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Immunoinformatic-Based Design of a Multi-Epitope Vaccine Against Heartland Virus

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Abstract

A developing tick-borne virus called Heartland virus (HRTV) is identified with febrile illness, and in a few cases, also with encephalitis. Most patients will recover fully but some are left with long-term fatigue and neurological sequelae. Recent epidemiologic data suggest increases in HRTV cases largely due to spreading tick populations and global climate change. There are still no vaccines nor specific antiviral treatments for the disease; a new prophylactic approach needs to be pursued against HRTV. This study used immunoinformatics to build a multi-epitope vaccination against HRTV. The goal of the HRTV nucleocapsid protein was to anticipate B- and T-cell epitopes. Identified epitopes were evaluated against conservancy, toxicity, allergenicity, and immunogenicity. Qualified epitopes were aligned to HLA alleles with the assurance of inducing robust immunity activation. These epitopes were assembled in a vaccine construct, with linkers and adjuvants enhancing immune response. Physicochemical analysis confirmed the vaccine's immunogenicity, stability, and safety. Toll-like receptor-8 (TLR-8) molecular docking and dynamics simulations confirmed the vaccine's receptor-binding effectiveness. Efficient expression was guaranteed by codon optimization and in silico cloning into an Escherichia coli plasmid. According to preliminary findings, the suggested vaccine is safe, immunogenic, and stable for preventing HRTV. However, to confirm its safety profile and protective effects, more in vitro and in vivo research is needed.

Keywords: Heartland Virus (HRTV), Epitope-based vaccine, Immunoinformatics, Nucleocapsid protein, Molecular docking, In silico analysis

Introduction

The recently discovered tick-borne virus known as Heartland virus (HRTV), has emerged as an important public health issue with respect to causing extreme illness and even death. Initially detected in the year 2009 in the state of Missouri, HRTV has continued to be registered in a variety of Midwestern and Southern American states in the USA, rendering a disease described as Heartland virus disease (Heartland). The disease also has influenza-like symptoms like fever, malaise, myalgia, and gastrointestinal symptoms, but is complicated by full-blown illnesses of thrombocytopenia, leukopenia, or even mortality in immunocompromised individuals and those with certain pre-existing ailments(1, 2, 3). Its case-fatality rate, as not known since it tends to be underreported, can however be conservatively estimated between 5-10%. This underreporting is due in large part

to the absence of general awareness among medical practitioners, the lack of readily available diagnostic tests, and the voluntary reporting of HRTV. The current diagnostic methods for HRTV are slow due to reliance on CDC testing. The lone star tick (*Amblyomma americanum*), the primary vector, is expanding its range because of favourable climate and increased host populations. (4). This geographic range extension has added to the possibility of increased HRTV occurrence and dissemination into new areas. Although the precise reservoir hosts of HRTV remain unclear, research is continued to determine the complete transmission cycle and potential animal reservoirs. The inability to conduct proper serosurveys prevents one from having the proper underestimation of the actual HRTV infection burden both in animals and humans. At present, no antiviral drugs or vaccines are available for HRTV. (5) Treatment remains supportive, primarily aimed at managing symptoms and addressing related complications. Although some agents, including favipiravir, have held promise in lab studies, they require additional research to demonstrate efficacy and safety in humans. Preventing HRTV infection relies heavily on the development of an effective vaccine. Current research is focusing on insights gained from similar viruses, like Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV). Ongoing efforts are investigating a range of vaccine approaches, including live-attenuated and multi-epitope subunit vaccines. (6).

Effective immunization is essential for preventing HRTV infection, and studies are already being conducted to take advantage of knowledge about related viruses, such as the severe fever with thrombocytopenia syndrome virus (SFTSV) (7). Among these initiatives is the investigation of several vaccination platforms, such as multi-epitope subunit vaccines and live-attenuated vaccines. The construction of a multi-epitope vaccine has the promise to be an effective means to circumvent the limitations placed by HRTV. Multi-epitope vaccines, which are made up of multiple immunogenic epitopes of different viral proteins, are able to elicit a better and broader immune response compared to single-epitope vaccines (8). This approach is especially suited to HRTV, as it is hoped that the vaccine will be able to target multiple viral proteins effectively and generate humoral as well as cellular immunity. A flexible tool for vaccine design, immune-informatics makes it possible to anticipate and select the optimal epitopes in silico, which expedites the vaccine development process and lessens the need for extensive experimental work (9). This approach is quite successful in creating vaccines against newly emerging infectious diseases and has already been applied successfully to other viruses, including SARS-CoV-2 (10). The goal of this effort is to employ immunoinformatics to create a new multi-epitope vaccine against HRTV based on the nucleocapsid protein. This vaccine will help avoid this new viral disease by improving the immune system. The procedure will include planning of adjuvant selection, population coverage, and optimization of linkers in order to achieve the maximum vaccine safety and efficacy (11).

Materials and Methods

Amino acid sequence retrieval and target protein structure analysis

The amino acid sequence of the membrane glycoprotein polyprotein of the Heartland virus (HRTV) was retrieved from the National Center for Biotechnology Information (NCBI) database in FASTA format, under the accession number WIF20428.1.The antigenicity of the targeted protein was analyzed by considering VaxiJen v2.0, an online tool accessible at (http://www.ddgpharmfac.net/vaxijen/VaxiJen/VaxiJen.html) which uses an arbitrarily selected threshold of 0.4 for determining the ability of a protein to induce an immune response. This step is vital for the identification of appropriate epitopes that can be chosen for inclusion in a multi-epitope vaccine design. (12, 13, 14).

Secondary Structure Prediction

The physico-chemical properties target protein for the Heartland Virus (HRTV) were tested using the ProtParam tool, available at (https://web.expasy.org/protparam/)Some of the essential information the software provides are instability, half-life estimations, and GRAVY. For predicting the secondary structure of the HRTV membrane glycoprotein polyprotein, the SOPMA tool (Self-Optimized Prediction Method with Alignment) was used. This predicted the four conformational states of the protein: alpha helix, beta sheet, turn, and coil. Usage of these tools is very instructive as to the probable structure of the protein, which will guide subsequent analysis of protein function and vaccine design. (15, 16).

IEDB-based B-cell epitope prediction

B-cell epitopes from the target protein sequence were predicted using a Bepipred linear epitope prediction tool available at the Immune Epitope Database (IEDB)(https://www.iedb.org/). The sequence of the target protein was then given as input to this tool, which in turn provided a list of putative B-cell epitopes according to their predicted binding affinity. The putative epitopes were further filtered on the basis of length, their position within the protein sequence, prediction scores, and threshold values for immunogenicity, among others. The selected epitopes were tabulated and depicted with figures to help in the design of a multi-epitope vaccine. This systematic approach ensures that the selected epitopes probably trigger a good immune response, which enhances the overall efficacy of vaccines. (17, 18).

Predicting Cytotoxic T Lymphocyte (CTL) Epitopes (MHC Class I)

The identification of cytotoxic T lymphocyte (CTL) epitopes is vital for vaccine development, since it provides a more rapid and less expensive option as against epidemiology-based epitope mapping in the laboratory(19).In this study, we applied NetCTL 1.2 software (http://www.cbs.dtu.dk/services/NetCTL/)to test a target protein sequence for the possible CD8+ T-cell epitopes restricted by MHC class I. In the analysis, twelve of the most frequent HLA class I alleles were presented: A1, A2, A3, A24, A26, B7, B8, B27, B39, B44, B58, and B62. The predictions were run with standard thresholds for proteasomal cleavage set at 0.15, TAP transport efficiency at 0.05, and epitope recognition at 0.75. Ten peptides with the highest binding scores to more than one HLA allele were subsequently selected as candidate CTL epitopes for further analysis. These candidate peptides were evaluated for immunogenicity and conservation first and then ranked according to their binding affinity scores. (20, 21).

Helper T lymphocyte (HTL) epitope prediction (MHC class II)

The identification of DRB-restricted CD4+ HTL epitopes was done using NetMHCII pan virtual screening, version 3.2(http://www.cbs.dtu.dk/services/NetMHCIIpan/). The following DRB1 alleles were interrogated: DRB1:01:01, DRB1:03:01, DRB1:04:01, DRB1:07:01, DRB1:08:03, DRB1:10:01, DRB1:11:01, DRB1:12:01, DRB1:13:02, DRB1:14:01, and DRB1:15:01, which cover about 95% of the human population worldwide. Therefore, the thresholds for percentile-rank scores were kept equal to 2% for strong binders and 10% for weak binders. The selection of candidates started with a list of 101 predicted strongly binding HTL epitopes, from which seven HTL epitopes were subsequently selected for shortlisting because they strongly bind to multiple HLA alleles. These seven HTL epitopes were then further evaluated with respect to their predicted immunogenicity, conserved sequences, and ability to mount an immune response, i.e., activation of CD4+ T cells. This way, a strong, population-wide, B-cell epitope, and T-cell epitope can be identified, which will be the basis for constructing a multi-peptide-plus-pathogen-derived vaccine.(22, 23)

Epitope selection and validation

A detailed investigation based on various parameters, such as antigenicity, allerge-

nicity, toxin activity, and transmembrane characteristics, was carried out upon the selected epitopes. To check for antigenicity, VaxiJen v2.0 was used to analyze the epitopes as potential antigens. The epitopes were then screened for possible allergenicity using the AllerTOP v.2.0 server (https://www.ddgpharmfac.net/AllerTOP/).Toxicity was examined using the ToxinPred server (http://crdd.osdd.net/raghava/toxinpred/). The transmembrane regions were predicted and thus identified by the TMHMM v.2.0 server (http://www.cbs.dtu.dk/services/TMHMM/). Non-toxic, non-allergenic epitopes are the ones predicted with antigenicity and exclusion from transmembrane regions that are further used in developing the vaccine. (24).

Screening for immunogenicity, allergenicity, and toxicity

In order to determine the safety and efficacy of the epitopes selected, bioinformatics tools, such as ToxinPred, AllerTOP, and VaxiJen, were utilized to verify their toxicity, allergenicity, and immunogenicity. Only epitopes that demonstrated strong immunogenic properties while displaying no toxicity or allergenicity were considered appropriate for multi-epitope vaccine development. The results of these evaluations and analyses are summarized in a table, reporting the results of each analysis. Specific prediction tools and the threshold values employed were selected with the intention of minimizing bias and maintaining high quality screening.(25).

Conservancy analysis

The conservancy of predicted B-cell and T-cell epitopes was analyzed with the IEDB conservancy analysis tool comparing selected epitopes across multiple viral strains at an identity threshold of ≥ 80%. Epitopes considered conserved were those conserved within 80% or more of sequences analyzed. To show the degree of conservancy for each epitope, the results were organized and summarized in table. (26)

Population coverage analysis

To check the conservation of B-cell and T-cell epitopes predicted and conserved among different protein variants from around the world, we have used the IEDB conservancy analysis tool (http://tools.iedb.org/conservancy/).Epitopes with 100% sequence conservation were selected for further analysis. Subsequently, the IEDB population coverage tool (http://tools.iedb.org/population/)was used with default parameters to analyze the global population coverage of the identified CTL epitopes and their MHC alleles. The population coverage analysis gave insights about the distribution of alleles of HLA in different global populations to estimate how efficacious the multi-epitope vaccine might be in different demographic groups. The results of the population coverage analysis have been presented in either tabular or graphical form for clarity. (27)

Molecular docking and 3d structural modeling

The selected T-cell epitope structures were built using PEPFOLD3 on the RPBS MOBYL portal, which generated five structural models for each epitope that were saved as PDB files. X-ray structures of HLA class I and class II alleles were sourced from the Protein Data Bank using 4U6Y for HLA-A01:01 and 1BX2 for HLA-DRB1*15:01. CASTp server (http://sts.bioe.uic.edu/castp/calculation.html) was used for receptor binding site identification. Flexible docking was performed with AutoDock Vina after converting receptor and ligand files into PDBQT format. Using PyMOL (v1.7.4.4), we were able to analyze docking poses of the receptor-epitope complexes to identify binding interactions and assess conformational stability. (28, 29, 30, 31)

Desired epitope construction using linkers and adjuvants

The selected epitopes were arranged systematically for the multi-epitope vaccine construction. A proper adjuvant was chosen, and

the sequence of steps included the adjuvant, CTL, HTL, and BCL epitopes. For its enhancement of immunogenicity, the L7/L12 ribosomal protein was employed as an adjuvant. Specific linkers were optimally utilized to connect each constituent to maintain their structural integrity and provoke the immune response stimuli in the vaccine antigen. The peculiar adjuvant sequence was joined through the EAAAK linker, whereas the CTL, HTL, and BCL epitopes were joined via the AAY, GPGPG, and KK linkers, respectively. (32, 33)

Prediction of immunogenicity, allergenicity, and stability

Various computational immunology tools were used to evaluate antigenic potential, allergenicity, toxicity, and physicochemical properties of the final vaccine construct. The antigenicity prediction was made using VaxiJen v2.0 with a threshold value of 0.4. Toxicity is assessed by ToxinPred, and allergens are defined by AllerTOP v.2.0. Regarding physicochemical properties, the ProtParam tool was used to predict molecular weight (Mw), isoelectric point (pI), instability index, and aliphatic index. These preclinical analyses were carried out to determine the immunogenicity, safety, and structural stability of the designed vaccine.(34).

3D structure prediction of vaccine construct

The secondary structure was predicted using SOPMA setting for an output width of 70, width of 17, and similarity threshold of 8. The default 3D structure of the vaccine was then generated through the trRosetta server (https://yanglab.nankai.edu.cn/trRosetta/). The predicted structure's accuracy and correctness were checked using Ramachandran plot analysis and PROCHECK software (https://servicesn.mbi.ucla.edu/PROCHECK/). The Galaxy Refine tool(http://galaxy.seoklab.org/)was then used to refine the model, leading to a more stable and reliable final 3D structure.

Toll-like receptor-8 (TLR-8) molecular docking

The Toll-like receptor 8 (TLR-8) plays key role in how the body deals with RNA viruses. To check its link with constructed vaccine, we got the TLR-8 crystal structure form (PDB ID: 3W3G, 2.3 Å Resolution) from the Protein Data Bank. Using theCLUSPRO server https://cluspro.bu.edu/login.php, docking was done to weigh how well it binds and stays. Before docking, both the vaccine and receptor were set by adding H atoms, setting charges, and marking rotatable bonds. The best docking pose, picked based on binding energy and interaction profile, showed key residues involved in immune activation. A visual representation was used to show main binding sites. (35)

Simulation of molecular dynamics

In order to confirm the conformational stability and structural flexibility and dynamic interactions of the vaccine–TLR-8 complex, MD simulations were carried out. Further, the simulation process was supported by iMODS server (http://imods.chaconlab.org/), through which the motion of the complex and the reliability of the structures could be reviewed during the course of the simulation.

Codon adaptation for escherichia coli expression

The multi-epitope vaccine was codon-adapted for enhanced expression efficiency with E. coli, using the JCat tool (https://www.jcat.de/). In this process, the gene sequence was altered in accordance with the codon usage of highly expressed genes in E. coli, so the amino acid sequence stayed exactly the same while potentially increasing the translation efficiency. (36, 37)

In silico restriction cloning in expression vector

The multi-epitope vaccine gene was cloned in silico into the pIB2 expression vector using SnapGene (https://www.snapgene.com/). The restriction sites were incorporated at either end (5′ and 3′) of the optimized gene for cloning. This in silico cloning simulation facilitates and confirms that the vaccine construct has been

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inserted into the vector correctly, in the right orientation, and with the correct sequence. (38, 39, 40)

Results and Discussion

Antigenic and structural features of target protein

The polyprotein sequence of glycoprotein found in the membrane of Heartland Virus, used as the target protein, was downloaded from NCBI and comprised 417 amino acids. Based on the tool VaxiJen v2.0, an antigenicity score was predicted as 0.4602, deeming it to be a potential antigen. ProtParam analysis revealed that it had a molecular weight of 45735.88 kDa and a theoretical pI of 6.66, indicating that it was positive. Forty-five of the residues were negatively charged (Asp + Glu), while forty-three were positively charged (Arg + Lys). While the aliphatic index (86.88) and GRAVY score (-0.006) were established, the protein was unstable according to the instability index (37.12). Secondary structure prediction by SOPMA showed 42.39% α-helices, 13.32% extended strands, 0.00% β-turns, and 44.29% random coils.

Expected t-cell and b-cell epitope sequences

The B-cell epitope was predicted by employing the Bepipred Linear Epitope Prediction tool available on the IEDB, which resulted in the identification of 10 probable epitopes (Fig. 1). One epitope, which exhibited 100% conservation and a peptide length of more than 9 amino acids, was selected. Various properties, including antigenicity, toxicity, topology, and allergenicity, were analyzed and are presented in Table 1. The epitope VFAPIKLAL scored the highest antigenicity score of 0.9557. This epitope has proven antigenic; moreover, it showed non-toxic and non-allergenic properties, which makes it an ideal vaccine candidate.

Table 1 shows the characteristics of the predicted MHC Class I epitopes, together with the antigenicity, allergenicity, toxicity, and topology.

Position	Hla class-1 alleles & supertypes	Peptide sequence	Antigenicity	Allergenicity	Topology	Toxicity
314	A1,A26,B58,B62	LVVVVMCCY	Antigen (0.4163)	Non-allergen	Inside	Non toxin
342	A24,B8,B39	VFAPIKLAL	Antigen (0.9557)	Non -allergen	Outside	Non toxin
276	B39,B58,B62	FSFDGHCIF	Antigen (0.8251)	Non-allergen	Outside	Non toxin

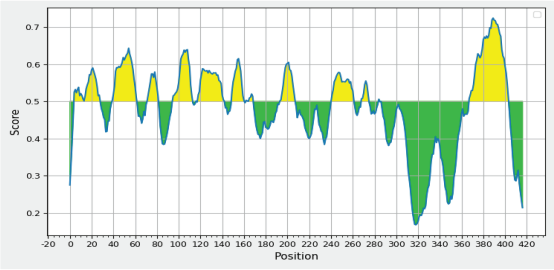


Fig1.Bepipred linear epitope prediction was used for predicting B-cell epitopes.

Prediction of t-cell epitopes

NetCTL (version 1.2) predicted 216 possible cytotoxic T-lymphocyte (CTL) epitopes (9-mer peptides) for ten HLA class I supertypes. Further screening for antigenicity, allergenicity, and topological affinity filtered these down to three 100% conserved peptides exhibiting binding affinities towards multiple HLA-class I alleles (Table 2). Of these, one, SELVSLSQSEFPDVC, displayed a high antigenicity value. Population coverage analysis via IEDB returned a global population coverage value of 52.39%, with South Asia (44.26%), Southwest Asia (40.37%), and East Asia (39.12%) exhibiting the highest regional coverages (Table 3, Fig. 2). NetMHCIIpan 3.2 predicted 20 HTL epitopes with strong binding affinity. From these, four highly conserved peptides with strong binding affinities to multiple HLA class II alleles were selected and optimized (Table 4, Fig. 3). The four epitope candidates were considered for inclusion in the vaccine.

Table 2.Antigenicity, allergenicity, toxicity, and topology parameters are predicted together with B cell epitopes.

S.no	Peptide sequence	Position	Antigenicity	Allergenicity	Toxicity	Topology
1.	RGTPVPEDVFSELVSLSQSEFPD	40-62	ANTIGEN 0.4619	Non-Allergen	Non toxicity	Outside

Table 3. Population coverage analysis based on selected MHC class I-restricted epitopes.

Population	Coverage Percentage
World	52.39%
East Asia	39.12%
North East Asia	31.28%
South Asia	44.26%
South East Asia	31.74%
South West Asia	40.37%

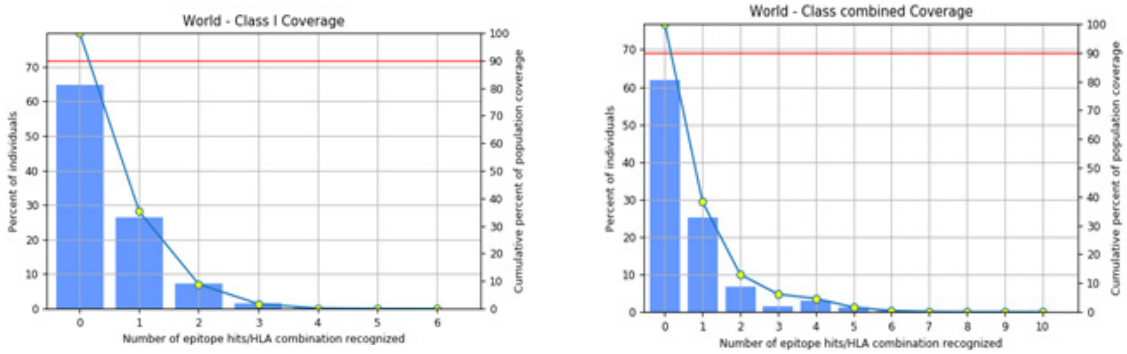
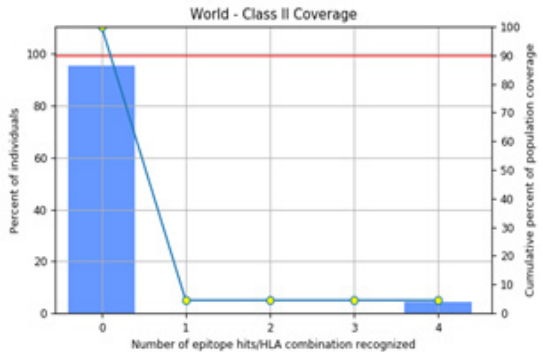


Fig 2. Evaluation of population coverages all around the world using predicted epitopes.



A) Population coverage analysis of epitopes restricted by MHC class II alleles.

B) Combined population coverage of epitopes restricted by MHC class I and II alleles.

Fig 3.

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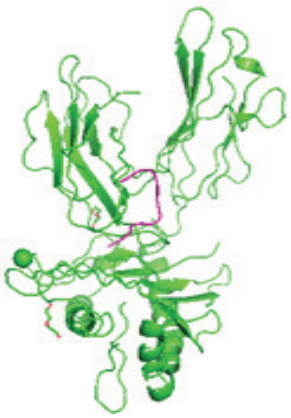

A) 1BX2 and Ligand interaction predicted in Autodockvina and Visualized in Pymol the Receptor

B) 4U6Y and Ligand interaction predicted in Autodock Vina and Visualized in Pymol the Receptor

Results of Molecular Docking

After modeling the 3D structures of CTL and HTL epitopes using the PEPFOLD server, molecular docking was conducted with AutoDockVina.Five models were generated for each epitope, and the top model from each was used for docking. Ten docked conformations were created for every one of the seven T-cell epitopes by docking them onto the 4U6Y and 1BX2 receptors. Visualization of the docked complexes was done using PyMol, which revealed significant interactions between receptors and their respective epitopes which can be seen in

Table 4. Prediction of MHC class II epitopes with Antigenicity, Allergenicity, Toxicity, and Topology.

Position	Peptide sequence	Subtypes	Antigenicity	Allergenicity	Topology
48	VFSELVSLSQSEFPD	DRB1_1201	Antigen (0.5133)	Non-allergen	Outside
49	FSELVSLSQSEFPDV	DRB1_1201	Antigen (0.7814)	Non-allergen	Outside
50	SELVSLSQSEFPDVC	DRB1_1201	Antigen (0.8506)	Non-allergen	Outside
219	VKIVTLTSELRSATV	DRB1_1201	Antigen (0.7458)	Non-allergen	Outside

Table 5.

S.No	Receptor	Peptide(Position)	Binding Affinity (Kcal/Mol)
1.	4U6Y	LVVVVMCCY(314)	-6.8
		VFAPIKLAL(342)	-7.2
		FSFDGHCIF(276)	-7.0
		VFSELVSLSQSEFPD(48)	-6.3
		FSELVSLSQSEFPDV(49)	-6.6
		SELVSLSQSEFPDVC(50)	-6.5
		VKIVTLTSELRSATV(219)	-6.3
2.	1BX2	LVVVVMCCY(314)	-7.7
		VFAPIKLAL(342)	-8.6
		FSFDGHCIF(276)	-10.1
		VFSELVSLSQSEFPD(48)	-8.9
		FSELVSLSQSEFPDV(49)	-8.5
		SELVSLSQSEFPDVC(50)	-8.1
		VKIVTLTSELRSATV(219)	-8.2

Table5.Selective epitopes' binding affinities with HLA-A*01:01 and HLA-DRB1* 15:01

Characteristics of the Vaccine Structure

The final vaccine has amino acids of 368 in length and is an integration of the L7/L12 adjuvant, CTL and HTL epitopes, and B-cell epitopes, all joined together by linker amino acids. VaxiJen v2.0 forecasted the vaccine with an antigenic score of 0.5038. It was, however, found to exhibit no toxicity or allergenicity. ProtParam revealed that its molecular weight was about 39.58 kDa, pI was 6.23, and the instability index was 37.43, indicating a hydrophilic and stable protein. Moreover, the GRAVY score of -0.335 and aliphatic index of 86.30 indicate that it is hydrophilic and stable, respectively. SOPMA analysis depicted the secondary structure as composed of 42.39% alpha helices, 13.51% extended strands, and 44.29% random coils (Fig. 4A). The initial 3D structure model (trRosetta) was then further refined using GalaxyRefine (Fig. 4B). Validation of the refined model with

```
SOPMA :
   Alpha helix      (Hh) :   156 is  42.39%
   3₁₀ helix        (Gg) :     0 is   0.00%
   Pi helix         (Ii) :     0 is   0.00%
   Beta bridge      (Bb) :     0 is   0.00%
   Extended strand  (Ee) :    49 is  13.32%
   Beta turn        (Tt) :     0 is   0.00%
   Bend region      (Ss) :     0 is   0.00%
   Random coil      (Cc) :   163 is  44.29%
   Ambiguous states (?)  :     0 is   0.00%
   Other states          :     0 is   0.00%
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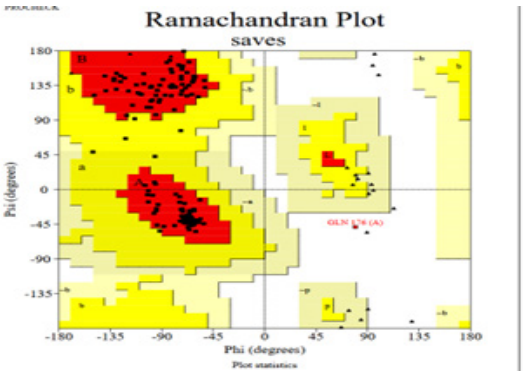
A) SOPMA version utilized for vaccine secondary structure analysis.

B) 3D model generated, predicted with tr Rosetta.



C) Tertiary structure validation with PRO CHECK using Ramachandran plot.

Fig 4. Vaccine Structure Prediction and Validation

PROCHECK and Ramachandran plot indicated higher accuracy, with 97.3% of residues lying in the favored region, 2.2% in allowed regions, and none in disallowed regions (Fig. 4C).

Simulation of Molecular Dynamics

Molecular dynamics simulations of the TLR-8–vaccine complex were carried out on iMODS (Fig. 5). The analysis also consisted of a B-factor plot comparing the flexibility of native and simulated structures (Fig. 6B), a deformability plot for locating flexible regions (Fig. 6A), and an eigenvalue of 3.874705e-06 indicating structural stability (Fig. 6C). Variance analyses

were carried out for individual and cumulative motions (Fig. 6D), with covariance maps show-

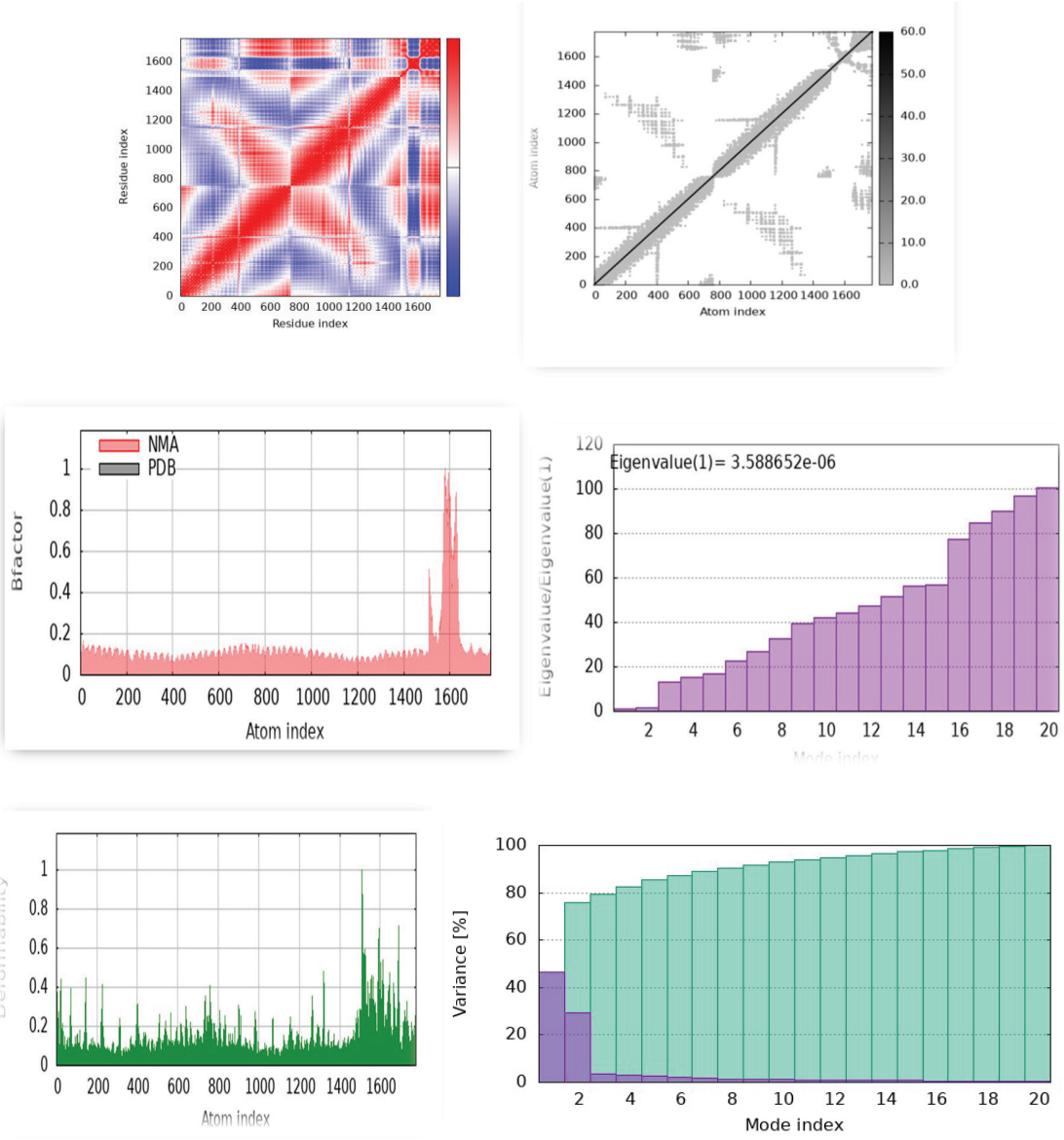


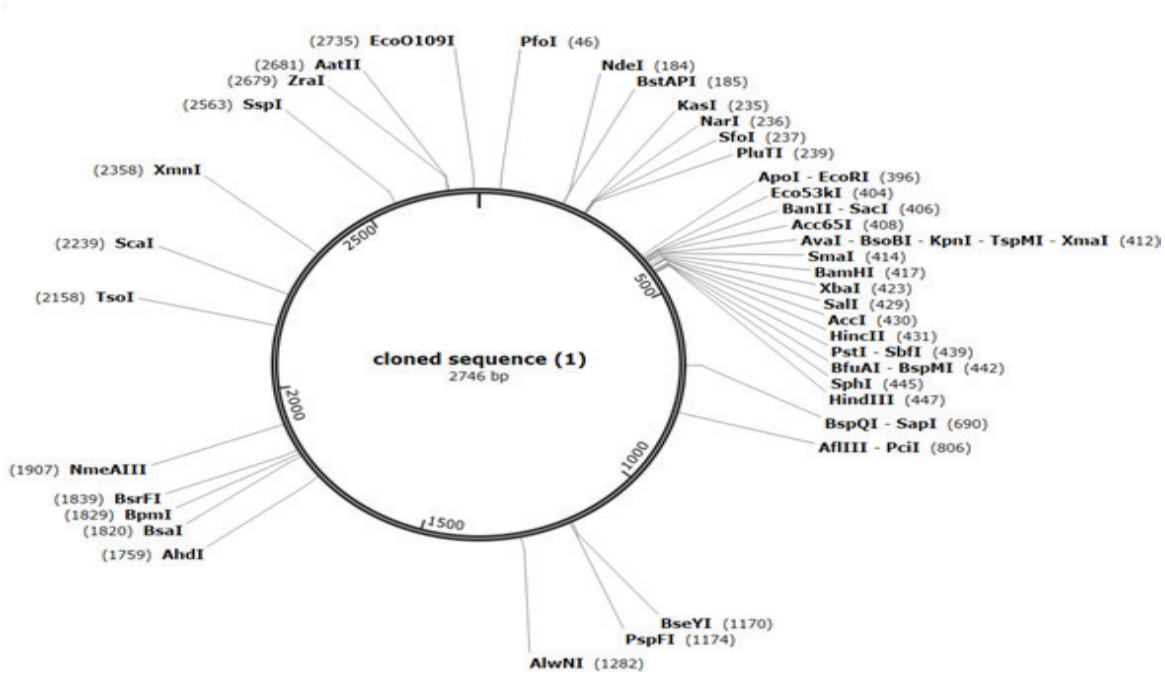
Fig 6. Maps and graphs showing the molecular movements of the vaccination complex and TLR-8. A) Deformability graph B) B-factor graph C) Eigenvalues D) Variance graph E) Covariance map F) Elastic Network

ing correlations of residue movements (Fig. 6E) and the elastic network map illustrating strong inter-residue interactions (Fig. 6F).

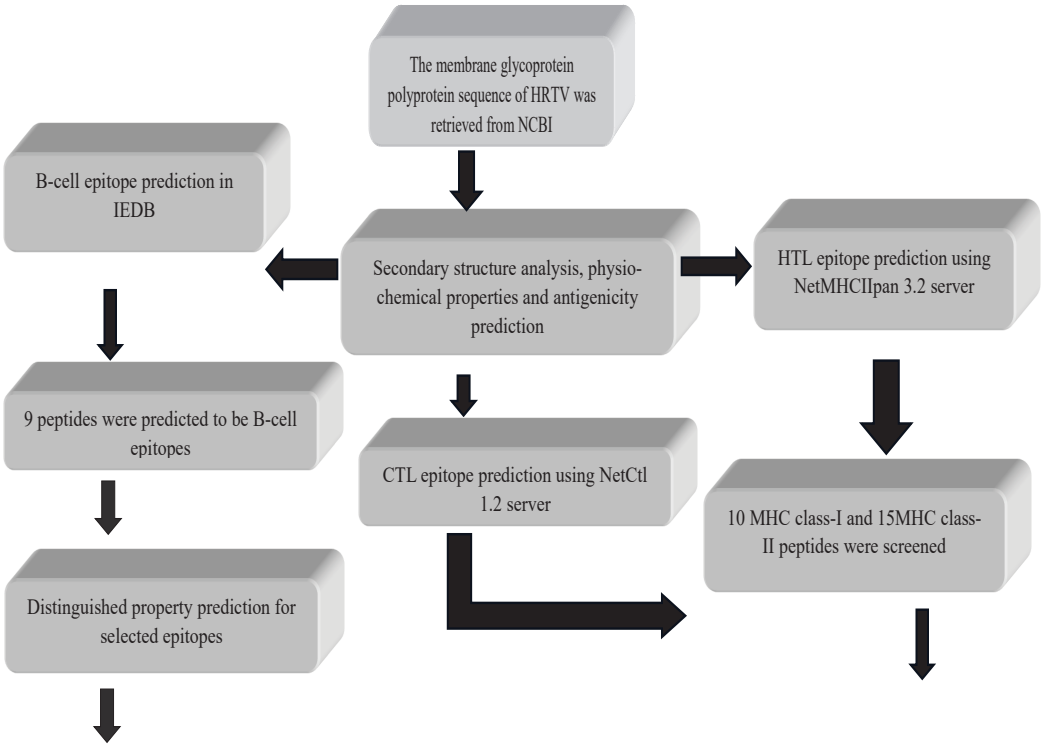
Fig 5.TLR-8 and designed vaccine interaction predicted in Cluspro server.

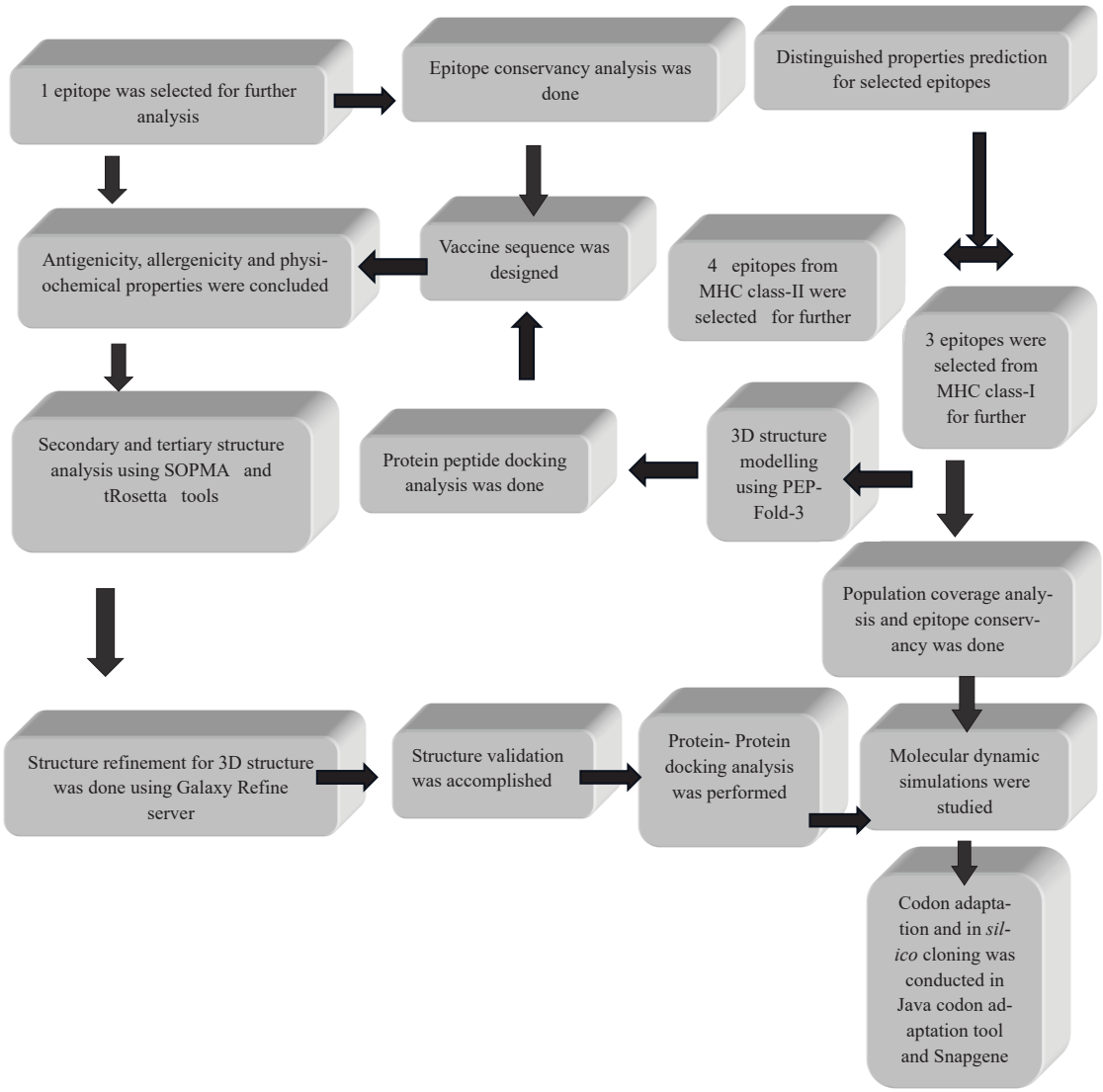
In Silico Cloning

Using the JCat program, codon optimization for E. coli K12 gave a 399-nucleotide sequence, with a GC content of 50.73% and a CAI value of 0.98, which suggests a high expression



potential. This optimized gene was designed with PspFI and BseYI restriction sites at the 5′ and 3′





ends, respectively, for ligation against the vector (Fig. 7). The recombinant plasmid in its final form contained 2746 bp (Fig. 8).

Fig7. pIB2 vector-based in silico restriction cloning of a vaccine

Fig 8. Flowchart illustrating the steps performed in vaccine construction

Discussion

Heartland virus is tick-borne diseases associated with the expanding range of *Ambly-omma americanum* (lone star tick); it does not have vaccines or antiviral therapies. Using im-munoinformatics approaches, a multi-epitope vaccine against the nucleocapsid protein of HRTV was developed. It comprises CTL, HTL, and B-cell epitopes for generating the cellular as well as humoral immune responses. Com-putational analysis suggested that the vaccine would be antigenic, stable, safe, non-allergenic, and non-toxic. Molecular docking and molec-

ular dynamics simulation studies suggested a strong binding affinity to TLR-8 and thus may be involved in immune activation. Further, codon optimization and in silico cloning confirmed that it would express well in E. coli, thus indicating feasibility for large-scale production.

In contrast to older techniques of vaccine manufacture, which may include an astronomical amount of laboratory procedures, multi-epitope vaccines are somehow better off. They are built with the highest possible specificity, eliminating the possibility of immune escape mutations. Furthermore, the incorporation of a variety of epitopes provides a broadspectrum immune response with minimal adverse effects. The addition of the L7/L12 ribosomal protein adjuvant also improves immunogenicity by activating TLR-8, which activates the NF-kB pathway, initiating cytokine induction and innate immune activation. Usage of linkers like EAAAK, AAY, GPGPG, and KK promotes better processing and presentation of epitopes, activating stronger immune responses. Furthermore, the insilico approach is more rapid and cost-effective than conventional vaccine production, enabling efficient identification and evaluation of promising epitope candidates.Once computer predictions are delivered that are worthy of consideration, experimental validation has to be ensured. Expression, stability, and immunogenicity of the vaccine need to be first confirmed using in vitro tests. Induction of immunity and protective efficacy must be evaluated in vivo in an animal model appropriate to the pathogen. If successful, clinical trials will be required to assess safety, immunogenicity, and long-term efficacy in humans. Success with this multi-epitope vaccine can provide a new prophylactic option for HRTV, preventing novel tick-borne viral diseases.

Conclusion

The study is focused on applying immunoinformatics and reverse vaccinology to design a potential multi-epitope vaccine targeting Heartland virus. For eliciting broad immunological responses, the vaccine candidate was engineered by combining highly conserved CTL, B-cell, and HTL epitopes. Its structural stability, non-toxicity, and immunogenicity was validated by computation models. Adequate contact with TLR-4 was evidenced by molecular docking and dynamics simulation, which suggests that it is able to induce immunological activation. Mass manufacturing was facilitated through codon optimization and in silico cloning, which also made it suitable for expression in Escherichia coli. Although the insilico results look promising, it is necessary to conduct in vitro and in vivo experimental work to validate the immunogenicity, stability, and protective potential of the vaccine design. Further efforts could improve its efficacy by focusing on adjuvant optimization and delivery optimization through viral vectors or nanoparticles.In addition, human population coverage studies must be conducted so that there is enormous HLA compatibility among different populations. If experimentally proved as established by computation, then such a multiepitope vaccine can be given as a prophylactic vaccine for HRTV and would prove of maximum value for the treatment of major novel tick-borne diseases.

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Conflict of Interest

The authors declare that no competing interest exists.

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