

RINGKASAN

Karakterisasi gen G virus rabies strain alam isolat Maros Sulawesi Selatan

Penyakit rabies merupakan salah satu jenis penyakit zoonosis yang menyerang susunan syaraf pusat. Rabies masih dianggap penyakit penting di Indonesia karena bersifat fatal dan dapat menimbulkan kematian serta berdampak psikologis bagi orang yang terpapar. Penyakit rabies tersebar luas di berbagai belahan dunia, termasuk Indonesia. Menurut data *World Health Organization* (WHO) rabies terjadi di 92 negara dan bahkan bersifat endemik di 72 negara. Penyakit rabies ini sangat penting adalah kenyataan bahwa selain bersifat fatal, penyakit ini penyebarannya di Indonesia makin lama cenderung meluas karena ada pulau yang sebelumnya bebas menjadi tertular (Soejoedono, 2005).

Setelah beberapa tahun berlalu sejak awal ditemukannya wabah rabies di Indonesia, telah cukup banyak penelitian penelitian yang dilakukan untuk mengkaji dan mengamati perkembangan virus ini baik di dalam negeri maupun diluar negeri. Masih sedikitnya penelitian yang bersifat berkelanjutan didalam pengamatan perkembangan genomik dari gen G virus rabies ini secara khusus. Penelitian ini memang diarahkan untuk dapat memberikan sedikit tambahan informasi tentang perkembangan genetika dan beberapa hal yang berkaitan dengan gen G virus rabies.

Sampel yang digunakan pada penelitian ini adalah otak anjing yang berasal dari Maros, Sulawesi Selatan. Kontrol negatif berasal dari otak anjing normal dan kontrol positif berasal dari isolat positif rabies. Tahap pertama yang dilakukan dalam penelitian ini adalah isolasi RNA total dari organ otak anjing dengan cara ekstraksi

menggunakan Trizol untuk mendapatkan RNA total virus rabies. Tahap kedua adalah amplifikasi DNA virus. Hasil isolasi diperoleh RNA total untuk selanjutnya disintesis menjadi cDNA menggunakan metode RT-PCR, kemudian cDNA hasil RT-PCR di amplifikasi dengan metode PCR menjadi DNA yang telah dilipatgandakan jumlahnya. Tahap ini menggunakan primer spesifik RG-3F dan RG-AR untuk mendeteksi dan memperoleh gen G. Tahap ketiga adalah sampel hasil PCR disekuensing sehingga dapat diketahui sekuen urutan nukleotida dari gen G virus rabies.

Hasil penelitian ini yaitu setelah diamplifikasi dengan menggunakan primer tertentu terhadap fragmen gen G virus rabies memiliki panjang 210 bp. Berdasarkan tingkat homologi memiliki kesamaan sekuen 93 % dengan isolat 03003INDO asal Indonesia tahun 2003, dan memiliki tingkat homologi sekuen 90,9 % dengan isolat Maros, Sulawesi Selatan tahun 2004.

Berdasarkan hasil penelitian ini dapat ditarik kesimpulan bahwa gambaran *amplicon* (produk PCR) terhadap fragmen gen G virus rabies isolat Maros Sulawesi Selatan dengan menggunakan primer tertentu memiliki panjang 210 bp. Terdapat perbedaan sekuen nukleotida fragmen gen G virus rabies isolat Maros Sulawesi Selatan, dibandingkan dengan virus rabies isolat Indonesia tahun 2003 memiliki tingkat homologi 93 % serta jika dibandingkan dengan virus rabies isolat Maros Sulawesi Selatan tahun 2004 memiliki tingkat homologi 90,9 %.

SUMMARY

The characterization of rabies virus G gene Natural Strain Isolate Maros, South Sulawesi.

Rabies is a kind of zoonosis disease which attacks central nervous system. Rabies is still considered as a common disease in Indonesia since it is fatal and deadly, and psychologically affects the infectious people. Rabies spreads throughout the world, including Indonesia. Based on the data from World Health Organization (WHO), rabies has contaminated 92 countries, and even it is endemic in 72 countries. This disease is serious because not only it is fatal but also the spread tends to expand, from an untainted isle to contaminated one.

A few years after the finding of rabies occurrence in Indonesia, many researches have been conducted to study and observe the virus growth either inside or outside the country. Not many continuing researches are exclusively done in the field of the observation on the genomic growth from G gene. This research is expected to give a piece of information about the genetic development and those related to the G gene of rabies virus.

In this research, the sample used is the brain of dog from Maros, South Sulawesi. The negative control from the brain of normal dog and the positive control from the positive isolate rabies. The first step in this research is the total RNA isolation from the organ of dog brain by extracting with Trizol to elicit the total RNA of rabies virus. The second step is the amplification of DNA virus. The result of isolation gained is the RNA total and is synthesized into cDNA using RT-PCR

method, and the cDNA from the result of RT-PCR is amplified using PCR method into DNA which has been doubled. This step uses specific primer of RG-3F and RG-AR to detect and gain G gene. The third step is the sample of PCR sequenced so that the series of nucleotides from G gene of rabies virus.

The result of this research which has been amplified by using certain primer toward the fragment of G-gene of rabies virus can reach 210 bp in length. Based on the homology level, the sequence has the similarity of 93% with isolate 03003INDO of Indonesia in 2003, and has the sequenced homology level of 90,9% with isolate in Maros, South Sulawesi in 2004.

Based on this research, it can be concluded that the description of amplicon (PCR product) toward the fragment of G gene of rabies virus isolate Maros, South Sulawesi by using certain primer which has 210 bp in length. There is a different sequence of nucleotide fragment of G gen rabies virus isolate Maros, South Sulawesi, compared to rabies virus isolate Indonesia in 2003 which had the homology level of 93%, and if it is compared to the rabies virus isolate Maros, South Sulawesi in 2004 which had the homology level of 90,9%.

ABSTRACT

The characterization of rabies virus G gene natural strain isolate Maros, South Sulawesi.

Rabies is one of zoonosis diseases which keep on terrorizing the tranquility of Indonesian people, and the disease is endemic. Rabies is caused by Rhabdovirus which reaches CNS through periphery nervous. This research is aimed at knowing how the sequenced description of nucleotide fragment of G gene rabies virus isolate Maros, South Sulawesi, and knowing whether there are any differences in the sequenced nucleotide fragment of G gene rabies virus isolate Maros, South Sulawesi. The sample was taken from the contaminated dog brain, and the screening was done after the FAT test in Maros, South Sulawesi. The total of RNA extraction was done by using Trizol and amplification fragment of G gene with primer RG-3F (nt 3984-4011) and RG-AR (nt 4165-4194) by using thermal cycler. The cDNA synthesis was done by using RT-PCR bead with denaturation at 94°C 2 minutes and was incubated at 42°C for 90 minutes. The result of RT-PCR was then done to amplify the DNA with early denaturation at 95°C for 5 minutes and followed with denaturation 94°C for 45 seconds, annealing 45°C for 45 seconds, extension 72°C for 10 minutes. The result of the research shows that primer RG-3F and RG-AR can amplify the fragment of G gene rabies virus isolate Sulawesi from the dog in South Sulawesi. Based on the result of this research, it can be concluded that the description of amplicon (PCR product) toward the fragment of G gene rabies virus isolate Maros, South Sulawesi by using certain primer which has 210 bp in length. There are sequenced nucleotide differences of fragment of G gene rabies virus isolate Maros, South Sulawesi, compared to rabies virus isolate Indonesia in 2003 which had the homology level of 93%, and if it is compared to rabies virus isolate Maros, South Sulawesi in 2004 which had the homology level of 90,9%.

Key Words : Rabies, glycoprotein gene, PCR, Sequencing