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Re-emergence of dengue virus serotype 3: A laboratory perspective from the private healthcare centre, Malaysia

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Abstract

Dengue has been raised in recurring cyclical patterns of outbreaks. We determined the predominant circulating dengue virus (DENV) serotypes from selected urban areas in Malaysia. A total of 1177 laboratory tests for multiplex Real-Time Polymerase chain reaction (RT-PCR) and socio-demographic details from suspected dengue cases from KPJ hospitals in Klang Valley, Malaysia, between 2017 to 2020 were reviewed. The odds ratio and Pearson Chi-square test were performed. Only 205 (17.4%) were confirmed as dengue and observed frequently among males. The young adult was most infected (n = 115/205; 56.1%). The majority of the cases were recorded in Shah Alam (n = 184/205). The population in Selangor had a higher risk (OR = 1.29; 95% CI = 0.7958, 2.0896) of contracting dengue. The predominant serotype was DENV-3 (n = 83) followed by DENV-2 (n = 65). There were three co-infections with different DENV serotypes detected [(DENV-2 and DENV-3: n = 2) (DENV-1 and DENV-3: n = 1)]. The DENV-3 was predominant throughout the years except in April, October, and November. The DENV-3 had 13 time-points of frequent seasonality in November and December 2017, March, June and July 2018, January, February, May to July and December 2020. It was evident that DENV-3 was the prevalent DENV serotype and was circulating for four years. The switching of predominant circulating serotypes from DENV-2 to DENV-3 provides substantial benchmark on the development of early warning system and prediction of future dengue epidemics in Malaysia, hence preparedness plans and multilayer interventional strategies can be executed.

Keywords: Re-emergence, Dengue, DENV-3, Private healthcare centre, Klang Valley, Malaysia

1. Introduction

Dengue is a mosquito-borne viral infection found particularly in semi-urban and urban areas of tropical and subtropical regions worldwide. The dengue virus (DENV) causing dengue is a single-stranded RNA virus with four established serotypes including DENV-1, DENV-2, DENV-3, and DENV-4 [1]. Dengue virus is transmitted by the bite of a female mosquito principally *Aedes aegypti* followed by *Aedes albopictus*.

Based on the report by World Health Organization (WHO), there were a total of 505,430 dengue cases in the year 2000, and this escalated to more than 5.2 million cases in 2019. This demonstrated that the rate had increased by more than eight folds over the last two decades [1]. Dengue remains endemic in more than 100 countries, of which 70% of the actual health burden is represented by the Asian region [1-6]. In the year 2019,

high dengue incidences were reported in the Philippines; 420,000 cases, Vietnam; 320,000 cases, Singapore; 16,100 cases, and Malaysia; 131,000 cases [1]. Hence, the incidence rate in Malaysia has almost doubled over the past 22 years from 1998 to 2019 [1-8]. This rise was paralleled to the rapid urbanization with the unintended creation of new vector breeding sites [8-11], of which the concentrated cases were noted within the highly urbanized states of Kuala Lumpur, Selangor, and Johor, representing 68% of cases between 2012 to 2016 [12].

Recent epidemiological studies reported that DENV-1 and DENV-2 were the predominant circulating serotypes within East Malaysia between 2016 and 2017 [8,12-14]. Harvie, et al. [13] reported that the DENV-2 was the predominant serotype in the Sibu and Miri division of Sarawak, whereas Aung, et al. [14] reported that the DENV-1 was the predominant serotype during a dengue outbreak in Kudat, Sabah. In Klang Valley, DENV-1 and DENV-2 were co-circulated and prevalent in 2011, 2012 and 2015 [13,15]. In relation to DENV-3, the last recorded outbreak occurred in 2002 based on the 31 years of DENV surveillance data from 1985 to 2016 [16]. Since then, only the DENV-1/DENV-2 outbreak cycle was observed in Klang Valley until 2016 [13,15,16].

As part of continuous surveillance efforts to combat the rising of dengue cases, it is essential to characterize the incidence pattern ecologically by performing periodic DENV serotypes detection [10] from 2017 onwards in Klang Valley. Data from the Klang Valley region could represent one of the urban areas which have been associated with high dengue cases [12]. More than 60% of the reported dengue cases in Malaysia were identified in the Klang Valley region, specifically in Mudin [17] Therefore, we aimed to determine the predominant circulating DENV serotypes within Klang Valley, which represents urban areas in Malaysia, by incorporating the data from one of the largest and established private health care facilities in Malaysia, known as KPJ hospitals.

2. Materials and methods

2.1 Study site

Klang Valley is an urban population and commerce hub in Malaysia located at the central part of the west coast of Peninsular Malaysia with an estimated eight million population (Figure 1) [18]. The region consists of major cities, including Kuala Lumpur, Petaling Jaya, Shah Alam, Subang Jaya, Klang, Rawang and Ampang Jaya (Figure 1). The climate is tropical, with temperatures ranging from 22°C to 35°C. It has high humidity between 65% to 83%, and year-round rainfall. The rainfall recorded between 2017 to 2020 ranges from 77 to 248 mm per month, with maximum rainfall recorded between March to May and October to December [19].

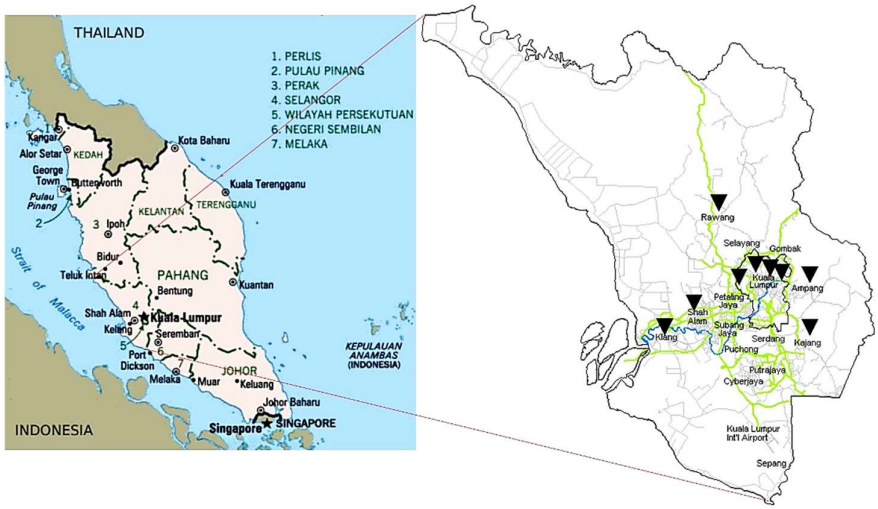

Figure 1 Location of the Klang Valley within the borders of the state of Selangor and the Federal Territory of Kuala Lumpur in the central part of the west coast of Peninsular Malaysia, where the focus area of study for the data catchment area of KPJ hospital. Reprinted from Map of Peninsular Malaysia [20,21].

2.2 Data collection

A total of 1177 requests of Real-Time polymerase chain reaction (RT-PCR) dengue differential testing at KPJ Lablink Medical Laboratory, Kuala Lumpur, Malaysia, were reviewed following dengue suspicion. The data were retrospectively gathered from January 1, 2017, to December 31, 2020. The report archived nine KPJ hospitals within the Klang Valley (Table 1). The main data were mainly obtained from the laboratory reports on

DENV serotypes using GenoAmp® RT-PCR Dengue differential testing (Mediven, Malaysia). It is a multiplex PCR platform used to detect DENV Serotype-1 (DENV-1), DENV Serotype-2 (DENV-2), DENV Serotype-3 (DENV-3) and DENV Serotype-4 (DENV-4). The GenoAmp® RT-PCR Dengue differential testing has 90% sensitivity and 100% specificity [22]. All requests for the multiplex PCR testing involved specimen collection on day 2 to day five of fever as recommended by the national and manufacturer's guideline. The types of dengue virus infection were grouped into non-dengue, single infection, and co-infection. Single infection is defined as dengue viral infection by either DENV-1 or DENV-2/3/4, whereas co-infection is defined as infection by two different DENV serotypes (e.g., DENV-2 and DENV-3).

The socio-demographic details and platelet count were also documented. The patient's age was categorized into infant (< 2-year-old), childhood (2 to 10-year-old), adolescent (11 to 17-year-old), young adult (18 to 40-year-old), adult (41 to 65-year-old), and elderly (> 66-year-old) based on WHO's classification [23].

Table 1 The list of KPJ hospitals in Klang Valley, Malaysia with the number of laboratory requests.

KPJ hospitals	No. of Request (%)
KPJ Ampang Puteri Specialist Hospital	197 (16.7)
KPJ Damansara Specialist Hospital	25 (2.1)
KPJ Kajang Specialist Hospital	26 (2.2)
KPJ Selangor Specialist Hospital	712 (60.5)
KPJ Tawakkal Specialist Hospital	122 (10.4)
KPJ Klang Specialist Hospital	13 (1.1)
KPJ Sentosa KL Specialist Hospital	2 (0.2)
KPJ Rawang Specialist Hospital	53 (4.5)
KPJ Lablink Central Laboratory Kuala Lumpur	27 (2.3)
Total	1177

2.3 Statistical analysis

A descriptive analysis on frequency and percentage of socio-demographic details and laboratory variables was performed using SPSS Statistics software (SPSS, IBM New York USA; version 21). The association between dengue and socio-demographics details with DENV serotypes was assessed by calculating the odds ratio (OR) framed by their 95% confidence intervals (CI). The Pearson Chi-Square test was employed to ascertain the significant association between the laboratory-confirmed DENV serotypes, socio-demographic characteristics, and the studied population. The results were presented by *p*-value and considered as significant when $p < 0.05$. The sample size for the present study was determined using a formula by Pourhoseingholi et al. [24], which revealed that 365 was the minimum sample size required to represent the urban area of Klang Valley. A total of 1177 requests in this study were sufficient to provide significant statistical power.

3. Results

3.1 Socio-demographic characteristics

The majority (n=712; 60.5%) of patients from the 1177 suspected dengue cases were from the KPJ Selangor Specialist Hospital, followed by KPJ Ampang Puteri Specialist Hospital (n=197; 16.7%) and KPJ Tawakkal Specialist Hospital (n=122; 10.4%) (Table 1). Most of the patients (92%) were in the young adult age group (n=628; 53.4%; median age=29), followed by the adult age group (n=329; 27.9%; median age=49) and the adolescent group (n=126; 10.7%; median age=15). The patients from the infant (median age=1), childhood (median age=8), and elderly age (median age=73) groups represent only 8% of cases reported in the present study (Table 2). There were 681 (57.9%) male and 496 (42.1%) female patients with the sex ratio of male: female of 1.4:1.0.

Table 2 Distribution of patients' age and gender subjected to RT-PCR dengue differential testing at KPJ hospitals from 2017 to 2020.

Age Group	n (%)		
	Total	Male	Female
Infant	17 (1.4)	8 (1.2)	9 (1.8)
Childhood	39 (3.3)	21 (3.1)	18 (3.6)
Adolescent	126 (10.7)	71 (10.4)	55 (11.1)
Young Adult	628 (53.4)	373 (54.8)	255 (51.4)
Adult	329 (27.9)	189 (27.7)	140 (28.2)
Elderly	38 (3.2)	19 (2.8)	19 (3.8)
Total	1177 (100)	681 (100)	496 (100)

Note: Infant (< 2-year-old), childhood (2 to 10-year-old), adolescent (11 to 17-year-old), young adult (18 to 40-year-old), adult (41 to 65-year-old), and elderly (> 66-year-old).

Of 1177 suspected dengue cases, only 205 (17.4%) cases were confirmed as dengue with the majority being a single infection (n=202) and the remaining (n=3) as co-infection. The dengue was prevalent among males (n=113) as compared to female patients (n=92) with a ratio of 1.2:1.0 (Table 3).

Table 3 Distribution of DENV serotypes in single and co- infection of dengue by RT-PCR dengue differential testing according to gender and age group from 2017 to 2020.

Serotype, n (%)	DENV-1	DENV-2	DENV-3	DENV-4	DENV-2 & DENV-3	DENV-1 & DENV-4	Non-dengue Cases, n (%)	Confirmed Cases, n (%)	Total, n (%)
Gender									
Male	21(3.1)	44 (6.5)	35(5.1)	10 (1.5)	2 (0.3)	1 (0.1)	568 (83.4)	113 (16.6)	681 (100)
Female	19 (3.8)	21 (4.2)	48 (9.8)	4 (0.8)	ND	ND	404 (81.5)	92 (18.5)	496 (100)
Total	40 (3.4)	65 (5.5)	83 (7.1)	14 (1.2)	2 (0.2)	1 (0.1)	972 (82.5)	205 (17.4)	1177 (100)
Age Groups									
Infant	ND	1 (5.9)	ND	ND	ND	ND	16 (94.1)	1 (5.9)	17 (100)
Childhood	2 (5.1)	3 (7.7)	2 (5.1)	2 (5.1)	ND	ND	30 (76.9)	9 (23.1)	39 (100)
Adolescent	6 (4.8)	7 (5.6)	8 (6.3)	ND	ND	ND	105 (83.3)	21 (16.7)	126 (100)
Young Adult	18 (2.8)	40 (6.4)	49 (7.8)	6 (0.9)	1 (0.2)	1 (0.2)	513 (81.7)	115 (18.3)	628 (100)
Adult	13 (3.9)	13 (3.9)	21 (6.4)	6 (1.8)	1 (0.3)	ND	275 (83.6)	54 (16.4)	329 (100)
Elderly	1 (2.6)	1 (2.6)	3 (7.9)	ND	ND	ND	33 (86.8)	5 (13.2)	38 (100)
Total	40 (3.4)	65 (5.5)	83 (7.1)	14 (1.2)	2 (0.2)	1 (0.1)	972 (82.6)	205 (17.4)	1177 (100)

Note: Infant (< 2-year-old), childhood (2 to 10-year-old), adolescent (11 to 17-year-old), young adult (18 to 40-year-old), adult (41 to 65-year-old), and elderly (> 66-year-old); ND: Not Detected.

3.2 Circulating DENV serotypes

The DENV-3 was found to be the predominant serotype (n=83/205; 40.5%), followed by DENV-2 (n=65/205; 31.7%), DENV-1 (n=40/205; 19.5%) and DENV-4 (n=14/205; 6.8%). The analysis revealed that DENV-1, DENV-2, DENV-3, and DENV-4 serotypes were frequently detected in the young adult age group between 18 to 40 years old (n=113/205; 55.1%), as compared to the adult age group (41 to 65-year-old) (n=54/205; 26.3%) (Table 3). The prevalence of DENV-3 was observed highest in young adults (n=49/205; 23.9%), followed by adult (n=21/205; 10.2%) with the median age of 29 and 49 years old, respectively.

The co-infection of dengue demonstrated two different DENV serotypes including (n = 2: DENV-2 and DENV-3) and (n=1: DENV-1 and DENV-3). These co-infections were detected in male young adult and adult age groups (Table 3). The majority of the reported circulating DENV serotypes (n=124/205; 60.4%) was from the KPJ Selangor Specialist Hospital located in Shah Alam, Selangor, followed by several cases from KPJ Ampang Puteri Specialist Hospital (n=40/205; 19.5%) and KPJ Tawakkal Specialist Hospital (n=14/205; 6.8%) (Table 4).

Table 4 Distribution of DENV serotypes in single and co- infections detected by RT-PCR dengue differential testing according to the region of KPJ hospitals from 2017 to 2020.

Serotype, n (%)	DENV-1	DENV-2	DENV-3	DENV-4	DENV-2 & DENV-3	DENV-1 & DENV-4	Non-dengue Cases, n (%)	Confirmed Cases, n (%)	Total, n (%)
APSH									
DSH	ND	3 (12)	3 (12)	1(4)	ND	ND	18(72)	7 (28)	25 (100)
KSH	ND	1 (3.8)	1 (3.8)	ND	ND	ND	24 (92.3)	2 (7.7)	26 (100)
SSH	23 (3.2)	42 (5.9)	51 (7.2)	6 (0.8)	1 (0.1)	1 (0.1)	588 (82.6)	124 (17.4)	712 (100)
TSH									
KLG	ND	1 (7.7)	2 (15.4)	1 (7.7)	ND	ND	9 (69.2)	4 (30.8)	13 (100)
STS	ND	ND	1 (0.5)	ND	ND	ND	1 (0.5)	1 (0.5)	2 (100)
RSH	2 (3.8)	1 (1.9)	4 (7.5)	ND	ND	ND	46 (86.8)	7 (13.2)	53 (100)
HQ	1 (3.7)	ND	5 (18.5)	ND	ND	ND	21 (77.8)	6 (22.2)	27 (100)
Total	40 (3.4)	65 (5.5)	83 (7.1)	14 (1.2)	2 (0.2)	1 (0.1)	972 (82.6)	205 (17.4)	1177 (100)
APSH	10 (5.1)	13 (6.6)	12 (6.1)	5 (2.5)	ND	ND	157 (79.7)	40 (20.3)	197 (100)
DSH	ND	3(12)	3 (12)	1(4)	ND	ND	18 (72)	7 (28)	25 (100)

ND, Not Detected; APSH, KPJ Ampang Puteri Specialist Hospital; DSH, KPJ Damansara Specialist Hospital; KSH, KPJ Kajang Specialist Hospital; SSH, KPJ Selangor Specialist Hospital; TSH, KPJ Tawakkal Specialist Hospital; KLG, KPJ Klang Specialist Hospital; STS, KPJ Sentosa Specialist Hospital; RSH, KPJ Rawang Specialist Hospital; HQ, KPJ Lablink Headquarters.

Based on Figure 2, the trends of the total confirmed dengue cases exhibited an increasing pattern of cases from December 2018 to February 2020. There was no evidence of the seasonal trend for DENV-1 (Figure 2A) and DENV-4 (Figure 2B), owing to the detection rate which was under the median for most of the time within four consecutive years. The detection rates for DENV-1 were observed to surpass the median detection rate on three-time points, and these were seen in May and June 2019, as well as February 2020. While the DENV-4 serotype was detected circulating starting from March 2019 onwards until November 2020.

The DENV-2 and DENV-3 had more apparent seasonal trends with detection rates exceeding the median on several time points. The DENV-2 showed eight-time points seasonality in December 2018, January to March 2019, June and July 2019, and October and November 2019 (Figure 2©). Meanwhile, DENV-3 showed more frequent seasonality with a total of 13 time-points which were November and December 2017, March, June and July 2018, January, February, May to July and December 2020 (Figure 2(D)). This implied that DENV-3 was emerged and replacing the previous DENV-2 and frequently circulates in contrast to DENV-2 within the study period.

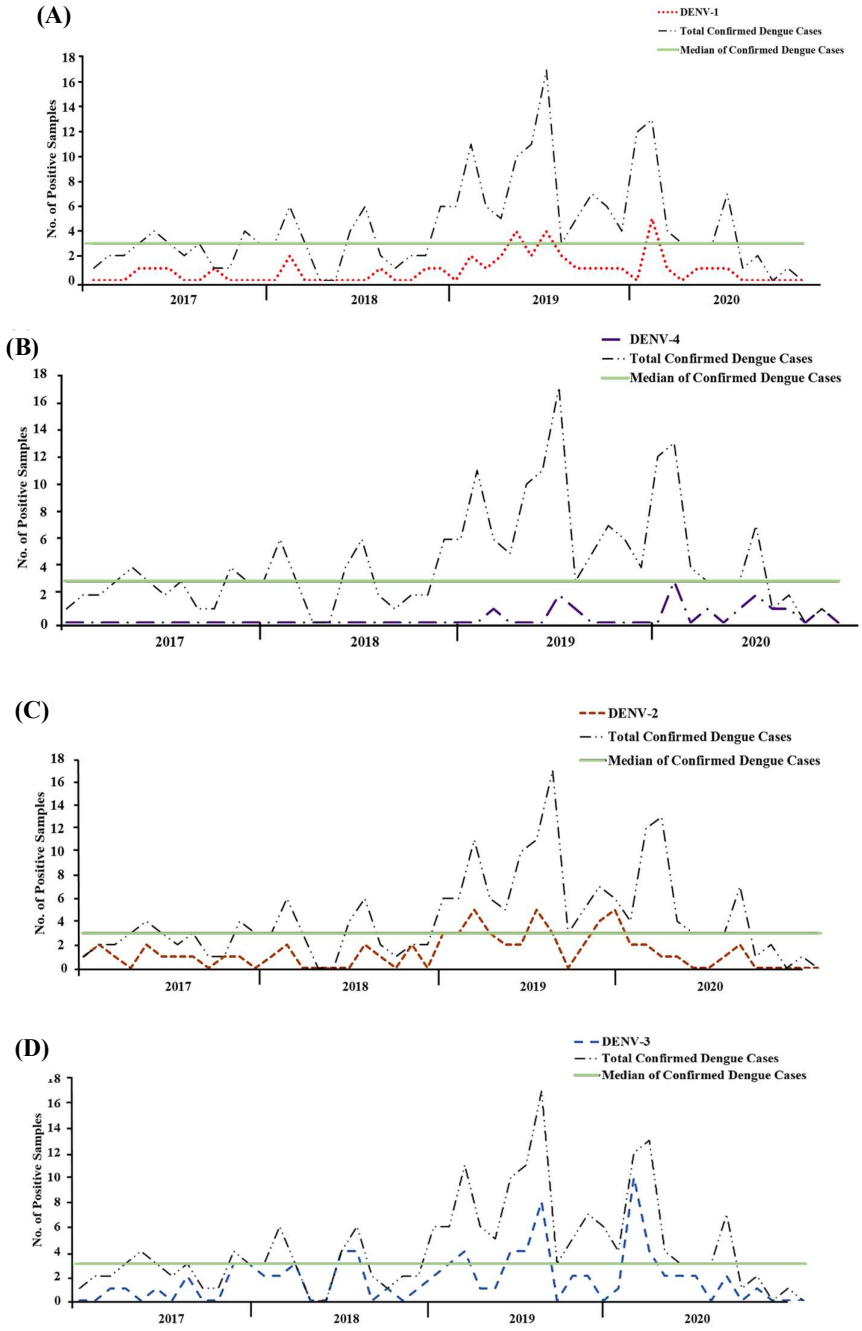

Figure 2 Trend and seasonality of dengue cases in KPJ hospital within the Klang Valley of Malaysia for four years from 2017 to 2020. Seasonality for DENV-1 (A), DENV-4 (B), DENV-2 (C) and DENV-3 (D) serotypes versus total confirmed dengue cases by RT-PCR.

3.3 Relationship of dengue with socio-demographic details and virus serotypes

Table 5 summarized the relationship between the confirmed dengue cases with socio-demographic details and DENV serotypes. There was no significant association found between confirmed dengue cases and socio-demographic details. However, a significant association between confirmed dengue cases and DENV serotypes ($p< 0.05$) was evident in the present study (Table 5). An increment of the odds ratio (OR > 1) in confirmed dengue cases was observed in Selangor (OR=1.29; 95% CI=0.7958, 2.0896), Malaysia. The risk of infection according to gender, age groups, and DENV serotypes revealed an OR < 1, indicating that the risks of getting infected with dengue were not much affected by age, gender, and DENV serotypes in the present study. Nonetheless, DENV-3 had a slightly higher odd ratio (OR=0.71; 95% CI=0.5278, 0.9441) in comparison to other DENV serotypes.

Table 5 Odds Ratios (OR) between confirmed dengue cases with socio-demographics detail and DENV serotypes.

Risk Factor	Confirmed Dengue, n = 205 (%)	Non-dengue, n = 972 (%)	OR	95% CI	*p*-value*
Gender					
Male	113 (55.1)	568 (58.4)	0.89	0.664 to 1.206	0.465
Female	92 (44.9)	404 (41.6)			
Age (Years)					
17 and below	31 (15.1)	151 (15.5)	0.97	0.644 to 1.473	0.901
18 and above	174 (84.9)	821 (84.5)			
State					
Selangor	184 (89.8)	842 (86.6)	1.29	0.796 to 2.090	0.302
Kuala Lumpur	21 (10.3)	130 (13.4)			
DENV Serotypes					
DENV-1	40	1137	0.31	0.220 to 0.447	0.000*
DENV-2	65	1112	0.47	0.347 to 0.640	0.000*
DENV-3	84	1093	0.71	0.528 to 0.944	0.019*
DENV-4	14	1163	0.07	0.043 to 0.128	0.000*

**p*-values were calculated by Pearson Chi-Square with a significance value of $p < 0.05$.

4. Discussion

A temporal trend in dengue incidence should be employed for continuous surveillance activities to ensure effective epidemiological control measures in dengue hyperendemic countries like Malaysia. Our findings on the predominant DENV serotypes within the Klang Valley, provide current and updated information on the circulating DENV serotypes from 2017 to 2020 which agreement with previous study [16].

It was noted that there were an increasing number of requests for RT-PCR dengue differential testing from Shah Alam, Selangor, owing to the escalation of suspected dengue cases in Ganesh [25] and the availability of the test offered by the private main laboratory which reflects the advantages of the RT-PCR including rapidity of the test and DENV strain identification. The significant stratification of dengue prevalence by age group, gender, and location reflects the influence of socio-demographic factors on the dengue incidence rate. The high male-to-female ratio in the dengue prevalence rate, particularly in the adult age group, indicates differences in their nature of work and the workplace between the males and females, leading to unequal exposure to the dengue [2,8].

The high number of dengue cases among young adult males in the present study is parallel to dengue incidence and epidemiological features in Singapore, Sabah, and Kelantan [5,8,10]. Most cases are among males aged between 25 to 44 years, and economically productive age range [5,8,10]. The same studies reported that dengue incidence occurred lowest in those below 10-year-old and the elderly within the 50 years age group [5,10]. They also reported a higher number of male patients than females throughout the 12 years of surveillance, with a male to female ratio of 1.5:1.0, which is consistent with our result (1.2:1.0). The high dengue incidence rate in males aged 15 and above were reported in four other culturally and economically diverse countries, namely the Philippines, Singapore, Sri Lanka, Cambodia and Malaysia [2].

The predominant laboratory spectrum of DENV serotypes reported for single (DENV-3) and co-infections here is unique as compared to the predominant serotypes reported in Sabah (DENV-1) and Sarawak (DENV-2) previously [13,14]. In contrast, the prevalence of DENV-4 has been scarcely reported within Southeast Asia [26]. However recent spatio-temporal study on DENV serotypes in Quang Nam Province, Vietnam, during the Outbreak in 2018 reported that the predominant circulating serotype was DENV-4 [27]. The divergence in the

reported predominant circulating DENV serotypes could be attributed to different geographical locations despite two previous and present studies using RT-PCR for DENV serotyping [13,14].

The difference might be related to switching predominant DENV serotypes in Malaysia from DENV-4 to DENV-1, then to the recent DENV-2 and DENV-3. Twelve years of a temporal trend on dengue clinical characteristics and the circulating DENV serotypes in Singapore revealed a cyclical epidemic pattern between 2004 to 2016 [5]. Singapore had an oscillation of predominant circulating serotypes between DENV-2 and DENV-1, in which older adults were more affected by DENV-2 than DENV-1 [5]. Similar outbreak cycles between DENV1 and DENV2 serotypes in Klang Valley, Malaysia, were recorded from 2003 until 2016 [16]. The DENV3 was prevalent in 1982 (41%), 1986 (78%), and 1992 to 1995 (55-93%) [16]. The last DENV-3 outbreak within Klang Valley, Malaysia, was recorded in 2002 [16].

Thus, the predominant DENV-3 reported in the present study signifies an early indication of the rise of DENV-3 serotype circulation in the Klang Valley, Malaysia. This finding corresponds to the DENV-3 recent report observed in Singapore as reported by the National Environment Agency in February 2020 [6]. Nevertheless, a larger-scale epidemiological study should be conducted to provide more comprehensive data on Malaysia's laboratory spectrum of the DENV serotypes. The evidence of the shift in DENV strains is crucial, as this may have implications in the clinical manifestation based on the previous report related to the reintroduction of a new DENV strain to the community may cause severe manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [28]. Furthermore, several local reports showed that the dengue outbreak could arise within four to six months after switching predominant circulating DENV serotypes due to the low population herd immunity against the new serotypes [10,14,17].

Concerning the factor that increases the risk of getting infected by DENV, our study revealed that the population in Selangor had a higher risk (OR=1.29) of contracting dengue compared to other cities within the Klang Valley, such as Kuala Lumpur. Furthermore, male patients aged 17 years or below with a fever had a likelihood between 89% to 97% (OR=0.89, 0.97, and 0.94) of being diagnosed with dengue. Besides that, in every confirmed dengue case, the likelihood of DENV-3 causing dengue was 71% (OR=71%) ($p < 0.05$).

Our time series analysis confirmed the re-emergence of DENV-3 and its seasonality for four consecutive years. We observed the recurring cycle of DENV-3 as the predominant serotype in Klang Valley, Malaysia, between 2017 and 2020 since the last reported outbreak in 2002 [16]. In the Klang Valley, the cyclical pattern of DENV serotypes was from DENV-1/DENV-2 in 2002 to 2016, while in 2017 to 2020, DENV-3/DENV-2 predominates and is evident in the present study.

Another interesting observation was detected in the present study, including the peak season of confirmed dengue which occurred in January to March and May to July, alternating with the rainfall between March to May and October to December. However, the correlation analysis between the rising dengue cases and rainfall could not be tested accordingly due to the unavailability of meteorology data between the periods, which is worth to be explored. Furthermore, these observations could imply an interplay between human, climate, and mosquito dynamics in navigating the transmission of mosquito-borne diseases, as reported by several studies [29-33]. Overall, the present spatio-temporal study has achieved its main objective. Nevertheless, it has limitations, including the lack of meteorological data on rainfall and clinical data to ascertain the relationship between DENV strains and severity of clinical manifestation. The incomplete serological data due to non-standardized requests from clinicians also restricts the comparison analysis between RT-PCR and other serological methods in laboratory diagnosis of dengue.

Despite all limitations discussed above, our finding provides a crucial benchmark which can be translated into recommendation of the public health management aspect of the dengue epidemic in the current national guideline. This may include the geographical distribution of the laboratory confirmed cases and DENV strains isolated, which will be reviewed weekly, and over time, allows public health epidemiologists and laboratorians to gain information for dengue transmission in their catchment area, while providing them with the real-time information. Simultaneously, the local municipal health authorities should be able to report on the mosquito surveillance in the catchment area accordingly, thus major epidemics can be controlled or prevented prior to peak transmission. Besides that, by knowing the shifting of DENV strains, further epidemic activity can be predicted and preparedness plans with intervention strategies would be appropriately outlined to control the predicted epidemic which is known as an early warning surveillance system that allow responsible authorities to implement early emergency response to reduce epidemic transmission, and reduce mortality.

5. Conclusion

Our study describes limited spatio-temporal circulating DENV serotypes based on secondary data obtained from the private laboratory in Klang Valley, representing the dengue epidemic in the Malaysian urban community. The predominant DENV-3 serotype from 2017 to 2020 emphasizes Malaysia's recurring cyclical patterns of significant dengue outbreaks. The presented spatio-temporal data here could be used to monitor the incursion and spread of dengue, particularly after switching the predominant dengue serotype that may lead to

an outbreak and severe life-threatening situation. An early warning system could be developed to determine the circulating and cyclic pattern of predominant serotypes leading to prompt decision-making in disease intervention to reduce the health burden of dengue in endemic countries such as Malaysia, hence highlight the importance of the present study.

6. Ethical approval

The present study was approved by the KPJ Clinical and Research Ethics Review Committee (CRERC/15092020) and Universiti Teknologi MARA (UiTM) Research Ethics Committee (REC/654/19). All patients' data analyzed were anonymized.

7. Acknowledgements

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