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Epitopes Prediction According to Glycoprotein Encoding Gene of Rabies Virus Local Isolates as Vaccine Candidate against Circulating Rabies Virus in Indonesia

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Abstract

Rabies is one of the zoonotic disease which exist in Indonesia. Vaccination using conventional seed is not capable to reduce the morbidity of rabies due to the unmatching between seed vaccine compared to circulating Rabies virus. Nine infected dog brain were collected from Sumatera, Sulawesi and Bali. They were processed into RT-PCR according to Glycoprotein encoding gene. Positive samples were processed into sequencing and molecular analysis. Epitopes of T and B cells were predicted. Two samples that were isolated from Sumatera showed high number of epitopes than others.

Key words : *RABV, Epitope predictions, peptide vaccination.*

Rabies disease is a zoonotic disease which is still emerging in Indonesia. It is caused by virus belonging to genus Lyssavirus from Rhabdoviridae family called RABV (MacLachlan *et al.*, 2011; Singh *et al.* 2017). RABV has an envelope protein called Glycoprotein (G) causing pathogenicity and induce host specific immunity (Singh *et al.*, *loc cit*). Vaccination using conventional seed has been done to control Rabies, but it still occurred in the field due to unmatching between seed vaccine compared to circulating virus (Susetya *et al.*, 2005).Vaccine based epitopes is usually used because it is capable to induce immune system, cheaper and decreasing the possibility of allergic response (Ahmed *et al.*, 2017).

Materials and Methods

Nine samples were collected from Sulawesi (RABV C3, RABV C4 and RABV C9), Bali (RABV 148, RABV 285, RABV 382), and Sumatera (RABV 391, RABV 438, RABV 533). RNA of the samples was extracted. They were processed into one-step RT-PCR using primer which amplify gene encoding glycoprotein (Table I). Annealing was used to amplify the samples at 50°C. The RT-PCR was run for 35 cycles. They were visualized through agarose gel electrophoresis. DNA ladder used was Azura PureViewTM 250bp DNA Ladder (Azura Genomics Inc.). Positive samples processed into sequencing. Prediction of T and B Lymphocyte cells were done using CD4 Immunogenecity Tools and Kolaskar-Tongaonkar.

Table I. Sequence of primer used for One-Step RT-PCR. The primer amplify gene encoding Glycoprotein (Yang *et al.*, 2011).

Primer	Nucleotide Sequences (5'-3')	Position	Sense	Primer Position
RVG1F	ATGGTTCTTCAGGCTCTCCTGTTTGT	3317-3342	+	28-53
RVG1R	GACTGACTTGTAGTGAGCATCGGC	4346-4369	-	1057-1080

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T and B cells epitopes prediction

Table II. Epitope prediction of RABVI_533.

	T cell epitope prediction				B cell epitope prediction		
No	**Peptide**	**Start**	**End**	**No.**	**Peptide**	**Start**	**End**
1	MVLQALLFVTFQPKT	1	15	1	KQKPTPTLL	23	31
2	LLFVTFQPKTDLLVQ	6	20	2	PRLRESTSARRQMH	36	49
3	DLLVQVQKQKPTPTL	16	30	3	EPHI	51	54
4	PTPTLLAMSPPRLRE	26	40	4	GMKSPCT	63	69
5	PRLRESTSARRQMHA	36	50	5	ELRPPRSPLS	79	88
6	GRWLVTPGMKSPCTI	56	70	6	Q	92	92
7	PLSSYLQASQSWTRT	86	100	7	QSWTR	95	99
8	LQASQSWTRTINPFT	91	105	8	NPF	102	104
9	SWTRTINPFTRESFL	96	110	9	NAQEQCHPPTALLTTTTPSG	113	132
10	SGCLKTLDWGLRVMS	131	145	10	SRGKRASKGSKTRGFV	152	167
11	CDIFTNSRGKRASKG	146	160	11	TSDETKWCS	202	210
12	GLYKSLKGACKLKLC	171	185	12	CLD	240	242
13	KLKLCGVLGLRLMDG	181	195	13	HYK	246	248
14	GVLGLRLMDGTWVAL	186	200				
15	PDQLVNLHDFHSDEI	211	225				

Table III. Epitope prediction of RABVI_438.

T cell epitope prediction				B cell epitope prediction			
No	**Peptide**	**Start**	**End**	**No.**	**Peptide**	**Start**	**End**
1	MVLQALLFVTSQPKT	1	15	1	QPKT	12	15
2	LLFVTSQPKTDLLVQ	6	20	2	KQKPTPTLL	23	31
3	DLLVQVQKQKPTPTL	16	30	3	PRLRESTSARRQMH	36	49
4	PTPTLLAMSPPRLRE	26	40	4	EPHI	51	54
5	PRLRESTSARRQMHA	36	50	5	GMKSPCT	63	69
6	GRWLVTPGMKSPCTI	56	70	6	ELRPPRSPLS	79	88
7	PLSSYLQASQSWTRT	86	100	7	Q	92	92
8	LQASQSWTRTINPFT	91	105	8	QSWTR	95	99
9	SWTRTINPFTRESFL	96	110	9	NPF	102	104
10	SGCLKTLDWGLRVMS	131	145	10	NAQEQCHPPTALLTTTTPSG	113	132
11	CDIFTNSRGKRAFMV	146	160	11	RG	153	154
12	RAFMVPQTLRFVDER	156	170	12	TSDETKWCS	202	210
13	PQTLRFVDERGLYKS	161	175	13	CLD	240	242
14	GLYKSLKGACKLKLC	171	185	14	HYK	246	248
15	KLKLCGVLGLRLMDG	181	195				
16	GVLGLRLMDGTWVAL	186	200				
17	PDQLVNLHDFHSDEI	211	225				

Reverse-Transcriptase Polymerase Chain Reaction

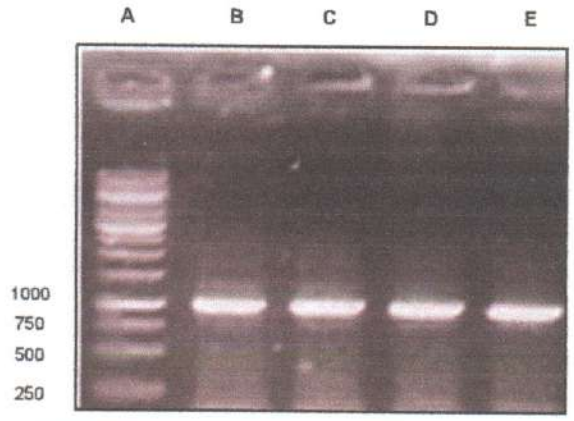

Fig 1. Visualization of product of RT-PCR under ultraviolet 302 nm. A as DNA Marker; B as positive control; C as as RABVI_533; D as RABVI_391; E as RABVI_

Results and Discussion

According to epitope predictions, samples that had high number of epitope were RABVI_533 and RABVI_438. They were isolated from Sumatera. T and B cells epitope that can be predicted from RABVI_533 was 15 and 13 respectively, while T and B cells epitope that can be predicted from RABVI_438 were 17 and 14, respectively. Epitopes are part of antigen which are capable to induce immunity of the host against antigen infection. The number of epitopes used in vaccine influence the protection rate of the host. Peptide based vaccine has many advantages such as reducing the possibility of allergy caused by unimmunogenicprotein or chemical compound in adjuvant (Ahmed *et al., loc cit*). It only uses part of pathogen capable to trigger activation of immunity (Reche *et al.*, 2015; Skwarczynski and Tolh 2016). Recently, local isolate is often used to control morbidity of several viral diseases such as Newcastle Disease (ND) (Dharmayanti *et al.*, 2014). Use of local isolate as vaccine candidate is a strategy to control Rabies disease in Indonesia (Susetya *et al., loc. cit*). According to the number of predicted epitopes, both samples RABVI_533 and RABVI_438 can be used as vaccine candidate. It needs further research to understand the effect of both candidate to induce specific immune system against circulating RABV, in Indonesia.

Summary

Rabies disease is an emerging disease in Indonesia. This study was done to predict the possibility of epitopes which appeared from RABV Indonesia isolate. Samples which showed high number of predicted epitope can be used as vaccine candidate against Rabies disease.

References

Ahmed, O.H., A. Abdelhalim, S. Obi, K.A.A. Elrahman, A. Hamdi, and M.A. Hassan.(2017) Immunoinformatic Approach for Epitope-Based Peptide Vaccine against Lagos Rabies Virus Glycoprotein G. *Immuno Res.***13(3).**

Dharmayanti, N.I., R. Hartawan, D.A. Hewajuli and R. Indriani. (2014) Phylogenic Analysis of Genotype VII of New Castle Diseases Virus in Indonesia*Afr. J. Microbiol. Res.***8(13)** : 1368 – 1374.

MacLachlan, N.J. and Dubois, E.J. (2011) Elsevier United Kindom : pp 324-331.

Reche, P., D.R. Flower, M. Fridskis-Hareli, and Y. Hosino. (2015) Peptide-Based Immunotherapeutics and Vaccines.*J IMMUNOL RES.*Article ID 349049

Singh, R., K. P. Singh, S. Cherian, M. Saminathan, S. Kapoor, G. B. M. Reddy, S. Panda, and K. Dhama. (2017) Rabies – epidemiology, pathogenesis, public health concerns and advances in diagnosis and control: a comprehensive review. *Vet Q.***37(1)** : 212–251.

Skwarczynski, M. and I. Toth.(2016) Peptide-based synthetic vaccines.*Chem Sci.***7** : 842-854.

Susetya, H.I. Naoto, M. Sugiyama, and N. Minamoto. (2005) Genetic Analysis of Glycoprotein Gene of Indonesian Rabies Virus.*Indones J Biotechnol.***10 (1)** : 795-800.