Satoto *et al. Kesmas: National Public Health Journal*. 2019; 13 (4): 189-194
DOI:10.21109/kesmas.v13i4.2709

Kesmas: National Public Health Journal

Occurrence of Natural Vertical Transmission of "Zika like Virus" in *Aedes aegypti* Mosquito in Jambi City

Transmisi Vertikal Alami Virus "Menyerupai" Zika dalam Nyamuk *Aedes Aegypti* di Kota Jambi

Tri Baskoro T Satoto*, Nur Alvira Pascawati, Wida Purwaningsih***, Hari Kusnanto Josef****, Purwono*****, Rumbiwati*****, Hermanto******, Sofia Mubarika*******, Roger Frutos**********

***Center for Tropical Medicine, Faculty of Medicine Universitas Gadjah Mada, Yogyakarta, Indonesia **Public Health Study Program, Faculty of Health Science, Universitas Respati, Yogyakarta, Indonesia ***Port Health Quarantine of Jambi Province, Indonesia ****Department of Public Health, Faculty of Medicine Universitas Gadjah Mada, Yogyakarta, Indonesia *****Department of Parasitology, Faculty of Medicine Universitas Gadjah Mada, Yogyakarta, Indonesia ******Health Polytechnic Jambi Province, Indonesia *******Department Histology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, ********University of Montpellier, France**

Abstract

Zika virus can be transmitted through mosquitoes such as *Aedes aegypti* and *Ae. albopictus.* During the transition period of 2014–2015, an outbreak of dengue was reported in Jambi City, during which several sufferers were screened positive for Zika virus infection by the Eijkman Institute. It was interesting to note that all of those positive for Zika virus infection were indigenous residents and none of them had a history of international travel. This descriptive analytic study with a cross-sectional design study was conducted to present an overview of *Aedes spp*. population using ovitrap and egg colonization methods and to detect the presence of Zika virus. Samples were analyzed using reverse transcription polymerase chain reaction for detection of Zika "like" virus and the mapping results were described. The Ovitrap Index was 44.74%, and examination of egg colonization collected from 40 neighborhoods revealed the presence of Zika "like" virus in samples obtained from the fourth neighborhood in Talang Bakung village. This result indicates the occurrence of natural vertical transmission of Zika "like" virus in *A*. aegypti mosquito in Jambi City, which potentially resulted in an outbreak.

Keywords: Transmission, vertical, Zika, *Aedes aegypti*

Abstrak

Virus Zika ditularkan oleh Nyamuk *Aedes aegypti* dan *Aedes albopictus.* Pada tahun 2014 sampai 2015 dilaporkan terjadi wabah demam berdarah di Kota Jambi, dimana beberapa penderita dinyatakan positif infeksi virus Zika dari hasil skrining lembaga Eijkman. Hal yang menarik adalah semua penderita yang positif virus Zika adalah penduduk lokal yang tidak pernah memiliki riwayat melakukan perjalanan international. Tujuan penelitian ini membuktikan keberadaan virus Zika dari nyamuk *Aedes spp.* melalui ovitrap dan kolonisasi telur yang berasal dari Kota Jambi. Penelitian ini merupakan penelitian deskriptif-analitik dengan desain *cross-sectional*. Sampel yang dikumpulkan dilakukan deteksi virus 'seperti' Zika menggunakan RT-PCR dan hasilnya digambarkan dalam bentuk pemetaan. Ovitrap Indeks sebesar 44,74% dan hasil pemeriksaan kolonisasi telur yang dikumpulkan dari 40 lingkungan ditemukan virus 'seperti' Zika pada sampel dari lingkungan 4 di Desa Talang Bakung. Kondisi ini mengindikasikan bahwa telah terjadi penularan vertikal alami virus 'menyerupai' zika dalam nyamuk *Aedes aegypti* di Kota Jambi, sehingga berpotensi memunculkan wabah.

Kata kunci: Penularan, vertikal, Zika, *Aedes aegypti*

How to Cite: Satoto TBT, Pascawati NA, Purwaningsih W, Josef HK, Purwono, Rumbiwati, Hermanto, Mubarika S, Frutos R. Occurrence of natural vertical transmission of zika "like" virus in aedes aegypti mosquito in an outbreak in Jambi City. Kesmas: National Public Health Journal. 2019; 13(3): 144-149 (doi:10.21109/kesmas.v13i3.1989)

***Correspondence**: Nur Alvira, Public Health Studies Program, Faculty of Health Science, Respati Yogyakarta University, A Building, Jalan Raya Tajem KM 1,5, Sleman, Indonesia, Phone: +62-817-6616928, E-mail: irha011859@yahoo.com*
Received : October 24th 2018
Revised : February 4th 2019
Accepted : March 26th 2019

Copyright @ 2019, Kesmas: National Public Health Journal, p-ISSN: 1907-7505, e-ISSN: 2460-0601, Accreditation Number: 30/E/KPT/2018, http://journal.fkm.ui.ac.id/kesmas

Introduction

Zika virus is transmitted by *Aedes spp.* Mosquitoes, and the infection could spread among humans as well.[1] Zika virus has a single-stranded ribonucleic acid (RNA) and belongs to the family Flaviviridae and the genus Flavivirus.[2,3] Since 2007, Zika virus has been an endemic in African regions and has been spreading to Yap Island of the Micronesian Pacific region and the Asian and Southern Pacific regions.[1] In the year 2016, the distribution of Zika virus had reached Northern America, the Carribbean, and Samoa of Oceanian regions. The World Health Organization (WHO) declared Zika virus as a lethally distributed virus in the American continent where 3–4 million people were infected.[4,5]

In the city of Jambi, there was a decrease in the number of dengue cases during 2014–2015, although the Eijkman Institute reported Zika-virus-positive patients.[6] Of the 103 patient case samples with a dengue fever diagnosis, one sample of JMB/JAMBI-185 was detected in a 27-year-old man who was under medication at the Jambi City Hospital and was declared to be positive for Zika virus. The patient showed symptoms of sudden high fever, headache, elbow, arthralgia knee, myalgia, and malaise for 2 days 6. However, the symptoms experienced by one patient did not exhibit certain general clinical characteristics of Zika virus infection, including maculopapular rash, conjunctivitis, and peripheral edema.[7]

Indonesia is a country that belongs to category 2 with respect to the transmission or infection of Zika virus through local mosquitoes, whereas Malaysia belongs to category 3, wherein Zika virus infection was transmitted through local mosquitoes during or before 2015.[8] Jambi province has been included in the Indonesia–Malaysia–Singapore growth triangle (IMS-GT) and the Indonesia–Malaysia–Thailand growth triangle (IMT-GT). The inclusion of Jambi province into these growth areas led to an increase in activity and mobility of population from Jambi province to the countries located in these areas, thereby affecting the vector mobility either directly or indirectly.

Identifying the potential vectors of Zika virus and dengue virus or other mosquito-borne diseases in a particular region could have important implications for understanding the occurrence of a disease. The *Aedes aegypti* mosquito can transmit dengue and Zika viruses, although *Ae. albopictus* has also been suspected to be a vector for transmitting Zika virus 1. According to the study conducted by the Eijkman Institute in Jambi City, all of those positive for Zika virus infection were found to be indigenous residents and none of them had a history of international travel. Such an incidence may be due to local mosquito-borne Zika virus transmission in Jambi City.[6]

Based on this background, this study was conducted to detect the presence of Zika virus in *Aedes spp.* mosquitoes and to map its distribution in Jambi City. The objectives of this study were to detect the presence of vertical transmission of Zika virus in *Aedes spp*. and to present an overview of *Aedes spp.* population through ovitrap capture and egg colonization of *Aedes spp.* mosquitoes in Jambi City.

Method

This descriptive study with a cross-sectional study design was conducted in the village of Talang Bakung, Paal Merah Subdistrict, Jambi City. Secondary data, including documents in the form of basic data during the process of preparation of the study, and reponeighborhoods of dengue cases were collected at the Jambi Health Office.

Primary data were collected in the field through the installation of an ovitrap, by capturing adult mosquitoes at the study site, and via field observations. The collected mosquito eggs were then hatched in the laboratory into adult mosquitoes, and the presence of virus was detected in 3-day adult mosquitoes. The study sample included adult mosquitoes that were captured directly at the study site and those obtained by egg-rearing of *Aedes spp*. trapped in the ovitrap. Samples were obtained using 310 installed ovitraps and by capturing adult mosquitoes. The Ovitrap Index (OI) was calculated, and mosquito eggs were colonized. The coordinate points of sample collection areas were documented for mapping using global positioning system (GPS). Adult mosquitoes captured at the study site were defeated by giving the cotton dipped in chloroform solution and then placed in an Eppendorf tube consisting of 1.5 ml of RNA stabilization solution until the mosquitoes submerged. Samples were brought in styrofoam boxes filled with ice pack or ice gel to keep them cool.

Examination of Dengue and Zika virus in *Aedes spp.* samples came from egg colonization and *Aedes spp.* mosquito-catching results at 10 neighborhoods with dengue fever cases. Each pooling consisted of maximum 25 *Aedes spp.* mosquitoes. Ribonucleic acid (RNA) was isolated from colonized eggs of mosquitoes, and Den3 derived from the serum of patients with dengue hemorrhagic fever (DHF) at Dr. Sardjito Central General Hospital was used as positive control. Dengue and Zika viruses were examined using one-step reverse transcription polymerase chain reaction (RT-PCR) method to determine virus serotypes against *Aedes spp*. mosquitoes based on pooling results.[9,10] The RT-PCR method has high sensitivity in amplifying RNA templates and is highly specific when using specific primers in complementary deoxyribonucleic acid (cDNA) synthesis. This technique is also extremely useful for detecting gene expression, RNA amplification before cloning and analysis, and diagnosing infective agents as well as genetic diseases. Synthesis of cDNA by the RT-PCR method was performed in a reaction mixture contai-

ning highly specific primers and free nucleotides (dNTP) at a maximum temperature of 42°–50°C for a minimum of 60 min.[9] The enzyme reverse transcriptase is used in the RT-PCR method to assist in synthesizing RNA into DNA. After cDNA synthesis, one-step RT-PCR product amplification was performed by electrophoresis using 1.5% agarose. A disadvantage of RT-PCR is that it can be used only for DNA amplification and not for studying functional proteins.[10]

Field data and results of virus serotype identification of dengue and Zika viruses were descriptively analyzed and presented in the form of tables and graphs. The technique of RT-PCR used for determining the presence of dengue and Zika viruses in mosquitoes is based on the description of fragments (bands) in the appropriate base pair (bp) after electrophoresis. The band sizes expected from the expected amplification results are 486 bp for DEN1, 119 bp for DEN 2, 290 bp for DEN 3, 389 bp for DEN 4, and 192 bp for Zika virus. Clear bands and corresponding band sizes in comparison with marker bands were considered to be positive and documented. The coordinates of sampling sites were documented in the form of a map using GPS.

Results

Among the 310 ovitraps installed in the village of Talang Bakung, more than 101 houses (65.16%) in 40 Neighborhoods were positive for *Aedes spp.* eggs, whereas 54 houses in 6 Neighborhoods were negative for Aedes spp. eggs (34.84%), as described in Figure 1.

The OI value in the village of Talang Bakung was 153 ovitraps (44.74%), comprising 64 ovitraps (36.99%) and 89 ovitraps (52.66%) from both indoor and outdoor sources, respectively. An area with an OI value of >10% is categorized as a potential region for experiencing outbreaks,[11] and the population abundance of Aedes spp. can be determined by analyzing the density of the number of eggs per ovitrap.[12] Results revealed that Talang Bakung village is a potential area of having an outbreak according to study conducted at the Elementary School in Yogyakarta.[13]

According to the nearest neighbor analysis, the distribution of ovitrap installation in Talang Bakung village was spread in clusters or groups with a z-score of –7.848495 and a p value of 0.00 as shown in Figure 2.

Clustered distribution results in a more rapid transmission of the virus carried by *Ae. aegypti* mosquitoes, which implies that it can speed up the transmission of dengue virus carried by *Aedes spp.* mosquitoes. The nearest positive ovitrap distance was 1.8 m, and the farthest distance was 410.2 m.

Based on the ovitrap installation results in Talang Bakung village, two species of mosquitoes were obtained, including *Ae. aegypti* and *Ae. albopictus* (Table 1), and some mosquitoes were still in the form of instar larvae I, II, III, IV, and pupae. There are 12 ovistrip that are not hatched by the number of mosquito eggs as much as 524 grains (6.46%).

Among 2316 adult mosquitoes that were reared, a total of 1223 (52.81%) were *Ae. aegypti* obtained from outdoor [530 (22.88%)] and indoor [693 (29.92%)] sources. The remaining 1093 mosquitoes were *Ae. albopictus* obtained from outdoor [1000 (43.18%)] and in-

Figure 1. Dots Indicate the Distribution of Ovitrap Installation, and Numbers Indicate the Neighborhoods in the Village of Talang Bakung

Table 1. Results of Hatching of Mosquito Eggs in the Pooling Process in the Laboratory

Species	Ovitrap Installation				Total	
	Outdoor		Indoor			
	Amount of eggs	Percentage	Amount of eggs	Percentage	Amount of Eggs	Percentage
Aedes aegypti	530	22.88	693	29.92	1.223	52.8
Aedes albopictus	1.000	43.18	93	4.02	1.093	47.2
Total	1.530	66.06	786	33.94	2.316	100

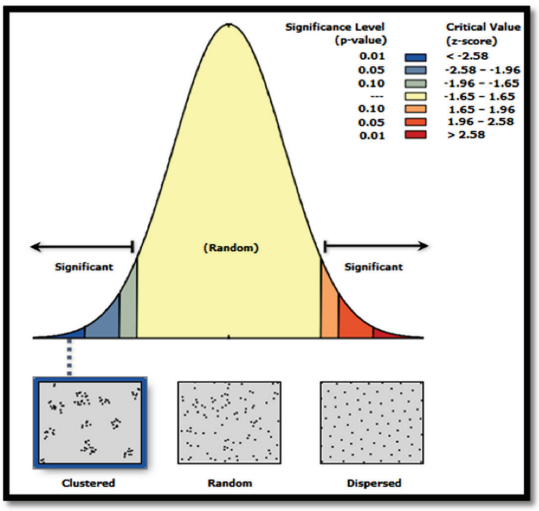


Figure 2. Distribution Pattern of Ovitraps in the Village of Talang Bakung

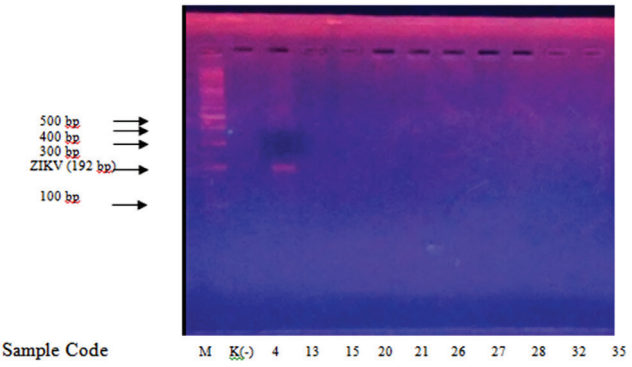
Figure 3. The Area Box Indicating the Vertical Transmission of Zika Virus in the Village of Talang Bakung

door [93 (4.02%)] sources.

Examination of dengue virus using the method described by Lanciotti *et al*,[14] demonstrated the absence of dengue virus in *Aedes spp.* mosquitoes in 10 neighborhoods, whereas examination of Zika virus using the RT-PCR method described by Balm method,[15] demonstrated that RT.4 was found to be positive for Zika virus in *Aedes spp.* mosquitoes (Figure 3).

The presence of Zika virus in RT.4 was discovered through egg colonization in 19 *Ae. aegypti* mosquitoes and in 6 tails of *Ae. albopictus.* In this study, 100 *Ae. aegypti* and 100 *Ae. albopictus* mosquitoes injected with Zika virus and fed with blood were used for the experiments.

There were 6 pooling of 69 pooling examined (consisting of 1738 first-generation *Ae. aegypti* F1 mosquitoes) using the immunofluorescence method obtained positive results containing Zika virus (Figure 4); however, with a 192 bp base pair, longer sequencing is needed to ensure that the virus found in *Aedes* mosquito is truly Zika virus. The results were found to be consistent with the study of Thangamani *et al*,[16] who reported that Zika virus



Note: M = Marker, K (–): Control Negative

Figure 4. PCR result from each RT and Zika Virus Detected in RT 4

can be transmitted vertically to *Ae. aegypti* mosquitoes and *Ae. albopictus* females.

Discussion

Results of this study indicate the high risk of transmission due to the widespread flying distance of female mosquitoes based on the results reported by Honório *et al*,[17] suggesting that females can fly at least 800 m in 6 days and, if infected, can potentially spread the virus rapidly. However, a study conducted in urban San Juan, Puerto Rico, demonstrated that female *Ae. aegypti* mosquitoes could travel at least 441 m from the releasing point within a few days.[18] Several studies mention that female *Ae. albopictus* (Skuse), which becomes another potential dengue vector, is also potential to transmit Zika virus.[19] Mosquito eggs obtained from indoor sources predominantly reared into *Ae. aegypti*, whereas those obtained from outdoor sources primarily reared into *Ae. albopictus*. *Aedes albopictus* are commonly found in outdoor environments, although they can also be found indoors. *Aedes albopictus* mosquitoes are similar to *Ae. aegypti* in terms of breeding place, although the former spp. tend to undergo oviposition in outdoor environments.[11] According to an earlier study,[20] some of the factors influencing the behavior of *Aedes spp.* in laying eggs include the color of the water reservoir, their daily-use water, humidity, temperature, and local environmental conditions.

Marchette *et al*,[21] stated that *Ae. aegypti* is one of the vectors that carries the identified Zika virus in Southeast Asia, and data regarding the interaction of mosquitoes that carry the Zika virus is also limited to Aedes mosquitoes.[22,23] This is also supported by the study of Wong,[24] who showed that the local strain of *Ae. albopictus* from Singapore also has the potential to transmit Zika virus. *Aedes albopictus* mosquito has been considered as a po-

tential vector of some important pathogens to humans and animals due to its biological and ecological plasticity. This species is also notoriously invasive and has a wide geographical distribution.[25] Results of the study conducted by the Eijkman Institute demonstrated that patients in Jambi City who were positive for Zika virus were indigenous people who had no history of traveling abroad.[6] It is possible that the sufferer could have been infected with the virus through local mosquito-borne Zika virus transmission.

Factors influencing the detection of dengue virus in *Aedes spp.* mosquitoes include the occurrence of viral RNA degradation, the time of arrest, epidemic events, and the presence of fumigation (fogging).[26] Dengue virus RNA is influenced by high or low temperatures, which can disrupt the mechanism of viral resistance inside the mosquito body. Chemical factors and a relatively short time of viremia are believed to affect the isolation of dengue virus in mosquitoes. Degradation of dengue virus RNA in mosquitoes leads to a lack of DNA amplification for detecting dengue virus serotypes.[26] Negative results can also occur due to improper fishing times when the case occurs and when the newly emerged mosquitoes from the pupa are captured early before they could suck blood. This is also due to the insufficient time of mosquito capture in the field or when there is no outbreak of DHF.[26,27]

Conclusion

Vertical transmission of "Zika like virus" was detected in *Ae. aegypti* mosquitoes collected from the fourth neighborhood (RT.4) in the village of Talang Bakung, Jambi City. Controlling this condition would require the development of *Ae. aegypti* vector control strategies to prevent the extensive spread of dengue fever cases and Zika virus through coordination between the Health Office and cadres involved in the surveillance of cases and vectors and by increasing the number of larva-monitoring cadres in the working area of Talang Bakung Public Health Center, Jambi City. Health promotion activities in the community regarding the areas where *Aedes spp*. mosquitoes could potentially breed must be implemented through mosquito nest eradication and periodic larva monitoring. The presence of dengue virus should be detected using the method described by Lanciotti *et al,* 14 on 7-day-old mosquitoes, which is a perfect method for detecting the virus in the mosquito body, and further confirmed using other PCR methods.

Acknowledgement

Deepest gratitude is directed toward the Health Office of Jambi City, Talang Bakung Primary Health Center, supervisors, and all related individuals supporting the research. INSINAS Research Funds No 4091/UN1-PIII/LT/Dit-Lit/2016 Ministry Education and Research of Indonesia.

References

1. Zanluca C, Melo VC, Mosimann AL, Santos GI, Santos CN, Luz K. First report of autochthonous transmission of Zika virus in Rio De Janeiro Brazil. Memórias do Instituto Oswaldo Cruz [Internet]. 2015[cited 2018 Mar 8]; 110 (4): 569–572. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4501423/pdf/0074-0276-mioc-110-4-0569.pdf
2. Kuno G, Chang G-JJ. Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika Viruses. Archives of Virologyl [Internet]. 2007 [cited 2018 Mar 8]; 152:687–696. Available from: https://link.springer.com/content/pdf/10.1007%2Fs00705-006-0903-z.pdf
3. Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak Bahia Brazil. Emerging Infectious Disease. 2015; 21(10):1885–1986.
4. Lanciotti, Robert S. Kosoy, Olga L. Laven JJ, Velez, Jason O. Lambert, Amy J. Johnson, Alison J. Stanfield, Stephanie M. Duffy MR. Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2008. Emerging Infectious Disease. 2008; 14(8):1232–1239.
5. Mark R. Duffy., Tai-Ho Chen MD, W. Thane Hancock, Ann M. Powers, Jacob L. Kool, Ph.D., Robert S. Lanciotti, Moses Pretrick BS, Maria Marfel, B.S., Stacey Holzbauer, Christine Dubray, Laurent Guillaumot, M.S., Anne Griggs, Martin Bel, Amy J. Lambert, Janeen Laven, Olga Kosoy. Zika Virus Outbreak on Yap Island, Federated States of Micronesia. New England Journal Medicine [Internet]. 2009 [cited 2018 Mar 8]; 360(24): 2536–2543. Available from: http://www.nejm.org/doi/pdf/10.1056/NEJMoa0805715
6. Aditya Perkasa, Frilasita Yudhaputri, Sotianingsih Haryanto, Rahma F. Hayati, Chairin Nisa Ma'roef, Ungke Antonjaya, Benediktus Yohan, Khin Saw Aye Myint, Jeremy P. Ledermann, Ronald Rosenberg, Ann M. Powers RTS. Isolation of Zika Virus from Febrile Patient, Indonesia. Emerging Infection Disease [Internet]. 2016 [cited 2018 Mar 8]; 22(5): 924–925. Available from: https://wwwnc.cdc.gov/eid/article/22/5/pdfs/15-1915.pdf
7. Bearcroft WGC. Zika Virus Infection Experimentally Induced In a Human Volunteer. Trans of The Royal Society of Tropical Medicine and Hygiene [Internet]. 1956 [cited 2018 Mar 8];50 (5): 442–448. Available from: http://www.icmr.nic.in/zika/publications/Zika virus experimentally induced.pdf
8. WHO. WHO | Zika situation report [Internet]. WHO. World Health Organization; 2016 [cited 2018 Mar 8]. Available from: http://www.who.int/emergencies/zika-virus/situation-report/28-july-2016/en/
9. Javois L. Immunocytochemical methods and protocols. [Internet]. Second. New Jersey: Humana Press; 1999 [cited 2018 Jun 3]. Available from: https://www.google.com/
10. Kendall, L.V., and Riley L. Reverse transcriptase polymerase chain reaction (RT-PCR). The American Association Laboratory Animal Science [Internet]. 2000 [cited 2018 Jun 1];39 (1):42. Available from: https://www.ingentaconnect.com/content/aalas/jaalas/2000/00000039/00000001/art00008?crawler=true
11. Lee HL. Aedes ovitrap and larval survey in several suburban communi-

ties in Selangor, Malaysia. Tropical Biomedicine [Internet]. 1992 Dec 5 [cited 2018 May 30]; 9(1):9–15. Available from: https://eurekamag.com/research/002/293/002293906.php

12. Fatmawati Titi, Ngabekti Sri PB. Distribusi dan kelimpahan populasi Aedes spp. Di Kelurahan Sukorejo Gunungpati Semarang berdasarkan Peletakkan Ovitrap. Unnes Journal Life Science [Internet]. 2014 [cited 2018 Jul 8]; 3(2): 130–139. Available from: http://studylibid.com/doc/972071/unnes-journal-of-life-science
13. Satoto TBT, Alvira N, Wibawa T, Diptyanusa A. Improvement to early warning system the transmission of Dengue fever through controlling potential factor in Public Elementary School At Yogyakarta. Kesmas National Public Health Journal. 2017; 11(4): 178-184
14. Lanciotti, R S Calisher, C H Gubler, D J Chang, G J Vorndam A V. Rapid detection and typing of Dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. Jornal of Clinical Microbiology [Internet]. 1992 Mar [cited 2018 May 28]; 30(3): 545–551. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1372617
15. Balm, Michelle N.D. Lee, Chun Kiat Lee, Hong Kai Chiu, Lily Koay, Evelyn S.C. Tang JW. A. Diagnostic polymerase chain reaction assay for Zika virus. Journal of Medicine Virology Internet]. 2012 Sep [cited 2018 May 30]; 84(9):1501–1505. Available from: http://doi.wiley.com/10.1002/jmv.23241
16. Tesh RB, Guzman H, Hart CE, Huang J, Thangamani S. Vertical Transmission of Zika virus in *Aedes aegypti* mosquitoes. American Jounal of Tropical Meicine and Hygiene [Internet]. 2016 November 2 [cited 2018 Jun 3]; 95(5):1169–1173. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27573623
17. Honório NA, Silva W da C, Leite PJ, Gonçalves JM, Lounibos LP, Lourenço-de-Oliveira R. Dispersal of *Aedes aegypt*i and *Aedes albopictus* (Diptera: Culicidae) in an urban endemic Dengue area in the State of Rio de Janeiro, Brazil. Memorias do Insituto Oswaldo Cruz [Internet]. 2003 Mar [cited 2018 Aug 9]; 98(2):191–198. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762003000200005&lng=en&tlng=en
18. Clark GG, Anderson RA, Amador MA, Reiter P. Short report: dispersal of *Aedes aegypti* in an urban area after blood feeding as demonstrated by rubidium-marked eggs. American Journal of Tropical Medicine and Hygiene [Internet]. 1995 Feb 1 [cited 2018 Aug 9];52(2):177–179. Available from: http://www.ajtmh.org/content/journals/10.4269/ajtmh.1995.52.177
19. Schatzmayr HG. Dengue situation in Brazil by year 2000. Memorias do Insituto Oswaldo Cruz [Internet]. 2000 [cited 2018 Aug 9];95(suppl 1):179–181. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762000000700030&lng=en&tlng=en
20. Cahyati WH, Sukendra DM, PS YD. Penurunan container index (CI) melalui penerapan ovitrap di Sekolah Dasar Kota Semarang. Unnes J Public Health [Internet]. 2016 Oct 28 [cited 2018 Jul 17]; 5(4): 330. Available from: https://journal.unnes.ac.id/sju/index.php/ujph/article/view/13965
21. Marchette NJ, Garcia R, Rudnick A. Isolation of Zika virus from *Aedes aegypti* mosquitoes in Malaysia. American Journal of Tropical Medicine and Hygiene [Internet]. 1969 May [cited 2018 Jun 1];18(3):411–415. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4976739
22. Boorman JP, Porterfield JS. A Simple technique for infection of mosquitoes with viruses; transmission of Zika virus. Transactions of The Royal Society of Tropical Medicine and Hygiene [Internet]. 1956 May [cited 2018 Jun 1]; 50(3):238–242. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13337908
23. Cornet, M., Robin, Y., Adam, C., Valade, M., Calvo MA 1979. comparison between experimental transmission of yellow fever and Zika Viruses in Aedes aegypti [arbovirus diseases, Ethiopian region, Senegal]. Cah ORSTOM Serie Entomol Medicale Parasitol [Internet]. 1979 [cited 2018 Jun 1];17: 47–53. Available from: http://agris.fao.org/agris-search/search.do?recordID=XE8036808
24. Wong P-SJ, Li MI, Chong C-S, Ng L-C, Tan C-H. Aedes (Stegomyia) albopictus (Skuse): a potential vector of Zika virus in Singapore. Turell MJ, editor. PLoS Negleted Tropical Diseases [Internet]. 2013 Aug 1 [cited 2018 Jun 1]; 7(8):e2348. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23936579
25. Zanluca C, Melo VCA de, Mosimann ALP, Santos GIV dos, Santos CND dos, Luz K. First report of autochthonous transmission of Zika virus in Brazil. Memorias do Insituto Oswaldo Cruz [Internet]. 2015 Jun 9 [cited 2018 Mar 8]; 110(4):569–572. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762015000400569&lng=en&tlng=en
26. Sasmono, T., Yohan, B., Setianingsih, T.Y., Aryati, Wardhani, P., Rantam FA. Identifikasi genotipe dan karakterisasi genome virus Dengue di Indonesia untuk penentuan prototipe virus. In: Prosiding InSiNas 2012. 2012. p. (422) 1-6.
27. Andriyani Y. Deteksi dan penentuan serotipe virus Dengue tipe-3 (Den-3) dari nyamuk *Aedes Aegypti* dengan menggunakan reverse transcriptase- PCR (RT-PCR) di Kota Medan [Internet]. USU, Medan; 2009 [cited 2018 Jun 1]. Available from: http://repository.usu.ac.id/handle/123456789/6230