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RESEARCH ARTICLE

Seroprevalences and their associated predictors of chikungunya, dengue, Japanese encephalitis and zika among forest fringe dwellers of Peninsular Malaysia

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ABSTRACT

Serological evidence has shown the presence of several mosquito-borne arbovirus infections among the inhabitants of the forest fringe areas of the tropics. Among these infections, Japanese encephalitis, dengue fever, chikungunya fever and Zika fever could be targeted for vaccination to overcome severe infection and limit the disease transmission. Seroprevalence data among this high-risk population are needed to provide an estimate of the potential cost-effectiveness of any vaccine programme targeting these infections. The present study was conducted at six indigenous people (Orang Asli) villages and FELDA (Federal Land Development Authority) settlements located at the forest fringes of Malaysia. All participants consented and provided blood samples and demographic data for the study. The blood samples were tested for the presence of antibodies against CHIKV, DENV, JEV and ZIKV individually using ELISA. Results obtained were also analysed to determine the predictors for CHIKV, DENV, JEV and ZIKV seropositivity. Among the 585 samples tested, 33.0% (N=193), 41.7% (N=244), 10.3% (N=60) and 21.0% (N=123) were positive for CHIKV IgG, DENV IgG, JEV IgG and ZIKV IgG, respectively. Approximately one-third (N=220, 37.6%) of the participants were tested negative for IgG antibodies against all four arboviruses. Age of participants and type of settlement were found to be a significant predictor for CHIKV, DENV, JEV and ZIKV seropositivity. Level of education was a significant predictor for CHIKV, DENV and ZIKV seropositivity. Gender, however, was not found to be a significant predictor for infection with any of these viruses. These findings reaffirmed the significant presence of infection involving these major arboviruses among the group of people living within the forest fringe areas of Peninsular Malaysia. Hence, any future consideration of vaccination for these infections must take into consideration the marginalized and underserved communities living at the forest fringe areas of the tropics where these infections are present.

Keywords: Infectious diseases; vector-borne; seroprevalence; arbovirus; forest fringe.

INTRODUCTION

Arboviruses are viruses transmitted by arthropod vectors such as mosquitoes and ticks. These diseases are mostly endemic in the tropics due to the conducive environment for the proliferation of the disease vectors and their animal hosts. Globally, at least 50 million arbovirus infections are reported to the World Health Organization each year (WHO, 2009). The figure is most likely an underestimation since most of the data were collected through passive surveillance which often do not include remote areas where healthcare facilities are limited. The actual figure could be around 104 million, signifying the importance of arbovirus infections in the tropics (Zeng *et al*., 2021).

The indigenous people of Peninsular Malaysia, commonly known as the Orang Asli (OA), are people who most commonly reside in OA villages located at forest fringes or in forests (Ministry of Rural and Regional Development Malaysia, 2014). Similarly, FELDA (Federal Land Development Authority) settlers reside in FELDA settlements located on forested lands cleared for plantation purposes. FELDA settlers were primarily engaged in the plantation sector while OA sustained themselves by working in plantations and foraging the forests for food and other resources (Bahrin, 1977; Howell *et al*., 2010; Tay *et al*., 2013). These forest fringe dwellers formed bulk of the marginalized and underserved communities that could be at increased exposure risks to spillover zoonotic infections in the tropics (Gottwalt, 2013).

CHIKV, DENV, JEV and ZIKV are among the major vector-borne pathogens found endemic in Malaysia (Ministry of Health Malaysia, 2019, 2023). Incidences of CHIKV and DENV infection are commonly reported throughout the year (Ministry of Health Malaysia, 2023). Whereas, incidences of JEV and ZIKV infection were scarce despite serological evidence suggesting otherwise (Khor *et al*., 2020). Mild JEV and ZIKV infections are often indistinguishable from DENV infections due to the similar clinical presentations at the early

phase of the infection where most will present with fever, malaise, nausea, loss of appetite and myalgia (Ioos *et al*., 2014; Ma'roef *et al*., 2020). While access to healthcare is often accessible to these communities, clinics within these areas are inadequately equipped to perform pathogen detection. Hence, the lack of reporting on JEV and ZIKV infections may have been caused by the limited access to laboratory detection for these pathogens.

Vaccines against a number of these arboviruses are available. Vaccines against Japanese encephalitis in particular received initial approval in 2009 (USFDA, 2019). The inactivated JEV strain SA14-14-2 has been widely used in countries such as Indonesia, South Korea and Thailand (Sohn, 2000; Liu *et al*., 2008). Vaccination against JE is also mandatory in Taiwan (Yang *et al*., 2006). Whereas Qdenga®, a live attenuated tetravalent dengue vaccine has been approved in Indonesia, European Union, and Brazil (Takeda, 2022). The Qdenga® is the second vaccine for dengue following the introduction of Dengvaxia® in 2015 (Shim, 2017). Several Chikungunya and Zika vaccine candidates have progressed to Phase 1 and 2 clinical trials setting the stage for possible introduction of these vaccines soon in the future (Schrauf *et al*., 2020). To ensure that these vaccines would benefit the intended population when introduced, additional data on the prevalence of CHIKV, DENV, JEV and ZIKV, especially in the high-risk populations are required. The present study, hence, intends to determine the seroprevalence of CHIKV, DENV, JEV and ZIKV among the communities living at forest fringes of Malaysia representing the marginalized and underserved population vulnerable to the infections.

MATERIALS AND METHODS

Ethics statement

This study protocol has been reviewed and approved by the Universiti Malaya Medical Centre-Medical Research Ethics Committee (UMMC-MREC) (MREC ID NO: 20161115-4602). Permissions were obtained from the Department of Orang Asli Development (JAKOA) (Ref No: JAKOA/PP.30.052Jld11(8)) and Federal Land Development Authority (FELDA) (Ref No: UM.TNC2/TIDREC/628), Malaysian government agency overseeing the conduct of the study in OA villages and FELDA settlements, respectively. All subjects or legal guardians of the subject consented to participate in this study.

Study sites, study population, and sample collection

A total of nine study sites were allocated for this study. These study sites were recommended by JAKOA and FELDA due to their willingness to participate in research studies. The study sites comprised of six OA villages (Donglai Baru, Paya Lebar, Tumboh Hangat, Sungai Perah, Tanah Runtuh and Buluh Nipis) and three FELDA settlements (Soeharto, Ijok and Jengka 8) located in central part of Peninsular Malaysia. OA and FELDA settlers aged more than 5 years old were thoroughly briefed before being invited to participate in this study. Approximately 5ml of blood specimen was obtained from consented participants through venipuncture. The blood samples were allowed to clot at room temperature, then centrifuged to separate serum from blood cells. The sera were aliquoted into separate tubes and stored in –80°C freezer for subsequent analysis. Demographic data (age, gender, level of education and type of settlement) were obtained from the participants through an administered questionnaire. Data were statistically analysed to determine if there were correlation between CHIKV, DENV, JEV and ZIKV seropositivity and the demographic data.

Serological detection of CHIKV, DENV, JEV and ZIKV immunoglobulin G (IgG) antibodies

The presence of CHIKV, DENV, JEV and ZIKV IgG antibodies in the serum samples were determined using the NovaLisa Chikungunya Virus IgG capture enzyme linked immunosorbent assay (ELISA) kit (NovaTec Immundiagnostica, Germany), SD Dengue IgG Capture ELISA (Standard Diagnostics, South Korea), JE Detect™ IgG ELISA kit (InBios International Inc., USA) and Anti-Zika Virus ELISA (IgG) ELISA kit (Euroimmun, Germany) respectively. All ELISA procedures were performed strictly according to the protocol provided by the respective ELISA kit manufacturers.

Statistical analysis

ELISA results and demographic data were analysed using the IBM SPSS Statistics v26 (IBM Corp, USA). Unadjusted logistic regression was used to assess the correlation between age, gender, level of education, and type of settlement (independent variables) and CHIKV, DENV, JEV, and ZIKV seropositivity (dependent variables). The data from OA villagers and FELDA settlers were analysed independently using logistic regression. Samples with equivocal JEV ELISA results were regarded as negative due to the potential cross-reactivity among flaviviruses. Samples with insufficient volume or incomplete demographic data were removed from the analysis.

RESULTS

A total of 602 participants consented to participate in the study. Due to insufficient sample volume and incomplete demographic data (N=17), only 585 participants from the six study sites were included in the analysis. The participants recruited consist of OA (N=330, 56.4%) and FELDA settlers (N=255, 43.6%) residing at the study sites (Table 1). Ten samples with equivocal results for JEV IgG were defined as negative instead to generate binary variable. The

Table 1. Demographic information of participants from OA villages and FELDA settlements

Type of settlement	Study site (District)	Main ethnicity	N	Age Mean ± SD	Female N (%)	Level of education* (%)
OA village	Buluh Nipis (Rompin)	Jakun	58	42.5 ± 12.7	35 (60.3)	Primary school (47.4)
	Tanah Runtuh (Rompin)	Jakun	25	26.7 ± 15.2	18 (72.0)	No formal education (52.2)
	Tumboh Hangat (Perak Tengah)	Semai	80	26.7 ± 16.2	54 (67.5)	Primary school (47.4)
	Sungai Perah (Perak Tengah)	Semai	111	34.9 ± 17.4	73 (65.8)	Primary school (48.6)
	Donglai Baru (Hulu Langat)	Temuan	27	28.4 ± 14.7	14 (51.9)	Secondary school (44.4)
	Paya Lebar (Hulu Langat)	Temuan	29	36.9 ± 13.9	23 (79.3)	No formal education (48.3)
	All OA villages	**330**	**330**	**34.1 ± 16.5**	**217 (65.8)**	**Primary school (42.9)**
FELDA settlement	Ijok	Malay	102	52.5 ± 16.7	62 (60.8)	Primary school (44.6)
	Jengka 8	Malay	105	51.7 ± 16.8	66 (62.9)	Primary school (39.0)
	Soeharto	Malay	48	45.8 ± 18.3	27 (56.3)	Primary school (44.1)
	All FELDA settlements	**255**	**255**	**50.9 ± 17.2**	**155 (60.8)**	**Primary school (42.1)**
All participants		**585**	**585**	**41.4 ±18.7**	**372 (63.6)**	**Primary school (42.6)**

*Level of education is a categorical variable with more than two levels. Only category with highest percentage was displayed.

mean age of participants was 41.4 years old (standard deviation = 18.7, range 5 to 82) and 63.6% (N=372) of them were female. Gender composition of participants from OA settlements and FELDA settlements was approximately the same, however, the mean age and level of education were significantly different ($p<0.01$). Participants from OA settlements (mean age ± SD = 34.1 ± 16.5; received tertiary education = 0.6%) has lower mean age and level of education as compared to participants from FELDA settlements (mean age ± SD = 50.9 ± 17.2; received tertiary education = 10.6%).

Serological tests performed revealed that 33.0% (N=193), 41.7% (N=244), 10.3% (N=60) and 21.0% (N=123) of the samples tested positive for CHIKV IgG, DENV IgG, JEV IgG and ZIKV IgG respectively (Table 2). At least 37.6% (N=220) of the participants tested negative for IgG antibodies for all four arboviruses. On the other hand, 31.3% (N=183) of the participants were seropositive for multiple arboviruses. Only 31.1% (N=182) of the participants were seropositive for just one of the four arboviruses tested.

Univariate logistic regression analysis performed revealed that type of settlement ($p<0.05$) and age ($p<0.01$) were significant predictors for CHIKV, DENV, JEV and ZIKV seropositivity (Table 3). An increase of 1 year in age reduced the risk for CHIKV seropositivity by 0.986 times, however, the risks for seropositivity for DENV, JEV and ZIKV increased by 1.023, 1.036 and 1.019 times, respectively. Volunteers from OA villages were 5.282, 1.513 and 3.886 times more likely to be seropositive than those from FELDA settlements for CHIKV, DENV and ZIKV, respectively. In contrast, volunteers from FELDA settlements were 3.136 times more likely to be seropositive for JEV than those from OA settlements. The level of education ($p<0.05$) was a significant predictor for CHIKV, DENV and ZIKV seropositivity. Having higher level of formal education was associated with a reduction in the likelihood of being seropositive for CHIKV, DENV and ZIKV. Since none of the tertiary educated volunteers were found to be seropositive for JEV and ZIKV, tertiary education was omitted from the analysis when logistic regression was performed. Gender was not found to be a significant predictor for any of the arboviruses.

Univariate logistic regression analysis performed solely on data from OA villager (N=330) revealed that age ($p<0.05$) were significant predictors for DENV, JEV and ZIKV (Table 4). The increase of age increases the risks of being seropositive for DENV, JEV and ZIKV by 1.066, 1.030 and 1.056 times respectively. The level of education ($p<0.05$) was a significant predictor for DENV and ZIKV seropositivity as well. Those who received formal education were about 50% (48.5% to 66.6%) less likely to be tested seropositive for DENV and

Table 2. Seroprevalence of CHIKV, DENV, JEV and ZIKV among OA and FELDA settlers of Malaysia

Type of settlement	Study site	N	Seroprevalence, N (%)			
			CHIKV IgG	DENV IgG	JEV IgG	ZIKV IgG
OA village	Buluh Nipis	58	27 (46.6)	36 (62.1)	9 (15.5)	19 (32.8)
	Tanah Runtuh	25	13 (52.0)	11 (44.0)	0 (0.0)	14 (56.0)
	Tumboh Hangat	80	30 (37.5)	23 (28.8)	1 (1.3)	6 (7.5)
	Sungai Perah	111	57 (51.4)	59 (53.2)	6 (5.4)	39 (35.1)
	Donglai Baru	27	17 (63.0)	12 (44.4)	1 (3.7)	9 (33.3)
	Paya Lebar	29	12 (41.4)	11 (37.9)	2 (6.9)	11 (37.9)
	All OA villages	**330**	**156 (47.3)**	**152 (46.1)**	**19 (5.8)**	**98 (29.7)**
FELDA settlement	Ijok	102	8 (7.8)	30 (29.4)	13 (12.7)	4 (3.9)
	Jengka 8	105	22 (21.0)	40 (38.1)	26 (24.8)	14 (13.3)
	Soeharto	48	7 (14.6)	22 (45.8)	2 (4.2)	7 (14.6)
	All FELDA settlements	**255**	**37 (14.5)**	**92 (36.1)**	**41 (16.1)**	**25 (9.8)**
All participants		**585**	**193 (33.0)**	**244 (41.7)**	**60 (10.3)**	**123 (21.0)**

Table 3. Univariate analysis on predictors for CHIKV, DENV, JEV and ZIKV seropositivity using logistic regression

Independent variables	Dependent variables, p-value (Odds ratio)			
	CHIKV IgG	DENV IgG	JEV IgG	ZIKV IgG
Age	<0.05 (0.986)	<0.05 (1.023)	<0.05 (1.036)	<0.05 (1.019)
Gender				
Male	Ref	Ref	Ref	Ref
Female	NS	NS	NS	NS
Type of settlement				
OA village	Ref	Ref	Ref	Ref
FELDA settlement	<0.05 (0.189)	<0.05 (0.661)	<0.05 (3.136)	<0.05 (0.257)
Level of education				
No formal education	Ref	Ref	Ref	Ref
Primary education	NS	<0.05 (0.549)	NS	<0.05 (0.426)
Secondary education	NS	<0.05 (0.414)	NS	<0.05 (0.291)
Tertiary education	<0.05 (0.050)	<0.05 (0.194)	NA	NA

Ref: Reference group.
NS: Not significant.
NA: Not applicable. Logistic regression cannot be performed for this category due to lack of seropositive participants (N=0).

Table 4. Univariate analysis on predictors for CHIKV, DENV, JEV and ZIKV seropositivity among OA and FELDA settlers using logistic regression

Independent variables	Dependent variables, p-value (Odds ratio)							
	OA (N=330)				FELDA (N=255)			
	CHIKV IgG	DENV IgG	JEV IgG	ZIKV IgG	CHIKV IgG	DENV IgG	JEV IgG	ZIKV IgG
Age	NS	<0.05 (1.066)	<0.05 (1.030)	<0.05 (1.056)	<0.05(1.025)	NS	<0.05 (1.026)	<0.05 (1.028)
Gender								
Male	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Female	NS	NS	NS	NS	NS	NS	NS	NS
Level of education								
No formal education	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Primary education	NS	<0.05 (0.490)	NS	<0.05 (0.515)	NS	NS	NS	NS
Secondary education	NS	<0.05 (0.334)	NS	<0.05 (0.375)	NS	NS	NS	NS
Tertiary education	NA	NA	NA	NA	NA	NS	NA	NA

Ref: Reference group.
NS: Not significant.
NA: Not applicable. Logistic regression cannot be performed for this category due to lack of seropositive participants (N=0) or insufficient observations in the category (N < 5).

ZIKV. Gender was not found to be a significant predictor for any of the arboviruses. When logistic regression was performed on data from FELDA settlers (N=255), age (p<0.05) was the only significant predictor (Table 4). The increase of age increases the risks of being seropositive for CHIKV, JEV and ZIKV by 1.025, 1.026 and 1.028 times respectively. Level of education and gender were not found to be significant predictors for any of the arboviruses.

DISCUSSION

This study found high CHIKV (33.0%) and DENV (41.7%) seropositivity rates among volunteers from OA and FELDA settlements. The high seropositivity observed were within expectation since both infections are endemic in Malaysia. Seropositivity of CHIKV and DENV reported in Malaysia ranges from 5.9% to 72.5% (Ayu *et al*., 2010; Azami *et al*., 2013; Khor *et al*., 2020) and 4.9% – 91.6% respectively (Muhammad Azami *et al*., 2011; Dhanoa *et al*., 2018; Abd-Jamil *et al*., 2020; Ng *et al*., 2022). The differences in seroprevalences reported were most likely due to different study populations. In comparison, moderate ZIKV (21.0%) and JEV (10.3%) seropositivity were observed. The moderate seropositivity for ZIKV and JEV were expected as only few incidences of these diseases have been documented in Malaysia (Kumar *et al*., 2018; Woon *et al*., 2019). Seropositivity found in other studies ranges from between 6.2% to 30.3% for ZIKV (Ngwe Tun *et al*., 2021; Khoo *et al*., 2022) and 22.4% to 48.4% for JEV (Nealon *et al*., 2019; Khor *et al*., 2020). Overall, the seroprevalences from this study were consistent to the earlier findings conducted elsewhere (Abd-Jamil *et al*., 2020; Khor *et al*., 2020). However, collectively the data showed increasing seropositivity for DENV and ZIKV among the OA over the years.

The seroprevalences of CHIKV, DENV, JEV and ZIKV were different between OA communities and FELDA settlements. These differences could be due to the different presence of mosquito species at the study sites. *Ae*. *aegypti* and *Ae*. *albopictus* are the two most common vectors for CHIKV, DENV and ZIKV (Saleeza *et al*., 2011, 2013). The high prevalence of CHIKV and ZIKV among the OA could reflect the higher presence of these mosquito species breeding sites (Chandren *et al*., 2015; Sabri, 2015). On the other hand, the absence of *Culex tritaeniorhyncus* and other *Culex* mosquito species resulted in the lower seroprevalence of JEV (Vythilingam *et al*., 1997). The clearing and infrastructure development of forested areas surrounding the OA settlements may have contributed to the reduction of Culex populations (Ministry of Finance Malaysia, 2011; Mayi *et al*., 2019). Whereas, these activities increased the potential breeding sites for *Aedes* spp. mosquitoes, which resulted in increased CHIKV, DENV and ZIKV transmission rate (Cheong *et al*., 2014).

Age was commonly reported as a significant factor for seroprevalence of arboviruses (Conlan *et al*., 2015, Vongpunsawad *et al*., 2017). Antibodies generated in response to previous infections can endure for years following the infection (Imrie *et al*., 2007), increasing the likelihood of seropositivity with age (Katzelnick & Harris, 2018). The result of this study varies from consensus, as age did not emerge as a significant predictor for both CHIKV and DENV seropositivity when the analysis was conducted separately for OA villagers and FELDA settlers. CHIKV seropositivity is uniformly distributed across age groups among OA villagers, while DENV seropositivity is evenly spread among age groups in FELDA settlers. This indicates that OA villagers were exposed to CHIKV from a young age, and similarly, FELDA settlers were exposed to DENV from a young age.

Antibodies induced by Flavivirus infections in general cross-react with each other across the different flaviviruses. The envelope protein (E) of Flaviviruses is often the main antigenic protein targeted by the antibody responses. Amino acid sequences of E were 40% – 79% identical across the mosquito-borne Flaviviruses (Hou *et al*., 2022). Despite the variations some of the epitopes located in E are conserved among different Flaviviruses, leading to cross-reactivity of antibodies (Chan *et al*., 2022). While the seropositive samples detected may be caused by cross-reactivity, previous study has shown that most flavivirus seropositive samples were able to neutralize the respective flavivirus (Khor *et al*., 2020). Hence, it is likely that most of the seropositive subjects found in this study were caused by actual exposure to the respective viruses.

Results from the present study provide important insights into the epidemiology of CHIKV, DENV, JEV and ZIKV among the forest fringe dwellers represented by the OA communities and FELDA settlers. A more comprehensive studies in other regions of the tropics amongst the forest fringe communities will need to be undertaken to obtain better estimate of the potential burden of the infections and the potential benefits should vaccination is considered for these arbovirus infections.

CONCLUSION

Results from the seroprevalence study among the forest fringe dwellers of Peninsular Malaysia found dengue and chikungunya were the most prominent arbovirus infections followed by zika and JEV. Age, type of settlement and level of education are the significant predictors of exposure to these arboviruses. This study highlights the potential health burden caused by arbovirus infections among the underserved communities in the tropics.

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Conflict of interest

The authors have declared no conflicts of interest.

REFERENCES

Abd-Jamil, J., Ngui, R., Nellis, S., Fauzi, R., Lim, A.L.Y., Chinna, K., Khor, C.S. & AbuBakar, S. (2020). Possible factors influencing the seroprevalence of dengue among residents of the forest fringe areas of Peninsular Malaysia. *Journal of Tropical Medicine* **2020**: 1019238. https://doi.org/10.1155/2020/1019238

Ayu, S.M., Lai, L.R., Chan, Y.F., Hatim, A., Hairi, N.N., Ayob, A. & Sam, I.C. (2010). Seroprevalence survey of Chikungunya virus in Bagan Panchor, Malaysia. *American Journal of Tropical Medicine and Hygiene* **83**: 1245-1248. https://doi.org/10.4269/ajtmh.2010.10-0279

Azami, N.A., Salleh, S.A., Shah, S.A., Neoh, H.M., Othman, Z., Zakaria, S.Z. & Jamal, R. (2013). Emergence of chikungunya seropositivity in healthy Malaysian adults residing in outbreak-free locations: chikungunya seroprevalence results from the Malaysian Cohort. *BMC Infectious Diseases* **13**: 67. https://doi.org/10.1186/1471-2334-13-67

Bahrin, T.S. (1977). FELDA 21 years of land development. FELDA 21 years of land development.

Chan, K.R., Ismail, A.A., Thergarajan, G., Raju, C.S., Yam, H.C., Rishya, M. & Sekaran, S. D. (2022). Serological cross-reactivity among common flaviviruses. *Frontiers in Cellular and Infection Microbiology* **12**: 975398. https://doi.org/10.3389/fcimb.2022.975398

Chandren, J.R., Wong, L.P. & AbuBakar, S. (2015). Practices of dengue fever prevention and the associated factors among the Orang Asli in Peninsular Malaysia. *PLoS Neglected Tropical Diseases* **9**: e0003954. https://doi.org/10.1371/journal.pntd.0003954

Cheong, Y.L., Leitao, P.J. & Lakes, T. (2014). Assessment of land use factors associated with dengue cases in Malaysia using Boosted Regression Trees. *Spatial and Spatio-Temporal Epidemiology* **10**: 75-84. https://doi.org/10.1016/j.sste.2014.05.002

Conlan, J.V., Vongxay, K., Khamlome, B., Jarman, R.G., Gibbons, R.V., Fenwick, S.G., Thompson, R.C.A., & Blacksell, S.D. (2015). Patterns of Flavivirus seroprevalence in the human population of Northern Laos. *American Journal of Tropical Medicine and Hygiene* **93**: 1010-1013. https://doi.org/10.4269/ajtmh.15-0072

Dhanoa, A., Hassan, S.S., Jahan, N.K., Reidpath, D.D., Fatt, Q.K., Ahmad, M.P., Meng, C.Y., Ming, L.W., Zain, A.Z., Phipps, M.E. *et al.* (2018). Seroprevalence of dengue among healthy adults in a rural community in Southern Malaysia: a pilot study. *Infectious Diseases of Poverty* **7**: 1. https://doi.org/10.1186/s40249-017-0384-1

Gottwalt, A. (2013). Impacts of deforestation on vector-borne disease incidence. *The Columbia University Journal of Global Health* **3**: 16-19.

Hou, B., Chen, H., Gao, N. & An, J. (2022). Cross-Reactive immunity among five medically important mosquito-borne Flaviviruses related to human diseases. *Viruses* **14**: 1213. https://doi.org/10.3390/v14061213

Howell, C.J., Schwabe, K.A. & Samah, A.H.A. (2010). Non-timber forest product dependence among the Jah Hut subgroup of Peninsular Malaysia's Orang Asli. *Environment, Development and Sustainability* **12**: 1-18.

Imrie, A., Meeks, J., Gurary, A., Sukhbaatar, M., Truong, T.T., Cropp, C.B. & Effler, P. (2007). Antibody to dengue 1 detected more than 60 years after infection. *Viral immunology* **20**: 672-675.

Ioos, S., Mallet, H.P., Leparc Goffart, I., Gauthier, V., Cardoso, T. & Herida, M. (2014). Current Zika virus epidemiology and recent epidemics. *Mᵎdecine et Maladies Infectieuses* **44**: 302-307. https://doi.org/10.1016/j.medmal.2014.04.008

Katzelnick, L.C. & Harris, E. (2018). The use of longitudinal cohorts for studies of dengue viral pathogenesis and protection. *Current Opinion in Virology* **29**: 51-61. https://doi.org/10.1016/j.coviro.2018.03.004

Khoo, H.Y., Lee, H.Y., Khor, C.S., Tan, K.K., Bin Hassan, M.R., Wong, C.M., Agustar, H.K., Samsusah, N.A., Rahim, S., Bin Jeffree, M.S. *et al.* (2022). Seroprevalence of Zika Virus among forest fringe communities in Peninsular Malaysia and Sabah: general population-based study. *American Journal of Tropical Medicine and Hygiene* **107**: 560-568. https://doi.org/10.4269/ajtmh.21-0988

Khor, C.S., Mohd-Rahim, N.F., Hassan, H., Tan, K.K., Zainal, N., Teoh, B.T., Sam, S.S., Khoo, J.J., Lee, H.Y., Lim, Y.A. *et al.* (2020). Serological evidence of DENV, JEV, and ZIKV among the indigenous people (Orang Asli) of Peninsular Malaysia. *Journal of Medical Virology* **92**: 956-962. https://doi.org/10.1002/jmv.25649

Kumar, K., Arshad, S.S., Selvarajah, G.T., Abu, J., Toung, O.P., Abba, Y., Yasmin, A.R., Bande, F., Sharma, R. & Ong, B.L. (2018). Japanese encephalitis in Malaysia: An overview and timeline. *Acta Tropica* **185**: 219-229.

Liu, W., Clemens, J.D., Kari, K. & Xu, Z.Y. (2008). Cost-effectiveness of Japanese encephalitis (JE) immunization in Bali, Indonesia. *Vaccine* **26**: 4456-4460. https://doi.org/10.1016/j.vaccine.2008.06.050

Ma'roef, C.N., Dhenni, R., Megawati, D., Fadhilah, A., Lucanus, A., Artika, I.M., Masyeni, S., Lestarini, A., Sari, K., Suryana, K. *et al.* (2020). Japanese encephalitis virus infection in non-encephalitic acute febrile illness patients. *PLoS Neglected Tropical Diseases* **14**: e0008454. https://doi.org/10.1371/journal.pntd.0008454

Mayi, M.P.A., Foncha, D.F., Kowo, C., Tchuinkam, T., Brisco, K., Anong, D.N., Ravinder, S. & Cornel, A.J. (2019). Impact of deforestation on the abundance, diversity, and richness of Culex mosquitoes in a southwest Cameroon tropical rainforest. *Journal of Vector Ecology* **44**: 271-281. https://doi.org/10.1111/jvec.12359

Ministry of Finance Malaysia. (2011). Estimated Federal Expenditure (2012). https://www.mof.gov.my/portal/arkib/expenditure/2012/ap2.pdf. Assessed 12 July 2023.

Ministry of Health Malaysia. (2019). *Situasi Semasa Japanese Encephalitis (JE) Di Malaysia: 17 July 2019.* https://www.moh.gov.my/index.php/database_stores/attach_download/337/1198. Assessed 06 July 2023.

Ministry of Health Malaysia. (2023). Situasi Semasa Demam Denggi Di Malaysia Tahun 2022. https://www.moh.gov.my/index.php/database_stores/store_view_page/17/2259. Assessed 06 July 2023.

Ministry of Rural and Regional Development Malaysia. (2014). Bilangan kampung dan penduduk Orang Asli mengikut negeri, 2014. http://www.data.gov.my/data/ms_MY/dataset/kampung dan penduduk orang asli mengikut negeri 2014. Assessed 06 July 2023.

Moro, M.L., Gagliotti, C., Silvi, G., Angelini, R., Sambri, V., Rezza, G., Massimiliani, E., Mattivi, A., Grilli, E., Finarelli, A.C. *et al.* (2010). Chikungunya virus in North-Eastern Italy: a seroprevalence survey. *American Journal of Tropical Medicine and Hygiene* **82**: 508-511. https://doi.org/10.4269/ajtmh.2010.09-0322

Muhammad Azami, N.A., Salleh, S.A., Neoh, H.M., Syed Zakaria, S.Z. & Jamal, R. (2011). Dengue epidemic in Malaysia: Not a predominantly urban disease anymore. *BMC Research Notes* **4**: 216. https://doi.org/10.1186/1756-0500-4-216

Nealon, J., Taurel, A.F., Yoksan, S., Moureau, A., Bonaparte, M., Quang, L.C., Capeding, M.R., Prayitno, A., Hadinegoro, S.R., Chansinghakul, D. *et al.* (2019). Serological evidence of Japanese Encephalitis virus circulation in Asian children from dengue-endemic countries. *The Journal of Infectious Diseases* **219**: 375-381. https://doi.org/10.1093/infdis/jiy513

Ng, R.J., Chong, Z.L., Abdul Mutalip, M.H. & Ng, C.W. (2022). Dengue seroprevalence and factors associated with dengue seropositivity in Petaling District, Malaysia. *International Journal of Environmental Research and Public Health* **19**: 7170. https://doi.org/10.3390/ijerph19127170

Ngwe Tun, M.M., Mori, D., Sabri, S.B., Kugan, O., Shaharom, S.B., John, J., Soe, A. M., Nwe, K.M., Dony, J.F., Inoue, S. *et al.* (2021). Serological evidence of Zika virus infection in febrile patients and healthy blood donors in Sabah, Malaysian Borneo, 2017-2018. *American Journal of Tropical Medicine and Hygiene* **106**: 601-606. https://doi.org/10.4269/ajtmh.21-0802

Sabri, S.N.A.S.M. (2015). Municipal solid waste management of indigenous community in Kampung Kuala Pangsun, Hulu Langat, Selangor. University of Malaya (Malaysia). https://studentsrepo.um.edu.my/id/eprint/6522

Saleeza, S.N., Norma-Rashid, Y. & Azirun, M.S. (2013). Mosquito species and outdoor breeding places in residential areas in Malaysia. *The Southeast Asian Journal of Tropical Medicine and Public Health* **44**: 963-969. https://www.ncbi.nlm.nih.gov/pubmed/24450233

Saleeza, S.N.R., Norma-Rashid, Y. & Sofian–Azirun, M. (2011). Mosquitoes larval breeding habitat in urban and suburban areas, Peninsular Malaysia. *International Journal of Bioengineering and Life Sciences* **5**: 599-603.

Schrauf, S., Tschismarov, R., Tauber, E. & Ramsauer, K. (2020). Current efforts in the development of vaccines for the prevention of Zika and Chikungunya virus infections. *Frontiers in Immunology* **11**: 592. https://doi.org/10.3389/fimmu.2020.00592

Shim, E. (2017). Cost-effectiveness of dengue vaccination in Yucatan, Mexico using a dynamic dengue transmission model. *PLoS One* **12**: e0175020. https://doi.org/10.1371/journal.pone.0175020

Sohn, Y.M. (2000). Japanese encephalitis immunization in South Korea: past, present, and future. *Emerging Infectious Diseases* **6**: 17-24.

Takeda (Takeda Pharmaceutical Company Limited). (2022). Takeda's QDENGA® (Dengue Tetravalent Vaccine [Live, Attenuated]) Approved for Use in European Union. https://www.takeda.com/newsroom/newsreleases/2022/takedas-qdenga-dengue-tetravalent-vaccine-live-attenuated-approved-for-use-in-european-union/. Assessed 11 July 2023.

Tay, S.T., Mohamed Zan, H.A., Lim, Y.A. & Ngui, R. (2013). Antibody prevalence and factors associated with exposure to Orientia tsutsugamushi in different aboriginal subgroups in West Malaysia. *PLoS Neglected Tropical Diseases* **7**: e2341. https://doi.org/10.1371/journal.pntd.0002341

USFDA (US Food Drug Administration). (2019). Package Insert and Patient Information – Ixiaro. https://www.fda.gov/media/75777/download?attachment. Assessed 17 November 2023.

Vongpunsawad, S., Intharasongkroh, D., Thongmee, T. & Poovorawan, Y. (2017). Seroprevalence of antibodies to dengue and chikungunya viruses in Thailand. *PLoS One* **12**: e0180560. https://doi.org/10.1371/journal.pone.0180560

Vythilingam, I., Oda, K., Mahadevan, S., Abdullah, G., Thim, C.S., Hong, C.C., Vijayamalar, B., Sinniah, M. & Igarashi, A. (1997). Abundance, parity, and Japanese encephalitis virus infection of mosquitoes (Diptera:Culicidae) in Sepang District, Malaysia. *Journal of Medical Entomology* **34**: 257-262. https://doi.org/10.1093/jmedent/34.3.257

World Health Organization (WHO). (2009). Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva: World Health Organization. https://www.who.int/publications/i/item/9789241547871

Woon, Y.L., Lim, M.F., Tg Abd Rashid, T.R., Thayan, R., Chidambaram, S.K., Syed Abdul Rahim, S.S., Mudin, R.N. & Sivasampu, S. (2019). Zika virus infection in Malaysia: an epidemiological, clinical and virological analysis. *BMC Infectious Diseases* **19**: 152. https://doi.org/10.1186/s12879-019-3786-9

Yang, S.E., Pan, M.J., Tseng, H.F. & Liau, M.Y. (2006). The efficacy of mouse-brain inactivated Nakayama strain Japanese encephalitis vaccine – results from 30 years experience in Taiwan. *Vaccine* **24**: 2669-2673. https://doi.org/10.1016/j.vaccine.2005.10.054

Zeng, Z., Zhan, J., Chen, L., Chen, H. & Cheng, S. (2021). Global, regional, and national dengue burden from 1990 to 2017: A systematic analysis based on the global burden of disease study 2017. *EClinicalMedicine* **32**: 100712. https://doi.org/10.1016/j.eclinm.2020.100712