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The detection of the Zika virus

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Abstract
Zika disease is an arthropod-borne disease that is most prevalent in tropical and sub-tropical countries. The diagnosis of this disease is made more difficult by its asymptomatic and non-specific symptoms. Ensuring accurate diagnoses of the Zika virus is a challenge because the virus can be found in a variety of examination samples depending on the onset of the disease. Hypotheses related to the transmission of this disease have been raised by many researchers because the transmission process is still uncertain. This article discusses the Zika virus and developments and its detection methods.

Keywords: Zika virus, detection methods, arthropod-borne disease

Introduction
Zika virus is a mosquito-borne diseases transmitted by *Aedes aegypti* mosquitoes. This virus was first isolated from monkeys in Uganda in 1947, and 5 years later identified in human. The outbreak was reported from the 1960s, 1980s, 2007, and the last was in 2015 in Brazil that reported an association between Zika virus infection and microcephaly. Its symptoms include fever, headache, conjunctivitis, rash, muscle and joint pain. Infection of Zika virus may be suspected based symptoms and recent history of travel to endemic area. Diagnosis of Zika virus infectioncan only be confirmed by laboratory test such as blood, urine, saliva and semen. This paper reviews the Zika virus, its development and its detection methods that have been used to detect the Zika virus.

Zika Virus Characteristic
The Zika virus belongs to the Flaviviridae family, from the flavivirus genus. This single chain RNA virus has a positive sense with three structured proteins (capsid, pre-membrane/membrane, envelopes) and 7 non-structured proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) [1, 2]. The virus was first discovered accidentally in a study aimed at detecting Yellow Fever in monkeys in the Zika Forests in Uganda in 1947. The study showed that the cross-neutralization tests on monkeys were different for Yellow Fever virus [3].
It then became clear that the Zika virus can infect humans through an intermediary vector resulting in a specific disease, which is called Zika disease. The first reported Zika virus infection in humans was in Nigeria in 1954 and was found in a serum sample of a girl during an outbreak of Yellow Fever [4]. Studies have revealed that a Zika infection is transmitted via certain mosquitoes, including forms of *Aedes sp*. (Ae) mosquitoes such as *Ae. furcifer, Ae. taylori, Ae. luteocephalus, Ae. aegypti* and *Ae. Hensilli* [5].

The Emergence of the Zika virus
When it first began to emerge, Zika disease was found in the tropics, where the reproduction of vector mosquitoes is optimal due to the seasons in such regions. In some areas of tropical countries such as Indonesia, the rainy season provides optimal conditions for the spread of *Aedes aegypti* mosquitoes. The first report on Zika disease in Indonesia was in 1978 as part of a study in Central Java. The study considered a small cluster of seven (two adults and five children) out of a total of 30 patients who tested positive for the Zika virus. Clinical manifestations arising from these patients included high fever, malaise, abdominal pain, dizzines and anorexia [6].
The first large-scale outbreak of the disease occurred in the Pacific Yap Islands in 2007 in which there were 49 confirmed cases and 59 cases of probable Zika virus infection [7, 8,].

Five years later, in 2012, there was a report of a Zika infection of a child in the Philippines [9]. At the same time, the same infection was found in French Polynesia, New Caledonia, and the Cook and Estonian Islands [10]. The most recent cases of Zika outbreaks were reported in the Pacific Islands of Martinique at the beginning of January 2016, on a greater scale. The reported incidences of Zika virus infections reached 1,000 cases per week [11].

Zika disease is known to have spread in several countries in Africa, Pacific Islands and Asia, particularly Southeast Asia. Several countries in Southeast Asia have reported cases of Zika disease, including the Philippines, Indonesia and Thailand. Between 2012-2014, there were several cases of Zika virus infections in both local people and tourists in Thailand. These patients exhibited symptoms such as fever, a red rash, conjunctivitis and atralgia, much like those found in other countries [12].

Zika disease reached the Americas with the first cluster of cases in Brazil reported in 2015. A total of 345 patients were tested for the disease, of whom 182 showed positive results of an infection [13]. Since then, cases have most often been found in North and South America [14, 15].

Zika Virus Phylogenetic

A phylogenetic study is usually carried out to determine the relationships between various incidences of Zika virus infections worldwide. An earlier study of Zika incidences showed that isolated samples from patients in Thailand had a closer correlation with comparative samples in French Polynesia compared to those originating from Canadian tourists in Cambodia. However, virus isolates are still in one part of the Zika virus pathway in Asia along with isolates from Micronesia and the Philippines [12]. The conclusion is that the Asia and Pacific strains are interconnected. This suggests that the strain had actually existed for a few years before it began to emerge [12].

Some positive samples of the Zika virus that do not have a history of traveling outside their province of origin [12]. This indicates the wide distribution of the Zika virus in Thailand [12]. A phylogenetic analysis demonstrates that Zika virus strains in the Philippines are closer to the strain that originated in Yap State, Micronesia [9].

Characteristics of Patients with Zika

Zika patients cover a wide range of ages and present with a variety of non-specific symptoms. For example, in Thailand, the age range of patients with Zika is 12-39 years (a mean of 25 years), and 10 to 60 years [12, 16]. Its symptoms resemble flu-like syndromes including a maculopapular rash, conjunctivitis, myalgia, rhinorrhea, headaches and arthralgia [12]. A Brazilian study of 61 people with suspected infections also showed similar symptoms, such as fever, a red rash, headaches, joint pain and conjunctivitis [14, 17]. The symptoms found in both places were highly comparable, although only two of the patients in Thailand complained of conjunctivitis [12].

Clinical symptoms of a neurological disorder were also reported in Zika patients on the Pacific Islands [10]. Two years later it was reported that two patients suffered from Guillain-Barre disease in the month following an outbreak of the Zika virus in Martinique. Both patients were found to be positively infected with the Zika virus following an examination of their urine samples. Guillain-Barre symptoms are characterized by weakness in the limbs, muscle numbness including in the facial muscles, swallowing disorders, and respiratory distress [11].

As previously mentioned, Zika disease can infect all age groups and both men and women, including pregnant women. A study in Brazil showed that pregnant women infected with the Zika virus demonstrated moderate symptoms of pain. These symptoms may vary and may include a maculopapular rash accompanied by itching, joint pain, conjunctival injection, headaches and fever [13]. It has been suggested that this viral infection may endanger the fetus, resulting in fetal death, fetal growth disturbance, abnormalities of central nervous system formation and microcephaly [13, 18].

Development of the Diagnosis of Zika

The wide range of non-specific clinical symptoms of Zika disease has hampered its diagnostic process. Patients infected with the Zika virus are mostly asymptomatic, or present with symptoms similar to Dengue virus infections [11]. Therefore, Zika disease can only be diagnosed after a series of investigations to detect the presence of DENV, CHIKV, rubella, and measles infections [12]. Moreover, the low and short phase of viremia of the Zika virus has contributed to the difficulties in securing a prompt diagnosis [19].

A diagnosis of Zika disease can be established using a serological method and a molecular examination. A serological examination aims to detect the presence of IgM or antibodies against the Zika virus while the molecular examination is used to detect viral RNA. Viral loads peak at the onset of symptoms but can be detected up to 3-5 days later with RT-PCR from saliva samples, nasopharyngeal swabs, urine or semen [19]. Most Zika virus detection attempts have used the molecular method of RT-PCR [11, 14, 20]. Other studies have also used serological methods to detect IgM with MAC-ELISA (IgM capture enzyme-linked immunosorbent assay), PRNT (Plaque Reduction Neutralization Test) or a combination of serological and molecular methods [12].

One thing to consider in detecting the virus serologically is the cross-reactivity of other flavivirus antibodies such as Dengue. The inconclusive results of MAC ELISA can be confirmed using a specific test with PNRT to distinguish the proximity of a virus' antibody. If PNRT is not available, the Zika virus can be presumed to be present when MAC ELISA is positive for Zika but negative for Dengue [21]. However, this method is rarely used due to its low accuracy, expense and time taken. The use of serological detection in Dengue-endemic areas is of course also less effective because 90% of the population has been exposed to flaviviruses.

The examination of patients suspected to have a Zika infection using the PCR method has been widely used and provides good results. There are a variety of PCR methods for detecting the Zika virus. The first PCR method used was the conventional PCR model. Zika virus RNA detection can be done using the RT-PCR method with good results. The method is not only able to identify the Zika virus but also to determine the virus level from the patient sample [12]. This RT-PCR method is very fast, taking less than 3 hours to provide specific results, with high sensitivity and specificity [22]. In addition, the combination of Zika virus detection from saliva, urine and blood samples may improve the sensitivity

of the examination [19].

Another method of Zika virus detection is with a viral culture using a variety of cell lines such as Vero cells, LLC-MK2 cells, MOS61, AP61 and C6 / 36 cells [23, 24]. The serological methods ELISA and PNRT were used to detect IgM against the virus and successfully confirmed 49 cases during the first outbreak of the human Zika virus in 2007 [7, 25].

Since 1980, the detection of the Zika virus in Brazil has been done using the RT-PCR method to detect the viral genes. This can be done during the acute phase of the disease for approximately 3-14 days [14, 20]. This process is difficult due to the possibility of cross-reactions with other viruses from different genera of flaviviruses. In addition, the viral RNA can only be detected during the short duration of the viremia phase [14].

The Zika virus can be identified through examining bodily fluids such as urine and blood (serum or plasma) samples, or indeed both [11, 13, 14]. The level of the Zika virus found in blood plasma during the acute phase is higher than in urine samples. From a total of 61 positive results of the Zika virus, 46 were identified from plasma alone, 37 from urine alone, and 28 were found in both urine and plasma samples five days after the onset of symptoms [14]. A study suggested that the Zika virus can still be found in urine up to the 20th day of the disease's onset [11, 20].

A case report in Italy showed that the Zika virus was also identified from a semen sample of a man traveling to Haiti. The sperm were examined 6 months after the first symptoms of a Zika infection occurred [26]. Approximately 17 days after the onset of symptoms, the Zika virus can still be found in saliva samples using the RT-PCR method with a 36.4 threshold cycle value, while serum samples were also positive in terms of IgM and IgG ZIKV [26].

Reports in 2014 showed that the Zika virus could be found in semen samples at days 27 and 64 after the onset of acute symptoms using the rRT-PCR method. In one case, the patient had a history of traveling to the Pacific islands [16]. This study indicated that the Zika virus can live and replicate in the semen in the absence of hematospermia. The result also suggested the possibility that the Zika virus can be transmitted through sexual intercourse [16]. A recent study has demonstrated that the Zika virus can also be detected from vaginal secretions. The virus was discovered on day 14 and from a complete blood sample until day 64 [17].

Many studies have suggested that the Zika virus can be vertically transmitted from mother to fetus during pregnancy. This is supported by the detection of viral RNA in the amniotic fluid during the pre-natal period, which can be found in the brain tissue or placenta shortly after an abortion or the post-natal period in a microcephalic child. Transmission during labor or the peri-partum period have also been reported with either mild or asymptomatic symptoms in the children. At the time of birth, viral Zika can be detected from cord blood with MAC ELISA and RT-PCR, but their sensitivity is not fully established [21].

Detection of Zika Virus in Vectors

Aedes sp. is known as the critical vector of Zika disease in most areas. However, other mosquito species such as *Mansonia uniformis*, *Anopheles coustani* and *Culex perfuscus* can also transmit the Zika virus [27]. The ability of vectors to transmit the virus varies depending on the species and the virus strains [28].

The detection of the Zika virus in this vector is necessary given the absence of specific vaccines and therapies. Successfully detected Zika virus strains from field mosquito samples in the Asia-Africa region using the rRT-PCR method, which can be done in less than 3 hours [22]. However, virus identification of vectors needs more validation in the laboratory to determine the capacity of vectors to transmit the virus to other hosts [29]. Therefore, it is necessary to further investigate the existence of the Zika virus in each region that has had reported cases. By doing so, it is possible to determine whether the Zika virus has been imported or is already widely distributed in the region concerned. Further investigations can be done using data mapping of the distribution of the Zika virus around the world and in Indonesia in particular.

Conclusion

This review has shown that Zika virus infection had various symptoms and suggesting potential connection with prenatal microcephaly and Guillain-Barre Syndrome. This review also discussed the current detection methods to detect Zika virus. Currently, less information is available regarding the Zika virus pathogenesis pathways compare to other flavivirus genus and host cellular response. So it needs further studies to aid the development of a robust detection assay.

References

1. Baronti C, Piorkowski G, Charrel RN, Boubis L, Leparc-Goffart I, de Lamballerie X. Complete coding sequence of Zika virus from a French Polynesia outbreak in 2013. Genome Announc. 2013; 2(3):e00500-14. Doi:10.1128/genomeA.00500-14.
2. Kuno G, Chang GJ. Full-lenght sequencing and genomic characterization of Bagaza, Kedougou and Zika Virus. Archives of Virology. 2007;152:687-696. http://dx.doi.org/10.1007/s00705-006-0903-z.
3. Dick GWA, Kitchen SF, Haddow AJ. Zika virus, Isolation and serological specifity. Transactions of The Roya Society of Tropical Medicine and Hygiene. 1952; 42(5):509-520.
4. Macnamara FN. Zika virus: A report on three cases of human infection during an epidemic jaundice in Nigeria. Transaction of the Royal Society of Tropical Medical and Hygiene. 1954; 48:139-145. http://dx.doi.org/10.1016/0035-9203(54)90006-1.
5. Cornet M, Robin Y, Chateau R, Heme G, Adam C, Valade M *et al*. Isolemet d'arboviruses au Senegal oriental a partir de mostiques (1972-1977) et notes sur l'epidemiologie des virus transmis par les aedes, en particulier du virus amaril. Cahiers OSRTOM Serie Entomologie medicale et Parasitologie. 1979; 17(3):149-163.
6. Olson JG, Ksiazek TG, Suhandiman, Triwibowo. Zika virus, a cause of fever in central Java, Indonesia. Transactions of The Roya Society of Tropical Medicine and Hygiene. 1981; 75:389-93.
7. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS *et al*. Zika virus outbreak on Yap Island, Federated State of Micronesia. The New England Journal

of Medicine. 2009; 360:2536-43.
8. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lamber AJ, Johnson AJ *et al*. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State Micronesia, 2007. Emerging Infectious Diseases. 2008; 14(8):1232-1239.
9. Alera MT, Herman L, Tac-An IA, Klungthong C, Rutvisuttinunt W, Manasatienkij W *et al*. Zika virus infection, Phillipines, 2012. Emerging Infectious Diseases. 2015; 21(4):722-724. DOI: http://dx.doi.org/10.3201/eid2104.141707.
10. Roth A, Mercier A, Lepers C, Hoy D, Duituturaga S, Benyon E. Concurrent outbreaks of dengue, chikungunya, and zika virus infections – unprecedented epidemic wave of mosquitoes borne viruses in the Pacific 2012-2014. Euro Surveillance. 2014; 19(41):20929. DOI:10.2807/1560-7917. ES 2014.19.41.20929 PMID:25345518.
11. Roze B, Najioullah F, Ferge J, Apetse K, Brouste Y, Cesaire R *et al*. Zika virus detection in urine from patients with Guillain-Bare syndrome on Martinique, January 2016. Euro Surveill. 2016; 21(9):pii=30154. DOI:http://dx.doi.org/ 10.2807/1560-7917. ES.2016.21.9.30154.
12. Buathong R, Hermann L, Thaisomboonsuk B, Rutvisuttinunt W, Klungthong C, Chinnawirotpisan P *et al*. Detection of Zika infection in Thailand 2012-2014. The American Journal of. Tropical Medicine Hygiene. 2015; 93(2):380-383. DOI:10.4269/ajtmh.15-0022.
13. Brasil P, Pereira JP, Moreira ME, Nogueira RMR, Damasoeno L, Wakimoto M *et al*. Zika virus infection in pregnant women in Rio de Janeiro. The New England Journal of Medicine. 2016; 374(24):2321-2334. DOI:10.1056/NEJMoa1602412.
14. Pessoa R, Patriota JV, de Souza M, Wahed AAE, Sanabani SS. Detection of Zika virus in Brazilianpatients during the first five days of infection – urine versus plasma. Euri Surveill. 2016; 21(30):pii=30302. Doi:http://dx.doi.org/10.2807/1560-7917. ES.2016.21.30.30320.
15. Centers for Diseases Control and Prevention (CDC). Travel Notice Zika virus. Atlanta: CDC. 2016. Downloaded at http://wwwnc.cdc.gov/travel/notice. Accessed on11 February 2017.
16. Atkinson B, Hearn P, Carter E, Aarons EJ, Simpson AJ, Brooks TJ *et al*. Detection of Zika virus in semen. Emerging Infectious Diseases. 2016; (22)5:940. DOI:http://dx.doi.org/10.3201/eid2205.160107.
17. Murray KO, Gorchakov R, Carlson AR, Berry R, Lai L, Natrajan M *et al*. Prolonged detection of Zika virus in vaginal secretions and whole blood. Emerging Infectious Diseases. 2017; 23(1):99-101. DOI: http://dx.doi.org/10.3201/eid2301.161394.
18. Rasmussen SA, Jamieson DJ, Honein MA, Peterson LR. Zika virus and birth defects-reviewing the evidenceof causality. The New England Journal of Medicine. 2016; 374(20):1981-1987.
19. Goeijenbier M, Slobbe L, Van der Eijk1 A, de Mendonça M, Melo, Koopmans MPG *et al*. Zika virus and the Current Outbreak : an overview. The Netherland Journal of Medicine. 2016. 74(3):104-109
20. Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of virus Zika in urine. Emerging Infectious Diseases Journal. 2015; 21:84-6.
21. Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika Virus. The New England Journal of Medicine. 2016;374:1552-63
22. Faye O, Diallo D, Weidmann M, Sall AA. Quantitative real-time PCR detection of Zika virus and evaluation with field-caught Mosquitoes. Virology Journal. 2013; 10:311
23. Haddow AD, Schuh AC, Yasuda CY, Kasper MR, Heang V, Huy R *et al*. Genetic characterization of zika virus strain: geographic expansion of the Asian lineage. Plos Neglected Trop Dis. 2012:6e1477. http://dx.doi.org/10.1371/journal.pntd.0001477.
24. Digoutte JP, Calvo-Wilson MA, Mondo M, Traore-Lamizana, Adam F. Continuous cell linesand immune ascitic fluid pools in arbovirus detection. Research in Virology. 1992; 143:417-422. http://dx.doi.org/10.1016/S0923-2516(06)80135-4.
25. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lamber AJ, Johnson AJ *et al*. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State Micronesia, 2007. Emerging Infectious Diseases. 2008; 14(8):1232-1239.
26. Nicastri E, Castilletti C, Liuzzi G, Lannetta M, Capobianchi MR. Persisten detection of zika virus RNA in semen for six month after symtoms onset in a traveller returning from Haiti to Italy, February 2016. Eurosurveillance. 2016; 21(32):pii=30314. DOI:http://dx.doi.org/10.2807/1560-7917.ES.2016.21.32.30314.
27. Diallo D, Sall AA, Diagne CT, Faye O, Faye O, Ba Y *et al*. Zika virus emergence in mosquitoes in southeastern Senegal, 2011. PLoS One. 2014; 9:e109442.
28. Liu Z, Zhou T, Lai Z, Zhang Z, Jia Z, Zhou G *et al*. Competence of *Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* Mosquitoes as Zika Virus Vectors, China. Emerging Infectious Diseases. www.cdc.gov/eid. 2017; 23:7.
29. Ayres FJ. Identification of Zika virus vectors and implications for control. Lancet Infect Dis. 2016. Published online February 4, 2016 http://dx.doi.org/10.1016/S1473-3099(16)00073-61