The first case of laboratory-confirmed dengue virus infection in Mimika, Papua province, Indonesia

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Abstrak

Latar belakang: *Dengue merupakan penyakit bersumber vektor yang berkontribusi cukup besar dalam menyebabkan masalah kesehatan baik di negara tropis maupun subtropis. Hingga saat ini virus dengue telah menyebar ke seluruh provinsi di Indonesia sejak pertama kali ditemukan di Surabaya pada tahun 1968. Kabupaten Mimika di propinsi Papua, Indonesia, merupakan daerah non-endemis dengue dan tidak pernah melaporkan munculnya kasus dengue. Walau begitu, pada tahun 2012 ditemukan 13 kasus tersangka dengue yang dirawat di Rumah Sakit Umum di Mimika. Studi ini bertujuan memberi gambaran karakteristik genetik virus dengue dari kasus terkonfirmasi (laboratory-confirmed) pertama di kabupaten Mimika, propinsi Papua, Indonesia.*

Metode: *Isolasi virus pada sel nyamuk C6/36, RT-PCR dan penentuan serotipe dilakukan untuk mengkonfirmasi adanya virus dengue (DENV) di dalam serum pasien tersangka dengue dari kabupaten Mimika, propinsi Papua, Indonesia. Sekuensing dan analisis pohon filogenetik terhadap* complete-coding sequence *(CDS) gen E dilakukan terhadap sampel yang telah positif DENV untuk penentuan genotipe virus.*

Hasil: *Sebanyak 4 kasus tersangka dengue terkonfirmasi positif DENV berdasarkan pemeriksaan RT-PCR, sedangkan 2 sampel berhasil dilakukan kultur pada sel C6/36. Hasil penentuan serotipe menunjukkan bahwa virus DENV dari kabupaten Mimika, propinsi Papua, Indonesia, termasuk ke dalam serotipe DENV 3. Analisis CDS gen E menunjukkan DENV 3 termasuk ke dalam genotipe I.*

Kesimpulan: *Studi ini melaporkan kasus pertama dengue yang terkonfirmasi secara laboratorium dari kabupaten Mimika, propinsi Papua, Indonesia, yang merupakan daerah non-endemis dengue. (**Health Science Journal of Indonesia 2016;7:1-6)***

Kata Kunci: *dengue, penentuan serotipe, penentuan genotipe, kabupaten Mimika*

Abstract

Background: Dengue is the most important vector-borne disease that poses serious health problem both in tropical and subtropical countries. Since the first outbreak in Surabaya in 1968, dengue infection has spread in all provinces in Indonesia. Mimika district in Papua province, Indonesia, is a non-endemic dengue area with no laboratory-confirmed case reported. However, until 2012 there were 13 suspected dengue infection admitted to local General Hospital in Mimika district, Papua province, Indonesia. This study described the genetic characteristics of first laboratory-confirmed dengue virus (DENV) infection in Mimika district, Papua province, Indonesia.

Methods: Viral isolation in C6/36 cell line, RT-PCR and serotyping were carried out to confirm the presence of DENV within serum patient of suspected DENV cases from Mimika district, Papua Province, Indonesia. Direct sequencing and phylogenetic analysis of complete coding sequence (CDS) of E gene was performed to the samples that have already confirmed positive DENV for viral genotyping.

Results: Four cases were confirmed to be DENV by RT-PCR while only 2 samples were able to be culture in C6/36 mosquito cell line. Serotyping confirmed that the DENV from Mimika district, Papua province were DENV3 serotype. The genotyping showed that the DENV3 from Mimika district were belonged to genotype I.

Conclusion: This study reported the first laboratory-confirmed of DENV cases in non-endemic dengue area, Mimika district, Papua Province, Indonesia. ***(Health Science Journal of Indonesia 2016;7:1-6)***

Keywords: dengue, serotyping, genotyping, Mimika district

Dengue infection remains the cause of health problem with high disease burden worldwide. This disease causes by Dengue Virus (DENV) that is transmitted from human to human both in urban and suburban areas by mosquito vector *Aedes aegypti* belonged to Aedes family.[1] To date, more than half of the world's population lives in dengue endemic area, where approximately 50 million DENV infections were reported annually and 500,000 cases were hospitalized due to Dengue Hemorragic Fever (DHF) alone.[2]

DENV genome consists of positive stranded RNA that has ~10.7 kb in length encoding 3 structural (C, prM/M, E) and 7 non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) proteins.[3] There are four antigenically distinct serotypes of DENV (DENV1-4) and each serotype is divided into several genotypes according to the nucleotide sequences.[4-8]

DENV has caused health public problem since its first outbreak report in Surabaya in 1968, with a total of 58 clinical cases and 24 deaths.[9] In addition, the largest incidence of DF and DHF in the South East Asia region since 2004 was reported in Jakarta.[10] Nowadays, DENV infection has occurred in all provinces in Indonesia.[11]

Mimika district in Papua has been recognized as non-endemic area of DENV since no laboratory-confirmed cases were reported. However, in mid-2012, there were suspected cases of dengue reported to local Hospital and the specimens were sent to National Institute of Health Research and Development of Health (NIHRD), Jakarta, for dengue virus examination and genetic characterization. The genetic information of these viruses is essential since these viruses obtained from non-endemic area.

This report described the genetic characteristics of the first cases of dengue found in Mimika district, Papua Province. Molecular analysis of dengue virus using bioinformatic analysis was performed for genotyping of the DENV from Mimika district, Papua Province.

METHODS

Specimen collection and DENV testing

In June 2012, Virology laboratory, NIHRD, received 13 serum samples of suspected dengue cases from Health office of Mimika District, Papua Province. All of the serum samples were obtained from suspected dengue patients admitted in local General Hospital in Mimika District. Four out of 13 serum samples were positive dengue IgM and IgG by rapid diagnostic test performed at the local General Hospital in Mimika District. However, NIHRD only received the specimens without any information from the Health office district. The demographic data such as age, gender, address and date of onset were not available from all of the samples. This become the limitation of this report as the epidemiological analysis could not be performed.

Identification and characterization of DENV were performed to all of serum samples in Virology laboratory, NIHRD, Jakarta. The serology confirmation was carried out using Panbio Dengue Duo ELISA (Alere). DENV serotyping to detect DENV nucleic acid on serum samples was performed using RT-PCR according to previous method by Lanciotti et.al.[5] after RNA extraction to obtain pure RNA from serum. Viral RNA was extracted using QiAmp Viral Mini Kit (Qiagen, Hilden, Germany) according manufacturer's instruction. RT-PCR was conducted using SuperScript™ III One-Step RT-PCR System with Platinum *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA). RT-PCR was performed on C1000 PCR Thermal cycler (Bio-Rad, Hercules, CA) in a total reaction volume of 25 μL. Nested PCR was carried out using KAPA TaqExtra HotStart ReadyMix PCR Kit (KAPA Biosystems) on positive RT-PCR amplicon with 100x dilution for DENV serotyping.

Virus culture

Serological or RT-PCR-positive serum samples were subjected to inoculation in C6/36 cell line. Monolayer of cells was inoculated with 200 ml of sera in 2 ml of RPMI medium supplemented with 2% of Fetal Bovine Serum (FBS), 2 mM of L-glutamine, 100U/ml of Penicillin, and 100mg/ml of Streptomycin. After incubation for 1 hour at 28^0C to allow virus attachment, medium was discarded and 3 ml of fresh medium was added. Infected cells were incubated at 28^0C for up to 14 days.

DNA Sequencing and phylogenetic analysis

DENV isolates harvested from C6/36 mosquito cell line were subjected for RT-PCR and further for direct sequencing of envelope (E) gene using overlapping primers that covered complete coding sequence of E gene. PCR product purification was

performed using QIAquick™ Gel Extraction Kit (QIAGEN, Hilden Germany) according to the manufacturer's instruction. Direct DNA sequencing was carried out using the Big Dye Terminator V.3.0 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) on ABI 3130xl Genetic Analyzer automatic sequencer (Applied Biosystems, Foster City, CA, USA).

The overlapping nucleotide sequences were edited, assembled and aligned using BioEdit Sequence Alignment Editor Ver 7.0.5.2.[12] Phylogenetic analyses were performed with Neighbor Joining using MEGA 5.0 with 1000 replicates. Known genotype sequences of DENV-3 from Indonesia and global sequence obtained from GenBank were included in the analysis as references. DENV-1, DENV-2, and DENV-4 sequences were included as outgroup sequences.

RESULTS

Four samples were positive using RT-PCR with only two samples were able to grow in C6/36 cell line. All of the samples were confirmed to be DENV-3 after serotyped using RT-PCR. Complete coding sequence of E gen (1479 bp) was obtained from one sample, Srt_Mimika2012. This sequence together with references sequences based on previous study[7] were subjected to phylogenetic tree construction. Phylogenetic analysis showed that the Srt_Mimika2012 belonged to DENV-3 genotype I with close relation with other sequences from Indonesia in 2008 and 2009 (Fig. 1).

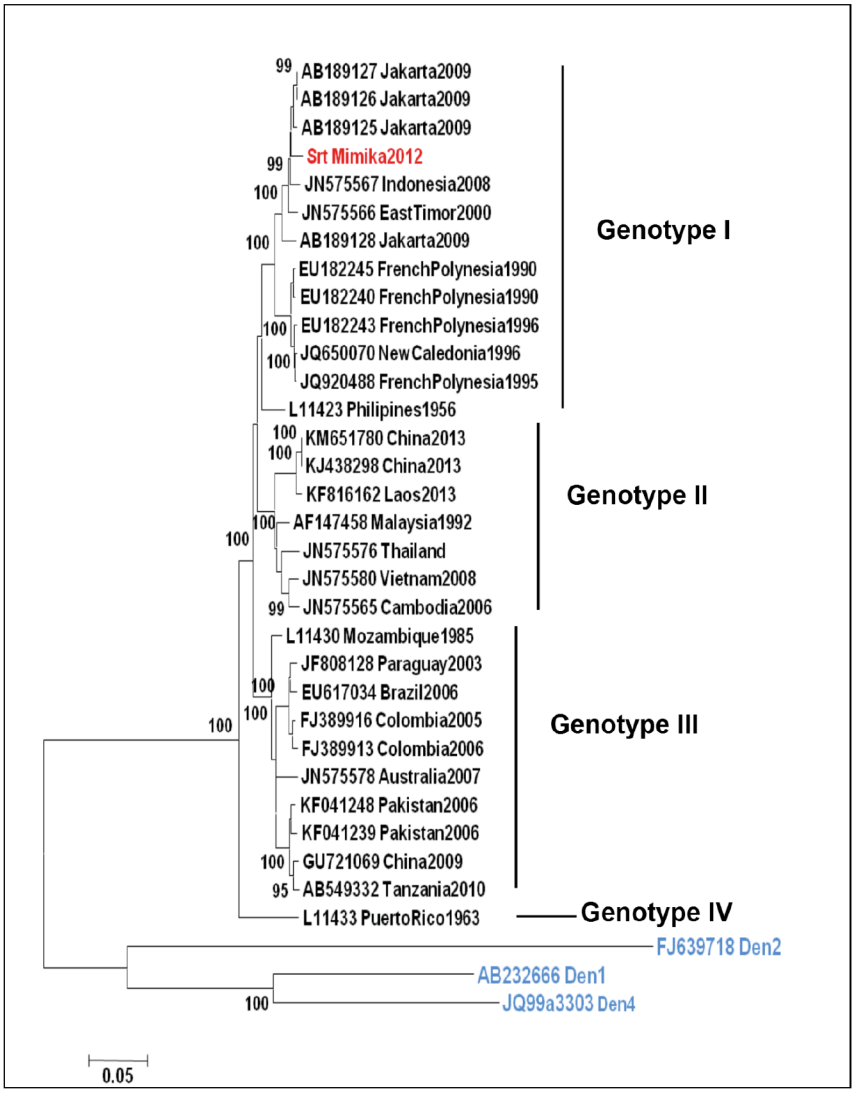

Figure 1. Phylogenetic tree of DENV-3. The sequence from Mimika (Srt_Mimika2012) was highlighted in red. The outgroup sequences (DENV-1, DENV-2 and DENV-4) were highlighted in blue.

Pair-wise nucleotide sequence alignment spanning the E gene of the Mimika strain to Jakarta 1988 strain showed an identity of 97% and a divergence of 2,7%, reflecting a total of 37 nucleotide changes between them. There were five differences of amino acid between sequence reference (Jakarta 1988) and Mimika district, shown in Table 1.

Table 1. The amino acid differences between Mimika sequence and reference sequence

E gene Position	19	124	301	377	399
AB600937 Jakarta indonesia 1988	T	S	L	V	M
Srt Mimika2012	K	L	S	I	I

All of the serum samples received in NIHRD, Jakarta, were lack of complete demographic data such as date of onset or detailed clinical symptoms'. Therefore demographic data was not described in this report.

DISCUSSION

Dengue infection has spread in all provinces in Indonesia and caused outbreaks annually. Several studies had reported dengue outbreak in several cities in Indonesia[9,10] in which both young age and older age groups were infected by DENV and had dengue hemorrhagic fever (DHF) incidence.[13] Mimika district is one of districts from Papua province, located in Irian Island, the Eastern part of Indonesian archipelago (Fig. 2). This area is known as non-endemic area of dengue since there was no data of laboratory-confirmed dengue cases reported from Mimika district, Papua province. Here we reported the first cases of laboratory-confirmed and genotyping of DENV taken from dengue outbreak in Mimika district, Papua Province, Indonesia, in 2012.

Figure 2. The map of Mimika District in Papua Province

Suspected samples from Mimika district were confirmed to be DENV-3 genotype I in which this serotype is predominant in other cities in Indonesia along with DENV-2.[11] The DENV-3 genotype I was already found in other cities in Indonesia[14,15] and also commonly found in South East Asia.[7] The Srt_Mimika2012 was clustered closely with other sequences from Indonesia, therefore it was possible that the Mimika virus was imported from other place since or before 2009.

Phylogenetic analysis confirmed that the genotype distributions of DENV strains circulating in each of the South East Asian Countries remained stable. DENV-3 genotype I was mainly distributed in South East Asia and Pacific although some studies also reported genotype III virus was introduced into South East Asian Countries and Southern China. This virus had caused dengue outbreak in recent years.[16]

The laboratory confirmation and genotyping of DENV from Mimika, Papua province, has informed health officer and government authorities concerning the DENV circulation in Mimika, Papua Province. According to the data obtained from Center Disease Control and Prevention, Ministry of Health, Republic of Indonesia, in 2012 to 2014 the incidence rate of dengue infection in Papua province is low. This data was provided with district health officers based on dengue cases found in primary health care or local hospital. However, since Malaria and HIV is the main health problem in Papua, dengue cases were not considered as priority in Papua province. Therefore the reported cases were low and many dengue infections were undetermined or under-reported.

The confirmation of dengue infection in local hospital is only based on clinical symptoms and trombocytopenia in which could be triggered by other viruses and may leads to missed diagnose. Therefore it is necessary to confirmed the dengue-suspected cases using laboratory testing for DENV antigen or DENV specific antibody detection. Among four samples out of 13 samples that were positive for DENV, only two samples were able to grow in C6/36 cell line and only one complete coding sequence of E gene was obtained from one sample. The possible reason for this is the low virus concentration in sera samples due to time of samples collection. The samples collection exceeding the acute phase where viremia had already passed can cause low titer of virus and affecting the examination results. However the disease phase from each patient in this report could not be determined since the date of onset was not available.

The authors acknowledged the lack of epidemiology data from each patient became the major limitation in this study. It is not known whether the cases were imported or emerge locally since address or travel history of patient admitted to the local General Hospital in Mimika District were not available. The epidemiological data such as the date of onset and demografic data may provide better understanding of the distribution of DENV in Mimika district, Papua province.

In conclusion, the first laboratory-confirmed cases of dengue from non-endemic dengue area in Mimika district, Papua province, Indonesia was DENV-3 genotype I.

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