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FULL PAPER

Computational analysis of Indonesian rabies vaccine enhanced by myeloid differentiation factor 88 (Myd88) genetic adjuvant

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Rabies remains a pervasive zoonotic disease in Indonesia, necessitating innovative strategies for vaccine development. This manuscript presents an *in silico* study focused on the construction of a novel plasmid-based DNA vaccine targeting the Indonesian rabies virus (RBV) glycoprotein, augmented with myeloid differentiation factor 88 (MyD88) as a genetic adjuvant. The glycoprotein of the RBV is a key immunogenic target due to its central role in viral entry and infection. MyD88, a crucial component of the innate immune system, has the potential to enhance the efficacy of vaccines through the activation of innate and adaptive immune responses. In this study, we employ computational biology techniques to elucidate the structural and functional interactions between the glycoprotein, MyD88, and host immune receptors, shedding light on the mechanisms by which MyD88 enhances vaccine-induced immunity. By leveraging *in silico* methods, this study contributed to the rational design of a plasmid-based DNA vaccine against rabies, with a particular focus on the Indonesian context. This approach has the potential to significantly reduce the incidence of rabies cases, enhance vaccine efficacy, and pave the way for future experimental validation and clinical trials.

KEYWORDS

Bioinformatics; Indonesia; medicine; rabies virus; vaccine development.

Introduction

Rabies remains a lethal zoonotic disease with a significant public health impact in many parts of the world, including Indonesia. While effective rabies vaccines have been developed and are available, there is a constant need for improvements in vaccine design to enhance efficacy and accessibility. Plasmid-based DNA vaccines have gained considerable attention due to their potential for inducing robust and long-lasting immune responses [1-5]. In the pursuit of enhancing the effectiveness of rabies vaccines, this study focuses on the construction of a novel plasmid-based DNA vaccine targeting the glycoprotein of Indonesian RBV, with myeloid differentiation factor 88 (MyD88) incorporated as a genetic adjuvant.

Rabies is primarily transmitted through the bite of infected animals, with dogs being the most common reservoir in Indonesia. Vaccination of both domestic animals and humans is a critical strategy to control the spread of the virus. The glycoprotein of RBV is a key antigenic target for vaccine development, as it plays a pivotal role in viral entry and is highly immunogenic. To enhance the immunogenicity of this plasmid-based DNA vaccine, we propose the incorporation of MyD88, a crucial innate immune system adaptor protein, as a genetic adjuvant. MyD88 has been demonstrated to activate various signaling pathways, leading to the production of pro-inflammatory cytokines and enhancing the adaptive immune response [6-8].

In silico studies have become indispensable tools in modern vaccine design, allowing researchers to predict and evaluate vaccine candidates before experimental testing. This *in silico* study leverages bioinformatics and computational biology techniques to assess the potential of the plasmid-based DNA vaccine construct. By analyzing the structural interactions between the glycoprotein, MyD88, and host immune receptors, we aim to elucidate the mechanisms by which MyD88 enhances the immune response against the RBV. Furthermore, this study explores the potential for personalized vaccine design by considering genetic variations in the Indonesian RBV, which may influence vaccine efficacy [9-11].

The development of an effective DNA vaccine against RBV, tailored to the Indonesian context, has the potential to significantly reduce the incidence of rabies cases in the region. In addition, the MyD88 inclusion as a genetic adjuvant offers a promising avenue to enhance vaccine-induced immune responses. This study represents a crucial initial step in the rational design of a novel rabies vaccine, providing insights into the structural and functional aspects of the vaccine construct and paving the way for future experimental validation and clinical trials.

Experimental

Data retrieval

Rabies virus (RBV) glycoprotein gene (1575 bp; Accession No.: AB115921.1) (Figure 1) retrieved from National Center for Biotechnology Information (NCBI), USA (https://www.ncbi.nlm.nih.gov/genbank/), and then the protein sequence obtained for further analysis [12]. This isolate was collected from Northern Sumatera, Indonesia and deposited by Susetya *et al.* [13].

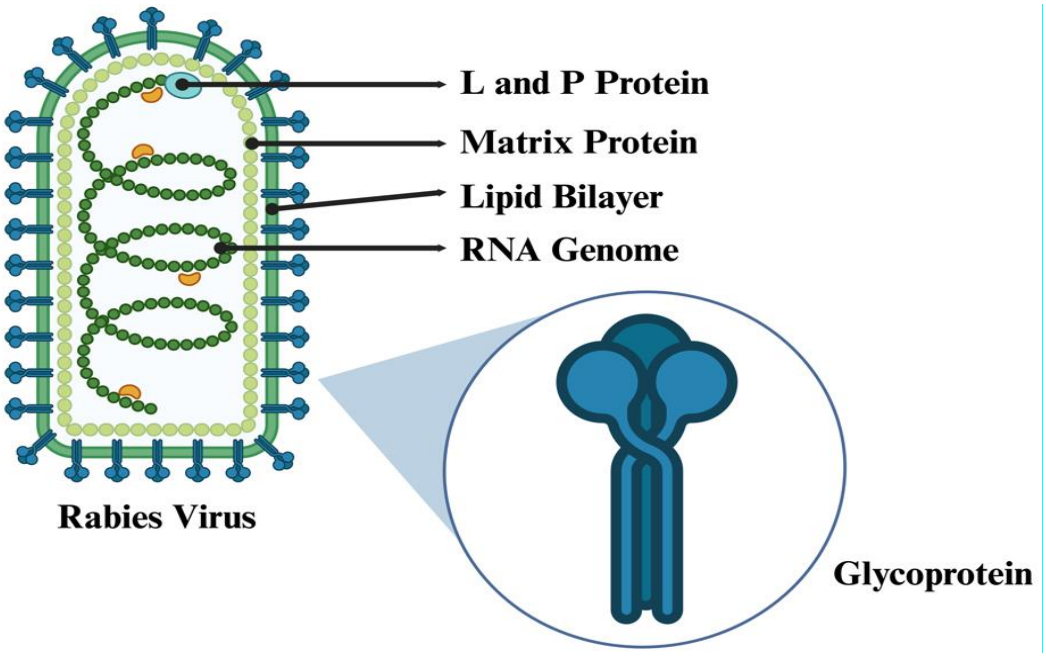


FIGURE 1 Rabies virus (RBV) structure and glycoprotein (blue)

HLA (Human Leucocytes Alleles) mapping

The target population of this study was Indonesia, and using Allele Frequency Net Database (http://www.allelefrequencies.net/) to obtain the alleles which associated in Indonesian. The HLA alleles which will be used are HLA-A*24:02, HLA-A*24:07, HLA-B*15:02, HLA-B*15:13, HLA-DRB1*12:02, and HLA-DRB1*15:0 [14,15].

B-Cell Prediction

B-cell will be predicted from antigen sequences using BepiPred-2.0 webserver (http://www.cbs.dtu.dk/services/BepiPred/). BepiPred-2.0's underlying random forest method was honed using epitopes discovered in antibody-antigen protein structures [16]. BepiPred-2.0's projected B-cell epitope mostly targeted the RBV's glycoprotein area [17].

Cytotoxic T lymphocytes (CTL) prediction

In this section, The CTL (Cytotoxic T Cell Lymphocytes) epitopes will be predicted using data from NetMHCpan-4.1 (https://services.healthtech.dtu.dk/services/NetMHCpan-4.1/). The type major antigens HLA-A*24:02, HLA-A*24:07, HLA-B*15:02, and HLS-B*15:13 were selected for the Indonesian population. Their main antigen will be assessed for toxicity using ToxinPred webserver (http://crdd.osdd.net/raghava/toxinpred/) with threshold 0 using SVM algorithm, allergenicity using AllerTop v2.0 (non alergen is the parameter) (https://www.ddg-pharmfac.net/AllerTOP/), and immunogenicity using VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) with threshold 0.4 [18].

Helper T lymphocytes (HTL) prediction

Each protein sequence generated from the glycoprotein domain of RBV to project the pan-specific binding of the extracted peptides to the acknowledged MHC class II alleles including the HLA alleles was combined to NetMHCIIpan in the previous section. NetMHCIIpan website (https://services.healthtech.dtu.dk/service.php?NetMHCIIpan-4.0) will be employed to predict the HTL. The peptides having a high affinity for the MHC class II alleles will be

moved on to the other website, IEDB Analysis Resource (http://tools.iedb.org/mhcii/), which involved IEDB MHC-II binding prediction [19].

The IEDB recommended 2.22 method was used to predict the peptide. The locus, alleles, and length specifications were all set to "Human, HLA-DR" for the species/locus. The uploaded alleles were DRB*12:02 and DRB*15:02, and the length of the peptides was 15 mers. To remove the strong binders, the obtained results were fed through a filter. The chosen epitopes were those that were carried over to the interferon-gamma-inducing ability projection (IFN) examination. Immunogenicity, allergenicity, toxicity, and conservancy were predicted after the MHC class I prediction. The output of this section still same with the CTL prediction, and positive IFN-gamma [20].

Peptide-protein docking

To conduct peptide-protein docking, the receptor will be used as a target proteins are B-cell receptor (BCR), HLA-A*24:02, HLA-A*24:07, HLA-B*15:02, and HLA-DRB1. While, the ligand will be from shortlisted peptides based on the criteria. The webserver will be executed from HPEPDOCK-2.0 (http://huanglab.phys.hust.edu.cn/hpepdock/), and the output will be the lowest interaction score result [21].

In silico cloning

We utilized the J-CAT tool (https://www.jcat.de/) to analyze codon optimization of vaccine constructs, employing *E. coli* as the source organism [22]. In this study, the expression vector pIRES was selected for cloning, and its nucleotide sequences were obtained from the Addgene vector database (https://www.addgene.org/vector-database/) [23]. Subsequently, *in silico* cloning was conducted using SnapGene v7.0 software (GSL Biotech LLC, California, USA) [24,25].

Results and discussion

Based on Figure 2 and Table 1, it indicates that the epitopes which potentially recognize B cells as antigenic peptides were “YTIPDKLGPWSPIDIH”, “TNFVGYVTTTFKRKHFRPTPD”, and “YNWKMAGDPRYEESLHNPYPDYHWLRTVKT” . While, the average of that B-cell prediction was 0.510, with the minimum of 0.202, and maximum of 0.698. Based on the results, it indicates that still in the threshold range.

Based on those criterias which mentioned in previous section, there are 6 candidates peptides which could be a CTL epitopes (Table 2) to construct RBV vaccine and able to recognize CTL immunogenic peptides, and “FVGYVTTTF” was a potential of epitope which will be interacted with HLA proteins because it mapped with all of alles from MHC class I. HLA-A*24:02, HLA-A*24:07, HLA-B*15:02, and HLA-B*15:13 alleles were the highest frequency in Indonesian population [14].

There are 3 candidates of HTL peptides (Table 3) which potentially can be construct RBV vaccine. It shortlisted based on the requirements, such as positive. “KESLVIISPSVADLD” epitope was mapped to all of HLA-DRB alleles from MHC class II (HLA-DRB1*12:02 and HLA-DRB1*15:02), “ELKVGYISAIKVNGF” was mapped to HLA-DRB1*12:02, and “MELKVGYISAIKVNG” was mapped to HLA-DRB1*15:02. Their epitopes was recognized by antigenic peptides of HTL. The alleles selected in HTL epitopes prediction were the highest frequency in Indonesian population [14].

From Table 4 and Figure 3, the molecular docking results were chosen based on the lowest interaction score which available in HPEPDOCK-2.0 webserver. 3D visualization in Figure 3 described that in different colour in minor represents the peptide, meanwhile the major colour represents the HLA proteins.

Visualization of 3D models were displayed based on the lowest interaction score in B cells, CTL (HLA-A and HLA-B), and HTL Based on the interaction results, "TNFVGYVTTTFKRKHFRPTPD" was the best interaction with B-cell receptor, and potential as B cells which recognize the antigenic peptides. "FVGYVTTTF" was a good interaction with HLA-A*24:02 with the score of -255.94, and HLA-B*15:02 with the score of -215.5, "HNPYPDYHW" had a score of -248.33, which a lowest score when interacted with HLA-A*24:07 protein, and "ELKVGYISAIKVNGF" was a lowest score of interaction which interacted with HLA-DRB1 protein, which was -218.34.

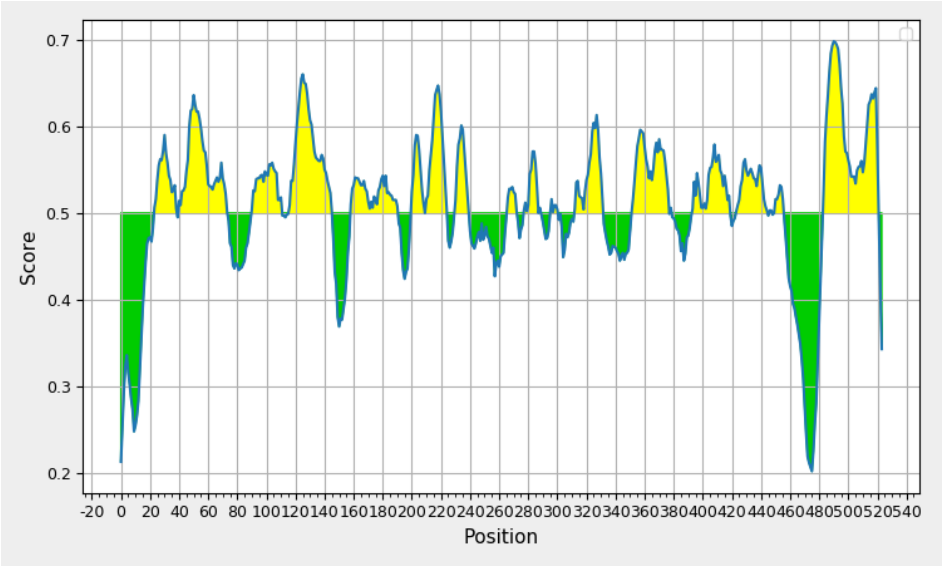


FIGURE 2 B-cell epitopes which represent the threshold of immunogenicity

TABLE 1 Shortlisted B-cell epitopes

Peptides	Length	Immunogenicity	Allergenicity	Toxicity
YTIPDKLGPWSPIDIH	16	1.2415	N	Non-Toxin
TNFVGYVTTTFKRKHFRPTPD	21	1.2007	N	Non-Toxin
YNWKMAGDPRYEESLHNPYPDYHWLRTVKT	30	0.6376	N	Non-Toxin

TABLE 2 Shortlisted CTL epitopes

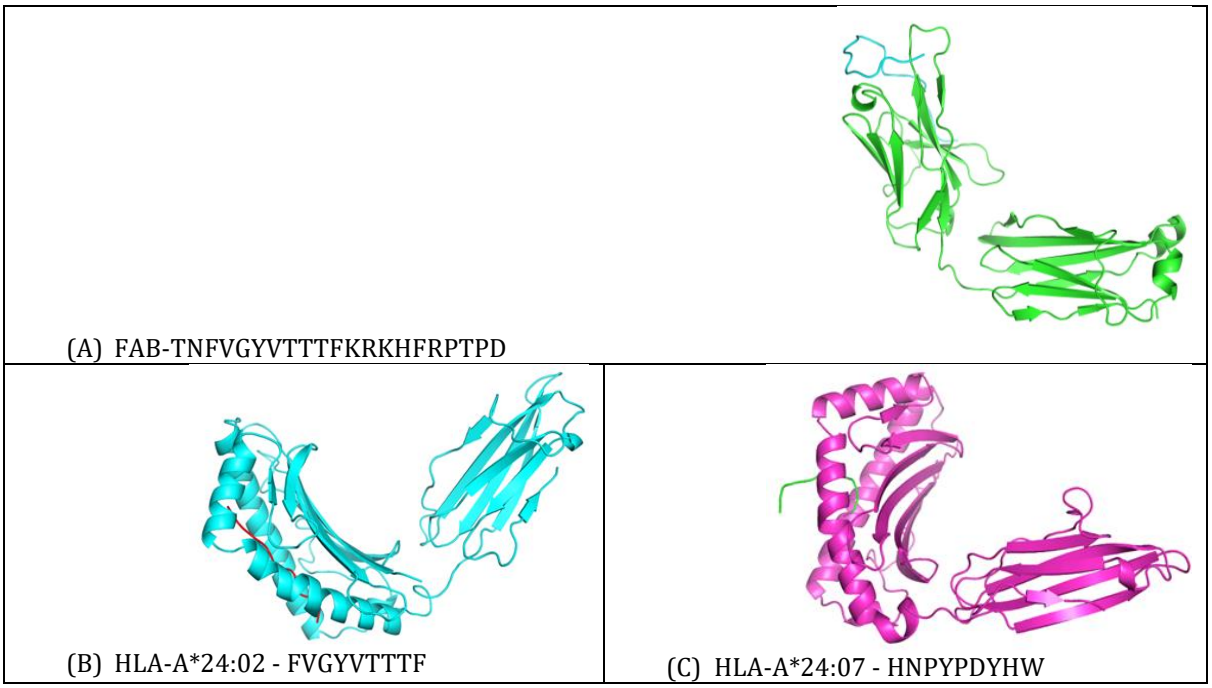
Peptide	Length	Score	Immunogenicity	Allergenicity	Toxicity	Alleles
LMDGTWVAL	9	0.34531	0.9293	N	Non-Toxin	HLA-B*15:02
GFVDERGLY	9	0.20406	0.7808	N	Non-Toxin	HLA-B*15:02
HPHVNGVFF	9	0.18088	0.4732	N	Non-Toxin	HLA-B*15:02, HLA-B*15:13
FVGYVTTTF	9	0.13946	0.7884	N	Non-Toxin	HLA-A*24:02, HLA-A*24:07, HLA-B*15:02, HLA-B*15:13
HNPYPDYHW	9	0.01854	0.9805	N	Non-Toxin	HLA-A*24:02, HLA-B*15:13
GVDLGLPNW	9	0.00546	0.9773	N	Non-Toxin	HLA-B*15:13

TABLE 3 Shortlisted HTL epitopes

Peptide	IFN	Immunogenicity	Allergenicity	Toxicity	Alleles
ELKVGYISAIKVNGF	0.12428592	0.7527	N	Non-Toxin	HLA-DRB1*12:02
KESLVIISPSVADLD	0.22573369	0.8870	N	Non-Toxin	HLA-DRB1*12:02, HLA-DRB1*15:02
MELKVGYISAIKVNG	0.51764118	0.9664	N	Non-Toxin	HLA-DRB1*15:02

TABLE 4 Molecular docking results which obtained from HPEPDOCK-2.0 Webserver

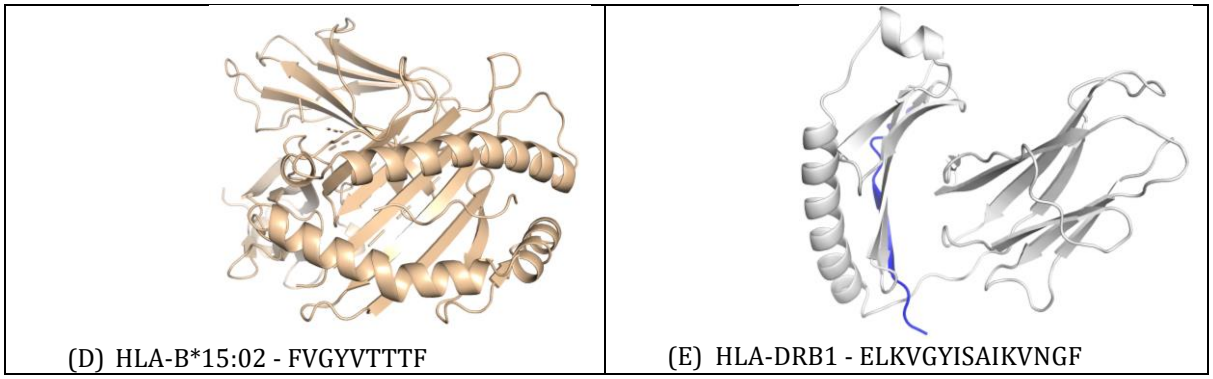
Protein (Receptor)	Epitopes (Ligand)	Score of Interaction
B-cell receptor (BCR)	YTIPDKLGPWSPIDIH	-190.05
B-cell receptor (BCR)	**TNFVGYVTTTFKRKHFRPTPD**	**-231.03**
B-cell receptor (BCR)	YNWKMAGDPRYEESLHNPYPDYHWLRTVKT	-229.19
HLA-A*24:02	**FVGYVTTTF**	**-255.94**
HLA-A*24:07	FVGYVTTTF	-243.08
HLA-A*24:07	**HNPYPDYHW**	**-248.33**
HLA-B*15:02	**FVGYVTTTF**	**-215.59**
HLA-B*15:02	GFVDERGLY	-163.26
HLA-B*15:02	LMDGTWVAL	-175.84
HLA-B*15:02	HPHVNGVFF	-204.3
HLA-DRB1	**ELKVGYISAIKVNGF**	**-218.34**
HLA-DRB1	KESLVIISPSVADLD	-191.12
HLA-DRB1	MELKVGYISAIKVNG	-188.04



(A) FAB-TNFVGYVTTTFKRKHFRPTPD

(B) HLA-A*24:02 - FVGYVTTTF

(C) HLA-A*24:07 - HNPYPDYHW



(D) HLA-B*15:02 - FVGYVTTTF (E) HLA-DRB1 - ELKVGYISAIKVNGF

FIGURE 3 Visualization of potential epitopes for RBV

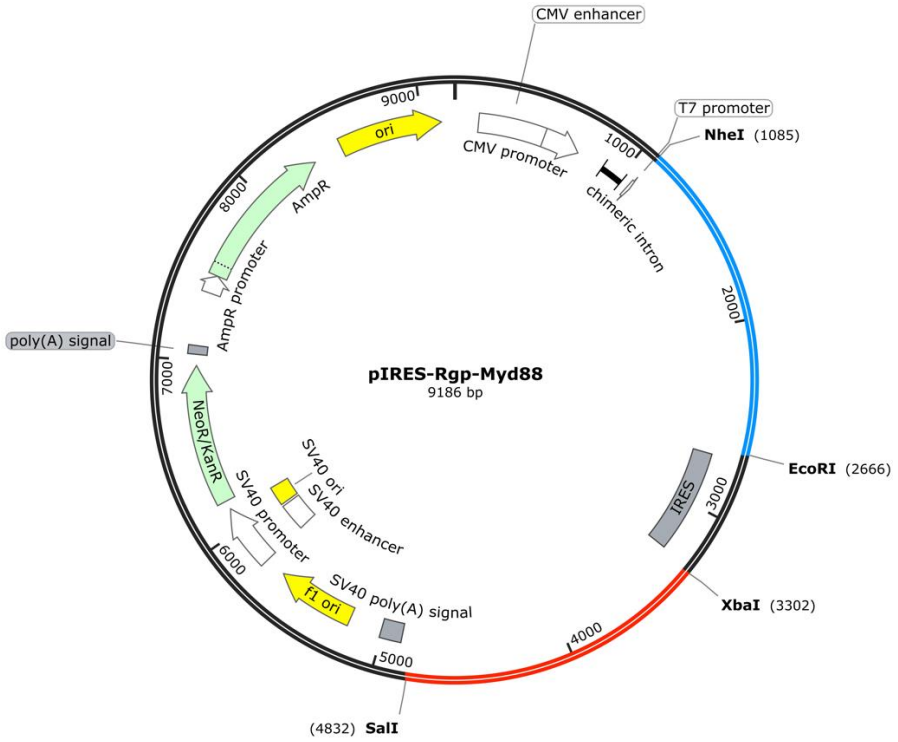


FIGURE 4 This schematic representation visually represents the in silico cloning of the vaccine candidate within the pIRES expression vector. The total size of the construct is labeled as 9,186 bp, and the genes of interest are depicted in red (MyD88; 1,546 bp) and blue (RBV glycoprotein; 1,596 bp) colors

Based on Figure 4, we visualized the result of *in silico* cloning of the plasmid-based DNA vaccine. Furthermore, the pIRES expression vector was utilized for the construction of a plasmid-based DNA vaccine incorporating a genetic adjuvant (MyD88), utilizing SnapGene v7.0 software for the cloning process. The total size of this construct candidate is 9,186 bp. In addition, the genes of interest depicted in red (MyD88; 1,546 bp) and blue (RBV glycoprotein; 1,596 bp) colors.

Basically, the MyD88 inclusion as a genetic adjuvant in plasmid-based DNA vaccine construct targeting the Indonesian RBV glycoprotein represents a significant advancement in rabies vaccine development. This approach aims to enhance the vaccine's immunogenicity by leveraging the innate immune system's critical mediator, MyD88. In this *in silico* report has revealed potential structural interactions between MyD88 and host immune receptors, suggesting that the

adjuvant effect of MyD88 could play a pivotal role in bolstering the adaptive immune response against the RBV [26,27].

Understanding the structural basis of the interactions between the glycoprotein, MyD88, and host immune receptors is of paramount importance. This knowledge provides critical insights into the mechanisms underlying MyD88's ability to enhance vaccine-induced immunity. By deciphering these structural interactions, we pave the way for more targeted and effective vaccine design strategies. It is crucial to consider the structural aspects as we move forward with experimental validation [28,29].

One notable aspect of this study is the consideration of genetic variations within the Indonesian RBV. By accounting for the genetic diversity of the virus, we open up the possibility of personalized vaccine design. This tailored approach has the potential to improve vaccine effectiveness, addressing the unique challenges posed by different viral variants in specific regions [30,31].

The utilization of *in silico* methods in vaccine design is a rational and cost-effective approach that accelerates the development process. It allows for the identification of potential interactions and prediction of vaccine candidates, thus reducing the time and resources typically associated with traditional vaccine development. This approach aligns with the need for rapid response to emerging infectious diseases [32,33].

Moreover, the incorporation of MyD88 in the vaccine construct may not only enhance the immune response against the targeted Indonesian RBV, but also have implications for cross-protection against other RBV strains. MyD88's role in immune activation could lead to broader and more robust protection, particularly relevant in regions with multiple RBV variants [35].

However, it is essential to emphasize that while our *in silico* study provides promising insights, further experimental validation is imperative before clinical translation. Laboratory and clinical trials will be necessary to assess the safety, immunogenicity, and efficacy of the proposed DNA vaccine. In addition, preclinical studies in relevant animal models are essential steps in evaluating the vaccine's potential [36-41].

The success of DNA vaccines not only relies on their design but also on the development of efficient delivery systems. Overcoming the challenges associated with DNA vaccine delivery, such as achieving efficient transfection and targeting antigen-presenting cells, is a critical aspect that needs to be addressed in future research.

Furthermore, navigating the regulatory approval process for DNA vaccines is a significant hurdle. Collaborations with regulatory agencies and adherence to established guidelines will be essential to advance the proposed vaccine from preclinical to clinical phases [42-44].

To assess the potential advantages and disadvantages of our proposed DNA vaccine, a comparative analysis with existing rabies vaccines, including traditional inactivated vaccines and newer recombinant protein vaccines, will be necessary [45]. Evaluating the cost-effectiveness and scalability of production will also be important considerations. Beyond its relevance to Indonesia, this study has global implications. The use of genetic adjuvants like MyD88 in DNA vaccines could revolutionize vaccine development strategies for a wide range of infectious diseases, ultimately contributing to global public health efforts [46,47]. Nevertheless, it is crucial to exercise caution, acknowledging the necessity for thorough experimental verification and adherence to regulatory standards before this novel method can influence efforts against rabies and other infectious illnesses.

Conclusion

To sum up, this *in silico* study provides a solid foundation for the rational design of a plasmid-based DNA vaccine against the Indonesian RBV. The incorporation of MyD88 as a genetic

adjuvant, coupled with insights into structural interactions and genetic diversity, offers exciting prospects for the development of more effective rabies vaccines. However, it is imperative to proceed with caution, recognizing the need for extensive experimental validation and regulatory compliance before this innovative approach can impact the fight against rabies and other infectious diseases.

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Authors' Contributions

Conceptualization: Arif Nur Muhammad Ansori and Nelson Chandra; Literature Study: Arif Nur Muhammad Ansori, Nelson Chandra, Teguh Hari Sucipto, Viol Dhea Kharisma, and Ahmad Affan Ali Murtadlo; Formal analysis and investigation: Arif Nur Muhammad Ansori, Nelson Chandra, Teguh Hari Sucipto, Evgeniy Kolesnik, Maksim Rebezov, Arli Aditya Parikesit, Putu Angga Wiradana, I Gede Widhiantara, Sukma Sahadewa, Fara Disa Durry, and Vikash Jakhmola; Writing original draft: Arif Nur Muhammad Ansori and Nelson Chandra; Funding acquisition: Rahadian Zainul; and Supervision: Arli Aditya Parikesit and Rahadian Zainul.

Conflict of Interest

The authors declare no conflict of interest with respect to the study, authorship, and/or publication of this article.

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