JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Epidemiological serosurvey of chikungunya fever post outbreak at Tanjung Sepat, Malaysia

Chee-Sieng Khor[1], Boon-Teong Teoh[1], Sing-Sin Sam[1], Hooi-Yuen Khoo[1], Noor-Syahida Azizan[1], AsmaAnati CheMatSeri[1], Kim-Ling Chin[1], Zur-Raiha Hamim[1], Noor-Adila Mohamed-Romai-Noor[1], Che-Norainon Yaacob[1], Juraina Abd-Jamil[1], Hai-Yen Lee[1], Yih-Harng Soh[1,2], Sazaly AbuBakar[1]

[1] Tropical Infectious Diseases Research and Education Centre (TIDREC), Universiti Malaya, Kuala Lumpur, Malaysia
[2] Department of Social and Preventive Medicine, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia

Abstract

Introduction: Chikungunya fever is a mosquito-borne viral disease that usually presents with prominent arthralgia. An outbreak of chikungunya fever was reported in Tanjung Sepat, Malaysia in 2019. The outbreak was limited in size with a low number of cases being reported. The present study sought to determine the possible variables that could have affected the transmission of the infection.

Methodology: A cross-sectional study involving 149 healthy adult volunteers from Tanjung Sepat was performed soon after the outbreak had subsided. All the participants donated blood samples and completed the questionnaires. Laboratory detection of anti-CHIKV IgM and IgG antibodies was performed using enzyme-linked immunoassays (ELISA). Risk factors associated with chikungunya seropositivity were determined using logistic regression.

Results: The majority (72.5%, n = 108) of the study participants tested positive for CHIKV antibodies. Only 8.3% (n = 9) of the participants out of all the seropositive volunteers had an asymptomatic infection. Participants who resided with a febrile ($p < 0.05$, Exp(B) = 2.2, confidence interval [CI] 1.3-3.6) or a CHIKV-diagnosed person ($p < 0.05$, Exp(B) = 2.1, CI 1.2-3.6) in the same household were found likely to be tested positive for CHIKV antibodies.

Conclusions: Findings from the study support that asymptomatic CHIKV infections and indoor transmission occurred during the outbreak. Hence, widespread community testing and indoor use of mosquito repellent are among the possible measures that can be implemented to reduce CHIKV transmission during an outbreak.

Key words: chikungunya; infectious diseases; vector-borne; seroprevalence; asymptomatic; Malaysia.

J Infect Dev Ctries 2023; 17(1):118-124. doi:10.3855/jidc.16613

(Received 23 March 2022 – Accepted 04 November 2022)

Introduction

Chikungunya fever, is a febrile illness caused by the chikungunya virus (CHIKV). The virus is transmitted to humans through the saliva of the CHIKV-infected female *Aedes* (*Ae*.) *aegypti* or *Ae*. *albopictus* mosquitoes during blood feedings [1]. The infected mosquitoes can feed on several persons throughout their adulthood [2] and travel up to 800 meters [3], thus, enabling the rapid spread of the disease. Typical chikungunya symptoms include fever, arthralgia, headache, myalgia and rash [4]. Its severity ranges from severe and persistent arthralgia which could last for years to mild fever with joint pain which subsequently disappears post-recovery [5]. Asymptomatic presentation of CHIKV infection has also been reported [4]. Asymptomatic yet infected individuals often escape detection, since they do not seek medical care unless they come to the hospital for other reasons. Thus only symptomatic cases are reported during an outbreak [6].

Chikungunya fever occurs worldwide and major outbreaks are reported more frequently in the tropical and sub-tropical regions where *Ae.* mosquitoes are abundant [7,8]. Among the more recent major chikungunya fever outbreaks is the outbreak in Thailand in 2018 with more than 15,000 reported cases [9,10]. Most of the chikungunya cases, however, were reported in the southern provinces bordering Malaysia. Intensified preventive and control measures to eradicate the mosquito populations and implementation of body temperature screening at entry points along the Malaysia-Thailand border [11] were among the steps introduced to contain the spread of the outbreak into Malaysia.

Despite the measures, a steep increase in the number of chikungunya fever cases was reported in the

state of Selangor, approximately 350 kilometers from the Thailand-Malaysia border. The number of chikungunya fever cases in Selangor increased exponentially from only two cases in 2018 to 184 cases in 2019 [12,13]. The chikungunya fever transmission in Selangor was presumed to have ended by mid-May 2019 since no further cases were reported till the end of the year [14,15].

Tanjung Sepat, a small town located in the southern part of Selangor (2°39.6051'N, 101°33.6115'E) was identified as the epicenter of the chikungunya fever outbreak in 2019 in Malaysia [16]. Considering that Tanjung Sepat had an estimated population size of 9,330 (annual population growth rate = 2.0%) [17] in 2019, the number of reported cases was only ~2.0% of the population. In a previous CHIKV outbreak in another small town, Bagan Panchor, Malaysia, more than 200 people were reported with symptoms of chikungunya representing at least 13.3% of the population. However, a subsequent serosurvey in the community showed that 55.6% of the study population had evidence of past CHIKV infection [4]. The low number of reported chikungunya cases could suggest that the chikungunya fever outbreak in Tanjung Sepat had a much lower reproduction rate (R_0) perhaps because the disease control and prevention measures were effective. It is also possible that the infection rate was simply grossly underreported due to misdiagnosis, milder symptoms not requiring laboratory confirmation of infection and a high prevalence of asymptomatic infections. Misdiagnosis of the infection is possible considering dengue is endemic in Malaysia. However, Tanjung Sepat is a rural coastal city not commonly associated with outbreaks of dengue and no dengue outbreaks were reported in the area during the chikungunya fever outbreak.

The study undertaken herein was to determine and assess the seroprevalence, asymptomatic rate, and potential risk factors of chikungunya fever cases in Tanjung Sepat, Selangor, post-2019 outbreak. The data obtained will be useful for our understanding of the extent of possible misdiagnosis of infection, symptomatic and asymptomatic infections during an outbreak and the risk factors associated with CHIKV seropositivity.

Methodology

Ethics Statement

The study protocol was approved by the Medical Ethics Committee of the Universiti Malaya Medical Centre, Malaysia (MEC Ref. No: 201956-7399) and conformed to the principles embodied in the Declaration of Helsinki. All subjects provided written consent to participate in the study voluntarily prior to enrollment.

Study population

Recruitment for the study was open to consenting Tanjung Sepat residents aged above 18 years old as approved by the Institutional Review Board (IRB) committee. Prior to the 2019 outbreak, CHIKV transmission was never reported in this population and chikungunya fever is not recognized as endemic in Malaysia. As such, all residents and participants were presumed to be seronegative for CHIKV antibodies. Residents who were clinically diagnosed with human immunodeficiency viruses, hepatitis B virus, or hepatitis C virus or were knowingly pregnant were excluded from this study as per approval by the IRB committee. The residents infected with human immunodeficiency viruses, hepatitis B virus, or hepatitis C virus were excluded to reduce the risks of contracting blood-borne diseases during blood handling. The pregnant women and residents aged below 18 years old were excluded from the study since they were categorized as among the vulnerable population by the local IRB.

Sample size

The reported chikungunya incidences were at ~2.0% of the total population (N = 184/9,330). The present study estimated the seroconversion rate at the study site to be one-fifth of the previous CHIKV study conducted for the outbreak in Bagan Panchor. The sample size required was calculated using 9,330 people as the population size with an estimated seroprevalence of CHIKV at 11% of the population, a 5% margin of error and a 95% confidence level. Using these parameters, the minimum sample size required as representative of the study population was 149 participants.

Parameters:

Estimated proportion of seropositive population (p): 11%

Population size (N): 9,330

Margin of error (e): 5%

Confidence level (z): 95%

Formula:

$$\text{MSSR} = \frac{\frac{z^2 \times p\,(1-p)}{e^2}}{1 + \left(\frac{z^2 \times p\,(1-p)}{e^2 N}\right)} = 148.05 \sim \mathbf{149}$$

Where: MSSR = Minimum Sample Size Required.

Recruitment and sampling activities

Information regarding the study was disseminated through local authorities a week prior to the on-site recruitment. Interested residents were invited to participate in the study by donating blood samples and completing a questionnaire. The samples were obtained from consented healthy adult volunteers from 26th to 27th of August 2019, more than three months after the last CHIKV cases were reported. About 5 mL of blood was obtained from each participant using blood tubes with a clot activator (BD, Franklin Lakes, USA). The samples were left to clot at room temperature for 30 minutes and centrifuged at 1000 x g for 10 minutes. Serum was aliquoted into tubes and stored in a -80 °C freezer until needed for analysis. The participants for the study also completed a given questionnaire detailing their history of CHIKV infection through self-reporting. The information on CHIKV diagnosis collected was either based on clinical presentation alone or clinical presentation with laboratory findings.

Detection of CHIKV IgM and IgG antibodies

The presence of CHIKV IgM and IgG antibodies in serum samples was determined using the commercial ELISA kit (EI 293a-9601M & EI 293a-9601G0, Euroimmun AG, Luebeck, Germany). The assays were performed strictly following the protocols provided by the manufacturer. Samples that tested positive for either IgM or IgG antibodies were considered as seropositive for CHIKV. Test for other infection was not performed as the availability of test kits were limited.

Statistical analysis

Statistical analysis of the ELISA results and questionnaire data were conducted using the Statistical Package for the Social Science (SPSS) v25 (IBM Corp., New York, USA). Binary Logistic Regression was used to test the association between age, gender, household size, febrile occupants and CHIKV-diagnosed occupants (independent variables) with CHIKV seropositivity as a dependent variable. Participants residing alone (household size = 1) and those that were unaware or uncertain of medical history (febrile, N = 2 and CHIKV-diagnosed, N = 14) of their household members were removed from the analysis. No missing data was recorded.

Results

Seroprevalence of CHIKV among residents in Tanjung Sepat

A total of 149 volunteers were recruited as participants for the study. All recruited participants were of legal age (> 18 years old), Tanjung Sepat residents, provided blood samples and responded to the questionnaire. Of the 149 participants recruited, 85 (57.0%) were female. The mean age of the participants was 58.25 years old (SD = 12.9, range = 18 to 88) and the median age was 59 years. Laboratory tests detected the presence of CHIKV antibodies in 108 (72.5%) participants. IgM antibodies, IgG antibodies and IgM & IgG antibodies were found in 46 (30.9%), 105 (70.5%) and 43 (28.9%) participants, respectively (Table 1). Further analysis was then performed using these laboratory results and information obtained from the administered questionnaire to unravel the possible risk factors for exposure to chikungunya.

False positive rate of CHIKV during Tanjung Sepat outbreak (2018 - 2019)

Among the 149 participants, 89 (59.7%) participants had clinically been diagnosed with CHIKV infection by their respective physicians (Table 2). Laboratory tests performed revealed that only 78 (87.6%) of those diagnosed with CHIKV infection tested positive for the presence of CHIKV antibodies. This suggested that 11 participants were clinically diagnosed with CHIKV infection but were serologically negative, representing a false positive rate of 12.4%. All the 11 volunteers presented with fever, however, 10 (90.9%) of them claimed they had joint pain, the hallmark symptom of CHIKV infection without developing anti-CHIKV IgG antibodies. The possible causative agents giving rise to the symptoms were not determined in this study.

The asymptomatic infection rate of CHIKV during the 2019 Tanjung Sepat outbreak

Positive CHIKV serology was detected in 50.0% (N = 30) of the participants who were not diagnosed with chikungunya fever. Among these 30 participants, 10 (33.3%) participants reported they had fever and joint pain, 11 (36.7%) participants had fever or joint pain and another 9 (30.0%) participants did not experience any

Table 1. Presence of IgM and IgG antibodies in recruited volunteers.

Antibodies		IgM, N (%)		Total
		Positive	Negative	
IgG	Positive	43 (28.9)	62 (41.6)	105 (70.5)
	Negative	3 (2.0)	41 (27.5)	44 (29.5)
	Total	46 (30.9)	103 (60.1)	149

febrile illnesses and arthralgia since the onset of the outbreak in Tanjung Sepat. With the absence of the typical chikungunya fever symptoms, these participants (N = 9/108) were categorized as having an asymptomatic infection. The asymptomatic infection rate for CHIKV infection in this study, hence, was ≈8.3%.

Transmissibility of CHIKV in Tanjung Sepat

Among the recruited participants, 138 (92.6%) participants shared housing with others living in Tanjung Sepat. The number of occupants per house ranged from 1 to 12 with a mean of 3.7 (SD = 2.0, median = 3). The majority (N = 99, 71.7%) of the participants who shared housing with multiple occupants tested positive for CHIKV antibodies. Among them, 52 (52.5%) and 37 (37.4%) participants lived with febrile occupants and/or CHIKV-diagnosed occupants during the outbreak period, respectively. Univariate analysis performed using logistic regression revealed that residing with a febrile occupant ($p < 0.05$, Exp(B) = 2.2, confidence interval [CI] 1.3-3.6) and a CHIKV-diagnosed occupant ($p < 0.05$, Exp(B) = 2.1, CI 1.2-3.6) were significant predictors for CHIKV seropositivity. An increase of one febrile occupant and/or one CHIKV-diagnosed occupant increased the risk for contracting CHIKV by 2.2 times and 2.1 times, respectively. Other independent variables investigated were found not to be significant predictors for seropositivity to chikungunya fever.

Apart from evaluating the risk of CHIKV infection among the Tanjung Sepat residents, data from the present study also revealed the possibility of CHIKV transmission to non-Tanjung Sepat residents. A total of 18 (12.1%) participants stated that their non-Tanjung Sepat acquaintances developed fever or joint pain after visiting Tanjung Sepat during the outbreak. However, this study was unable to confirm the serostatus of the mentioned non-Tanjung Sepat residents. Further study will be needed to investigate the transmission of CHIKV beyond the Tanjung Sepat locality during the outbreak.

Discussion

Widespread CHIKV infections have been reported in many regions of the world [5-6,10]. In some parts, including Malaysia, the CHIKV outbreak tends to be sporadic [19], limited in size and localized [19-22]. It is currently not known if this limitation is simply a reflection of the limited study sample size or due to the under-reporting of cases [23]. Here, a post-outbreak investigation using serological assays revealed the presence of CHIKV antibodies in 72.5% of the study participants. This seroprevalence rate is much higher than the seroprevalence rate (55.6%) reported from a previous sporadic CHIKV outbreak in Malaysia [4]. It is also higher than seroprevalence studies reported in the neighboring countries where 14.2-61.3% of the study population was seroconverted [24,25]. The differences observed could be a reflection of sampling bias due to the eagerness of the recently affected population to participate in the study. It could also be due to variations in the sensitivity and specificity of the different serologic assays used [26]. Interference due to cross-reactivity with other alphaviruses, however, should be negligible if any since serological evidence suggests the low activity of other alphaviruses among the general population in Malaysia [27]. Though the study was supposedly undertaken as a post-outbreak investigation, the findings that 30.9% of the volunteers were positive for IgM and negative for IgG, suggest that acute CHIKV infections were still active in the Tanjung Sepat community during the study period which was at about 3 months after the last official reporting of a chikungunya fever case [14], suggesting a possible ongoing endemic infection.

It was also noted in the study that about 40% of the participants were not previously diagnosed with

Table 2. Diagnosis, clinical manifestation, living conditions and serological findings of volunteers recruited.

Self-reported information	Seropositive	Seronegative	Total
	N (%)		N (%)
Diagnosed	78 (87.6)	11 (12.4)[1]	89 (59.7)
Asymptomatic	-	-	-
Symptomatic	78 (87.6)	11 (12.4)	89 (100.0)
Not diagnosed	30 (50.0)[2]	30 (50.0)	60 (40.3)
Asymptomatic	9 (30.0)[3]	21 (70.0)	30 (50.0)
Symptomatic	21 (70.0)	9 (30.0)	30 (50.0)
Living alone	9 (81.8)	2 (18.2)	11 (7.4)
Shared housing	99 (71.7)	39 (28.3)	138 (92.6)
With CHIKV-diagnosed occupants	37 (88.1)	5 (11.9)	42 (30.4)
With febrile occupants	52 (86.7)	8 (13.3)	60 (43.5)

[1] False positive; [2] Undetected CHIKV incidence; [3] Asymptomatic infection rate is at 8.3% (Asymptomatic infection/seropositive samples).

CHIKV, and among these about 50% were found to be seropositive. This highlights the issue of underreporting of chikungunya fever cases in most outbreaks which probably contribute to the pressing difficulty in controlling the transmission of the infection worldwide [23,28-29]. Underreporting of chikungunya incidences can occur due to several reasons. Firstly, diagnosis is often done solely based on clinical presentations without complementary laboratory testing and this could result in misdiagnosis as noted in the present study where more than 10% of the febrile patients diagnosed with chikungunya fever did not have serological evidence of an infection. Chikungunya fever, however, is often misdiagnosed as dengue, especially in regions where dengue is endemic [30,31]. Secondly, CHIKV-infected individuals may not know they are infected because of asymptomatic infection or they opted not to seek medical treatment due to the mildness of the symptoms [32,33]. Therefore, these incidences were not captured in a passive surveillance database. An infected person presenting with no clinical symptoms could in principle still harbor the virus and upon being bitten by mosquitoes could facilitate transmission to the next person in subsequent feeding [34]. Studies on chikungunya fever have shown a significant proportion of asymptomatic infections ranging from 14.0-27.7% during past outbreaks [29,35,36]. In the present study, a calculated asymptomatic rate of slightly above 8% was obtained. As such, active surveillance during an outbreak has to be carried out by performing widespread laboratory testing of the affected community with an emphasis on the detection of symptomatic and asymptomatic individuals.

Results from the present study identified for the first time that residing with febrile occupants and/or CHIKV-diagnosed occupants was an important risk factor for contracting chikungunya fever. This finding highlights the importance of indoor transmission of CHIKV infection. This finding is in agreement with the involvement of *Ae. aegypti* mosquitoes in the transmission of CHIK Vas vector since the mosquito is predominantly found indoors and they are in general anthropophilic, preferentially feeding on humans, with multiple blood meals in a single gonotrophic cycle [37-39]. No entomological data, however, was available to support this assertion for the Tanjung Sepat outbreak. Nonetheless, through contact tracing, the CHIKV circulating in Tanjung Sepat were found to be of East/Central/South African (ECSA) strains from Southern Thailand. The ECSA strains were reported to have E1:K211E and E1:226A mutations which have been suggested to confer increased CHIKV infectivity, dissemination and transmission in *Ae. aegypti* [16,40].

Findings from the present study point to possible approaches to mitigate the spread of chikungunya fever during an outbreak. In addition to the recommended use of personal protective measures which include the use of topical mosquito repellent, bednets and insecticide-treated clothing [41-43], effort must be taken to detect those with an asymptomatic infection, especially among households and this can be done by widespread testing of the affected population. Other vector control methods such as residual spray and thermal fogging that require the involvement of pest control specialists or public health departments are among the options to consider in managing the CHIKV outbreak [44,45].

Conclusions

Findings from the present study suggest that the magnitude of the chikungunya fever outbreak in Tanjung Sepat in 2018-2019 was much higher than the actual reported number of confirmed cases. Factors contributing to the low reporting may include underdiagnosis of the infection and asymptomatic infections. The study further identified the spread of CHIKV infection within a household as an important risk factor during an outbreak. Hence, widespread testing for chikungunya in the community during an outbreak especially among household contacts of the positive individual, could help to identify those who are also asymptomatically infected.

Acknowledgements

The authors would like to thank all volunteers from Tanjung Sepat and Village Community Management Council (Kampung Tanjung Sepat Indah) for their cooperation in facilitating the sample collection activities.

Funding Information

This study is funded in parts by the Higher Institution Centre of Excellence (HICoE) program: MO002-2019 and Fundamental Research Grant Scheme (FRGS): FP014-2019A from Ministry of Education Malaysia.

Authors' Contribution

CSK, BTT, SSS, HYL, YHS and SAB contributed to the conception of the work and interpretation of the findings. CSK, BTT and SSS coordinated the sampling activities CSK, BTT, SSS, HYK, NSA, AACMS, KLC, ZRH, NAMRN, CNY and JAJ contributed to the sample collection. CSK, BTT and HYK performed laboratory tests. CSK, BTT and SAB performed the statistical analysis and drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

References

1. Vazeille M, Mousson L, Martin E, Failloux AB (2010) Orally co-infected *Aedes albopictus* from La Reunion island, Indian Ocean, can deliver both dengue and chikungunya infectious viral particles in their saliva. PLoS Negl Trop Dis 6: e706.
2. Harrington LC, Fleisher A, Ruiz-Moreno D, Vermeylen F, Wa CV, Poulson RL, Edman JD, Clark JM, Jones JW, Kitthawee S, Scott TW (2014) Heterogeneous feeding patterns of the dengue vector, *Aedes aegypti*, on individual human hosts in rural Thailand. PLoS Negl Trop Dis 8: e3048.
3. Honorio NA, Silva Wda C, Leite PJ, Goncalves JM, Lounibos LP, Lourenco-de-Oliveira R (2003) Dispersal of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in an urban endemic dengue area in the State of Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 2: 191-198.
4. Ayu SM, Lai LR, Chan YF, Hatim A, Hairi NN, Ayob A, Sam IC (2010) Seroprevalence survey of chikungunya virus in Bagan Panchor, Malaysia. Am J Trop Med Hyg 6: 1245-1248.
5. Borgherini G, Poubeau P, Jossaume A, Gouix A, Cotte L, Michault A, Arvin-Berod C, Paganin F (2008) Persistent arthralgia associated with chikungunya virus: a study of 88 adult patients on Reunion Island. Clin Infect Dis 4: 469-475.
6. Velasco JM, Valderama MT, Lopez MN, Chua Jr D, Rene Latog I, Roque Jr V, Corpuz J, Klungthong C, Rodpradit P, Hussem K (2015) Chikungunya virus infections among patients with dengue-like illness at a tertiary care hospital in the Philippines, 2012–2013. Am J Trop Med Hyg 6: 1318.
7. Weaver SC and Forrester NL (2015) Chikungunya: evolutionary history and recent epidemic spread. Antiviral Res: 32-39.
8. Kamal M, Kenawy MA, Rady MH, Khaled AS, Samy AM (2018) Mapping the global potential distributions of two arboviral vectors *Aedes aegypti* and *Ae. albopictus* under changing climate. PLoS One 12: e0210122.
9. Tuite AR, Watts AG, Khan K, Bogoch, II (2019) Countries at risk of importation of chikungunya virus cases from southern Thailand: a modeling study. Infect Dis Model: 251-256.
10. Outbreak News Today (2019) Thailand: chikungunya cases up 500 in past week, nearly 10,000 dengue cases reported in 2019. Available: http://outbreaknewstoday.com/thailand-chikungunya-cases-500-past-week-nearly-10000-dengue-cases-reported-2019. Accessed: 05 May 2021.
11. Bernama (2019) Kedah acts following chikungunya outbreak in southern Thailand. Available: https://www.bernama.com/en/news.php?id=1684983. Accessed: 05 November 2021.
12. Abdullah NH (2019) Current situation of chikungunya in Malaysia - 15 January 2019. Available: https://www.moh.gov.my/index.php/database_stores/attach_download/337/1122. Accessed: 05 November 2021.
13. Abdullah NH (2019) Current situation of chikungunya in Malaysia - 30 May 2019. Available: https://www.moh.gov.my/index.php/database_stores/attach_download/337/1174. Accessed: 05 November 2021.
14. Abdullah NH (2019) Current situation of chikungunya in Malaysia - 21 May 2019. Available: https://www.moh.gov.my/index.php/database_stores/attach_download/337/1166. Accessed: 05 September 2022.
15. Abdullah NH (2019) Current situation of chikungunya in Malaysia - 24 December 2019. Available: https://www.moh.gov.my/index.php/database_stores/attach_download/337/1278. Accessed: 05 September 2022.
16. Haji-Ibrahim HK (2019) Statement of the early incident of fever and joint pain in Tanjung Sepat, Kuala Langat, Selangor. Available: https://www.facebook.com/236299813121124/posts/kenyataan-akhbarkementerian-kesihatan-malaysia-kenyataan-awal-kejadian-demam-dan/2017895001628254. Accessed: 05 November 2021.
17. Department of Statistics, Malaysia (2021) Population distribution by local authority and mukims 2010. Available: https://www.mycensus.gov.my/index.php/census-product/publication/census-2010/681-population-distribution-by-local-authority-and-mukims-2010. Accessed: 05 November 2021.
18. Bi Q, Wu Y, Mei S, Ye C, Zou X, Zhang Z, Liu X, Wei L, Truelove SA, Zhang T (2020) Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. Lancet Infect Dis 8: 911-919.
19. Kumarasamy V, Prathapa S, Zuridah H, Chem YK, Norizah I, Chua KB (2006) Re-emergence of chikungunya virus in Malaysia. Med J Malaysia 2: 221-225.
20. Lam SK, Chua KB, Hooi PS, Rahimah MA, Kumari S, Tharmaratnam M, Chuah SK, Smith DW, Sampson IA (2001) Chikungunya infection - an emerging disease in Malaysia. Southeast Asian J Trop Med Public Health 3: 447-451.
21. AbuBakar S, Sam IC, Wong PF, MatRahim N, Hooi PS, Roslan N (2007) Reemergence of endemic chikungunya, Malaysia. Emerg Infect Dis 1: 147-149.
22. Noridah O, Paranthaman V, Nayar SK, Masliza M, Ranjit K, Norizah I, Chem YK, Mustafa B, Kumarasamy V, Chua KB (2007) Outbreak of chikungunya due to virus of Central/East African genotype in Malaysia. Med J Malaysia 4: 323-328.
23. Ngwe Tun MM, Inoue S, Thant KZ, Talemaitoga N, Aryati A, Dimaano EM, Matias RR, Buerano CC, Natividad FF, Abeyewickreme W, Thuy NT, Mai LT, Hasebe F, Hayasaka D, Morita K (2016) Retrospective seroepidemiological study of chikungunya infection in South Asia, Southeast Asia and the Pacific region. Epidemiol Infect 11: 2268-2275.
24. Vongpunsawad S, Intharasongkroh D, Thongmee T, Poovorawan Y (2017) Seroprevalence of antibodies to dengue and chikungunya viruses in Thailand. PLoS One 6: e0180560.
25. Johnson BW, Goodman CH, Holloway K, de Salazar PM, Valadere AM, Drebot MA (2016) Evaluation of commercially available chikungunya virus immunoglobulin m detection assays. Am J Trop Med Hyg 1: 182-192.
26. Andy J and Rudnick A (1967) Ecology of dengue and other arboviruses of Malaysia. Available: https://apps.dtic.mil/sti/pdfs/AD0655288.pdf. Accessed: 11 November 2021.
27. Pacheco O, Martinez M, Alarcon A, Bonilla M, Caycedo A, Valbuena T, Zabaleta A (2017) Estimation of underreporting of chikungunya virus infection cases in Girardot, Colombia, from November, 2014 to May, 2015. Biomedica 4: 507-515.
28. Ndeffo-Mbah ML, Durham DP, Skrip LA, Nsoesie EO, Brownstein JS, Fish D, Galvani AP (2016) Evaluating the effectiveness of localized control strategies to curtail chikungunya. Sci Rep: 23997.
29. Moro ML, Gagliotti C, Silvi G, Angelini R, Sambri V, Rezza G, Massimiliani E, Mattivi A, Grilli E, Finarelli AC, Spataro N, Pierro AM, Seyler T, Macini P, Chikungunya Study G (2010) Chikungunya virus in north-eastern Italy: a seroprevalence survey. Am J Trop Med Hyg 3: 508-511.

30. Velasco JM, Valderama MT, Lopez MN, Chua D, Jr., Latog R, 2nd, Roque V, Jr., Corpuz J, Klungthong C, Rodpradit P, Hussem K, Poolpanichupatam Y, Macareo L, Fernandez S, Yoon IK (2015) Chikungunya virus infections among patients with dengue-like illness at a Tertiary Care Hospital in the Philippines, 2012-2013. Am J Trop Med Hyg 6: 1318-1324.
31. Dinkar A, Singh J, Prakash P, Das A, Nath G (2018) Hidden burden of chikungunya in North India; a prospective study in a tertiary care centre. J Infect Public Health 4: 586-591.
32. Sharp TM, Roth NM, Torres J, Ryff KR, Perez Rodriguez NM, Mercado C, Pilar Diaz Padro MD, Ramos M, Phillips R, Lozier M, Arriola CS, Johansson M, Hunsperger E, Munoz-Jordan JL, Margolis HS, Garcia BR, Centers for Disease C, Prevention (2014) Chikungunya cases identified through passive surveillance and household investigations--Puerto Rico, May 5-August 12, 2014. MMWR Morb Mortal Wkly Rep 48: 1121-1128.
33. Sissoko D, Ezzedine K, Moendandze A, Giry C, Renault P, Malvy D (2010) Field evaluation of clinical features during chikungunya outbreak in Mayotte, 2005-2006. Trop Med Int Health 5: 600-607.
34. Appassakij H, Khuntikij P, Kemapunmanus M, Wutthanarungsan R, Silpapojakul K (2013) Viremic profiles in asymptomatic and symptomatic chikungunya fever: a blood transfusion threat? Transfusion 10 Pt 2: 2567-2574.
35. Sissoko D, Moendandze A, Malvy D, Giry C, Ezzedine K, Solet JL, Pierre V (2008) Seroprevalence and risk factors of chikungunya virus infection in Mayotte, Indian Ocean, 2005-2006: a population-based survey. PLoS One 8: e3066.
36. Sergon K, Yahaya AA, Brown J, Bedja SA, Mlindasse M, Agata N, Allaranger Y, Ball MD, Powers AM, Ofula V, Onyango C, Konongoi LS, Sang R, Njenga MK, Breiman RF (2007) Seroprevalence of chikungunya virus infection on Grande Comore Island, union of the Comoros, 2005. Am J Trop Med Hyg 6: 1189-1193.
37. Chen CD, Benjamin S, Saranum MM, Chiang YF, Lee HL, Nazni WA, Sofian-Azirun M (2005) Dengue vector surveillance in urban residential and settlement areas in Selangor, Malaysia. Trop Biomed 1: 39-43.
38. Barrera R, Bingham AM, Hassan HK, Amador M, Mackay AJ, Unnasch TR (2012) Vertebrate hosts of *Aedes aegypti* and *Aedes mediovittatus* (Diptera: *Culicidae*) in rural Puerto Rico. J Med Entomol 4: 917-921.
39. Scott TW, Amerasinghe PH, Morrison AC, Lorenz LH, Clark GG, Strickman D, Kittayapong P, Edman JD (2000) Longitudinal studies of *Aedes aegypti* (Diptera: *Culicidae*) in Thailand and Puerto Rico: blood feeding frequency. J Med Entomol 1: 89-101.
40. Intayot P, Phumee A, Boonserm R, Sor-Suwan S, Buathong R, Wacharapluesadee S, Brownell N, Poovorawan Y, Siriyasatien P (2019) Genetic characterization of chikungunya virus in field-caught *Aedes aegypti* mosquitoes collected during the recent outbreaks in 2019, Thailand. Pathogens 3: 121.
41. Van Roey K, Sokny M, Denis L, Van den Broeck N, Heng S, Siv S, Sluydts V, Sochantha T, Coosemans M, Durnez L (2014) Field evaluation of picaridin repellents reveals differences in repellent sensitivity between southeast Asian vectors of malaria and arboviruses. PLoS Negl Trop Dis 12: e3326.
42. Debboun M and Strickman D (2013) Insect repellents and associated personal protection for a reduction in human disease. Med Vet Entomol 1: 1-9.
43. Londono-Renteria B, Patel JC, Vaughn M, Funkhauser S, Ponnusamy L, Grippin C, Jameson SB, Apperson C, Mores CN, Wesson DM, Colpitts TM, Meshnick SR (2015) Long-lasting permethrin-impregnated clothing protects against mosquito bites in outdoor workers. Am J Trop Med Hyg 4: 869-874.
44. Chung YK, Lam-Phua SG, Chua YT, Yatiman R (2001) Evaluation of biological and chemical insecticide mixture against *Aedes aegypti* larvae and adults by thermal fogging in Singapore. Med Vet Entomol 3: 321-327.
45. Manica M, Cobre P, Rosa R, Caputo B (2017) Not in my backyard: effectiveness of outdoor residual spraying from hand-held sprayers against the mosquito *Aedes albopictus* in Rome, Italy. Pest Manag Sci 1: 138-145.

Corresponding author

Sazaly Abubakar, Ph.D.
Tropical Infectious Diseases Research & Education Centre (TIDREC),
Level 2, High Impact Research Building, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.
Tel: +603-79676670
E-mail: sazaly@um.edu.my

Conflict of interests: No conflict of interests is declared.