Inapparent dengue in a community living among dengue-positive *Aedes* mosquitoes and in a hospital in Klang Valley, Malaysia

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**ABSTRACT**

The public health burden of dengue is most likely underreported. Current dengue control measures only considered symptomatic dengue transmission. Hence, there is a paucity of information on the epidemiology of inapparent dengue. This study reports that many people have been unknowingly exposed to dengue infection. Almost 10% and 70% of individuals without any history of dengue infection and living in a dengue hotspot, in Selangor, Malaysia, were dengue IgM and IgG positive respectively. When dengue-positive mosquitoes were detected in the hotspot, 11 (6.3%) of the 174 individuals tested were found to have dengue viremia, of which 10 were asymptomatic. Besides, upon detection of a dengue-infected mosquito, transmission was already widespread. In a clinical setting, it appears that people living with dengue patients have been exposed to dengue, whether asymptomatic or symptomatic. They can either have circulating viral RNA and/or presence of NS1 antigen. It is also possible that they are dengue seropositive. Collectively, the results indicate that actions taken to control dengue transmission after the first report of dengue cases may be already too late. The current study also revealed challenges in diagnosing clinically inapparent dengue in hyperendemic settings. There is no one best method for diagnosing inapparent dengue. This study demonstrates empirical evidence of inapparent dengue in different settings. Early dengue surveillance in the mosquito population and active serological/virological surveillance in humans can go hand in hand. More studies are required to investigate the epidemiology, seroprevalence, diagnostics, and control of inapparent dengue. It is also crucial to educate the public, health staff and medical professionals on asymptomatic dengue and to propagate awareness, which is important for controlling transmission.

1. Introduction

Dengue fever is a growing public health problem worldwide, with an estimated 390 million infections per year (World Health Organization, 2019). Likewise, the occurrence of dengue in Malaysia is high. As of 30th November 2019, 119 524 cases with 162 deaths have been reported this year (Ministry of Health Malaysia, 2019). Owing to the absence of anti-dengue drugs, vector control remains the mainstay of the dengue control programs in many countries (Chang et al., 2011), although recent evidence has indicated the ineffectiveness of current measures (Esu et al., 2010; Lau et al., 2017; Morrison et al., 2008). In addition, the current versions of dengue vaccines do not provide satisfactory levels of protection against the disease (Capeding et al., 2014).

Around 300 million of the estimated 390 million dengue infections per year are subclinical (Bhatt et al., 2013). Dengue virus infection has been known to present as clinically inapparent infection. People with inapparent dengue infection may have no clinical manifestations of typical dengue infections, or present as an illness that is mild and is not associated with a visit to a health care provider nor an illness related to absence from school or work. Thus, inapparent dengue infection may not be detected by surveillance programs as most programs collect data...
of visits to a health care provider or hospitalization as an indicator of dengue illness. Therefore, inapparent dengue infection contribute to the burden of dengue infection that goes undetected and hence “inapparent”.

Yet, there is a lack of information on the epidemiology of inapparent dengue disease. It is highly likely that inapparent dengue plays a role in the maintenance of dengue transmission in the absence of an epidemic, and may account for 84% of all dengue transmissions (Bosch et al., 2018). In Thailand, of the 5.7% rate of total virus infection in school students, 87% of these were either asymptomatic or mild (Burke et al., 1988). Symptomatic secondary dengue infection and inapparent dengue may also have nearly equal incidence among elementary students (Endy et al., 2002). Even though the prevalence of inapparent dengue varies by geographical location, time, and demography (Alera et al., 2016; Chastel 2012; Endy et al., 2002; Montoya et al., 2013; Wang et al., 2015), it has been reported that such individuals (which include asymptomatic, pre-symptomatic, and sub-clinical cases) can still transmit dengue to mosquitoes (Duong et al., 2015). Furthermore, relative to symptomatic dengue, inapparent dengue appears to be more infectious to Aedes mosquitoes (Duong et al., 2015). These phenomena constitute a major cause for concern since the public health burden of dengue is likely to be much larger than expected, and that current dengue control measures have only taken into account the symptomatic population.

In Malaysia, the seroprevalence of dengue ranges from 28–94% (depending on the subset of population being studied), and over 90% of individuals will invariably have a history of dengue seropositivity as early as the age of 45 (Chew et al., 2016; Dhanoa et al., 2018; Muhammad Azami et al., 2011). However, all relevant local researches published to date have only quantified subclinical dengue retrospectively based on dengue IgG – an immunological biomarker of dengue which persists in the blood for about a year post-infection. Concurrently, it has been reported that almost 9 of 10 seemingly-healthy individuals who were dengue-seropositive did not recall having a previous dengue infection (Dhanoa et al., 2018), thus corroborating the overwhelming majority of subclinical cases over symptomatic ones. However, since Aedes can only transmit the disease if it has bitten a viremic person within a certain period (usually from days −2 to 6 of the onset of fever) (Duong et al., 2015), only a small subset of all inapparent dengue cases actually contributes to the transmission of the disease. Accordingly, to improve the clinical relevance of inapparent dengue cases, seromarkers of recent dengue like NS1 and IgM should be employed.

With reference to the above discourse, dengue monitoring requires both active and passive surveillance. In line with that, the current study has attempted to estimate the occurrence of recent inapparent dengue in different settings using several types of tests. This will guide future studies on the early detection, transmissibility, infectiousness, diagnostics, and control of dengue.

2. Material and methods

2.1. Study population

This cross-sectional study was conducted with the aim of estimating the field and clinical occurrences of inapparent dengue in different populations (Table 1). To investigate the baseline exposure (seroprevalence) of residents to dengue, venous blood samples were collected from residents living in a dengue hotspot in Damansara Damai (3.1930°N, 101.5923°E), Petaling Jaya, Selangor, Malaysia, where an ongoing cluster randomized controlled trial (ClinicalTrials.gov ID: NCT03799237) on early dengue surveillance is being carried out (Liew et al., 2019).

To investigate the occurrence of clinically inapparent dengue among residents living around dengue-positive Aedes mosquitoes, the residents from 4 apartments in the intervention arm of the above trial
were sampled within the same week of detection of a dengue NS1 antigen-positive *Aedes* in these apartments (Table 1). The samplings were performed in tandem with the intervention of the trial, which is during notification of dengue-positive mosquitoes in the neighbourhood and education on dengue prevention. No intervention is carried out in the other 4 apartments which are in the control arm. Depending on consents, dengue NS1 antigen tests were done door-to-door, specifically the residential units within 20 m radius from where the dengue-positive mosquito was found and/or in a pre-determined place where the community was gathered. Upon written consent, details like age, any recent signs and symptoms, history of dengue infection and family history of dengue infection were taken. Individuals positive for dengue NS1 antigen on the spot were advised to take precautionary measures to protect themselves and others from mosquito bites and to seek medical attention if symptoms occur.

To investigate the occurrence of inapparent dengue among people living with dengue patients, the healthy kin (who do not show any sign and symptom of dengue) of suspected or confirmed dengue patients at the Primary Care Clinic of a tertiary referral center, University of Malaya Medical Center (UMMC), Kuala Lumpur, Malaysia were enrolled (Table 1). Sampling was performed on as many kin as available at that time and space for each patient. Kin denoted all individuals who lived in the same house/unit as the suspected/confirmed-dengue individuals, as well as visitors who have stayed at the latter’s houses/units for the last 14 days (Stoddard et al., 2013). Suspected dengue was defined as the acute onset of high grade fever of usually 2–5 days or more, along with at least 2 of the following symptoms: headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands, rashes and mild haemorrhagic manifestation (Ministry of Health Malaysia, 2017). Confirmed dengue was defined as a case compatible with clinical description of dengue and laboratory confirmed with any of the following: detection of dengue NS1 antigen, detection of dengue IgM and IgG in a single sample and PCR.

The type of samples collected and the subsequent tests performed (Table 1) differed from one setting to another, due to logistics, costs and the consents given by the subjects. An individual was deemed to have recent inapparent dengue if PCR, rapid dengue NS1 test and/or dengue IgM test was positive.

### 2.2. Ethics statement

Collection of blood samples from the residents at Damansara Damai (MRECID No.: 2018525-6321) and next of kin of suspected or confirmed dengue patients and the patients themselves present at Primary Care Clinic, UMMC (MRECID No.: 201895-6659) were approved by the Institutional Medical Research Ethics Committee, University Malaya Medical Center, Kuala Lumpur, Malaysia.

### 2.3. Dengue serology of samples

Detection of dengue IgM and IgG levels from serum/plasma were performed using STANDARD F IgM/IgG Fluorescence Immunoassay tests, on the STANDARD F200 Fluorescent Immunoassay system (SD Biosensor, Gyeonggi-Do, South Korea) according to the manufacturer’s instructions. The system was calibrated using the Calibration Set prior to usage. Analyses of the results also followed those of the manufacturer’s instructions and cut-off indices. According to the manufacturer (https://www.labmark.eu/data/system/attachments/sdbiosensor-katalog-f.pdf), the test has a sensitivity of 98% and specificity of 99% with reference to ELISA. An evaluation has found the STANDARD F dengue IgG and IgM concordance rate of positivity to be 100% and 80% respectively for acute infection as compared to ELISA and 70% for IgG for past infections, while specificities of dengue IgG and IgM were 83.5% and 91.7% respectively (Zammarchi et al., 2019).

On the other hand, approximately 3 drops of finger-pricked blood or 100 μL of venous blood were used directly on a STANDARD Q Dengue NS1 Ag test or STANDARD Q Dengue Duo kit (SD Biosensor, Gyeonggi-Do, South Korea), and 3 blood spots were made on a Whatman filter paper. As per the manufacturer’s instructions, results were interpreted within 15 to 20 min. The appearance of the test band, with the control band, indicates the presence of dengue NS1 antigen, IgG or IgM in the sample.

### 2.4. Real-time reverse transcription PCR (real-time RT-PCR) and nested RT-PCR for detection and serotyping of dengue viruses

RNA was extracted from at least 1 blood spot or 250 μL of plasma/whole blood (if available) in 750 μL of TRizol (Life Technologies, USA) according to the manufacturer’s instructions. For detection of clinically inapparent dengue among communities living around dengue-positive *Aedes* mosquitoes, the extracted RNA was assayed using a universal single probe real-time RT-PCR method described by Alm et al. (2014). Four μL of RNA was used in a 20 μL reaction, using the 2x qPCR Probe 1-Step Go kit (PCR Biosystems, London, UK), with 900 nM of each primer and 200 nM of probe. Amplification and detection were performed in a Bio-Rad CFX96 Real-Time PCR detection system. The thermal cycling started with a 10 min reverse transcription step at 50 °C, followed by inactivation of reverse transcription/initial denaturation at 95 °C for 2 min, and 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 30 s.

To serotype the dengue virus, the RNA of the samples underwent a nested RT-PCR using primers described by Klungthon et al. (2015). The initial RT-PCR was performed using PCRBio 1-Step Go RT-PCR Kit (PCR Biosystems, London, UK), whereby 200 nM of each primers and 5 μL of RNA were used in each 25-μL reaction. The cycling conditions were as follows: reverse transcription at 55 °C for 10 s, initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation (95 °C for 10 s), annealing (55 °C for 10 s), and extension (72 °C for 30 s). From there, multiplex PCR was performed using NBE Multiplex PCR 5X Master Mix (New England Biosystems, USA), with 0.2 μM of each primers and 1 μL of the RT-PCR product in 25 μL reaction. The cycling reactions comprised initial denaturation at 95 °C for 1 min, followed by 30 cycles of denaturation (95 °C for 30 s), annealing (55 °C for 1 min), and extension (68 °C for 1 min); as well as final extension at 68 °C for 5 min. Gel electrophoreses were performed using 1.5% agarose gels; the presence of a 482, 119, 280, and 392 bp bands denoted the presence of dengue virus (DENV) serotype 1, 2, 3 and 4 respectively.

### 3. Results

#### 3.1. Inapparent dengue is common in all 3 settings

One hundred and eight residents from the 8 apartments provided venous blood for dengue serology. Their age range was 18–76 years; 62 (57.4%) were male, 43 (39.8%) were female, and 3 (2.8%) did not mention their gender in the questionnaire. Overall, 10.2% and 70.4% of the sampled residents were dengue IgM and IgG positive respectively (Table 2). The seropositivity rates directly translate into the seroprevalence of dengue. Specifically, the positivity rates of the IgM and IgG tests imply the prevalence of dengue from day 6–90 of infection and from day 7 to around 1 year after infection respectively. Fifteen (13.9%) participants reported a past history of dengue, with the times of diagnoses ranging from 3 months to 29 years prior to sampling. Among the 93 individuals without any history of dengue, 9 (9.7%) and 65 (69.9%) were dengue IgM and IgG positive respectively. These show that many individuals have had recent or past exposure to dengue without their knowledge, an indication that inapparent dengue could be fairly common in dengue hotspot.

Of the 174 individuals sampled when dengue NS1 antigen-positive *Aedes* mosquitoes were detected, one had symptoms suggestive of dengue. Therefore, only 173 healthy individuals were included and real-time RT-PCR managed to detect dengue virus RNA in 10 (5.8%) of
these 173 individuals (Table 2). Only one of the individuals with viremia was also positive in the dengue NS1 test.

A total of 39 kin of patients with suspected or confirmed dengue were sampled. Among the 39 kin, 18 were excluded as they did not fit the inclusion criteria. Hence, only 21 kin who were without any symptoms suggestive of dengue nor past history of dengue, were included for data analysis. With regards to their age, the range was 20–56 years, median 39 years, and interquartile range 17 years. Thirteen were males and the rest females. Only 1 was staying with a family member who was diagnosed with dengue within 2 weeks prior to the time of sampling. This person was tested to be dengue IgM positive, but was dengue NS1 and IgG negative. With respect to Table 2, all NS1-positive individuals had negative dengue IgM results. Meanwhile, 2 and 1 of the IgG-positive individuals were also positive for NS1 and IgM respectively. As such, the prevalence of recent asymptomatic dengue among the patient’s next of kin was 23.8%.

Overall, seemingly healthy people living around dengue-positive Aedes mosquitoes and people living with suspected or confirmed dengue patients, had acute dengue infection or had been exposed to dengue (Table 2). Direct comparisons between the sampling populations may not be realistic due to the differences in sampling size and setting. If comparisons were to be made, only results of the dengue NS1 tests and PCR can be used. In this case, a higher percentage of people living with suspected or confirmed dengue individuals (14.3%) present with acute infection than among people living around dengue-positive Aedes mosquitoes (5.8%).

### 3.2. When dengue-positive mosquitoes are detected, dengue infections are already widespread, and most are asymptomatic

Besides the 10 individuals with inapparent dengue mentioned above, another individual with symptoms were also found to be positive for dengue viremia. Therefore, of the 174 individuals sampled when dengue-positive mosquitoes were detected, 11 (6.3%) were found to have dengue (Table 3). The dengue NS1 antigen test only detected one asymptomatic 29-year-old male with negative history and family history of dengue. Moreover, while dengue IgG test was positive and IgM test turned out negative, dengue virus serotype 2 was detected in his whole blood. Apart from that, all the other 173 individuals were negative for dengue NS1 antigen (Table 3).

Of the 10 individuals who were asymptomatic, only 5 were tested for dengue IgM and IgG as they provided blood. It is interesting to note that, while they have viremia and are IgG positive, none of these asymptomatic individuals were dengue IgM positive (Table 3). Besides, upon detection of an infected mosquito, transmission was already widespread, as asymptomatic infections were found throughout a 17-storey condominium or circulating among 2 floors in a particular 5-storey residential block in a densely-packed apartment (Table 3).

### 3.3. Exposure to dengue among people living with dengue patients

In addition to the asymptomatic kin with inapparent dengue shown in Tables 2, 4 kin with fever and/or symptoms suggestive of dengue were also detected. It can be seen that all of them have indications of dengue infection, with 2 of them confirmed by PCR (Table 4). Thus, it appears that kin living with dengue patient are likely to be exposed to dengue too, whether asymptomatic or symptomatic. They can either have circulating viral RNA and/or presence of NS1 antigen. It is also likely that they are dengue seropositive.

### 4. Discussion

The results from this study clearly show the occurrence of clinically inapparent dengue in settings or populations, which have been less studied in the context of dengue transmission. These settings include dengue transmission when infected mosquitoes are present, and the population living with dengue-infected family members. The data also demonstrates that many people are and have been unknowingly exposed to dengue, and that the extent of dengue transmission has been underestimated.

The most important finding is the detection of asymptomatic individuals with dengue viremia living around infected mosquitoes. These individuals are capable of transmitting the virus even though they appear healthy (Duong et al., 2015). It is also obvious that transmission of dengue has already been ongoing at that point of time, and widespread. This means, actions taken to control dengue transmission after the first report of dengue cases as in the current reactive dengue control program, are already too late and likely futile. Dengue would have already spread throughout the communities. This emphasizes the necessity of early and proactive dengue surveillance/control (Lau et al., 2017; Liew et al., 2019). Indeed, vector control such as insecticide treated material, personal repellents and indoor residual spray if implemented early in an outbreak, can still prevent many people from being infected (Achee et al., 2015). In order to do that, surveillance on the vector population and ecology are more appropriate than surveillance on epidemiological indices (Schwab et al., 2018). Moreover,

<table>
<thead>
<tr>
<th>Sample population</th>
<th>Number of samples positive for dengue tests / total number of samples collected (percentage positive)</th>
<th>Number of samples positive for the given tests (percentage positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG</td>
</tr>
<tr>
<td>Residents living in a dengue hotspot (baseline seroprevalence)</td>
<td>79/108 (73.1%)</td>
<td>76 (70.4%)</td>
</tr>
<tr>
<td>Residents living around dengue-positive Aedes mosquitoes</td>
<td>10/173 (6.3%)</td>
<td>NA</td>
</tr>
<tr>
<td>Kin of suspected or confirmed dengue patients</td>
<td>5/21 (23.8%)</td>
<td>4 (19.0%)</td>
</tr>
</tbody>
</table>

NA, results not available as not everyone provided venous blood for dengue IgG and IgM tests.

* Dengue IgG or IgM positive.

** Finger-prick blood positive for dengue NS1 antigen and/or IgM.
while using dengue incidences or notified cases in real-time monitoring of dengue occurrences have its advantages, the idea that this real-time indicator for dengue occurrences is an early warning indicator (Rizwan et al., 2018), can be considered a fallacy.

From a scientific perspective, the method of dengue surveillance employed in this study which uses dengue-positive mosquito as an index, will enable us to locate dengue asymptomatic individuals. This can be a method for use in apparent dengue surveillance or for epidemiological purposes. The outcome of such surveillance adds on to a more accurate measure of human disease risks, aiding the identification of potential threats and better targeting of surveillance and control, thereby enhancing dengue control measures to reduce outbreaks. These factors can also be incorporated in an early warning system in addition to environmental, climate, vector and host based data (Racloz et al., 2012).

Reports on the overall prevalence of dengue are numerous, and the figures vary widely depending on the sociodemographic characteristics and the method(s) of detection. A review of published articles revealed the seroprevalence of dengue (based on indirect IgG ELISA) throughout various urban sites in Malaysia to be 61–92% (Chew et al., 2016; Dhanoa et al., 2018) while rural sites have reported a figure of 28–91% (Chew et al., 2016). According to Dhanoa et al. (2018), 87.1% of individuals did not recall having any previous (symptomatic) dengue infection. Albeit the small sample size, our results corroborate with the above. Overall dengue IgG seroprevalence of the community living in a dengue hotspot was 70.4% and almost 70% of these individuals were unaware of their previous exposure to dengue. This suggests that people living in a dengue-prone area in Malaysia may invariably be infected by dengue at some point in their lives, without their knowledge. It is also not surprising to find people living with dengue patients to be exposed or infected by dengue. They could be two times more likely to be infected (Ly et al., 2019; Martínez-Vega et al., 2015; Yoon et al., 2012).

The current study also revealed some challenges of diagnosing clinically inapparent dengue in hyperendemic settings. The combination of extrinsic and intrinsic incubation periods of dengue gives rise to a wide range of durations. Coupled with the fact that asymptomatic dengue individuals do not provide the "luxury" of disease onset as a guide to the kinetics of the disease, there is a need for more researches into the kinetics of asymptomatic dengue. There is currently no gold standard for detecting inapparent dengue. As it is, diagnosis of dengue is a conundrum on its own, as there is no test that is considered an ideal dengue diagnostic method (Gyawali and Taylor-Robinson, 2017; Peeling et al., 2010). The detection of DENV RNA or dengue NS1 antigen can be performed during the acute phase of the infection until 8–9 days after onset of fever, after which viral RNA or virus detection typically becomes negative. Serological methods though useful, are not confirmative diagnosis and have their own limits due to the varying levels of the antibodies in different conditions. Dengue IgM is detected earlier (5 or more days after onset of illness) than IgG (from 10 to 15 days) during primary infection while in secondary infections, IgG titre increases rapidly and IgM is at lower titres (Peeling et al., 2010; Teoh et al., 2016). Therefore, sensitivities of the tests are affected by the time of specimen collection (before, during, after onset) and the baseline immunity of an individual.

According to limited studies, although dengue NS1 antigen is detected in the blood as early as viral RNA, NS1 test has lower sensitivity (35.3%) for non-symptomatic individuals compared to dengue infected individuals. Furthermore, NS1 tests have lower sensitivity to secondary infections (Duyen et al., 2011; Teoh et al., 2016), possibly due to the shorter duration of NS1 antigen circulating in the blood during secondary dengue infections than in primary infections (Duyen et al., 2011) or the detection of NS1 antigen during acute secondary infection is compromised by pre-existing virus–IgG immunocomplexes (Peeling et al., 2010). Dengue NS1 antigenemia and its detection, are also influenced by the infecting dengue serotype and baseline immunity, causing the varying sensitivities of different diagnostic tests

<table>
<thead>
<tr>
<th>Sample ID / residential location</th>
<th>Dengue serotype</th>
<th>NS1 IgM IgG</th>
<th>Symptoms</th>
<th>Dengue history [Year(s) ago]</th>
<th>Kin's dengue history [Year(s) ago]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARB01 / Block A, 6th Floor</td>
<td>DENV 2 &amp; DENV 3</td>
<td>−ve −ve +ve</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>PARB03 / Block A, 17th Floor</td>
<td>DENV 2</td>
<td>−ve −ve +ve</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>PARB12 / Block A, 3rd Floor</td>
<td>DENV 2</td>
<td>−ve −ve +ve</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>PARB14 / Block A, 2nd Floor</td>
<td>DENV 2</td>
<td>−ve −ve +ve</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>HARP07 / Block B, 1st Floor</td>
<td>DENV 2</td>
<td>−ve −ve +ve</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>HARa40 / Block Q, 2nd Floor</td>
<td>DENV 2</td>
<td>−ve −ve +ve</td>
<td>No</td>
<td>Yes (5 years)</td>
<td>No</td>
</tr>
<tr>
<td>HARa46 / Block F, 1st floor</td>
<td>DENV 2</td>
<td>−ve −ve +ve</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>HARa47 / Block F, 1st floor</td>
<td>DENV 2 &amp; DENV 3</td>
<td>−ve −ve +ve</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>HARa48 / Block F, 1st floor</td>
<td>DENV 2</td>
<td>−ve −ve +ve</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>HARa49 / Block F, Ground Floor</td>
<td>DENV 2 &amp; DENV 3</td>
<td>−ve −ve +ve</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>HARa50 / Block F, Ground Floor</td>
<td>DENV 2 &amp; DENV 3</td>
<td>−ve −ve +ve</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>SURD07 / Block B, 1st Floor</td>
<td>DENV 2</td>
<td>−ve −ve +ve</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 3

Details of dengue-positive individuals by real-time RT-PCR when dengue-positive Aedes was detected.

−ve, negative; +ve, positive; DENV 2, dengue virus serotype 2; DENV 3, dengue virus serotype 3. NA, not available; these individuals did not consent to providing venous blood, thus dengue IgG and IgM tests were not performed.
lower sensitivities for DENV2 (Blacksell et al., 2012; Duong et al., 2011). Population density is an important factor, and it has been found that some NS1 tests have relatively lower sensitivity for DENV2 (Table 3); DENV2 and 3 are responsible for the infections in the study area. Based on our findings, mosquitoes were dengue NS1 negative, but with viremia. Perhaps this is because of low sensitivity of the dengue NS1 test. In some cases, IgM may even persist longer in capillary blood than in venous blood (Kuno et al., 2011). Furthermore, in our small number of samples, despite having viremia or being NS1 positive, it was found that most asymptomatic individuals whom we managed to test were dengue IgG positive, but negative for dengue IgM. Perhaps, they have not yet mounted an IgM response (Ly et al., 2019) or it is more likely that they were experiencing a secondary dengue infection (Ambrose et al., 2017). While there is a stronger IgM response during primary DENV infections, IgM-positive rates in secondary infections are lower compared to that of IgG, that is measurable even on the first day of onset. In some cases, IgM may even be undetectable, as discussed in Gyawali and Taylor-Robinson (2017); and Palomares-Reyes et al., 2019. However, serological tests may be able to detect more dengue infections, as they have a wider window for detection compared to PCR or NS1 tests which are useful mostly during the acute phase of the infection. Both PCR and NS1 tests have been shown to have decreasing positive detection of secondary infections compared to primary infections, a possible attribute of a faster and earlier clearance of viruses by memory antibodies during secondary infections (Teoh et al., 2016). Serological tests, though affected by primary or secondary infections, can still provide positive results during and after the acute phase of dengue infection.

Clearly, there is no one best method for diagnosing inapparent dengue, except virus isolation or nucleic acid detection via RT-PCR, which gives a confirmatory diagnosis and indication of active transmission. However, these, too need to be done within the correct window of time during the course of infection. The mass-detection of symptomatic dengue will undoubtedly require cheap, simple, efficacious, rapid, and ready-to-use test instruments, using as little blood sample as possible. Accordingly, a more reliable parameter (e.g. time of exposure to infected mosquito) has to be developed in order to facilitate predictions of subsequent outbreaks or dissemination of dengue. A field-deployable rapid molecular technique for detection of dengue viral RNA would be most useful. Such techniques are already in the pipeline as reviewed in Rodriguez-Manzano et al. (2018).

Capillary blood was mostly used in the current study because it may be of higher sensitivity for diagnosis at early phase of the disease (Matheu et al., 2014). Despite that, the positive dengue NS1 results obtained in capillary blood but not in venous blood remain questionable. However, dengue virus and viral particles have been found to persist longer in capillary blood than in venous blood (Kuno et al., 1991; Matheu et al., 2007). Therefore, it remains inconclusive, if the use of capillary blood is as good as or better than the venous blood. A study is currently ongoing to determine that by collecting a larger sample size.

With respect to the above discourse, asymptomatic dengue screenings, if at all they are implemented, will be more likely to benefit the community, as a whole rather than the affected individuals per se, since these individuals may not be in direct danger of contracting the complications of dengue unlike their symptomatic counterparts. Additionally, even though individuals with asymptomatic dengue are made aware of their disease status and their risk of major anamnestic reactions in the event of a repeat infection by a dengue virus strain, it is practically impossible to completely avoid exposure to mosquitoes for the remainder of their lives. Ergo, the aforementioned screenings will probably provide greater public health benefits than individual benefits.

In addition, community participation in dengue control and prevention is very important and one cannot just depend on health authorities to do the preventive measures. Thus, the community can be involved such as setting and observing simple mosquito traps while dengue surveillance in the mosquito and human population can be carried out by health authorities, with cooperation from the community. It is envisaged that this may at least reduce dengue mortality to a great extent. The control of Aedes vectors, also invariably helps in controlling chikungunya or Zika.

Lastly, it has to be noted that results from the current study have to be compared with care, as the methods used from one setting to the other differ, and the sample size is also not similar. For example, although a higher percentage of people living with suspected or confirmed dengue individuals were found to present with acute infection than among people living around dengue-positive Aedes mosquitoes, this may not be a true reflection of the scenario. Samples were performed depending on consent. Not everyone was sampled within the 20 m radius from where the mosquito was found. Furthermore, not all kin of the patients were sampled. Only kin who were present with the patients during the visit to the hospital were sampled. More importantly, children below the age of 18 were excluded from the study.

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**Table 4**

Details of dengue patients' symptomatic kin.

<table>
<thead>
<tr>
<th>Individual</th>
<th>Past dengue</th>
<th>Family history of dengue</th>
<th>Rapid tests</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No</td>
<td>No</td>
<td>– ve</td>
<td>– ve</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>Yes</td>
<td>+ ve</td>
<td>– ve</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>No</td>
<td>+ ve</td>
<td>– ve</td>
</tr>
<tr>
<td>4</td>
<td>Yes (3 years ago)</td>
<td>No</td>
<td>– ve</td>
<td>– ve</td>
</tr>
</tbody>
</table>

– ve, negative; + ve, positive; NA, not available.

* Results from capillary blood.

" Results from venous blood.

**Table 5**

Positivity rates of dengue NS1, PCR, dengue IgM and IgG of dengue patients’ asymptomatic next of kin using capillary versus venous blood (N = 9).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Capillary blood (n, %)</th>
<th>Venous blood (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS1</td>
<td>3 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>PCR</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Dengue IgM</td>
<td>1 (11.1%)</td>
<td>3 (33.3%)</td>
</tr>
<tr>
<td>Dengue IgG</td>
<td>3 (33.3%)</td>
<td>3 (33.3%)</td>
</tr>
</tbody>
</table>

* Direct capillary blood was used on the dengue NS1, IgM, and IgG rapid tests, while bloodspots were used for PCR.

(Duyen et al., 2011; Rodriguez-Manzano et al., 2018). In fact, NS1 with dengue IgG/IgM tests or RT-PCR combined with these tests were more sensitive for dengue diagnosis, especially secondary dengue infections (Duong et al., 2011; Teoh et al., 2016).

Indeed, the use of NS1 tests alone may not be sensitive enough to detect all asymptomatic cases as shown in the study. This could be due to the individuals experiencing asymptomatic infections or secondary infections, which could be quite prevalent in hyperendemic countries such as Malaysia, but this was not determined in the study. Results from the kin of dengue patients revealed some of them to be dengue NS1 positive, but with no viremia. This is very much possible as NS1 antigen can persist longer in the blood even after viremia is cleared. On the other hand, the asymptomatic residents living around dengue-positive mosquitoes were dengue NS1 negative, but with viremia. Perhaps this is a case of low sensitivity of the dengue NS1 test. Based on our findings (Table 3), DENV 2 and 3 are responsible for the infections in the study population, and it has been found that some NS1 tests have relatively lower sensitivities for DENV2 (Blacksell et al., 2012; Duong et al., 2011). Furthermore, in our small number of samples, despite having viremia or being NS1 positive, it was found that most asymptomatic individuals whom we managed to test were dengue IgG positive, but negative for dengue IgM. Perhaps, they have not yet mounted an IgM response (Ly et al., 2019) or it is more likely that they were experiencing a secondary dengue infection (Ambrose et al., 2017). While there is a stronger IgM response during primary DENV infections, IgM-positive rates in secondary infections are lower compared to that of IgG, that is measurable even on the first day of onset. In some cases, IgM may even be undetectable, as discussed in Gyawali and Taylor-Robinson (2017); and Palomares-Reyes et al., 2019. However, serological tests may be able to detect more dengue infections, as they have a wider window for detection compared to PCR or NS1 tests which are useful mostly during the acute phase of the infection. Both PCR and NS1 tests have been shown to have decreasing positive detection of secondary infections compared to primary infections, a possible attribute of a faster and earlier clearance of viruses by memory antibodies during secondary infections (Teoh et al., 2016). Serological tests, though affected by primary or secondary infections, can still provide positive results during and after the acute phase of dengue infection.

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5. Conclusions

This study demonstrates empirical evidence of inapparent dengue in different settings. Dengue transmission is already widespread when dengue-infected mosquitoes are detected. Asymptomatic dengue has extensive epidemiological consequences. Relying on notifications of symptomatic dengue underestimates cases, and further delays control efforts in areas at risk for dengue virus transmission. Early detection of dengue in mosquitoes and active serological/virological surveillance in humans can be used in hand in hand. It is also timely and crucial to educate the public, health staff and medical professionals on asymptomatic dengue and to propagate awareness, which is central to prevention of transmission. In fact, if resources are available, it would not be implausible to also screen the people living with dengue patients for dengue.

More studies are required to investigate the epidemiology, seroprevalence, diagnostics, and control of inapparent dengue. They need to take into account the incubation periods, infectious period, immunological kinetics, cost-effectiveness, and community-related factors (e.g. receptivity to venuplexis) of the disease, among others.

Declaration of Competing Interest

All authors declare that they have no competing interest.

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Supplementary materials
