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Original Article

Community prevalence and distribution of dengue virus serotype based on antibody neutralization assay in Jakarta, Indonesia

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Abstract

Background Dengue infection is still rising globally despite the implementation of preventive efforts in many endemic countries. Monitoring the circulation of dengue virus (DENV) serotypes is not performed routinely in the Indonesian national surveillance program, primarily due to high cost and effort.

Objective To evaluate the distribution of DENV serotypes based on serological profile and neutralizing antibody level against all four DENV serotypes in Jakarta, Indonesia.

Methods This cross-sectional study was performed as part of a dengue vaccine effectiveness study, 10 years after a dengue vaccination program was initiated. It was conducted in five community public health centers in Jakarta in subjects aged 12 years and above who had not received the dengue vaccine. We collected serum samples and DENV neutralizing antibody titers were measured using a plaque reduction neutralization test (PRNT).

Results Eighty healthy subjects with a median age of 15 (range 12-27) years were enrolled. The highest median antibody titer was that to DENV-2 [898 (range 29-91558) 1/dil], followed by that to DENV-3 [297.5 (range 10-36091) 1/dil], DENV-1 [288 (range 0-68237) 1/dil], and DENV-4 [164 (range 0-35812) 1/dil]. Neutralizing antibodies against the four DENV serotypes were found in all the 5 districts studied in Jakarta. A multitypic neutralizing antibody profile was observed in the majority (74/80 subjects; 92.5%). Three subjects were naïve.

Conclusion All four dengue serotypes are widely circulating in Jakarta based on neutralizing antibody detection in the community, with the highest neutralizing antibody titer being against DENV-2, followed by DENV-3, DENV-1 and DENV-4. **[Paediatr Indones. 2024;65:; DOI: https://doi.org/10.14238/pi65.2.2024.10-6**].

Keywords: *dengue; neutralizing antibody; serotype; seroprevalence; PRNT, Jakarta, Indonesia*

Dengue infection is arguably the most important mosquito-borne viral disease in tropical and subtropical countries. The global burden of dengue has been rising for over 60 years and affects populations in over half the world.[1] Dengue virus (DENV) consists of four serotypes, which can be transmitted from human to human by several species of the Aedes genus of mosquitos. The primary vector of the dengue virus is the species *Aedes aegypti*, which dwells in tropical and subtropical regions worldwide.[2] Any of the four virus serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) can induce specific, but not cross-protective long-term immunity and all may cause severe dengue.[3]

According to the *World Health Organization* (WHO) 2021 report, the number of dengue cases in the last two decades has increased eight-fold globally, from 505,430 cases in 2000, to 2.4 million

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cases in 2010 and 5.2 million cases in 2019. The global burden was estimated at 3.9 billion people are at risk of infection with dengue viruses , of which 96 million were clinical dengue, in 128 countries. Dengue infection is a leading cause of pediatric morbidity and mortality in some Southeast Asian countries.[4] The WHO reported that the highest dengue incidence was in Brazil, followed by Indonesia.[5]

Growth of the human population, urbanization, modern transportation, and increases in the range and density of mosquitoes spurred on by global warming have led to the increased incidence of dengue worldwide, despite prevention efforts in vector control, intervention with Wolbachia bacteria, and the approval of the dengue vaccine in several countries.[6] Epidemiological studies in endemic areas are needed to determine changes in DENV serotype circulation in endemic countries. Indonesia is a highly endemic country with all dengue serotypes, with DENV-2 was reported to be the predominant serotype in a limited number of studies.[7-10] We aimed to identify the circulation of DENV serotypes in 5 areas of Jakarta to provide data for further development of vaccines and diagnostic approaches.

Methods

This cross-sectional study was performed to measure the prevalence and titers of serotype-specific neutralizing antibodies against the four DENV serotypes using an in-house plaque reduction neutralization test (PRNT). This undertaking was part of a study on the effectiveness of the dengue vaccine 10 years after vaccination was conducted in five district public health centers (PHC), each district representing Central, East, North, West, and South Jakarta, namely Senen, Jatinegara, Koja, Tambora, and Pasar Minggu in the Jakarta Special Capital Region, Indonesia. Healthy subjects aged 12-27 years were enrolled from June to December 2022.

The inclusion criteria were healthy subjects aged 12-27 years from the communities served by the five district PHCs who were willing to participate and/or whose parents provided informed consent. Exclusion criteria were those who had received the dengue vaccine, suffered from an acute infection within seven days of study recruitment, had been diagnosed with immune system disorders such as human immunodeficiency virus (HIV) or primary immunodeficiency, or had received long-term corticosteroid treatment (more than two weeks). We also excluded people whose addresses could not be located and those who had moved from the area.

Consecutive sampling was performed to recruit subjects. Blood specimens (3-5 mL) were drawn using venipuncture; sera were separated and frozen at -80°C before tested using the *PRNT50* assay. Neutralizing antibody data were used to describe the historical dengue serotype circulation and their distribution according to age and PHC location. Retrospective clinical, nutritional status, and demographic data were recorded to support the analysis.

Dengue plaque reduction neutralization test (*PRNT50*) was used to identify and measure the neutralizing antibodies. The assay was performed based on optimized and validated *PRNT50* assay for the detection of neutralizing antibodies to four serotypes of DENV.[11] Briefly, serum samples were heat-inactivated at 56°C and tested separately for each DENV serotype in four different PRNT runs. Vero cells (*CCL-81* from ATCC) were grown in minimum essential medium (MEM) (*Gibco-Thermo Fisher Scientific*, CA, USA), supplemented with 5% heat-inactivated fetal bovine serum (FBS) (*Gibco-Thermo Fisher Scientific*, CA, USA), 2 mM L-glutamine, and 1% antibiotic/antimycotic (*Gibco-Thermo Fisher Scientific*, CA, USA) at 37°C with 5% CO_2. The challenge viruses were as described previously.[7] For virus detection, mouse monoclonal flavivirus group antibody D1-4G2-4-15(4G2) (*GeneTex*, Irvine, CA, USA) was used. Alkaline phosphatase-conjugated goat anti-mouse IgG (*Jakcson Immunoresearch Laboratories*, West Grove, PA, USA) was used as the secondary antibody. Positive and negative dengue antibody serum controls from healthy adult donors in Indonesia were included in each assay to ensure the accuracy and validity of the tests.

The neutralization titer (*PRNT50*) of the test serum sample was determined to be the reciprocal of the highest serum dilution at which virus infectivity was reduced by 50% compared to the average plaque count of the challenge virus control. This was calculated using a four-point linear regression method. Since the initial serum dilution in the assay was 1:5, the theoretical lower limit of quantitation

for the assay was a titer of 10 (reciprocal dilution). As recommended by the WHO; a cut-off of >10 1/dil was considered to be positive.[11]

Descriptive statistics were used to summarize age, district, and neutralizing antibody titers. Chi-square, Fisher's exact, and non-parametric tests were performed using *SPSS version 26* (*IBM*, Armonk, New York, USA). Dengue serotype-specific PRNT profiles were categorized as naïve (no previous infection), monotypic (infection with one serotype), or multitypic (infection with multiple serotypes). The study was approved by the Health Research Ethics Committee of the Faculty of Medicine Universitas Indonesia and the Jakarta Special Capital Region Health Service.

Results

Eighty healthy subjects from five district PHC areas agreed to participate in the study. The median age of subjects was 15 (range 12-27) years. There were more male subjects, with a male-to-female ratio of 42:38. Subjects were distributed across the five districts as follows: Senen (16 subjects), Jatinegara (16 subjects), Tambora (16 subjects), Koja (16 subjects), and Pasar Minggu (16 subjects). The majority of subjects (64/80; 82%) had good nutritional status.

Dengue serotype-specific neutralizing antibody (nAb) titers were obtained from all 80 subjects. *PRNT50* detected all four DENV-serotype specific nAb in most subjects. Seventy-four (92.5%) subjects had nAb against more than one serotype (multitypic) (**Figure 1**). Three subjects had a monotypic nAb profile: two individuals had nAb against DENV-3 and one against DENV-4. Three individuals were nAb-naïve; one was aged 14 years, and two were aged 18 years.

Next, we mapped the distribution of neutralizing antibody prevalence against four DENV serotypes based on PRNT data according to the residential address of the subjects in the five districts in Jakarta province. As shown in **Table 1**, all four DENV serotypes were circulating in all five districts in Jakarta, with similar proportions of each serotype observed in all five districts.

Overall, the DENV-2 serotype significantly induced the highest titers of nAb in the population, while DENV-4 induced the lowest titer (**Figure 2A** and **Table 2**). To assess whether subjects' age impacted the immunological response against DENV infection, we stratified the nAb titers by age group of 12-17 years (adolescence) and 18 years and above (adults). As shown in **Figure 2B-E** and **Table 2**, there were no significant differences in DENV serotype distribution between the two age groups. Statistical analyses showed that there was no significant difference when neutralization antibody titers were stratified by gender, PHC sites, and nutritional status (data not shown).

We also analyzed the nAb titers in subjects' serum specimens. Serotype-specific nAb responses varied among the population in the districts, as

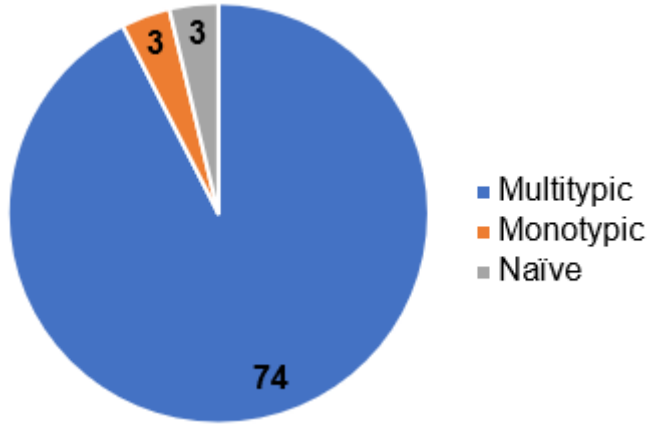

Figure 1. Dengue neutralizing antibody profile in 80 healthy individuals aged 12-27 years in Jakarta. Dengue serotype-specific PRNT profiles were categorized as naïve (no previous infection), monotypic (infection with one serotype), or multitypic (infection with multiple serotypes).

Table 1. Distribution of serotype-specific dengue neutralizing antibody in five districts in Jakarta province (N=80)

Districts	Number of subjects based on serotype-specific dengue neutralizing antibody
Central Jakarta (Senen), n(%)	
DENV-1	15 (23.4)
DENV-2	15 (23.4)
DENV-3	15 (23.4)
DENV-4	15 (23.4)
East Jakarta (Jatinegara), n(%)	
DENV-1	15 (23.4)
DENV-2	15 (23.4)
DENV-3	16 (25.0)
DENV-4	15 (23.4)
West Jakarta (Tambora), n(%)	
DENV-1	15 (23.4)
DENV-2	15 (23.4)
DENV-3	14 (21.9)
DENV-4	15 (23.4)
North Jakarta (Koja), n(%)	
DENV-1	12 (18.8)
DENV-2	12 (18.8)
DENV-3	14 (21.9)
DENV-4	14 (21.9)
South Jakarta (Pasar Minggu), n(%)	
DENV-1	14 (21.9)
DENV-2	15 (23.4)
DENV-3	15 (23.4)
DENV-4	12 (18.8)

There were 16 subjects in each district who underwent antibody assessment for the 4 serotypes, hence we had 16x4=64 as denumerator. One subject might be negative to certain serotype.

shown in **Figure 3**. Notably, across all districts, DENV-2 exhibited the highest PRNT titers, with most pronounced ones found at Senen, where median DENV-2 titer was 1,400 1/dil. This was significantly higher than the other serotypes within this district, as DENV-1, DENV-3, and DENV-4 had much lower titers. Similar phenomena were observed in other districts where consistently higher antibody titer was induced by DENV-2 compared to those caused by DENV-1, DENV-3 and DENV-4, respectively.

Discussion

This study provides an updated assessment of the circulating DENV serotypes based on serological prevalence in 5 PHC areas in Jakarta, Indonesia. A previous dengue seroprevalence study was conducted in 14 provinces in Indonesia including Jakarta in 2014,[9] therefore, this study provides the most current seroprevalence data. Our findings revealed that all four DENV serotypes are currently circulating, with DENV-2 predominating. The highest titer of serotype-specific dengue neutralizing antibodies was found against DENV-2 in the 80 healthy subjects who resided in five districts in Jakarta, although the prevalence of seropositive subjects was slightly higher towards DENV-3 than the other serotypes (**Table 1**). The highest titer of neutralizing antibody against DENV-2 was consistent with a previous finding of dengue seroprevalence in Indonesia.[7] In previous studies in Indonesia, DENV-2 was reported as the predominant dengue serotype associated with both mild and severe dengue cases.[7,9,10] Other studies showed a trend that the predominant serotype tends to vacillate between DENV-2 and DENV-3.[12-14]

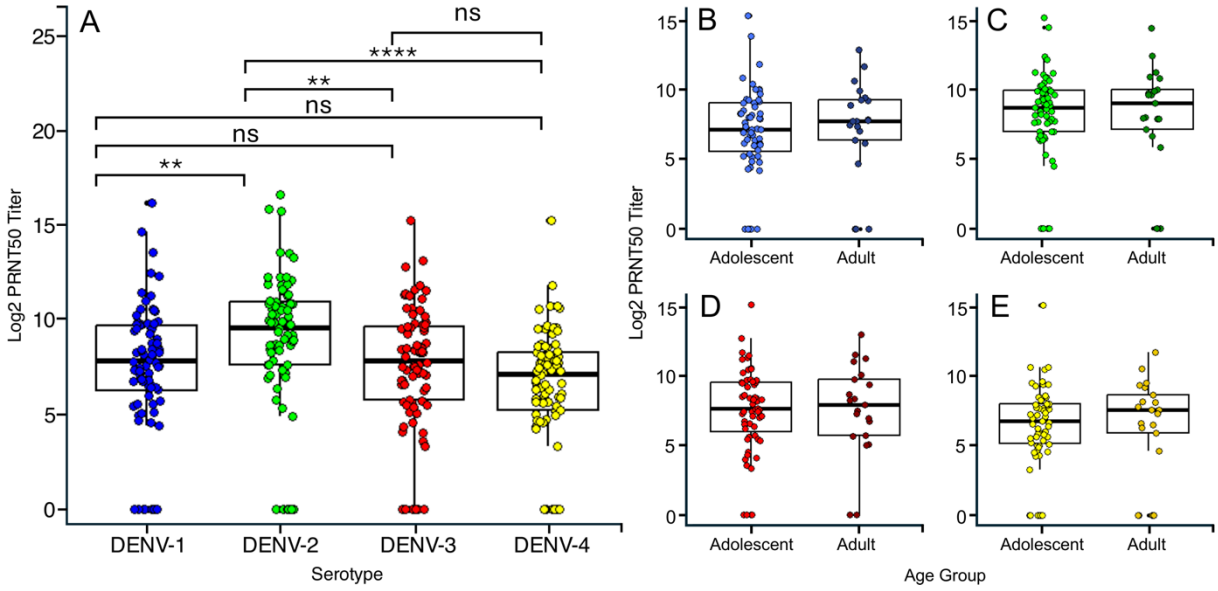

Figure 2. (A) Dengue neutralizing antibody titers according to serotype. The overall titers of nAb in healthy subjects (n=80) resided in five districts in Jakarta. The nAb titers in adolescents and adults according to serotype: (B) DENV-1 (ns), (C) DENV-2 (ns), (D) DENV-3 (ns), and (E) DENV-4 (ns). Statistical significance was determined by non-parametric Kruskal-Wallis test; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns: not significant

Table 2. Titers of DENV neutralizing antibodies measured using PRNT50 according to age group

Serotype	Median (range) of dengue nAb geometric mean titer (GMT) 1/dil			
	Overall (n=80)	12-17 years (n=59)	>18 years (n=21)	P value
DENV-1	288 (0-68,237)	182 (0-68,237)	281 (0-11,449)	0.447
DENV-2	898 (29-91,558)	703 (0-91,558)	882(0-50,965)	0.789
DENV-3	297.5 (10-36,091)	205 (0-36,091)	246 (10-8,304)	0.818
DENV-4	164 (0-35,812)	109 (0-35,812)	191 (0-3,446)	0.515

GMT=geometric mean titer

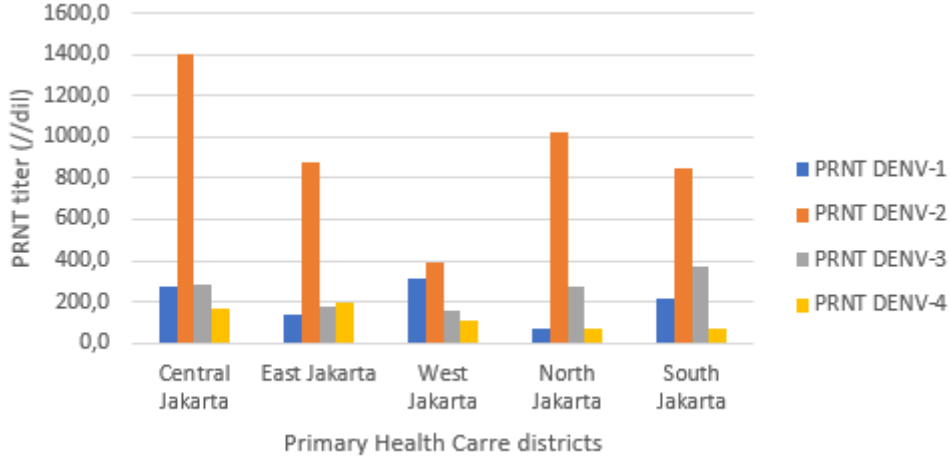

Figure 3. Mean titers of serotype-specific dengue nAb against four DENV serotypes in the five districts in Jakarta

The fact that multitypic neutralizing antibodies were identified in most of the study population indicates that a majority of individuals had been exposed to multiple DENV serotypes over time. This is consistent with the endemic prevalence of dengue in Indonesia, where repeated exposure to different serotypes is common.[7,9] The high prevalence of multitypic antibody profiles (92.5%) in this study suggests substantial prior exposure to dengue virus in the population, which is concerning, as secondary infections with a different serotype are a known risk factor for severe dengue.[14]

Interestingly, the lowest neutralizing antibody titer detected was against DENV-4, which may have been due to a lower prevalence of this serotype or due to a potentially less robust immune response in the population.[8,10] This finding could have implications for vaccine development and public health strategies, as the different circulation of DENV serotypes could affect the efficacy of dengue vaccines designed to protect against all four DENV serotypes.[15,16]

The identification of all four DENV serotypes in all the five districts re-emphasises the need for continuous and comprehensive serotype-specific surveillance to monitor changes in dengue virus circulation. Such data are crucial for anticipating potential outbreaks and for adjusting vector control strategies and dengue vaccine implementation accordingly.[17,18]

The prevalence of multitypic profiles in the population implies that future vaccine strategies in this region may need to prioritize formulations that are effective across all four DENV serotypes, most especially against DENV-2.[6] Additionally, public health measures should consider the risk of severe dengue due to secondary dengue infection, which underscores the importance of early detection and prompt treatment.[5,19,20] These findings also highlight the importance of maintaining serotype-specific surveillance for dengue viruses, vector control, and the search for an effective vaccine that confers immunity to all dengue serotypes.[21,22] Further studies should focus on understanding the clinical implications of these serotype distributions and on evaluating the long-term efficacy of current and emerging dengue vaccines in this population.

Our findings confirmed that all four DENV serotypes are circulating in Jakarta, with highest neutralizing antibody titer detected against DENV-2, followed by DENV-3, DENV-1 and DENV-4. These findings bring to attention the need for continuous monitoring to manage and control dengue transmission, help in refining vaccine strategies, and prepare for potential outbreaks.

Conflict of interest

None declared.

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References

1. Messina JP, Brady OJ, Golding N, Kraemer MUG, Wint GRW, Ray SE, *et al*. The current and future global distribution and population at risk of dengue. Nat Microbiol. 2019;4:1508-15. DOI: https://doi.org/10.1038/s41564-019-0476-8
2. Carrington LB, Simmons CP. Human to mosquito transmission of dengue viruses. Front Immunol. 2014;5:290. DOI: https://doi.org/10.3389/fimmu.2014.00290
3. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, *et al*. Dengue: a continuing global threat. Nat Rev Microbiol. 2010;8:S7-16. DOI:https://doi.org/10.1038/nrmicro2460
4. Bhatt S, Gething PQ. Brady OF, Messina JP, Farlow AW, Moyes CL, *et al*. The global distribution and burden of dengue. Nature. 2013;496:504-7. DOI: https://doi.org/10.1038/nature12060
5. World Health Organization. Global strategy for dengue prevention and control 2012-2020. 2nd edition. Geneva: World Health Organization; 2012.
6. Wilder-Smith A, Ooi EE, Vasudevan SG, Gubler DJ. Update

on dengue: epidemiology, virus evolution, antiviral drugs, and vaccine development. Curr Infect Dis Rep. 2010;12:157-64. DOI: https://doi.org/10.1007/s11908-010-0102-7

7. Sasmono RT, Taurel AF, Prayitno A, Sitompul H, Yohan B, Hayati RF, *et al.* Dengue virus serotype distribution based on serological evidence in pediatric urban population in Indonesia. PLoS Negl Trop Dis. 2018;12:e0006616-26. DOI: https://doi.org/10.1371/journal.pntd.0006616
8. Prayitno A, Taurel AF, Nealon J, Satari HI, Karyanti MR, Sekartini R, *et al.* Dengue seroprevalence and force of primary infection in a representative population of urban dwelling Indonesian children. PLoS Negl Trop Dis. 2017;11:e0005621-37. DOI: https://doi.org/10.1371/journal.pntd.0005621
9. Sasmono RT, Santoso MS, Pamai YWB, Yohan B, Afida AM, Denis D, *et al.* Distinct dengue disease epidemiology, clinical, and diagnosis features in western, central, and eastern regions of Indonesia, 2017-2019. Front Med. 2020;7:582235. DOI: https://doi.org/10.3389/fmed.2020.582235
10. Dewi BE, Nainggolan L, Sudiro TM, Chenderawasi S, Goentoro PL, Sjatha F. Circulation of various dengue serotypes in a community-based study in Jakarta, Indonesia. Jpn J Infect Dis. 2021;74:17-22. DOI: https://doi.org/10.7883/yoken.JJID.2019.431
11. Timiryasova TM, Bonaparte MI, Luo P, Zedar R, Hu BT, Hildreth SW. Optimization and validation of a plaque reduction neutralization test for the detection of neutralizing antibodies to four serotypes of dengue virus used in support of dengue vaccine development. Am J Trop Med Hyg. 2013;88:962-70. DOI: https://doi.org/10.4269/ajtmh.12-0461
12. Lardo S, Utami Y, Yohan B, Tarigan SM, Santoso WD, Nainggolan L, *et al.* Concurrent infections of dengue viruses serotype 2 and 3 in patient with severe dengue from Jakarta, Indonesia. Asian Pac J Trop Med. 2016;9:134-40. DOI: https://doi.org/10.1016/j.apjtm.2016.01.013
13. Seto D, Budianti A, Dewi BE, Cucunawangsih, Chandra R, Chaidir J, *et al.* Association between dengue virus serotypes and type of viral infection in Department of Child Health, Cipto Mangunkusumo hospital, Jakarta, Indonesia. Dengue Bulletin. 2011;35:206-14. https://www.researchgate.net/publication/235513485_Association_between_dengue_virus_serotypes_and_type_of_dengue_viral_infection_in_Department_of_Child_Health_Cipto_Mangunkusumo_Hospital_Jakarta_Indonesia#fullTextFileContent
14. Harapan H, Michie A, Yohan B, Shu PY, Mudatsir M, Sasmono RT, *et al.* Dengue viruses circulating in Indonesia: a systematic review and phylogenetic analysis of data from five decades. Rev Med Virol. 2019;29:e2037. DOI: https://doi.org/10.1002/rmv.2037
15. Coller B, Clements D. Dengue vaccines: progress and challenges. Curr Opin Immunol. 2011;23:391-8. DOI: https://doi.org/10.1016/j.coi.2011.03.005
16. Hou J, Ye W, Chen J. Current development and challenges of tetravalent live-attenuated dengue vaccines. Front Immunol. 2022;13:840104. DOI: https://doi.org/10.3389/fimmu.2022.840104
17. Ramadona AL, Lazuardi L, Hii YL, Holmner Å, Kusnanto H, Rocklöv J. Prediction of dengue outbreaks based on disease surveillance and meteorological data. PLOS ONE. 2016;11: e0152688 DOI: https://doi.org/10.1371/journal.pone.0152688
18. Kusriastuti R, Sutomo S. Evolution of dengue prevention and control programme in Indonesia. Dengue Bull. 2005;29:1-7. https://iris.who.int/bitstream/handle/10665/164013/dbv29p1.pdf
19. World Health Organization. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever, revised and exopanded. 2nd ed. India: WHO, Regional Office for South-East Asia; 2011. https://iris.who.int/bitstream/handle/10665/204894/B4751.pdf?sequence=1&isAllowed=y
20. Cavallier P, Tarantola A, Leo Y, Lover A, Rachline A, Duch M, *et al.* Early diagnosis of dengue disease severity in a resource-limited Asian country. BMC Infect Dis. 2016;16:512-9. DOI: https://doi.org/10.1186/s12879-016-1849-8
21. Wilder-Smith A. Dengue vaccine development: status and future. Bundesgesundheitsbl. 2020;63:40-4. DOI: https://doi.org/10.1007/s00103-019-03060-3
22. Plennevaux E, Sabchareon A, Limkittikul K, Chanthavanich P, Sirivichayakul C, Moureau A, et al. Detection of dengue cases by serological testing in a dengue vaccine efficacy trial: utility for efficacy evaluation and impact of future vaccine introduction. Vaccine. 2016;34:2707-12. DOI: https://doi.org/10.1016/j.vaccine.2016.04.028