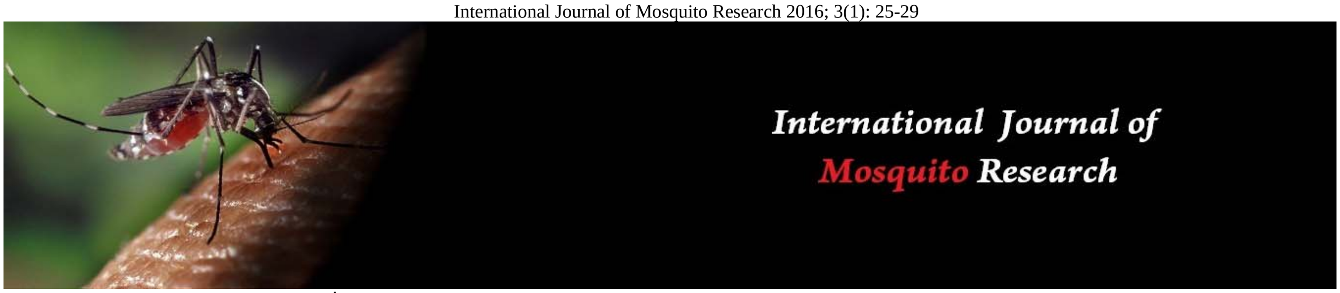

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Water quality characteristics of dengue vectors breeding containers

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Abstract
Dengue fever is an infectious mosquito borne disease and continues to be one of the most important public health problem. To date, the most widely used method to prevent dengue outbreak is attacking the mosquitoes breeding site. This study was to assess the water quality conditions of the breeding containers which may affect the oviposition preferences of dengue vectors (DVs) mosquitoes. An ecological study was conducted on the species distribution of the DVs mosquitoes with the water quality condition of the breeding containers. The most efficient container types for DVs mosquitoes are container identity 9 (CID$_9$ = L$_e$: 12.98) and followed by the groups of tyres (CID$_6$ = L$_e$: 11.21), which are the most productive container types. Both efficient breeding container types had moderately turbid water, and contaminated with the manifestation of algae. All water samples (*n*=91) collected were tested for physicochemical parameters and was further analyzed using water quality index (WQI) in order to assess the water quality of the breeding containers. The adaptation to poor and polluted water quality in dengue vectors mosquito larval habitat is related to pollutant from domestic and industrial exposure. Most mosquito species examined appear to breed effectively from this kind of habitat and further research on their adaptation must be done in the future.

Keywords: Dengue, Water Quality Index (WQI), *Aedes* abundance, productivity

1. Introduction

Dengue is classified as one of water-associated disease as the water acts as a media of transmission, although occurs indirectly. Water-associated diseases account for approximately 10% of the global disease burden, representing a significant source of morbidity and mortality worldwide. These infections are spread by waterborne agents, vectors carrying viruses and parasites like dengue, and water contact diseases like schistosomiasis [1-3]. Another essential point is the density of dengue vector (DVs) mosquitoes related to the climate conditions [4-8]. Thus, knowledge in habitat selection of DVs mosquitoes is vital in order to implement vector control program [9-11]. In dengue active area, it is crucial to monitor DVs mosquitos' abundance and its distribution to predict the dengue epidemics [12, 13]. Water conditions, location, presence of larval food, location, and shades are the determining factors of breeding sites of dengue vectors [14]. Study on the vectors abundance with the water condition specifically on the water quality will provide better understanding in complexity of dengue outbreak. Water quality index (WQI) provides a convenient means of summarizing large numbers of water quality data, for the ease of communication to a general audience and will aid in establishment of priorities by providing quantitative data on overall water quality in regularly sampled water bodies [15] instead of assessing the breeding site in specific parameter. WQI provides a clear picture on overall state of the water including biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solid (TSS), pH, dissolved oxygen (DO), and also ammonia nitrogen (AN) presence in the water in a single and understandable number, which can be further classified into the level of cleanliness of the water.

It is vital to eliminate the DVs mosquito from the larval stage by several anti-larval measures. In order to execute that strategy, knowledge in habitat selection of DVs mosquitoes is important and vital [10, 11]. Therefore, this study was conducted to provide a better input on the knowledge in habitat selection of DVs mosquitoes by accessing the water quality of the breeding sites and exploring the correlation of those physical factors to the prevalence of DVs mosquitoes especially in the water quality aspect which is known as the breeding site of the vectors.

2. Materials and methods

A cross-sectional entomological survey of water-holding containers which supporting mosquito breeding was conducted in ten selected localities in Subang Jaya, Malaysia from December 2013 to January 2014. This study was conducted due to the limited knowledge on the specific water quality parameters of the DVs breeding sites. The curiosity has increased on the water quality characteristics of the breeding sites, which reflect the preferences of DVs mosquitoes to oviposit their eggs. This study consists three main phases (Phases 1: to identify the locality based on the epidemiological data of DF cases; Phase 2: Field sampling includes observation of the breeding sites characteristics and *in-situ* measurement of several water quality parameters; Phase 3: Extended water quality analysis at the laboratory and larval identification) in order to studied the water quality characteristics of DVs breeding sites.

2.1 Study area

In this study, Subang Jaya area in Selangor, Malaysia was selected as the main research site to study the water quality characteristics of DVs breeding sites. The selection of this area was justified by several factors. First it has a high population density in Selangor and has a significant public health implication in relation to the control and prevention of dengue. The main landscape characteristics of this area are the urban areas, which are rapidly developed. The major land-use in this area is residential (housing), commercial and industrial area. Furthermore, forested area and construction area also has shown the rapid changes trend and this might influence the trend of dengue distribution

2.2 Mosquito sampling

The selection of the localities was based on the constant occurrence of DF cases for five years (2010-2014). A house to house and peri-domestic area survey was carried out in order to detect mosquito larval breeding sites which reflect the infestation level with *Aedes* larvae in the localities.

All water-holding containers were visually inspected for the presence of water with immature mosquitoes (pupae and larvae) (Figure 1). All the water-holding containers detected during the survey were counted and classified into 13 categories based on the container identity (CID). CID were given to the containers observed at the sampling sites were CID_1: drums; CID_2: water reservoir; CID_3: buckets; CID_4: flower vases and flower pots bases; CID_5: earthen pots; CID_6: Tires; CID_7: Plastic bowls and pots, oil can, galloon jar and water bottled; CID_8: plastic sheets, plastic water pools and bags; CID_9: types of glass, which are unused aquarium, glass jars and ceramics; CID_{10}: group of metal; CID_{11}: discarded appliances, discarded foams, shoes, and helmets; CID_{12}: dust carriers and bins, baby baskets and CID_{13}: ant guards and trays.

Legend:

CID	Interpretation	CID	Interpretation
1	Drums	8	Plastic sheet
2	Water reservoir	9	Glass
3	Buckets	10	Group of metal
4	Flower vases	11	Discarded appliances
5	Earthen pots	12	Dust carrier and bins
6	Tyres	13	Ant guards and trays
7	Plastic bowl		

Fig 1: Categories of water-holding containers based on the CID

Specimen of immature mosquitoes were kept in plastic bottles and labelled with date of collection before being transported to the laboratory. The larvae and dead pupae were counted and identified to species under a compound microscope according to the standard keys [16]. The number of larvae and pupae were recorded along with the container types and added to a database for subsequent analyses.

2.3 Measurement of water quality parameters

In order to assess the water quality characteristics of DVs breeding sites, six parameters were considered in this assessment which include physicochemical parameters. A physical analysis of water in the breeding habitat was performed *in-situ*. The parameters involved are turbidity (NTU), pH, and dissolved oxygen (mg/L) by using appropriate instrument. The collected water samples were then further analysed for other parameters which include chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS), and ammoniacal nitrogen (AN) which follow the standard method from USEPA methods for chemical analysis [21]. Calculations were performed not on the parameters themselves but on their sub-indices. The sub-indices for each parameters were named SIDO, SIBOD, SICOD, SIAN, SISS and SIpH accordingly. Finally, water quality index (WQI) tool were used to evaluate the water quality of DVs breeding sites. The six resulting values were then entered into an establishment formula to arrive the WQI score and it was calculated using the formula:

$$\textbf{WQI} = 0.22 \times SI\ DO + 0.19 \times SI\ BOD + 0.16 \times SI\ COD + 0.15 \times SI\ AN + 0.16 \times SI\ SS + 0.12 \times SI\ pH \quad (1)$$

Where SI is refers to sub-index for each parameters. The summation of the weight ages for all the sub-indices must have a value of the unity. The value of 100 is the highest possible score and denotes high quality of the water and zero is the lowest.

3. Results

A total of 91 samples were collected at 10 areas where details on sampling localities and species are tabulated in Table 1. based on the result obtained, *Ae. albopictus* was identified as the most dominant species (63.7%) as compared to *Ae. aegypti* (7.7%) and mixed breeding (28.6%).

Table 1: Distribution and abundance of *Aedes species* larvae collected

Location	Species composition per breeding sites			Total samples
	Ae. albopictus	***Ae. aegypti***	**Mixed Breeding**	
PJS13	5	0	1	6
Lagoon Apartment	1	0	3	4
Low Cost Flat PJS 7	7	2	1	10
Arcadia Apartment	2	0	3	5
Angsana Flat	4	1	4	9
SS15 Commercial	2	1	2	5
SS19 Terrace Area	8	0	3	11
Kampung Sri Aman	14	0	1	15
Kampung Kenangan	11	2	7	20
Puchong Perdana Terrace	4	1	1	6
	58 (63.7%)	7 (7.7%)	26 (28.6%)	91

3.1 Dengue vectors breeding site characteristics

Larval productivity (L_p) and efficiency (L_e) of dengue vector breeding containers were identified for each water-holding container types (Table 2). The most efficient group of water-holding containers for the development of immature DVs are types of aquarium unused glass jars and ceramics (L_e: 12.98), followed by tires (L_e: 11.21), and the plastic sheets (L_e: 7.30). The efficiency of breeding sites was calculated from productivity over prevalence of each type of breeding containers.

Table 2: Larval productivity and efficiency of dengue vector breeding containers

CID[1]	Container types	Larvae			No. Positive container	Productivity[4]	Prevalence of container[5]	Effiency[6]
		n	**Average[2]**	**Maximum[3]**				
1	Drums	42	14	22	3	2	0.462	4.33
2	Water Reservoirs	65	22	36	3	3	0.462	6.49
3	Buckets	229	18	72	13	11	2.003	5.49
4	Flower vases	3	3	3	1	1	0.154	6.49
5	Earthen pots	77	19	35	4	4	0.616	6.49
6	Tires	365	33	87	11	19	1.695	11.21
7	Plastic bowls	476	19	115	25	25	3.852	6.49
8	Plastic sheets	163	20	48	8	9	1.233	7.30
9	Glass	185	37	78	5	10	0.770	12.98
10	Group of metal	62	12	25	5	3	0.770	3.89
11	Discarded appliances	149	17	37	9	8	1.387	5.77
12	Dust carriers and bins	63	21	25	3	3	0.462	6.49
13	Ant guards, trays	32	16	24	2	2	0.308	6.49
TOTAL		1911						

*Note
1 CID, container identity; PC, positive container.
2 Average no. of larvae in an individual container.
3 Maximum no. of larvae in an individual container.
4 Productivity = no. of larvae X 100/all larvae.
5 Prevalence of container = no. of positive containers X 100/all containers.
6 Efficiency = productivity/prevalence of containers

3.2 Water quality parameter based on DVs mosquitoes breeding containers

Table 3 shows the average of water quality parameters for respective containers from CID 1 to 13. It is interesting to note that in all six parameters of this study, it shows that percentage of larvae is higher in lower readings of each parameter excluding pH and DO parameters. This study found that the DVs mosquitoes prefer to oviposit either in neutral or slightly alkaline water. Different results shown in DO content which the results of immature of dengue vectors are in higher percentage if the DO is high and vice versa.

Table 3: Water quality characteristics of DVs breeding containers

CID	Water Quality Parameters (Average)						Total larvae (*n*)	Larvae productivity (%)
	pH	DO	COD	BOD	TSS	AN		
1	7.72	7.92	396.67	271.67	616.67	2.23	42	2
2	7.79	5.78	490.00	335.55	333.33	2.25	65	3
3	7.63	6.79	533.69	366.00	388.46	1.33	229	11
4	7.50	7.75	110.00	77.00	50.00	1.06	3	1
5	7.50	7.86	459.50	318.50	337.50	0.92	77	4
6	7.62	7.82	816.36	560.18	1159.09	2.05	365	19
7	7.43	7.61	654.28	447.76	568.00	1.68	476	25
8	7.79	6.42	1074.38	736.50	1025.00	2.26	163	9
9	7.94	5.74	394.00	269.60	1400.00	4.47	185	10
10	7.53	5.72	826.00	582.60	1502.00	3.51	62	3
11	7.97	7.23	630.44	431.33	1078.89	1.98	149	8
12	7.25	6.72	473.33	304.33	403.33	1.62	63	3
13	6.98	7.06	1155.00	788.50	200.00	3.08	32	2

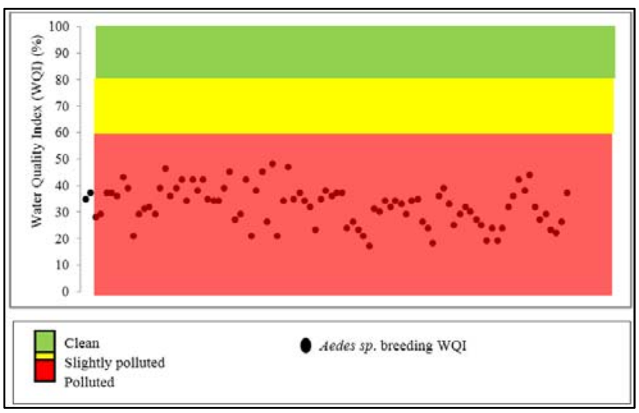

Fig 2: Distribution of DVs breeding containers water samples according to respective water quality index (WQI)

The water quality parameters were calculated for the WQI in representing the status of water quality characteristics of the DVs mosquitoes breeding container. WQI indices from 1-100 was used to represent its water quality characteristics. Figure 1 illustrates the water quality characteristics of DVs breeding containers by using different color code to represent the quality of the water (red color: WQI=0-59 (polluted); yellow color: WQI: 60-79 (slightly polluted) and green: WQI: 80-100 (clean). The respective class for the WQI range was based on water quality criteria in describing water quality condition which can be easily understood by general population[22]. Based on the result, the water quality characteristics of DVs mosquitoes are considered polluted for all types of water holding containers.

4. Discussion

Most of the mosquitoes select their breeding sites accordingly with their preferred characteristics that may help them to survive and for their population dynamics [8-10]. There are physical and chemical factors that can affect the sites for mosquitoes that can be attractants and deterrent for those mosquitoes. Certain condition of the site can be favoured by most species while other species may not. A study have shown that several chemical properties of the larval habitat in peri-domestic area are related to vegetation as well as different content of physicochemical characteristics which may affect the larval development and survival [17].

Variety of the containers that are categorizes as man-made containers and natural sites would be the preferable habitat for DVs mosquitoes. Female *Aedes aegypti* prefers to lay their eggs in domestic containers with a majority of discarded receptacles, water storage containers and tyres, wells, cements tanks and sinks [18]. Size of the container can have different number of predator where small container will have fewer predators or even not existed since smaller container may create smaller number of prey that might be too small to support trophic levels higher than filter feeding mosquitoes in that particular habitat. Based on the result obtained from larval productivity (L_p) and efficiency (L_e) of dengue vector breeding containers indicates that most of the DVs mosqutoes prefers to breed in small containers. This finding collaborates with the study by Sunahara *et al.* [19, 20], which highlighted that small size of container habitat can be vital characteristics that control the community structure of mosquitoes and predator. Besides that, the co-existence of *Aedes aegypti* and *Aedes albopictus* in breeding containers is likely attribute to the abundance of suitable container that are favorable to all container-breeding mosquitoes and the availability of shade and sufficient organic material for larval feeding.

In the present study, the habitat characteristics of DVs mosquitoes breeding containers were conducted by measuring the water quality of positive breeding containers using water quality index (WQI). This study revealed that from the total number of 91 breeding containers surveyed in dengue hotspot areas, most of the DVs mosquitoes (*Aedes albopictus*) prefers to breed in polluted water. The water has become polluted due to the storage of the water in the container for long period with ambient relative humidity and temperature, which favor for the DVs mosquitoes to breed. The results found here reinforce that *Aedes albopictus* can thrive in a variety of habitat with fresh water, brackish water or any water (clear, turbid or polluted), which are used for drinking or washing. *Aedes albopictus* is more commonly in outside areas such as in open spaces with shaded vegetation and suitable breeding sites such as car tyres and garbage dumps.

5. Conclusion

As a conclusion the types of the containers, water quality, and conditions of water containers may reflect the oviposition preferences of pregnant females of DVs mosquitoes. Selection of breeding habitat by mosquitoes is a critical factor for survival and population dynamics and indirectly poses vital implication for the control of mosquitoes. Therefore, knowledge in habitat selection of DVs mosquitoes is vital in order to implement a sustainable vector control.

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