Short Report: West Nile Virus Documented in Indonesia from Acute Febrile Illness Specimens

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Abstract. We report the presence of West Nile virus in a cryopreserved, dengue-negative serum specimen collected from an acute fever case on Java in 2004–2005. The strain belongs to genotype lineage 2, which has recently been implicated in human outbreaks in Europe.

The Indonesian archipelago has been predicted to be a “hotspot” for the emergence of zoonotic and vector-borne pathogens.¹ Dengue (DENV), chikungunya (CHIKV), and Japanese encephalitis (JEV) are some of the arthropod-borne viruses (arboviruses) known to cause febrile illness in Indonesia; however it is suspected that other relatively uncommon arboviruses are also causing disease. Currently, there is only limited data on the etiology of febrile illnesses in Indonesia.² As part of an effort to build capacity to detect the etiologies of underlying acute febrile illnesses in Indonesia, we have begun an analysis of cryopreserved specimens collected in an earlier study of acute febrile illness, which had previously tested negative for hantaviruses and DENV.

Archived samples were selected from an acute febrile illness study that enrolled hospitalized suspected hantavirus patients at two hospitals in Bandung, West Java, Indonesia during 2004–2005.³ Samples were collected from patients ≥ 10 years of age with fever of unknown etiology and at least one of the following symptoms: 1) hemorrhagic manifestations, 2) platelet count $< 100,000/\text{mm}^3$, 3) renal insufficiency, 4) liver dysfunction, or 5) non-cardiogenic pulmonary edema. Serum samples were collected from patients at admission to the hospital and at discharge. Collections were made under institutional review board approvals from the National Institutes of Health Research and Development, Indonesian Ministry of Health, and the U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia. The samples were originally tested for hantavirus and dengue using reverse transcription-polymerase chain reaction (RT-PCR) and in-house and commercial immunoglobulin M (IgM) and IgG enzyme-linked immunosorbent assay (ELISA) tests (Focus Diagnostics, Cypress, CA).

Of 406 cases enrolled, 249 had evidence of recent DENV infection and one had evidence of hantavirus infection. Infecting etiologies for the remaining 157 cases were negative for both DENV and hantavirus. We tested 154 acute specimens from these cases, which had been preserved at -80°C , for other arboviruses. Initial testing was performed by using group/family-specific primers in conventional RT-PCR assays followed by gel electrophoresis of the resulting amplicons.⁴ The flavivirus group primer testing resulted in four positive samples (265-bp amplicon products), which were further tested with virus-specific RT-PCR reactions and immunofluorescent assay of Vero cells infected with either DENV

or JEV. The purified nucleic acid of one flavivirus-positive sample that was negative for both DENV and JEV in the additional testing was subjected to nucleotide sequencing at the Eijkman Institute with the same primers used to generate the PCR product. A 242 basepair sequence from the NS5 gene was generated from the original 265 bp PCR amplicon. Genetic comparisons revealed the closest match (99% nucleotide identity) with the first West Nile virus (WNV) strain isolated (B956), an isolate from Uganda within lineage 2.⁵ Phylogenetic analyses confirmed the relationship of the Indonesian strain with other lineage 2 WNV sequences (Figure 1).

The WNV positive sample came from a 15-year-old boy admitted for systemic febrile illness with epistaxis, gastrointestinal symptoms, elevated serum transaminases, leucopenia, and thrombocytopenia. No neurological symptoms were reported and the patient was discharged after full recovery. Culture of the serum sample was attempted; however, the sample did not produce cytopathology in Vero cells propagated for 10 days.

West Nile virus, a zoonotic, mosquito-transmitted arbovirus belonging to the *Flaviviridae* family, is reported to be the most common cause of epidemic viral encephalitis in the United States. Phylogenetic analysis has supported the presence of two major genetic lineages. The lineage 1 viruses have typically been associated with large outbreaks and thus are considered to be more virulent than the lineage 2 strains.⁶ However, several recent outbreaks in Europe have been caused by lineage 2 WNV strains.^{7–9} Thus, it is possible that more recent lineage 2 strains are emerging with increased levels of virulence. The Indonesian strain might follow this trend but data regarding neurovirulence from Indonesia are lacking. The close phylogenetic relationship of the Indonesian strain with those from Uganda rather than strains from Australia is somewhat unexpected as there is relatively less movement of people and goods between Africa and Indonesia. Grouping of isolates, however, does not necessarily correlate with the geographic distribution of the virus¹⁰ and may be a further indicator of the recent widespread movement of pathogenic arboviruses.

WNV is considered a serious threat to public health and known to cause large outbreaks of epidemic encephalitis in Europe and North America with significant morbidity and mortality.^{11,12} WNV has not been previously isolated in Indonesia, but a serological study detected the presence of antibody to this virus in Lombok, Indonesia.¹³ Our study associates detection of WNV nucleic acid in Indonesia with human illness. Given that these samples were collected several years ago, the possibility that WNV may be currently circulating in

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FIGURE 1. A 184 basepair ns5 nucleotide sequence fragment from Bandung, Indonesia (sample HTV236-01) was aligned with other West Nile virus (WNV) sequences from GenBank using BioEdit. The maximum likelihood tree was constructed by using software MEGA5. Bootstrap values are expressed as a percentage of 1,000 replicates.

Indonesia warrants further study. The clinical spectrum caused by WNV varies dramatically from inapparent infection (~80% of cases) to non-neurological febrile illness (~20% of cases), to the most severe form including neuroinvasive disease (< 1%).¹⁴ Neuroinvasive disease cases have a mortality rate of about 10% and often display chronic manifestations.

There has been limited laboratory capacity in Indonesia to detect arboviruses other than dengue and the role of CHIKV, WNV, JEV, and other vector-borne viruses, such as Zika virus, as causes of febrile illness or more serious encephalitis has likely been underestimated. Development of rapid and simple molecular diagnostic tests combined with the establishment of dedicated research facilities in Indonesia will lead to an increased understanding of both endemic and emerging pathogens. With this recent detection of WNV in a febrile human in Indonesia, it is clear that WNV should be considered a serious threat to public health in Southeast Asia. Enhanced surveillance studies in humans, vectors and animals, and epidemiological surveys are warranted in Indonesia.

Received July 31, 2013. Accepted for publication November 27, 2013.
Published online January 13, 2014.

Acknowledgments: We acknowledge Universitas Padjadjaran, Bandung, the National Institute of Health Research and Development, Ministry of Health; and U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia for their support in conduction of research and specimen archiving.

Financial support: This study was supported by the USAID Emerging Pandemic Threat (EPT) Program and the U.S. Centers for Disease Control and Prevention.

Disclaimer: The findings and conclusions in this report are those of the authors only, and do not necessarily reflect the views of their respective institutions.

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