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THE SEQUENCING OF RABIES VIRUS G GENE ISOLATE MAROS, SOUTH CELEBES

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ABSTRACT

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 attacks the central nervous system. This research is aimed at knowing how the sequenced description of nucleotide fragment of G gene rabies virus isolate Maros, South Celebes, and knowing whether there are any differences in the sequenced nucleotide fragment of G gene rabies virus isolate Maros, South Celebes. The sample was taken from the contaminated dog brain, and the screening was done after the FAT test in Maros, South Celebes. The total of RNA extraction was done by using Trizol and amplification fragment of G gen with primer RG-3F (nt 3984-4011) and RG-AR (nt 4165-4194) by using thermal cycler. The result of the research shows that primer RG-3F and RG-AR can amplify the fragment of G gen rabies virus isolate Maros, South Celebes from the dog. Based on the result of this research, it can be concluded that the description of sequenced nucleotide differences of fragment of G gene rabies virus isolate Maros, South Celebes, 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 Celebes in 2004 which had the homology level of 90,9%.

Keywords: rabies, gen glycoprotein, sequencing

INTRODUCTION

Rabies is an acute infectious disease of the central nervous system caused by rabies virus. Rabies is also called hydrophobia. Rabies comes from the Latin "rabere" that have a sense of anger or in other words have a hot-tempered nature, "rabere" also comes from the previous language of Sanskrit language "rabhas" significant violence. The Greeks adopted the word "Lyssa" which also means "madness". Rabies is a symbol for a disease that attacks the dog and make the dog like crazy (mad Dog) (Wilkinson, 2002).

According to data from the World Health Organization (2008) Rabies occurs in 92 countries and even is endemic in 72 countries. Another thing that makes rabies is very important is the fact that in addition are fatal, the disease is spreading in Indonesia, the longer tend to spread because there are islands that were previously free become infected.

Rabies infected areas which had been only a few provinces only, has expanded into other areas previously free are: West Sumatera, Central Java and East Java (1953), North Sumatra and North Sulawesi (1956), South Sulawesi (1958), South Sumatra (1959), Lampung (1969), Aceh (1970), Jambi and Yogyakarta (1971), Bengkulu, Jakarta and Central Sulawesi (1972), East Kalimantan (1974) and Riau (1975). Recently Rabies continues to spread to areas previously free become infected with the island of Flores (1998) The island of Ambon and Seram Island (2003), Halmahera and Morotai (2005) Ketapang (2005) and Buru Island (2006) and the island of Bali, Island Bengkalis and

Rupat Island in Riau Province (2009). Currently, rabies-free province of Riau Islands Province, Bangka Belitung, West Nusa Tenggara, Papua and West Papua (Soejoedono, 2005).

According to Fenner et al., (1993) that the virus has great genetic diversity. genetic diversity occurs through natural selection acting on the viral genome that change continuously as a result of mutation, recombination or merging back. In other cases, mutations that affect the virion surface epitopes of proteins very frequently occurs when the virus replicates in the presence of antibodies in the host body.

Nucleotide differences can occur because of the influence of time and geographic area. The use of PCR method based on gene G has been done by many researchers, especially for diagnostic purposes because it is one of the rare gene mutations. In 2003, Bourhy et al., Successful amplification of rabies virus G genes derived from brain 03003INDO dog isolates from Indonesia, by PCR (Polymerase Chain Reaction) (Bourhy et al., 2008).

Based on this background that encourages researchers conducted a study to detect rabies virus G gene fragment in Maros, South Sulawesi with primers RG-3F and RG-AR. There was little research that is both sustainable in the observation of the development of genomic gen rabies virus G is specifically, and therefore this study aimed to provide little additional information about the development of genetics and some aspects related with the gene G rabies virus.

MATERIAL AND METHODS

This study is a non-experimental descriptive. Descriptive aims to identify characteristic gene fragment G. Molecular characteristics observed by rabies virus protein preparation, amplification and sequencing the gene fragment G nucleotide. Research carried out for three months, starting August 1, 2010 to December 15, 2010, at the ex Laboratory of Virology and Immunology Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Airlangga University, Laboratory of Molecular Biology, Faculty of Veterinary Medicine, Airlangga University and conducted at the Centre Veterinaria Farma (PUSVETMA).

Sample

The sample of brain research is a dog infected with rabies virus and have been tested positive by fluorescent antibody test technique (FAT) and Sellers. The samples originated from Central Veterinary (BBVet) Maros, South Sulawesi, which was obtained through Dr. Suwarno drh., M.Sc. Samples of 2 samples and for comparison purposes that is not normal dog brains infected with rabies virus as much as 1 sample and the brain as a negative control dog rabies virus infected as many as 1 sample as positive control, bringing the total sample consists of 4 samples. Positive control derived from isolates that were isolated by Dr Sulawesi. Suwarno, drh., M. Si in 2004.

Materials Research

Materials used in this study is Primary gene G (RG-3F and RG-AR), agarose, ethidium bromide, TAE buffer, DNA markers, loading buffer, buffer NT, Nucleospin @ Extract II Column, buffer NT3, Big dye Terminator X Purification kit.

Research Tools

The tools used in this study were pipette pastuer, micropippet, Eppendorf tubes, measuring cups, cork shelf, laminar flow hood, pipettes, microcentrifuge

(Beckman), translluminator-UV, gel electrophoresis apparatus (Bio-Rad), vortex, Centrifugasi, agarose electrophoresis apparatus (Bio-Rad), white tip, blue tip, yellow tip, mortar and penggerusnya, bulb, tube erlenmayer, tape, Machine Sequencer AB Applied Biosystem 3130 Genetic Analyser HITACHI,.

Synthesis of cDNA by RT-PCR

Synthesis of cDNA was performed using RT-PCR beads. RNA was isolated disentrifuge for 15 minutes at a temperature of 40C with a speed of 12,000 rpm and supernatant removed and dried for 10 minutes. Solution is then added with Nuclease Free Water (NFW) 10-100µl. A total of 5µl RNA, 2µl primers RN-3 and 43µl Nuclease Free Water (NFW) is inserted into the RT-PCR bead so that the total volume of 50µl. RT-PCR beads in-spindown and then incorporated into the PCR thermocycler with denaturation at 940C 2 minutes and incubated with a temperature of 420C for 90 minutes. Results RT-PCR and then inserted into the ice and stored at-200C until used again.

DNA Amplification

Results cDNA synthesis was then performed DNA amplification (PCR) using Ready to Go PCR kit (Amersham). Bead PCR coupled with 5 µl RT-PCR product, 2 µl l primer (20 pmol) primer RG-3F, 2 µl RG-AR primers, 16 µl NFW, so the total volume of 25 µl. Bead-spindown and then inserted the PCR machine with initial denaturation of 95 °C 2 min and denaturation followed by 94 °C for 1 minute, anealing 55 °C 2 min, extension 72 °C final extension 7 minutes and 72 °C for 10 minutes, stage 2 to 4 performed by 40 cycles. The PCR product was then inserted into the ice and stored at -20 °C until use (Suwarno, 2005). Selection of primers for amplification of cDNA by PCR method are selected based on research Suwarno (2005).

Table 1. Primer Used for Amplification of Gene G

Name	Sequence	Location
RG-3F	5′-cctgcagrcttgcggatttgttgacg -3′	3984 - 4011
RG-AR	5′-caacaaggtgctcaatttcgtctgagcgaa -3′	4165 - 4194

Sequencing

Sequencing is done by machine AB Applied Biosystem Sequencer 3130 Genetic Analyser automated HITACHI using big dye terminator X TM Solution TM kit and SAM Solution (Applied Biosytems). Vortex first Big Dye Terminator X until homogeneous, then enter the SAM Sol 4.5 times the volume cycle sequencing into the well and enter the Big Dye Terminator X once the volume of the cycle sequencing so that the total volume of 20 µl. Vortex for 30 minutes and centrifuged 1000 g / RCF for 2 minutes. Then on the move into the plate and inserted into the machine with a program sequencer 96 °C 1 min as initial denaturation, then 96 °C and 10 seconds, 50 °C 5 seconds and 60 °C 4 min by 25 cycles. Results of sequence then the machine-loading in the sequencer and the results read by the monitor in graphical form alogram (Sambrook and Russell., 2001).

RESULTS AND DISCUSSION

Amplification of rabies virus G gene DNA

The PCR amplification process that converts cDNA into rabies virus DNA using primers RG and RG-AR-3F able to produce the G gene fragment with a length of 210 bp. After electrophoresis and read under ultraviolet light with a wavelength of 302nm, length amplicon from the G gene fragment.

The success of this research is based on the suitability of efficiency and optimization of primers and PCR process. Non-specific primers can cause teramplifikasinya other regions in the genome that are not targeted or otherwise does not exist of amplified genomic regions. Optimization of PCR is also required to produce the desired character. This optimization involves denaturation and annealing temperature of DNA in the PCR machine. Low denaturation temperature can cause double strand DNA is not open so it is not possible occurrence of new DNA polymerization. Primer annealing process on the pieces of DNA that have been opened requiring optimum temperature, because the temperature is too high can lead to amplification or vice versa does not happen too low temperatures cause the primer attached to the other side of the genome that are not side homologny; consequently to many areas not specifically amplified in the genome them. Annealing temperature (annealing) is determined based on the primers used are influenced by the length and composition of the primary.

PCR products were observed by gel electrophoresis using agarose gel or polyacrylamide gel and observed by UV-transiluminator. The amplified gene fragment nucleotide length G generate 210 bp. The use of primers 3F and RG-RG-AR is very compatible with the G gene nucleotide sequences of natural strains of the virus reaction from Sulawesi.

G protein-coding genes in charge of attaching the virus to the host cell membrane (Conzelmann et al., 1999). G protein-coding genes also serves to induce virus neutralizing antibody (VNA) and the protection of experimental animals were injected in intraceberal Rabies virus (Fenner et al., 1993).

Rabies virus G gene sequencing

Results of PCR to further sequenced by using the RG-AR that can be known nucleotide sequence of rabies virus G gene. The results of sequencing of samples with the code 2307 from Maros, Sulawesi, with referrals coming from 03003INDO isolates from Indonesia which was isolated in 2003 by Bourhy et al. (2008) and positive control. Originated from Sulawesi isolates isolated in 2004 by Suwarno (2005) in his dissertation.

Results PCR for subsequent sequencing using RG-AR primers that can be known nucleotide sequence of rabies virus G gene. The reading of the results of electrophoresis bands starting from the farthest. This tape shows the size of the smallest DNA and is a chain that ends with the incorporation of dNTPs in the first position of the mold, for example is on line A, the first nucleotide in sequence A. The next tape that moves the furthest after the first band will be in accordance with the DNA molecule one nucleotide longer than the first tape, for example the path T, then up to this stage of the sequence that forms the AT. So forth so that the whole can be read (Sambrook el al., 2001).

In this study, the nucleotide sequence of the gene fragment sequencing G at nt position 3984 - 4194 (210 nucleotides) of natural strains from Maros, South Sulawesi have mutations compared to isolates from Indonesia by Bourhy 03003INDO et al., In

2003. Sequencing study of 2010 isolates from Maros, South Sulawesi, this shows the level of 90.9% homology with Positive Controls are isolated by Suwarno of 2004 isolates from Sulawesi. When compared with the referent of 03003INDO isolates from Indonesia in 2003, these sequencing results showed 93% homology level. If the Positive Control is isolated by Suwarno isolates from Sulawesi in 2004 compared with the referent 03003INDO isolates from Indonesia in 2003 showed 94.3% homology level.

In this study sequence base sequence of N base there sekuencing result of nucleotide bases (or nucleobases refers to the section on DNA and RNA that may be involved in base pairing, mainly cytosine, guanine, adenine (DNA and RNA), thymine (DNA) and uracil (RNA), respectively abbreviated C, G, A, T, and U. In genetics, nucleotide bases are usually only referred to as a base or bases N (N stands for nitrogen, because it has a beratom amine group nitrogen). Since A, G, C, and T appear in the DNA, these molecules dsebut DNA bases, while the A, G, C, and U is called RNA bases.

Table 2. Sequence of rabies virus G gene homology

	812	822	832	842	852
Referen	caccatttgg	Tctcatctga	cgtctgaagt	gcaacccatg	ttccatccat
Kontrol+	**********a	**********	t********t*	**g********	****g*****
Sample 2.507	**********	*t**g*****	**********	**********	**********
Sample 5.452	NgaNN***NN	aNNtgcaacN	*a*gNtNcaN	T*cNtaaN*c	*aaNNcNNNN
	862	872	882	892	902
Referen	aagtctaagt	Ccaagaaccc	cacatagctt	cagcttgcat	gccccttta
Kontrol+	**********	**t*******	**********	**********	**t*******
Sample 2.507	g**c******	*****g****	**********	*********c	**********
Sample 5.452	acccNNNNNN	N*ctt*g*tt	gcatgcccct	tttaga*ac*	tgta*aagcc
	912	922	932	942	952
Referen	gagacttgta	Caagcctctt	tcatctacaa	atccacaagg	cctgaagg
Kontrol+	**********	**********	**********	**********	********
Sample 2.507	**********	**********	**g**a****	****g*****	********
Sample 5.452	tcttcgtc**	ac*aatc*gc	aag***g**gg	gNNNtNNNNa	aNcN***N

(*) Indicates there are similarities with the referent.

This research is used referen Rabies virus isolates 03003INDO isolated from dogs in 2003 in Indonesia by Bourhy et al., The result of this southern Sulawesi, Maros isolates showed 93% homology compared to the referent isolate 03003INDO 2003. Mutations occur with a change in just a few nucleotides so-called point mutation or point mutation. In the 812 nucleotide sequence of nucleotides seemingly referen c t replace nucleotides in the results of this research. And nucleotide sequence of the 815 looks a substitute nucleotide g. Nucleotide sequence of the 851 looks a substitute nucleotide g. to 854 nucleotide sequence shown replaces nucleotides c. t Nucleotide sequence of the 866 looks a substitute nucleotide g. To 890 nucleotide sequence shown

replaces nucleotides c. t Nucleotide sequence of the 923 looks a substitute nucleotide g. To 926 nucleotide sequence shown replaces nucleotides a. t A 935 nucleotide sequence shown replaces nucleotides g of the results of this research.

Positive control of this research comes from Sulawesi in 2004 isolates were isolated by Suwarno (2005). From the results of the study compared with positive control shows the difference of a few nucleotides. It also demonstrates the existence of point mutations from isolates Sulawesi in 2004 as a positive control compared with the results of the study of 2010 isolates of Sulawesi.

Sequencing isolates Maros South Sulawesi in 2010 has a kinship with rabies virus isolates 03003INDO isolated from dogs in Indonesia by Bourhy et al., (2008). As shown in Table 5.1 Sequence of rabies virus G gene homology follows:

Result sequence shown in table 5.2 above shows the homology between isolates of the study sample Maros, South Sulawesi, compared with the referent isolate that was isolated from Indonesia 03003INDO Bourhy et al., In 2003. and compared with positive control derived from isolates that were isolated by Suwarno Sulawesi in 2004. the percentage of nucleotide homology of rabies virus G gene shown in Table 5.3 below:

Table 3. Percentage of Homology Nucleotide rabies virus G gene.

	Referen	Kontrol Positif	Sampel
Referen	-	94,3 %	93 %
Kontrol Positif	-	-	90.9 %

CONCLUSION

Based on the results of this study can be concluded that there are differences in nucleotide sequence fragment of the G gene of rabies virus isolates Maros South Sulawesi, compared with rabies virus isolates Indonesia in 2003 has a high homology of 93% and when compared with the rabies virus isolates Maros, South Sulawesi in 2004 had 90.9% homology level..

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