

Research Report

QUICK DIAGNOSIS OF JAPANESE ENCEPHALITIS FOR NEW DIAGNOSED EMERGING DISEASE USING PCR TECHNIQUE IN SURABAYA, INDONESIA

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ABSTRACT

Background: Japanese encephalitis (JE) is a viral disease that considered as zoonotic disease, which transmitted through mosquito vectors that had JE virus. Mainly caused by the mosquito *C. Tritaeniorhynchus* (the most important vector is the mosquito *Culex*, which feeds on cattle in preference to human). JE virus disease can also cause disturbances in the central nervous system eg. brain, bone marrow, and meninges which has serious impact on public health. This disease has been reported from Japan, Korea, Taiwan, India, Myanmar, Thailand, Western Pacific and Southeast Asia to Indonesia. However, the incidence of this disease in Indonesia has not been well known in various animal species or humans. *Aim:* The purpose of this study is to develop rapid diagnostic examinations on patient diagnosed JE virus in Surabaya by using PCR (Polymerase Chain Reaction). Because, JE disease can lead to dead-end at the patient if not treated immediately. *Method:* The research methods, extraction method, PCR (1st RT-PCR and 2nd Nested PCR) are conducted using Japanese encephalitis PCR detection kit. *Result:* The results of the examination showed that 2 out of 17 people (11,765%) are positive with PCR bands 227 bp (basepair). This diagnostic technique to determine and to deal with early onset of the disease. Solutions for preventive actions can be started from the termination of the cycle vectors to vaccination measures. *Conclusion:* For his own medical factors given to reduce fever and swelling and reduce the pain.

Key words: Japanese encephalitis, PCR, New Emerging Disease, preventive, Indonesia

ABSTRAK

Latar belakang: Japanese encephalitis (JE) adalah penyakit virus yang dianggap sebagai penyakit zoonosis, yang ditularkan melalui vektor nyamuk yang memiliki virus JE. Terutama disebabkan oleh nyamuk *Culex C.* (vektor yang paling penting adalah nyamuk *Culex*, yang menyusu pada sapi dalam preferensi untuk manusia). Penyakit virus JE juga dapat menyebabkan gangguan pada sistem saraf pusat misalnya: otak, sumsum tulang, dan meninges yang memiliki dampak serius pada kesehatan masyarakat. Penyakit ini telah dilaporkan dari Jepang, Korea, Taiwan, India, Myanmar, Thailand, Pasifik Barat dan Asia Tenggara ke Indonesia. Namun, kejadian penyakit ini di Indonesia belum dikenal di berbagai spesies hewan atau manusia. *Tujuan:* Tujuan dari penelitian ini adalah untuk mengembangkan pemeriksaan diagnostik cepat pada pasien didiagnosis virus JE di Surabaya dengan menggunakan PCR (Polymerase Chain Reaction). Karena, penyakit JE dapat menyebabkan buntu pada pasien jika tidak segera diobati. *Metode:* Metode penelitian, metode ekstraksi, PCR (1st RT-PCR dan 2nd Nested PCR) dilakukan menggunakan Japanese ensefalitis PCR deteksi kit. *Hasil:* Hasil pemeriksaan menunjukkan bahwa 2 dari 17 orang (11,765%) positif dengan PCR band 227 bp (basepair). Teknik diagnostik ini untuk mengetahui dan menangani onset awal disease. Solusi untuk tindakan pencegahan dapat dimulai dari penghentian vektor siklus tindakan vaksinasi. *Kesimpulan:* Untuk faktor medis sendiri diberikan untuk mengurangi demam dan pembengkakan dan mengurangi rasa sakit.

Key words: Japanese encephalitis, PCR, New Emerging Disease, pencegahan, Indonesia

INTRODUCTION

Japanese encephalitis (JE) is a disease can cause brain inflammation in animals and humans which can be transmitted from animals to the human through mosquito bites. This disease has become widespread in parts of East Asia such as Japan, Korea, Siberia, China, Taiwan, Thailand, Laos, Cambodia, Vietnam, Philippines, Malaysia, Indonesia, Myanmar, Bangladesh, India, Sri Lanka, and Nepal. In Indonesia, JE case was first reported in 1960. Japanese encephalitis virus is part of the Flaviviridae family. This virus has the envelope (about 50 nm) with a small lipoprotein that surrounds the nucleocapsid core protein and consists of a single chain of RNA. JE virus is related to West Nile virus and St. Louis encephalitis viruses. On the outer layer are formed by (E) protein and act as protective antigen. This helps in the entry of the virus into the cell¹. JE disease in humans is a way of ending the cycle of transmission (dead - end), because viraemia in humans occurs only a few hours which is difficult to spread further to other people. Human disease can result in death if not treated properly. Wei and Gautama, in the same year reported that the most vulnerable age among children infected with JE is between 5 to 9 years.²³

In Asia, with around 50,000 cases and 10,000 deaths per year in children below 15 years of age. The JEV has shown a tendency to extend to other geographic regions. Case fatality averages 30% and a high percentage of the survivors are left with permanent neuropsychiatric sequelae. JE is a disease of public health importance because of its epidemic potential and high fatality rate. In endemic areas, the highest age-specific attack rates occur in children of 3 to 6 years of age. Approximately one third of patients die, and half of the survivors suffer severe neuropsychiatric sequelae from the disease.¹⁰

The Clinical symptoms commonly shown in the case of Japanese encephalitis is usually a non - specific symptom such as fever, followed by headache, vomiting, and decreased level of consciousness. Because the tissue covering the brain and spinal cord become infected and swollen, the patient will usually experience stiffness in the neck and painful. Then within two or three days, the patient began to experience the effects of swelling on the brain. These effects may include interference with balance and coordination, paralysis on several groups of muscles, tremors, seizures, and disturbances in consciousness.⁴ Patients also experience dehydration and weight loss. If the patient can survive with the pain, the fever will drop down about day 7, and the symptoms will begin to rise again approximately on day 14. Meanwhile there are also people who will continue to have a very high fever and get even worse. In this case, the symptoms will usually be followed by coma and then death occurs within 7–14 days. However, the area also quite a few patients who had recovered but was followed by permanent disability due to brain damage⁴.

Some reports suggest that children and teenagers are prone to this disease. In Thailand, allegedly 40 of 100,000

children to adolescents aged 5–25 years suffering from this disease. In addition, it was reported also that a lot of JE cases occur in rural areas. By all means this case epidemiology in the Northern Vietnam, northern Thailand, Korea, Japan, Taiwan, China, Nepal and northern India more common in summer sat. Within the area of southern Vietnam, southern Thailand, Indonesia, Malaysia, Philippines, Sri Lanka, and southern India, JE cases occur sporadically throughout the year. This disease also has been reported to cause behavioral abnormalities. In some children the clinical symptoms that appeared to be a single seizure, followed by a rapid recovery of consciousness. The symptoms of seizures are a common cause shaking on digits or mouth, eye deviation, nystagmus, excess salivation, or irregular respiration.⁴

In Indonesia the first time in the case of JE in serological report which occur in humans in 1999 in Bali. Examination of serum specimens from 12 patients with clinical diagnosis of viral encephalitis, meningitis or dengue hemorrhagic fever (DHF) found two of them positively infected with Japanese encephalitis.¹¹ JE cases in humans were also reported in some areas, namely in West Sumatra, West Kalimantan, Yogyakarta, Central Java, East Java, West Nusa Tenggara, East Nusa Tenggara and Papua.⁵

A recent report there are even reported cases of JE virus infection in tourists who holiday in Bali. The tourists traveling 3 weeks to Java and Bali, including vacation stricken rural to rural. Last week of March was spent in Bali. After returning home, the patient complained of fatigue and 5 days later he fell ill with numbness in both clengan, and can not use a knife and fork while eating. He also vomited and fell to the floor several times, can not stand by itself. When admitted to the hospital on the same night, the patient was febrile (39.18° C), but in general good condition. The next day he became confused and do not understand simple questions or instructions. Test results show the conditions that lead to the condition encephalitis JE.¹²

Based on the background of the above, the study is to conduct a quick diagnostic on Japanese Encephalitis using PCR techniques among patients in Surabaya, Indonesia. Some above incident, due to the lack of knowledge about the disease is accompanied by a rapid diagnostic examination for checking the disease and So far as JE is a viral disease, then there is no treatment to stop or slow the progression of the virus. Treatment can only be done in a way that is symptomatic relieve symptoms seen each patient. The aims of the study are to get a quick diagnose of Japanese encephalitis virus using PCR techniques, and to be able to diagnose JE virus in Surabaya to treat early-infected patients from JE disease. The method is by using PCR technique (Japanese Encephalitis Virus Detection Kit).

Action is one step vaccination is effective in preventing the disease. Generally, vaccines are given to children to adolescents under 17 years USIS in JE endemic areas. For tourists or travelers visiting endemic areas of JE can also take advantage of this vaccine as a precautionary measure. Another preventive measure is to efforts to control mosquito populations.¹³ and factors are the main

factors for the prevention of outbreaks of JE virus cope in endemic areas.

MATERIALS AND METHODS

The samples are considered as positive when there is a band that emerged in 227 bp. The samples are mainly from whole blood, serum, CSF. The samples are extracted. First PCR conducted starting with reverse transcription reaction cycle in 45° C for 30 minutes, then continue with 30 cycles of denaturation in 94° C for 45 second, annealing process in 72° C for 60 second, extension phase in 72° C for 60 second, and finally 1 cycle of final extension in 72° C for 5 minutes. Second PCR was conducted after the first finish. Starting with pre-denaturation 1 cycle in 94° C for 2 minutes, continue with denaturation 30 cycle in 94° C for 30 second, annealing in 50° C for 30 second, extension in 72° C for 30 second, and final extension 1 cycle in 72° C for 5 minutes. After the PCR process is completed, we continue to electrophoresis with agarose gel for 30 min (110V).

RESULT AND DISCUSSION

There are total 17 patients has been examined in Institute of Tropical Disease (ITD) Universitas Airlangga. 2 person was found positive with JE virus. The PCR result showed positive JE virus.

JE virus was first discovered in Indonesia proved by HA and HI antibody tests. In the tropics, JE virus continues to circulate among mosquitoes, birds and pigs.⁶

The mechanism of transmission of JE virus in humans occurs because of mosquito *Cx. tritaeniorhynchus* were supposed to be zoophilic population becomes heavy or sudden there was an increase of mosquito populations and thus be forced even this mosquito bite humans around him. Also, it can also occur because of the number of pigs suffering from viraemia (virus containing JE) virus became much so that in nature reserves increased and easily transmitted to humans. Age, JE vector, *Culex* mosquitoes, ranged between 14–21 days and *Culex* flight distance can reach more than 3 km. *Culex* generally breed in stagnant water overgrown with plants such as rice fields and irrigation channels, shallow ditches or ponds that are not used. In pigs, viraemia occurs during 2–4 days and is followed by the formation of antibodies in the first period of up to 4 weeks. JE virus can cross the placenta depends on the gestation and JE virus strains. Fetal death and mummification can occur when the JE infection takes place at 40–60 days gestation. While the JE infection after 85 days gestation, abnormalities caused very little. JE incubation period in humans ranges from 4 to 14 days.¹³

Japanese encephalitis virus is quite a new problem in public health, especially in Indonesia as the cases still not many but could be pandemic based on the availability of the vectors of disease. Precaution may be a good way to

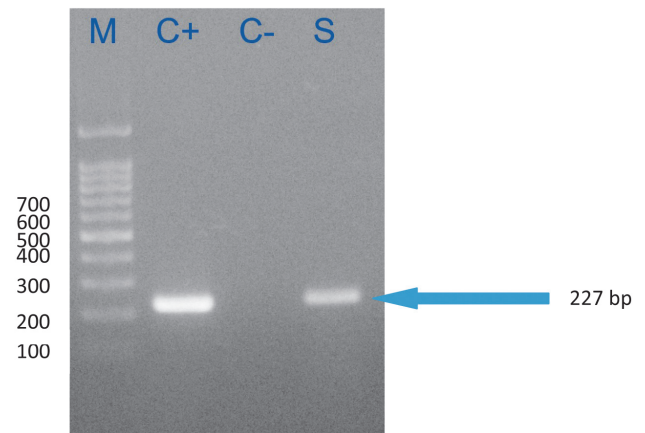


Figure 1. Can be seen as well as the positive control samples are at base pair 227 bp which indicates that the samples was positive JEV.

be applied to both vectors, source of transmission (pigs), human and living environments.

First way is by against the vector (Mosquito), by using insecticides to kill adult mosquitoes and larvae and try to remain the hygiene of water in our home. Second way is against the source of transmission (pig), by giving vaccination to infantile pigs, and make sure that the case of the pigs are surrounded by wire netting, sprayed insecticides, and should be free of mosquitoes. And the third way is prevention to ourself, by getting vaccination. It is an act that should be done 1 month before the time of transmission and addressed to people who have a high risk for getting the infection. Due to the one-month period in an environment of high risk people can take precautions as a whole (the three preventive measures), as well as stop the line of the spread of the virus. Alongside, we could also use mosquito repellent during sleep or during activity.

CONCLUSION

JE disease is a viral disease that is zoonotic and disturbing the public, which causes agents and vectors and animal reservoir potential. The existence of JE disease itself can be seen with the examinee 17 peoples in Surabaya 2 peoples JE disease was detected. Then made an attempt to overcome these problems. Any suggestions with regard to the presence of JE control such depth research on JE in humans in Indonesia in order to know the region spread in Indonesia.

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