RAPID DETECTION AND PHYLOGENETIC ANALYSIS OF WEST NILE VIRUS AS ZOONOSIS NEW EMERGING DISEASE INPATIENT SWITH FEVER OF UNKNOWN ORIGIN (FUO) IN SURABAVA

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RAPID DETECTION AND PHYLOGENETIC ANALYSIS OF WEST NILE VIRUS AS ZOONOSIS NEW EMERGING DISEASE IN PATIENTS WITH FEVER OF UNKNOWN ORIGIN (FUO) IN SURABAYA

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ABSTRACT

This study aims to perform a rapid detection and phylogenetic analysis of WNV in the serum of patients with clinical isolates with Fever of Unknown Origin. Serum samples in Surabaya came from 2 patients who had been examined by a Specialist Doctors In the June-July, 2013, with symptoms of fever, headache, sore throat, back pain, myalgia, arthralgia, fatigue, conjunctivitis, pain, anorexia, nausea , abdominal pain, diarrhea, and respiratory symptoms. Running RNA extraction procedure and nested PCR PCR is run in accordance with protocols west nile virus detection kit from Intron. Similarly, primers used are designed specifically for the west nile virus. Interpretation of PCR products using 1% agarose gel (306 bp), and then performed a homology analysis using Genetix Mac Ver. 8.0 with reference WNV isolates from the gene bank (JF920741.1.gnu). This research demonstrated the effective PCR methods for the rapid detection of WNV and diagnosis of WNV infection from clinical isolates represent a breakthrough in the clinical world to many cases of infectious disease that has not been diagnosed (Fever Unknown Origin = FUO). Based on homology analysis, This sample showed 95% with WNV were reported in the gene bank (JF920741.1.gnu).
Keywords : PCR, West Nile Virus, FUO, Patients, Mosquitos

INTRODUCTION

West Nile virus (WNV) is an infectious disease that is new emerging diseases, mcfequito-borne and has been reported to be found throughout Europe, Russia, Africa, Southwest Asia, India and allegedly including Indonesia. West nile virus is transmitted by mosquitoes of the genus Culex, especially that has been infected with WNV. WNV is a member of the Japanese encephalitis antigenic complex of the genus Flavivirus, family Flaviviridae (Gubler, 2007).

According Smithburn *et al* **cited** by Gubler **(2007)** that **WNV was first** isolated from blood specimens obtained from patients in the province of West Nile of Uganda in **1937**. From that time until the fall of **1999**, **WNV** is considered relatively unimportant as human and animal pathogens. The virus was enzootic throughout Africa, West and Central Asia, Middle East, and the Mediterranean, with occasional extension into Europe (Hayes, **1989).** A subtype of WNV (Kunjin) is also found in Australia. Epidemiological characteristics for the period 1937-1999, WNV infection only occurs occasionally in humans, horses, and birds and is generally asymptomatic or mild, while the associated neurological disease and death are rarely reported (Gubler, **2007;** Murgue *et al*, **2001;** Murgue *et al*, **2002**). One exception was a small outbreak of WNV infection in the Camargue, area of France in 1960 at the expected associated with severe and fatal neurological disease in horses and **humans.** Long intervals between **epidemics,** and the perception that WNV is not an important issue related to public health, and the lack of reporting of epidemics and / or epizootics in the Mediterranean region opened the realization that the emerging epidemic of WNV infection is strongly associated with severe neurological disease and can be fatal, especially in the case of 1990.

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West Nile Virus (WNV) can survive in the environment because of the arthropod vectors that transmit the virus between vertebrate hosts. The main vector for WNV in the United States is the Culex pipiens mosquitoes usually breed in urban areas and prefer to bite birds. The main line of West Nile virus infection in humans is through the bite of an infected mosquito. Mosquitoes are infected when they bite infected birds, then WNV mosquito can circulate in the blood for several days. WNV finally entered the salivary glands of mosquitoes. Furthermore, the virus can be transmitted to humans and animals (Peterson *et al*, 2003). WNV has been detected in many species of wild birds, including the American crow.

Imad *et al* (2005) quoting from various sources report that during the year 2002 reported that a WNV outbreak of 2002, can be transmitted through organ transplantation and blood transfusion, one case was reported through organ transplants and one suspected case of transmission through breastfeeding. Reported two cases of WNV infection in laboratory workers.

WNV incubation period is estimated to range between 3 to 14 days. WNV infection is generally mild and often clinically unapparent (Campbell *et al*, 2002; Gubler, 2007). It is estimated that 20% of those infected will show mild symptoms, including fever, headache, and general hospital for 3 to 6 days (Sejvar *et al*, 2003; Peterson *et al*, 2003; Gubler, 2007) An estimated one in 150 patients infected with WNV show symptoms of meningoencephalitis. Patients who have a risk of serious infections and deaths including immunocompromised, elderly, and very young, with symptoms including severe headache, high fever, neck stiffness, stupor, disorienttation, coma, tremors, convulsions, muscle weakness, and abnormal movements (Sejvar *et al* , 2003; Peterson *et al*, 2003; Gubler, 2007; Klein *et al*, 2002)

According Guebler (2007) quotes from several sources reported that the vitritis and chorioretinitis have been found in patients with WNV infection. Some patients with WNV infection present with acute flaccid paralysis similar to polio. A minority of patients with severe disease developed into a maculopapular or morbilliform rash involving the neck, trunk, arms, or legs. According to data from the CDC 2002, the mortality rate is estimated at 7%, with most deaths related to complications of meningoencephalitis. Most patients who survive this disease will have a complete recovery, but some patients with meningoencephalitis with acute flaccid paralysis will require long-term rehabilitation.

Diagnosis of WNV infection is based on clinical symptoms and laboratory tests specific. WNV, or other arboviral diseases such as St. Louis encephalitis, should be considered in people suffering from encephalitis or meningitis in summer or early fall. Total number of leukocytes in peripheral blood is generally reported normal or slightly elevated with lymphocytopenia. Sometimes found hyponatremia, especially in patients with encephalitis. Examination of cerebrospinal fluid (CSF) may show pleocytosis, usually with a predominance of lymphocytes. Protein is universally increased. Glucose is usually normal (Sejvar *et al*, 2003; Peterson *et al*, 2003; Guebler, 2007; Klein *et al*, 2002). The most efficient diagnostic method is detection of IgM antibodies to WNV in CSF within 8 days of onset using the IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA). Detection of IgM antibodies in the CSF are more specific to central nervous system infection because IgM antibodies do not cross the blood-brain barrier. The finding that WNV IgM antibody may persist in the blood for up to 500 days to make a single blood sample testing problem. Thus, acute and recovered blood samples needed for confirmation.

However in Indonesia have not been many reports of cases of WNV which has infected humans and in mosquitoes. On the other hand there are many reported cases of the infection have no clear diagnosis or Fever of Unknown Origin. This study aims to perform a rapid detection and phylogenetic analysis of WNV in the serum of patients with clinical isolates with Fever of Unknown Origin.

MATERIAL AND METHODS

The research was conducted at the Institute of Tropical Disease, Airlangga University. Serum samples in Surabaya came from 2 patients who had been examined by a Specialist Doctors In the June-July, 2013, with symptoms of fever, headache, sore throat, back pain, myalgia, arthralgia, fatigue, conjunctivitis, pain, anorexia, nausea, abdominal pain, diarrhea, and respiratory symptoms. Running RNA extraction procedure and nested PCR PCR is run in accordance with protocols west nile virus detection kit from Intron. Similarly, primers used are designed specifically for the west nile virus. Interpretation of PCR products using 1% agarose gel (306 bp), and then perfôrmed a homology analysis using Genetix Mac Ver. 8.0. The study was carried out in accordance with ethical clearance by RS. Unair.

RESULTS AND DISCUSSION

The results showed that serum PCR positive patients contain the infection with WNV. DNA PCR product nucleotide with length 104 bp and the results of homology analysis showed 95% with WNV were reported in the gene bank (JF920741.1.gnu) (figure 3)

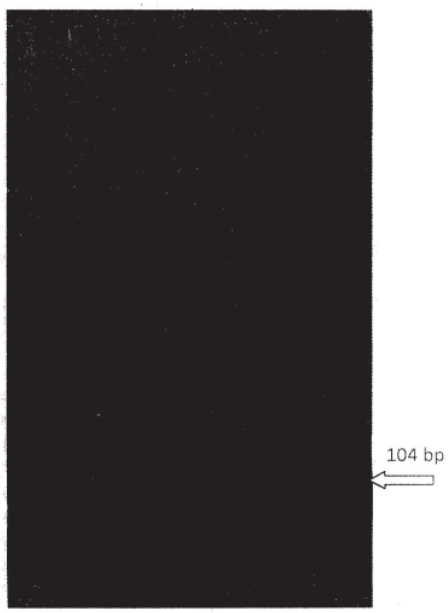

Figure 1. PCR products of west nile virus from clinical isolates in Surabaya in the 1% gel electrophoresis

CONCLUSION

This research demonstrated the effective PCR methods for the rapid detection of WNV and diagnosis of WNV infection from clinical isolates represent a breakthrough in the clinical world to many cases of infectious disease that has not been diagnosed (Fever Unknown Origin = FUO). Based on homology analysis, This sample showed 95% with WNV were reported in the gene bank (JF920741.1.gnu).

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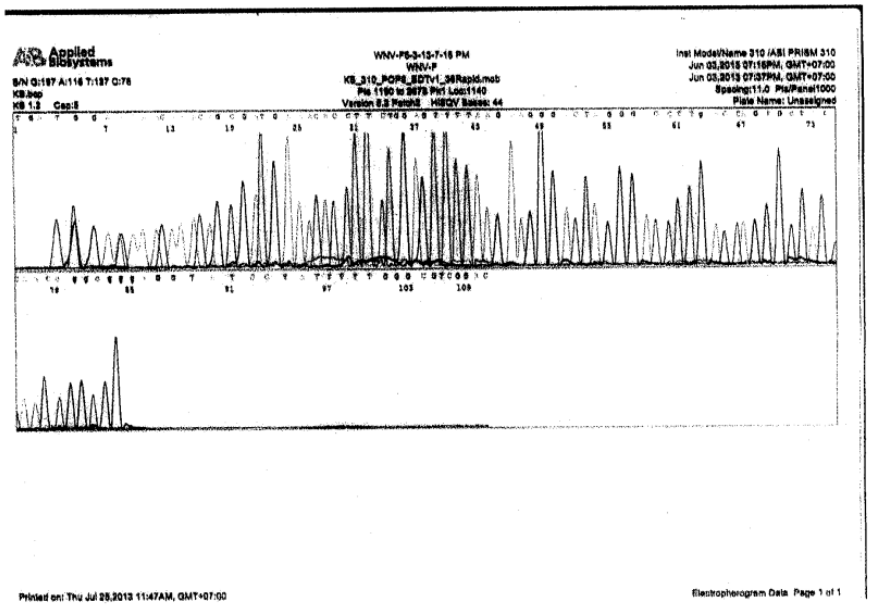

Figure 2. Sequencing results of west nile virus from clinical isolates in Surabaya

[GENETYX : Homology Data]
Date : 2013.08.15
Query Sequence
File Name :
Sequence Name : WNV-F6-3-13-7-16
Sequence Size : 111
Target Sequence
File Name : JF920741.1.gnu
Sequence Name : JF920741.1.gnu
Sequence Size : 80
Query Range: 5 - 85
Sbjct Range: 1 - 80
81 bp, INT.Score: 194, OPT.Score: 290
Identity: 77 / 81 (95%)
Similarity: 77 / 81 (95%)
Strand: Plus / Plus

```
Query 5   GGAAAAACAAACAGCGATGAAACACCTTCTGGAGTTTTAAGAAGGGACTAGGGACCTTGA  64
Sbjct 1   GAACAAACAAACAGCGATGAAACACCTTCT-GAGTTTTAAGAAGGAACTAGGGACCTTGA  59

Query 65  CCAGTGCTATCAATCGGCGGG 85
Sbjct 60  CCAGTGCTATCAATCGGCGGG 80
```

Figure 3. Homology analysis of west nile virus from clinical isolates in Surabaya

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GENERAL COMMENTS

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