Implications of the COVID-19 lockdown on dengue transmission and the occurrence of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in Malaysia

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Abstract

The impact of movement restrictions (MRs) during the COVID-19 lockdown on the existing endemic infectious disease dengue fever has generated considerable research interest. We compared the curve of weekly epidemiological records of dengue incidences during the period of lockdown to the trend of previous years (2015 to 2019) and a simulation at the corresponding period that expected no MRs and found that the dengue incidence declined significantly with a greater magnitude at phase 1 of lockdown, with a negative gradient of 3.2-fold steeper than the trend observed in previous years, indicating that the control of population movement did reduce dengue transmission. However, starting from phase 2 of lockdown, the dengue incidences demonstrated an elevation and earlier rebound by 4 weeks and grew with an exponential pattern. Together with our data on *Aedes* mosquitoes, we proposed a stronger diffusive effect of vector dispersal that led to a higher rate of transmission. From the result of the *Aedes* survey using human landing caught (HLC), we revealed that *Aedes albopictus* is the predominant species for both indoor and outdoor environments, with the abundance increasing steadily during the period of lockdown. We only recovered *Aedes aegypti* from the indoor environment, which is relatively fewer than *Ae. albopictus*, by contrasting their population growth, which suggested that *Ae. albopictus* invaded and colonized the habitat of *Ae. aegypti* during the period of lockdown. These findings would help authorities review the direction and efforts of the vector control strategy.

Author summary

COVID-19 pandemic is taking hold globally and dengue fever transmission is not on the top of the list of concerns. With a partial lockdown implemented by Malaysia on 18 March, we postulate the movement restrictions (MRs) of people in large-scale would hamper the regular dengue transmission and aim to reveal the impact of MRs on both dengue incidences and *Aedes* mosquitoes. We showed a significant decline of dengue incidences at the beginning of lockdown but later rebounded at an earlier time and higher rate compared to the corresponding period of previous years. Our result also reviews how adaptive the *Ae. albopictus* with the movement of the host, as the human contained in the house, the abundance of the mosquitoes increased significantly during the period of lockdown. We also suggest that *Ae. albopictus* could be the key substitution vector that contributes significantly to dengue virus circulation, and therefore, the vector control direction and strategies should be redesigned.

Introduction

Lockdown has been commonly implemented all over the world to halt COVID-19 transmission [1]. This is to control human movement, as physical proximity is a key risk factor for the transmission of SAR-CoV-2 [2]. Although the physical proximity of humans shapes the spatial spread of a pathogen, it refers mostly to directly transmitted infectious diseases [3] such as COVID-19, but the effect on indirectly transmitted infectious diseases such as dengue fever (DF) remains unclear. By using an agent-based transmission model, Reiner et al. [4] indicated that the socially structured movement of humans caused a significant influence on dengue transmission, although the infection dynamics were hidden by the diffusive effect of mosquitoes. Falcón-Lezama et al. [5] used a mathematical model to evaluate the effect of people's day-to-day movement on the dengue epidemic and concluded that the vector-host and spatial connectivity posted epidemic risk. To simulate the actual situation, Stoddard et al. [6] used contact-site cluster investigations in a case-control design to review the risk of dengue infection by human movement and argued the importance of movement restriction in managing the spatiotemporal dynamics of dengue virus. However, the previous experimental configurations were far from the real situation, especially when the mobility of the population on a large scale is not feasible to demonstrate the direct effect on dengue transmission.

Dengue fever (DF) is the most prevalent mosquito-borne disease in the world [7]. For the past two decades, DF has been the central public health problem for Malaysia, and the endemic dengue in Malaysia is seasonal, with variable transmission and prevalence patterns affected by the large diversity in rainfall and spatial variation [8]. The major transmission periods of DF occur from June to September, following the main rainy seasons. The minor transmission period is from September to March, following monsoon rains sessions that bring higher precipitation and lead to the greater potential breeding ground for the vector [9]. Since the end of the minor peak and the start of major peaks of DF transmission often coincides with the duration of the COVID-19 lockdown, we are particularly interested in the impact of movement restrictions on the endemic of dengue transmission and vector occurrence. With the imposition of the COVID-19 partial lockdown in Malaysia, about 90% of people were restricted to their homes, and 10% essential workers were allowed to carry out their daily activities [10]; thus, the unprecedented large-scale movement restriction highly potentially spurred dengue endemics.

The spatial distribution of the DF vectors *Aedes aegypti* and *Aedes albopictus* are potentially affected by movement restrictions (MRs). Both *Ae. aegypti* and *Ae. albopictus* are highly anthropophilic, in which *Ae. aegypti* almost exclusively rely on human blood and *Ae. albopictus* is an aggressive and highly adaptive species that can easily colonize the habitat of other mosquitoes in urban areas [11]. Numerous studies [11-13] have shown that the occurrence of *Aedes* mosquitoes is significantly different from the human population density, strongly supporting the idea that vector-parasite interactions depend on the spatial distribution of the host. In addition, the spatial distribution of the host also influenced the behavior of the vectors, and previous studies [14-15] identified a shift of the *Ae. albopictus* habitat to an indoor environment where the species usually inhabit the forest or are mostly vegetative and cause interspecies competition with other existing mosquito species, such as *Ae. aegypti*. Therefore, when the COVID-19 partial lockdown restricts humans in mostly indoor environments with minimum outdoor activities, the occurrence of mosquitoes is desirable.

We study the effect of the physical proximity restriction on humans during the COVID-19 lockdown in Malaysia on two main variables:

Dengue transmission trend. One of the goals in this study is to understand the impact of MRs on the trend of dengue transmission. We conduct a temporal analysis for dengue incidences from 2015 to 2019 and construct a simulation that expects no MR practice and statistically compares the actual trend with the trends of previous years and simulation to evaluate the level of heterogeneity.

The occurrence of vectors. We do not know the extent to which movement restriction influences vector dynamics and therefore aim to obtain data on the abundance and distribution of *Aedes* mosquitoes during a period of lockdown by conducting sampling in the indoor and outdoor environments of an area that was historically a dengue hotspot.

Methods

Data collection

We started the analysis from the data of 2015, which recorded the second-highest dengue incidence after 2019 [16]. We retrieved the data from the official press statement and the Dengue Surveillance System developed by the Vector-Borne Disease Section, Ministry of Health (MOH), Malaysia, to monitor dengue transmission using remote sensing (RS) –

iDengue [17], under the supervision of Remote Sensing Agency Malaysia. This surveillance system monitors dengue transmission across the country by updating dengue fevers reported in every hospital and medical institution on a daily basis, and all notified cases were followed up by the relevant health authorities for case verification before being recorded in the registry of the Dengue Surveillance System.

Temporal analysis of dengue incidences during partial lockdown

We observed the previous temporal pattern of dengue incidences and found that the period of the COVID-19 partial lockdown (March 18 to June 9, 2020) coincided between the end of minor (March-May) and the start of major (June-Sept) fluctuations of dengue transmission (Fig. 1). Therefore, to understand the heterogeneity of the trend of weekly incidences due to the partial lockdown, we compared the trend with two reference trends, namely, simulation and mean weekly incidence from 2015-2019. Both references were without the interference of city lockdown and population movement control. To construct a simulation, we applied auto-regressive integrated moving average (ARIMA) models, which are advantageous for modeling the time-based dependent configuration of a time series [18] and are commonly applied for epidemiological surveillance. We trained the models by using weekly dengue incidences from the dataset from 2015 to 2019 and the means of the incidences. To select the best-fitting dataset to construct the weekly trend of dengue incidence during the COVID-19 partial lockdown of Malaysia, the simulated model before lockdown (weeks 1 to 12 of 2020) was correlated with the actual weekly trend of dengue incidences by using Spearman rank correlation at the significance level of 0.05 (SPSS 17.0, IBM Corp. IBM SPSS Statistics for Windows, Version 17.0. Armonk, NY: IBM Corp.), and the dataset with the strongest correlation was selected to further develop the simulated trend during the partial lockdown. To refine the ARIMA model, we finetuned the parameter (p,d,q) [p is the order of autoregression, d is the degree of differencing, q is the order of the moving-average model] based on the model proposed by previous studies [19-20]. The best-fitting model was selected based on the lowest values of the normalized Bayesian information criterion (NBIC) and the root mean square error (RMSE) [20]. Furthermore, we divided the time series of weeks 1 to 24 of 2015-2020 into eight stages (two prelockdown periods, five phases during the partial lockdown, and postlockdown) according to the announcement from the Malaysia government [21] (Fig. 1 and Table 1) and compared the actual weekly dengue incidences during the period of partial lockdown with the those of the simulation and previous years (2015-2019) using two-way ANOVA with two independent variables, namely, years and stages. Because the trends follow an open-up parabolic pattern, we further distinguish the pattern by comparing the slopes for the stages to study the rates of decline and endemic incline.

Fig 1. Dengue endemic growth from years 2015 to June of 2020. Eight stages: Pre2, Pre-lockdown 2; Pre1, Pre-lockdown 1; P1, Phase 1; P2, Phase 2; P3, Phase 3; P4, Phase 4; P5, Phase 5; Post, Post-lockdown

Table 1 Division of Eight Stages of time series for data analysis

Stages	Phases	Terms	Time
1	Pre-2		19 February to 3 March
2	Pre-1		4 March to 17 March
3	P1*	MCO	18 March to 31 March
4	P2	MCO	1 April to 14 April
5	P3	MCO	14 April to 28 April
6	P4	MCO	29 April to 12 May
7	P5	CMCO	13 May to 9 June
8	Post	RMCO	10 June to 31 August

MCO - Movement Control Order
CMCO - Conditional Movement Control Order
RMCO - Recover Movement Control Order
*for 2020, week 12 remarks the starting of COVID-19 partial lockdown

Mosquito abundance and distribution

Site selection. To understand dengue transmission, we have to assess the abundance and distribution of *Aedes* mosquitoes during the host MR, and we are particularly interested in the spatial distribution of the vector for indoor and outdoor environments due to the possibility of a decrease in artificial breeding sites reported at the beginning of lockdown [22] and the host contained in their housing area. The sites were selected from a residential area, namely, Taman Bukit Jambul, Bayan Lepas, Penang Island, Malaysia (GPS - 5°20'06.6"N 100°17'18.7"E), which is located within the Southwest Penang Island District and was a dengue hotspot area in 2005 and 2017 [23]. Due to the restriction of movement, the sampling was conducted within a 10-km radius from the home of the participant [24]. The sampling was assessed at five outdoor (n=5) and indoor (n=5) locations covering an area of 12.11 hectares (Fig. 2).

Fig. 2 Sampling locations at Taman Bukit Jambul, Penang Malaysia

Human landing catch (HLC). For high anthropophilic mosquitoes, the human landing catch (HLC) method is the most effective sampling method [25], although it poses the risks of the human contracting the mosquito-borne pathogen, especially at the location where dengue and chikungunya are endemic. We obtained consent from the participant for the first author (male, blood group A+, 33 years old, BMI 24.22 kg/m^2), who is a trained medical entomologist, to conduct the sampling at the location. The caught was performed in the early morning from 7:30 A.M. to 10 A.M. with the left arm and both legs exposed without any artificial chemical (e.g., lotion and body shampoo) interference; the mosquitoes were collected by a manual aspirator and transferred to a sealed container. Mosquitoes were killed by freezing, counted, and identified using taxonomy keys. The counts of *Ae. aegypti* and *Ae. albopictus* from indoor and outdoor locations for the eight stages of the time series during partial lockdown were compared by using two-way ANOVA with post hoc LSD.

Mosquitoes taxonomy. We focused on two main vectors of dengue in Malaysia – *Ae. aegypti* and *Ae. albopictus* in which contribute to the majority of dengue incidences [26]. The identification of mosquitoes was assisted with a device – *Aedes* Detector that able to enlarge the specimen with high resolution. In addition to the common distinguish features - the lyre-shaped markings on *Ae. aegypti* and the white stripe marking on the thorax of *Ae. albopictus*, we also focused on the scutellum and clypeus-pedicel parts of the mosquitoes that consisted of distinctive white scales on the mesepimeron on *Ae. aegypti*.

Results

Temporal analysis of dengue incidences during partial lockdown

Most of the studies on the growth of dengue incidences in Malaysia have focused on the total cases reported annually, in which the proposed model may not be sensitive and flexible enough to predict growth and propose necessary management. To our knowledge, this study is the first to report a simulation model with weekly dengue incidences in 2020, including the period of COVID-19 partial lockdown in Malaysia. To select the best-fitting simulation model, Table 2 shows the comparison of the NBIC, RMSE, and MAPE of three ARIMA models, and the best-fitting forecast model for dengue incidences is ARIMA (1,1,0) due having the lowest NBIC and RMSE. The dataset of the mean weekly incidences of dengue incidences from 2015 to 2019 was selected to conduct the simulation because the strongest correlation ($r = 0.909$, $p<0.001$) was obtained for weeks 1 to 12 of 2020 (before partial lockdown) with the actual incidences trend. As seen in Fig. 3, when we generated a simulated model on the trend of weeks 12 to 24 of 2020, which coincided with the COVID-19 partial lockdown period in Malaysia, the actual dengue incidence trend was significantly diverted and demonstrated a strong negative correlation compared with the simulated trend ($r = -0.944$, $p<0.001$), which implied that the actual dengue incidence trend significantly disobeyed the simulation, which presumed no lockdown, indicating that movement control greatly impacted dengue transmission in Malaysia.

Table 2 ARIMA model evaluation

Model	(0,1,0)	(1,1,0)	(1,1,1)
NBIC	9.44	9.38	9.46
RMSE	107.80	100.97	101.07
MAPE	4.11	3.77	3.73
R^2	0.89	0.91	0.91

NBIC = Normalized Bayesian Information Criterion
RMSE = Root Mean Square Error
MAPE = Mean Absolute Percentage Error

Fig. 3 Simulation of dengue incidence by using mean of 2015 to 2019 weekly incidences and correlation between the simulated and the actual trend before and the duration of partial lockdown. ULC, upper confidence level; LCL, lover confidence level.

To further analyze the changes in dengue incidence trends due to the partial lockdown, we divided the time series of endemical weeks into eight stages as described in Table 1, and Fig. 4 describes the comparison of the eight stages for previous years (mean weekly incidence of 2015-2019), the simulated trends and actual dengue incidences during the COVID-19 partial lockdown, and the slope for the particular stages. Although many researchers have indicated that the dengue incidences in 2020 were lower than those in 2019 [16], when we averaged the weekly incidences of the previous five years (2015-2019), 2020 had significantly higher dengue incidences at prelockdown 1 and 2. Nevertheless, the slopes between the dengue incidences of the previous years and those of the year 2020 during the period of prelockdown (1 and 2) are fairly the same. When Malaysia imposed phase 1 of the partial lockdown, the slope declined dramatically, which was 319% steeper than in previous years. This provides a strong implication that movement control during partial lockdown significantly reduced the reported dengue incidences. Although at phases 2 to 4, the incidences in 2020 were significantly lower than those in previous years, when we compared the stages for the slope to become positive (which indicates an upsurge in dengue incidences), this change in slope occurred in 2020 two stages (4 weeks) earlier than in previous years; specifically, a positive slope was obtained at phase 3 in 2020 compared to in previous years, in which a positive was obtained at phase 5. Furthermore, at phase 5, the steepness of the slope of the year 2020 spiked from phase 4 to postlockdown compared to previous years, with the steepness increasing from 22% to 227%, suggesting a significant increase in dengue transmission.

Fig. 4 Analysis of variance (ANOVA) of dengue incidences of mean, simulation and year 2020 for eight stages of COVID-19 partial lockdown in Malaysia and the slope for the stages. Pre-LD-2, Pre-lockdown-2; Pre-LD-1, Pre-lockdown-1; P1, Phase 1; P2, Phase 2; P3, Phase 3; P4, Phase 4; P5, Phase 5; Post-LD, post-lockdown.

Distribution of mosquitoes

To study one of the factors that contributes to the spread-out of dengue transmission during the COVID-19 partial lockdown, we assessed the abundance and distribution of vectors during the partial breakdown. Fig. 5 shows the temporal numbers of *Ae. albopictus* collected from the outdoor area of the sampling location during the period of partial lockdown. *Ae. albopictus* is the predominant species in the outdoor area, with no *Ae. aegypti* was caught. The abundance of *Ae. albopictus* showed slight fluctuation patterns during the partial lockdown but still demonstrated a strong linear increment ($R^2 = 0.7199$) throughout the eight stages of partial lockdown. In general, the total number of mosquitoes caught indoors was significantly lower than that outdoors, and we reported the occurrence and abundance of *Aedes* mosquitoes in the indoor environment during COVID-19 and found that both *Ae. aegypti* and *Ae. albopictus* were caught indoors, with the abundance of *Ae. aegypti* being relatively low and plateauing throughout phase 3 to post-LD. However, *Ae. albopictus* demonstrated higher abundance and exponential growth with the population during the same corresponding period (Table 3).

Table 3 Total Aedes mosquitoes caught from indoor environment of sampling location

Phases	*Aedes aegypti*	*Aedes albopictus*
P3	0	4
P4	3	2
P5	4	9
Post	2	12

P3, Phase 3
P4, Phase 4
P5, Phase 5
Post, post-lockdown

Our objectives did not include the correlation between dengue incidences and mosquito's abundances. This is due to these few reasons – (1) The extent of dengue transmission is determined by multiple factors, exp. the level of herd immunity in the population, the virulence of virus, survival, feeding behavior of vectors, etc.; (2) Non-aligned of sampling timeline and location between the dengue incidences and entomological data, the participant started the outdoor sampling on 22 March (phase 1 of lockdown), and therefore no data for the pre-lockdown is available. Furthermore, due to the rules of movement restriction, indoor sampling could only happen at the lobby (ground floor)

Fig. 5 Total counts of Aedes albopictus for the dates and phases of COVID-19 partial lockdown at the outdoor environment of sampling location.

Discussion

The factors that contribute to dengue transmission are multifaceted, and the spatial variation in the contact rates of the host and vector are probably the most important factors for the dynamics of DENV [27]. With the MRs imposed due to the COVID-19 pandemic, we can investigate the effect of the large-scale MRs of the host on two interrelated variables: dengue transmission and spatial distribution of *Aedes* mosquitoes. We analyze the dengue incidence trends by comparing their significant differences among the stages before, during, and after the lockdown to those of the same corresponding periods for previous years and simulation. We first reported evidence that the MRs of the COVID-19 partial lockdown significantly influenced the weekly dengue incidence trend in Malaysia. Our findings provide direct evidence from analysis and extend the studies of Reiner et al. [4] and Falcón-Lezama et al. [5], which demonstrated that people's movement affected dengue transmission by using a simulation model. The early decline in dengue incidences was also reported in India, with dengue cases dropping by 50% compared to previous years. The decline of incidences at the beginning of the lockdown could have occurred for several reasons: (1) fewer hosts available outdoors and therefore less vector-host contact, as *Ae. aegypti* and *Ae. albopictus* are exophilic [28]; (2) the alteration of the environment and relatively fewer artificial breeding sites for the vector due to less solid waste from humans [22]; and (3) the limited movement of infected patients due to the COVID-19 partial lockdown.

Unfortunately, our analysis showed that the dengue incidences rebounded earlier and spiked up at a higher rate than in previous years, indicating that the large-scale MRs of the population are not sustainable in controlling the spread of dengue. The finding is compatible with the situation in Singapore, which has had the most serious dengue outbreak in seven years [29], and an agent-based simulation model study by Jindal and Rao [30] that showed a significantly higher risk and severity of dengue transmission after the COVID-19 partial lockdown. The stay-at-home situation makes the host available most of the time in the indoor environment and optimizes the biting activities for endophagic *Ae. aegypti* to transmit the virus. In contrast to Harrington et al. [31], who argued that people, rather than mosquitoes, rapidly move the virus within and between rural communities and places due to the limitation of the flight range of female *Ae. aegypti*, our result of *Aedes* mosquitoes revealed that the element of vector dispersal plays a more crucial role in spreading the virus. We also proposed the idea of the vector *Ae. Albopictus*, which showed increased abundance during the MRs on people posted to a stronger diffusive effect of vector dispersal and therefore caused earlier rebound and a higher rate of dengue transmission during lockdown. We also suggest that *Ae. albopictus* could be the key substitution vector that contributes significantly to dengue virus circulation, and therefore, the vector control direction and strategies should be redesigned.

With no current entomological data about female adult *Ae. albopictus* in the corresponding period with lockdown, we refer to Rozilawati et al. [12] and Rahim et al. [32], who studied the seasonal abundance of *Ae. albopictus* in Penang by sampling eggs, the Ovitrap index, the container index (CI), the house index (HI) and the Breteau Index (BI). Their results demonstrated that the indexes of *Ae. albopictus* for the corresponding period of phase 2 to 4 of lockdown should be lower, in contrast to our finding that the abundance of *Ae. albopictus* increased steadily from phases 1 to 5. There are several reasons for the increase in *Ae. Albopictus*. First, as proposed by the WHO [33], the upsurge of *Aedes* mosquitoes may be due to the southwestern monsoon (end of May to September), which brought a higher frequency of precipitation and higher humidity and temperature, and therefore, a higher breeding rate for the mosquitoes. Second, a minimum centralized vector control program can be conducted due to the stay-at-home policy. It is relevant to any method that is intended to reduce dengue incidences by reducing, but not eliminating, *Aedes* mosquito populations. Before that, researchers [34] have associated the index of the temporal vector with dengue occurrence, and the relationships between *Aedes* mosquito density and DENV transmission indexes for *Ae. aegypti* density are correlated with the prevalence of human dengue infections but are relatively weakly correlated with the incidences, indicating that other factors were involved in determining the incidence pattern. This is supported by the participant during the *Aedes* survey when a fogging activity was observed on May 28, 2020 (phase 5 – conditional movement control order), and total *Ae. albopictus* was significantly lower on May 29, 2020 (Fig. 5), but the mosquitoes caught afterward remained elevated in general.

Furthermore, our findings showed the presence of both *Ae. agypti* and *Ae. albopictus* from an indoor environment but no *Ae. aegypti* from the outdoors, indicating that *Ae. albopictus* is better adapted to a sudden change in the environment, such as the duration of lockdown when most of the hosts shift to the indoors. With the consistent growth rate of the indoor and outdoor populations, we postulate that *Ae. albopictus* invades the habitat of *Ae. aegypti* and showed a high possibility of colonizing the habitat. Our result is consistent with Nur Aida et al. [35] and Dieng et al. [15], who found that they could increase the invasiveness of *Ae. albopictus* by obtaining a high number of egg and mosquito counts from the indoor environment of Penang Island. Previous studies [11, 13, 36] from other countries have also reported the aggressive invasive behavior of *Ae. albopictus*, which shared the habitat with other native or existing mosquitoes, including *Ae. aegypti*, which are commonly predominant in indoor environments [37]. Due to the restriction of traveling during the period of lockdown, our results provided limited area coverage, but when considering the scale of the study as a semifield assessment, the results propose a few important discoveries of vector distribution and occurrence during the MRs of lockdown.

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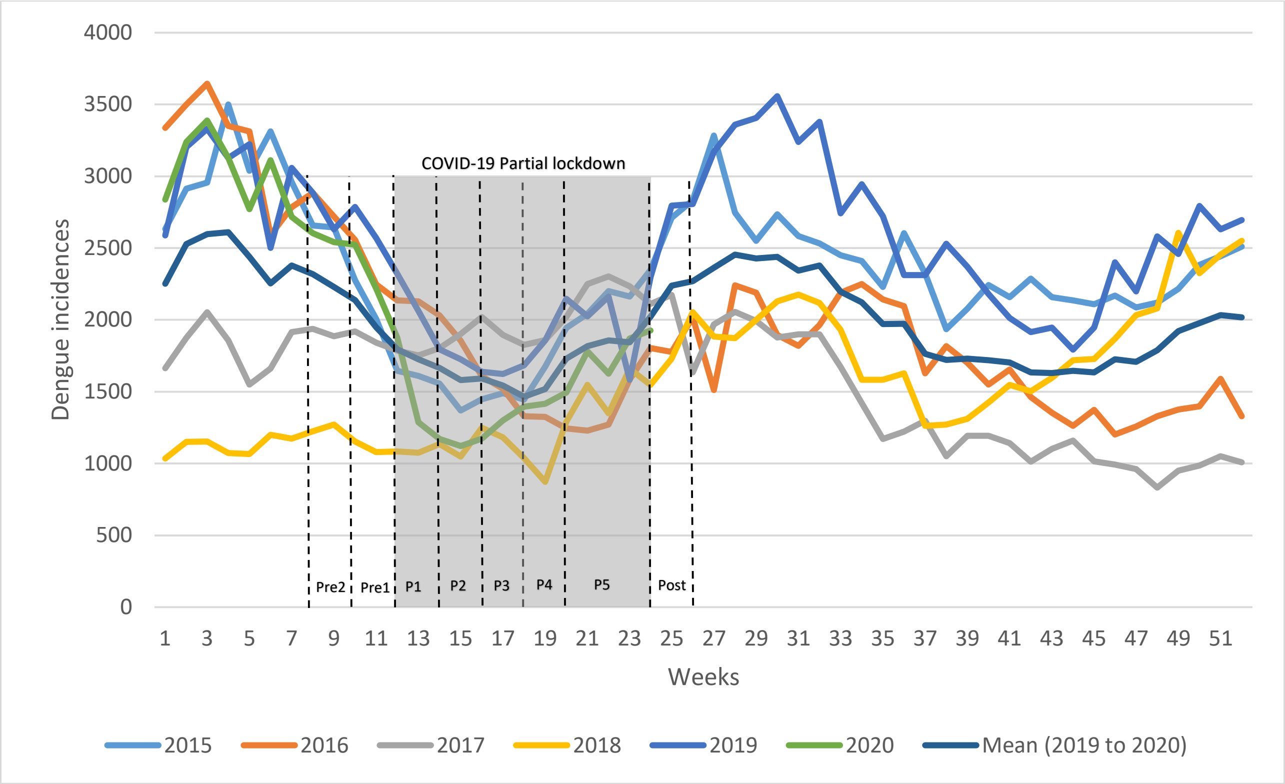


Fig 1. Dengue endemic growth from years 2015 to June of 2020. Eight stages: Pre2, Pre-lockdown 2; Pre1, Pre-lockdown 1; P1, Phase 1; P2, Phase 2; P3, Phase 3; P4, Phase 4; P5, Phase 5; Post, Post-lockdown

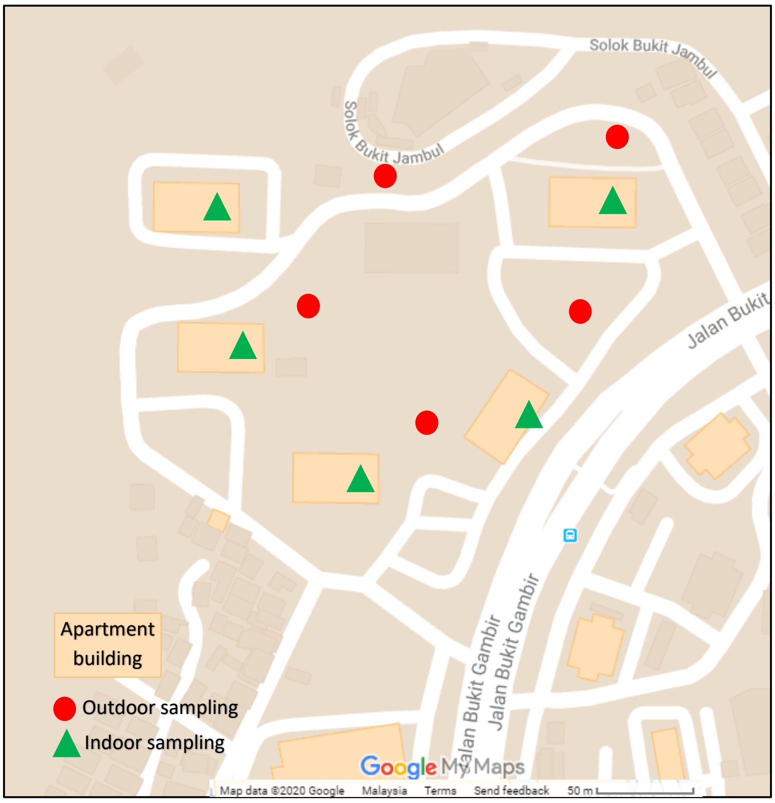


Fig. 2 Sampling locations at Taman Bukit Jambul, Penang Malaysia

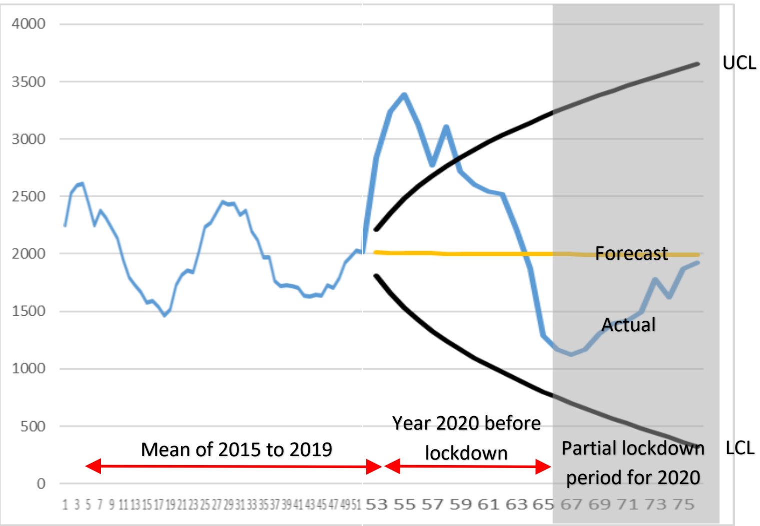
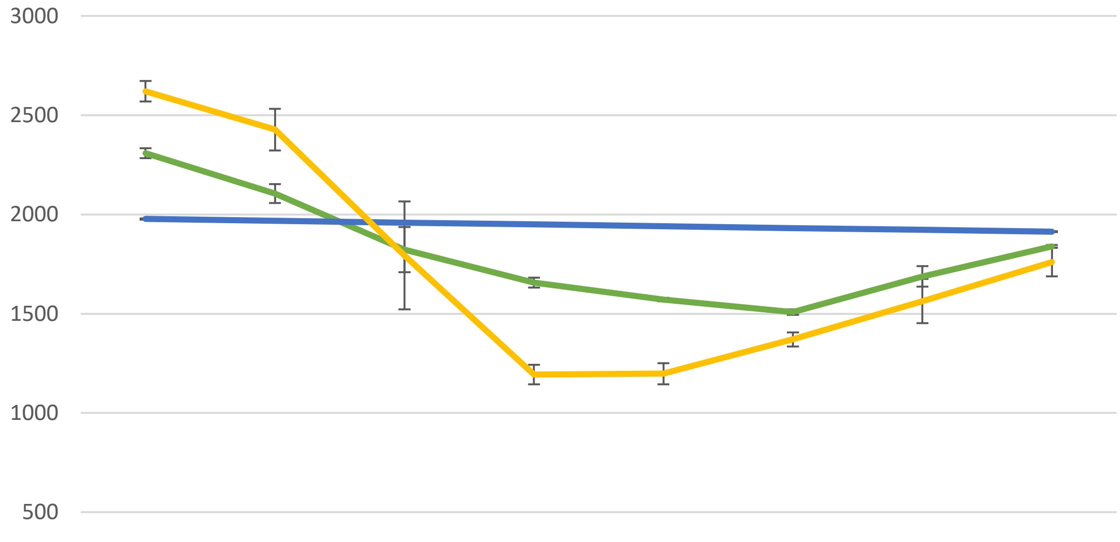


Fig. 3 Simulation of dengue incidence by using mean of 2015 to 2019 weekly incidences and correlation between the simulated and the actual trend before and the duration of partial lockdown. ULC, upper confidence level; LCL, lover confidence level.



	Pre-LD-2	Pre-LD-1	P1	P2	P3	P4	P5	Post-LD	
Mean (year 2019 to 2020) 🟩	-74.1	-140.6	-111.2	-73.2	-19	-11.9	149.5	13.6	SLOPE
Simulation 🟦	-0.22	-0.22	-0.22	-0.22	-0.22	-0.22	-0.22	-0.22	
Year 2020 🟨	-88	-161	-466	-83	90	57	183.5	44.5	

Fig. 4 Analysis of variance (ANOVA) of dengue incidences of mean, simulation and year 2020 for eight stages of COVID-19 partial lockdown in Malaysia and the slope for the stages. Pre-LD-2, Pre-lockdown-2; Pre-LD-1, Pre-lockdown-1; P1, Phase 1; P2, Phase 2; P3, Phase 3; P4, Phase 4; P5, Phase 5; Post-LD, post-lockdown.

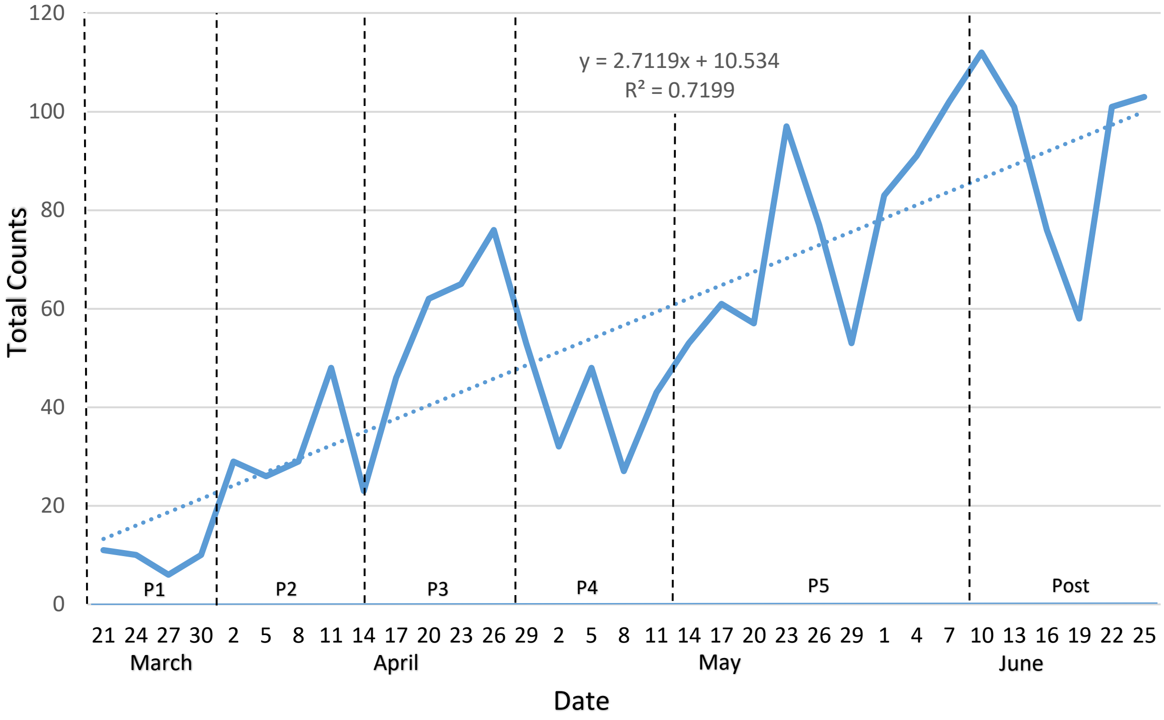


Figure 5 Total counts of *Aedes albopictus* for the dates and phases of COVID-19 partial lockdown at the outdoor environment of sampling location.