening hours emit a certain odor, sweat and exhale carbon dioxide at higher concentrations. Human odor contains a complex combination of chemicals that are used by mosquitoes to detect the presence of a host (26). Moreover, the emitted carbon dioxide induces the female mosquito to linger around the area. *Aedes aegypti* mosquitoes respond to certain scents and are able to detect these scents from 36 meters away (26). Thus, there is a great possibility of mosquitoes from outside the school compounds finding their way to the students, resulting in an increase of mosquitoes.

In schools without hostels, students leave the school after classes end, creating a quiet and desolate environment. Furthermore, the number of eggs was lowest in Week 1 because the school had just re-opened after the Movement Control Order (MCO). This data proved that the presence of students in the hostel had stimulated the rise in or presence of the dengue vector population.

After school hours, students from schools without hostels leave the school area. However, students staying in the schools carry on with other activities. These activities including sports, that causes an increase in body heat. Actively feeding female mosquitoes are able to detect the students' body heat (29, 30), using their visuals and also when in close range (26). Mosquitoes are sensitive to the rise and fall of temperature, allowing them to respond to thermal signals and temperature changes from their hosts (26). As a result, the mosquito's abundance is more likely to grow in schools with hostels than those without. This study did not include the temperature and humidity change considering the almost constant climate throughout the year.

**Relationship between Positive ovitrap index (POI) and Mean egg per trap (MET)**

The hostel areas have a higher percentage of becoming a breeding ground for mosquitoes. This is due to the presence of water tanks used to store water. These tanks need to be covered well to prevent mosquito breeding in high numbers (22). The dormitory, surau, and dining hall are other common structures in schools with hostels. As water is used in these areas, mosquitoes may successfully hatch from their eggs when exposed to the water (25). Each type of mosquito has a different preference for their breeding sites. For example, *Aedes aegypti* mosquitoes prefer clean water containers, e.g. toilets, potted plants with stagnant water, and containers. On the contrary, *Aedes* albopictus mosquitoes prefer breeding outside the house, like in a tree trunk with stagnant water (17, 25).

Furthermore, the laundry room, clothesline, and bathroom are places that may house stagnant water in several ways (for example on the floor or in a bucket). If the still water is not flushed away, it attracts the vectors to lay their eggs. This observation is consistent with a study by Dieng et al. (27), which found that *Aedes* mosquitoes have a longer life span indoors. This is why mosquito breeding sites have been identified as one of the factors that contribute to the occurrence of dengue virus vectors in school environment, especially in the hostels and dormitories (28). Therefore, cleaning the rooms and bathrooms should be a vital practice for students staying in hostels and dormitories.

According to Rahim et al. (31), the frequency of new dengue cases significantly decreased after the implementation of Movement Control Orders (MCOs). Only 583 cases of DF were reported until March 2021, whereas 4,218 cases were reported until March 2020 (14). Specifically, there was an 86.2% decrease in dengue cases from 2020 to 2021. The MCO prohibited large-scale movements and gatherings of people. During the MCO, all schools were closed, and the students were having home-based teaching and learning (termed by the acronyms PdPR from the Malay word). This reduced the number of human activities within the school environment. Since there were fewer hosts outside their dwelling, vector-host interactions were decreased. This slowed the virus's transmission in the region. As learning was conducted remotely from January until the first week of March of 2021, Week 1 of the study recorded the lowest number of positive ovitraps and egg counts from both schools with hostels and without hostels. This finding coincides with a report by Rahim et al. (31) that explained that limitations in human movement slowed the development of DF in Malaysia.

By the end of the study, it can be concluded that all the schools are in areas of high population of *Aedes* mosquitoes. Our discovery of a large number of active breeding sites near schools has its ramifications. Schools can increase their vector control efforts by focusing on these active breeding sites. High-visibility prevention techniques with low maintenance are strongly suggested for the schools. For the elimination of potential larval breeding sites, personal hygiene and health education are essential first steps in modern mosquito-borne disease control (8, 32). It has been shown that a partnership of social participants and environmental management can drastically reduce vector density and increase the efficiency of conventional dengue control measures (33). Consequently, community participation in managing the dengue epidemic can be strengthened and promoted through proactive health education using appropriate outreach programs.

Health education for dengue management in schools or public workplaces may sometimes lack the resources needed to sustain a consistent program. Thus, local authorities and the school management could devise a mitigation plan to reduce the dengue cases in the community. School-based education programs and social mobilization are suggested for implementation. Furthermore, fun and relatable efforts are better for involving students. For example, the 'search and destroy' activity can help destroy breeding containers. The exercise teaches the students to care for their surroundings, to help prevent the mosquito from breeding. Additionally, policies must be in place to ensure that the school administrations comprehensively execute vector control measures at schools, including classrooms and outdoor play spaces.

## CONCLUSION

From this study, it is evident of the presence of mosquitoes within school areas. Schools with hostels have a higher distribution and abundance than schools without hostels. Finally, the findings show that it is important to include schools in dengue monitoring programs, to have a higher impact on community dengue preventive and control efforts. Control of mosquito population relies on commitment, population awareness, and understanding of breeding circumstances.


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