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**Author(s):** Sarvan Kumar Guguloth, Narendar Malothu, Umasankar Kulandaivelu, Koteswar Rao GSN, Anka Rao Areti, Sadhana Noothi  
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**Author(s):** Amaq Fadholly, Arif N. M. Ansori, Viol D. Kharisma, Jola Rahmahani, Martia R. Tacharina  
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**RESEARCH ARTICLE**

## **Immunobioinformatics of Rabies Virus in Various Countries of Asia: Glycoprotein Gene**

**Amaq Fadholly<sup>1</sup>, Arif N. M. Ansori<sup>1</sup>, Viol D. Kharisma<sup>2</sup>, Jola Rahmahani<sup>3</sup>,  
Martia R. Tacharina<sup>3\*</sup>**

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### **ABSTRACT:**

The purpose of this study was to analyze the genomic information of the glycoprotein gene of rabies virus (RABV) in several countries of Asia. The genomics information of vaccine virus strain and some local isolates were retrieved from GeneBank<sup>®</sup> (National Center for Biotechnology Information, USA). Ten genomics informations are consist of ERA vaccine virus, two viruses from Indonesia, a virus from Myanmar, India, Nepal, South Korea, Japan, Sri Lanka, and Iran. This bioinformatics process the ten genomics data in order to find pattern in 1,575 bp of glycoprotein gene of RABV. In conclusion, this study have revealed the data of homology, B-cell epitope prediction, genetic distance, and molecular phylogenetic analysis of RABV in various countries of Asia. We indicated that the glycoprotein, the immunogenic surface protein of RABV, has important part for vaccine design.

**KEYWORDS:** Bioinformatics, *Lyssavirus*, glycoprotein, rabies, vaccine.

### **INTRODUCTION:**

Rabies is an ancient lethal zoonotic disease that is caused by *Lyssavirus* called RABV which is a member of Rhabdoviridae. Rabies is invariably fatal in human and mammals for over 4000 years in all across the continents<sup>1</sup>. Dogs are the most important reservoir for rabies and around 99% in human cases of dog bites<sup>2</sup>. Rabies were also detected due to exposure to rabid cats and wildlife. The disease is often reported as a disease of poverty, ignorance in some circumstances and misinformation<sup>3</sup>.

The rabies situation in Asia is characterized by high rate of human mortalities. Only few countries in Asia (Hong Kong, Japan, Malaysia, Singapore, Taiwan) have eradicated rabies through vaccination and stray programs in dogs<sup>4</sup>. World Health Organization (WHO) of South, Southeast, East Asia region stated that there are eleven countries (Bangladesh, Bhutan, India, Indonesia, Korea, Maldives, Myanmar, Nepal, Srilanka, Thailand, Timor-Leste) and eight of them are endemic for rabies. Rabies cases in Indonesia are progressively increase the status from low to medium rabies endemic country. It is emerging problem because previously many islands of Indonesia considered free of rabies<sup>5</sup>. Therefore, a joint project of WHO-OIE-FAO plans to eliminate rabies cases worldwide by the year 2030<sup>6</sup>.

RABV encodes five proteins: the nucleoprotein (N), phosphoprotein (P), matrixprotein (M), glycoprotein (G), and RNA polymerase (L). G protein is a surface protein in RABV that has high immunogenic potency. Hence, G protein is the target protein in rabies vaccine<sup>7</sup>. The attenuated live vaccine strain Evelyn-Rokitnicki-

Abelseth (ERA) and Flury low egg passage vaccine are the most used antigenic properties that estimated to be sufficient to block rabies transmission in the body<sup>8</sup>. The properties can also be used as viral characterization to collect the diversity of *Lyssavirus* genus, enhanced surveillance and sequencing technologies<sup>9</sup>.

However, this study applied bioinformatics analysis to reveal the data of homology, pathotype, B-cell epitope prediction, and genetic distance with phylogenetic tree of RABV in Southeast Asia through a database of isolates in GeneBank<sup>®</sup>. The result of this study can be used as a data reference for vaccine virus design study to eliminate the rabies.

**MATERIAL AND METHODS:**

**Rabies Virus Isolates:**

All isolates were adopted from Gen Bank<sup>®</sup> (National Center of Biotechnology Information, USA) (Table 1.)

**Table 1. Rabies virus isolates**

1	AF406693.2	ERA	2005
2	EU086151.1	Indonesia	2011
3	AB115921.1	Indonesia	2003
4	EU086129.1	Myanmar	2008
5	KR105372.1	India	2015
6	JX944593.1	Nepal	2013
7	DQ076105.1	Korea	2005
8	AB817146.1	Japan	2013
9	EU086156.1	Sri Lanka	2008
10	JX987734.1	Iran	2013

**Nucleotide Sequence Preparation:**

RABV nucleotide sequences (glycoprotein gene: 1575 bp) from all isolates were adopted from GenBank<sup>®</sup> (National Center of Biotechnology Information, USA). Multiple sequence alignment of nucleotide sequences were analyzed with BioEdit vers. 7.

**Sequence Homology:**

The ten isolates of RABV we used in this study were aligned and the homology analysis were identified using Needleman-Wunsch Global Align Nucleotide Sequences of NCBI.

**Prediction of B Cell Epitop and Protective Antigens:**

The antigenic determinant or epitope of whole G protein gene were used IEDB Online Web server. The part of these antigen that binds to a specific antigen receptor on the surface of mamalian B cell were predicted using Vaxi Jen v2.0.

**Genetic Distance and Molecular Phylogenetic Analysis:**

Genetic distanced were analyzed using MEGA X with the Kimura two-parameter method. Molecular phylogenetic were obtained by maximum likelihood as phylogenetic tree using a bootstrap test on 1000 replication with Tamura-Nei substitution model.

**RESULTS AND DISCUSSION:**

The results of homology analysis were count to compared with ERA as vaccine strain. are presented in Table 2. The higher scores of homology showed closer genetic relationship. The isolates of RABV that closest with ERA was isolate of Iran/Middle-East (92%), followed by India/South (89%), Nepal/South (87%), Korea/East (86%), Indonesia/Southeast and Japan/East (84%), Sri Lanka/South (83%). Two isolates from Indonesia actually got mutation or different variance, showed in homology scores that the isolates were not identically 100%. Some of the isolates showed no significant similarity found like Indonesia/Southeast-Nepal/South, Indonesia/ Southeast-Korea/ East, Myanmar/ Southeast-Iran/ Middle-East, and Nepal/South-Japan/ East.

**Table 2. The results of homology analysis of rabies virus using Needleman-Wunsch Global Align Nucleotide Sequences (NCBI, USA).**

Isolates	Homology scores (%)									
	1	2	3	4	5	6	7	8	9	10
<b>ERA (Vaccine)</b>	100									
<b>Indonesia</b>	84	100								
<b>Indonesia</b>	84	97	100							
<b>Myanmar</b>	83	88	88	100						
<b>India</b>	89	85	85	85	100					
<b>Nepal</b>	87	-	-	84	87	100				
<b>Korea</b>	86	-	85	85	87	91	100			
<b>Japan</b>	84	85	85	85	85	-	85	100		
<b>Sri Lanka</b>	83	84	84	84	84	84	84	92	100	
<b>Iran</b>	92	85	85	-	85	88	87	85	84	100

In addition, all protein's sequences of glycoprotein were selected from NCBI website to get conserved region of RABV. We used ten conserved epitopes that have affinity of binding B cell while there are eight epitopes presence antigenic and only two epitopes as non-

antigenic, showed in Table 3. Isolate of Sri Lanka/South has the highest predictive score, 23.96 and 'GNQAGSKQHGLEGTGRKVSVTSQLGKVI' as potential antigenic peptide, followed by Korea/East, 22.91, Indonesia/ Southeast, 21.12 and

‘VNRPKSTQHNLGGTGRKVSVTSQSGKVI’, ‘NRAESIQHSSGETGRKVSVTSQNGR’ as potential antigenic peptide. Both isolates of Myanmar/Southeast and India/South have the same prediction score as non-antigenic while the lowest prediction score was another Indonesia isolate, 11.19 but still as potential antigenic peptide.

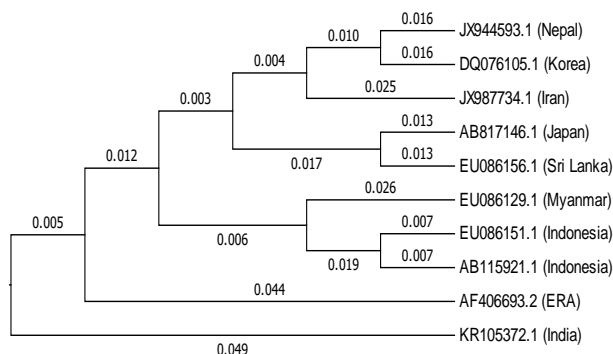
**Table 3. The results of B-cell epitope prediction analysis and antigenicity of rabies virus isolates using Immune Epitope Database and Analysis Resource (IEDB) and VaxiJen v2.0.**

Country	Sequence	Score	Antigenicity
ERA (Vaccine)	KMAGDPRYEESLHNPYPDY	18.01	Antigen
Indonesia	NRAESIQHSSGETGRKVSVTSQNGR	21.12	Antigen
Indonesia	HSLGETGRKVSVTSQNGR	11.19	Antigen
Myanmar	KMAGDPRYEESLHNPYPD	17.45	Non
India	KMAGDPRYEESLHNPYPD	17.45	Non
Nepal	HRPKSTQHGLGGTGRKVSVTSQSGKVI	20.68	Antigen
Korea	VNRPKSTQHNLGGTGRKVSVTSQSGKVI	22.91	Antigen
Japan	NQAGSKQHGLEGAGRKVSVTSQ	17.11	Antigen
Sri Lanka	GNQAGSKQHGLEGTGRKVSVTSQNGKVI	23.96	Antigen
Iran	RSESTQRSLLGGAGRKVSI TSQGGKVI	19.32	Antigen

Identification of B-cell epitopes is high importance for many medical applications including disease control and vaccine development. B-cells can neutralize pathogenic molecules by targeting them with extreme specificity using receptors expressed on their surface or antibodies via interaction between binding site as paratope and a specific region of the antigen as epitope<sup>10</sup>. This identifying is a great interest for understanding disease etiology, immune monitoring, developing diagnosis assay and designing epitope-based vaccines. A good epitope commonly has more than nine peptides<sup>11</sup>. In this case, all isolates have more than nine peptides which is good as an epitope then immunogenicity determined how the peptides become potential antigen or non-antigen.

The results of molecular phylogenetic as genetic distance analysis (*p*-analysis) will be correlated to vaccination results. If the genetic distance of field viruses is quite far from the vaccine virus it will affect the effectiveness of vaccination because the antibodies introduction determined the repeat vaccination program. Isolates from Nepal/South, Korea/East and Iran/Middle-East have close genetic relationship as well as other isolates from Japan/East and Sri Lanka/South. Uncommonly Korea and Japan as country in East Asia got separated group of genetic relationship while Myanmar and Indonesia as country in Southeast Asia got the same group of genetic

relationship. Isolates from India/South was quite far from the other isolates and ERA as vaccine virus still collaterated to other isolates except India/South.



**Figure 1. The results of molecular phylogenetics analysis of rabies virus.**

Phylogenetics is known as a field related to biological sciences that provide data of biological evolution<sup>4</sup>. However, the possibility of mutation or combination of a field virus and vaccine virus should be analyzed used genome sequences of the isolate<sup>12</sup>. To the next study, isolates from Korea, Japan, and India must be carried more than one sequence to compare the result of this study and gain the glycoprotein gene data. On the other hand, previous study revealed the genetic relationship between RABV in Asia based on N gene<sup>13</sup>.

The importance of selecting RABV in this study was based on many contributed high mortality rates to produce rabies vaccine. This data has shown that the establishment of glycoprotein gene of RABV is still needed as antigenic peptide and vaccination against viral diseases is very important for protection, due to the lack of appropriate consuming antiviral drugs and high cost<sup>14</sup>. The challenges associated with rabies vaccines are exciting because they have potential to reveal fundamental features of immune memory against antigenically variable pathogens and prime for vaccine development.

In addition, vaccination is a highly effective approach to disease control in veterinary health care. Vaccine is a molecular or supramolecular agent that is an enhanced adaptive immune response to re-infection by pathogenic agents through the potentiation of immune memory. Vaccination ultimately mitigates the effect of subsequent infection and disease. Thus, the immune system recognizes vaccine agents as foreign, then destroys and remembers them. Furthermore, genomics has revolutionized vaccine development study. The ability to sequence the whole genome of a virulent microorganism has led some to screen *in silico* for the most probable protective antigens before undertaking confirmatory experiments. Apart from obvious advantages, such as



speed and low cost, the success of this approach is dependent on the accuracy of antigen prediction, and many bioinformatics tools are available to facilitate this process<sup>15</sup>.

### CONCLUSION:

In conclusion, vaccine development is an important issue. This study has revealed the data of homology, B cell epitope prediction, genetic distance, and the molecular phylogenetic analysis of RABV in various countries of Asia. Furthermore, the results of glycoprotein gene of RABV are still exist and indicated that this protein is functionally important as a part for another rabies vaccine virus design, antibody cross-reactivity, and *in silico* antibody library screening.

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### CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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