Volume 3, Number 2, 2026
Pages 244-272
Bioscience Insights
ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)
BiosciInsights.com

IN-SILICO BASED VACCINE DESIGNING FOR HEPATITIS E VIRUS

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Article Information

Article History

Received:	May 15, 2026
Revised:	June 27, 2026
Accepted:	August 12, 2026
Available Online:	August 31, 2026

Keywords:
HEV, In-silico/ computational vaccine, Epitopes

Abstract

Globally, acute viral hepatitis is most frequently caused by the hepatitis E virus. In addition to other HEV-related viruses, there are eight genotypes of Hepatitis E virus ranges from HEV-I to HEV-VIII. It has different human and animal hosts. With significant aquatic outbreaks in impoverished nations and zoonotic foodborne diseases in industrialized countries, transmission pathways differ by genotype and geographic location. A severe infection can be increased in pregnant women, recipients of organ transplant, patients with pre-existing liver disease, and immunologically weak patients. A lot of research has been published to date that points to the development of an in-silico vaccination against the Hepatitis E virus. The current work aims to create a computational vaccination model of HEV based on epitopes. The FASTA format yielded a 477 amino acid sequence with the primary accession AOA896W9U2. The ORF2 protein's secondary structure and physiochemical characteristics were determined using PSIPRED and Protparam. The VaxiJen 2.0 server and AllerTop predicted the allergenicity and antigenicity of a selected protein. The Immune Epitope Database (IED) predicted B and T cell epitopes. The constructed sequence of the vaccines was submitted to assess their toxicity and solubility using ToxinPred and SoluProt. Following physiological and structural prediction, ClusPro was used to submit the vaccination for docking analysis. Following vector and gene of interest cloning, the vaccine's expression in the body was examined in the last stage. The current study is an attempt in the addition to already reported literature in the market regarding in-silico techniques for HEV vaccine construction.

INTRODUCTION

Hepatitis E is a liver disease. Hepatitis E causes inflammation in liver. Hepatitis E virus is the causative agent of Hepatitis E. HEV was first identified in 1983 by investigators looking into an epidemic of unexplained hepatitis among Soviet soldiers in Afghanistan (Kamar et al., 2014). The viral genomic sequence was identified in 1990 (Lemon & Walker, 2019). Hepatitis E is present all throughout the world. However, it is more prevalent in Sub-Saharan Africa and Asia, where not enough facilities of pure drinking water, adequate sanitation, self-hygiene, basic health care are present. In developed nations, occasional and cluster instances of hepatitis E have been documented as a result of consuming raw or undercooked animal flesh. (Sooryanarain & Meng, 2019). Hepatitis E virus is currently emerged as a significant zoonotic virus with several animal sources, including deer, camel, swine, rabbit and rat (B. Wang & Meng, 2021). HEV-I, HEV-II, HEV-III, and HEV-IV belongs to the genus Paslahepevirus and belong to the subfamily Orthohepevirinae.

Genotypes I and II have only been discovered in humans and produce widespread waterborne epidemics in endemic areas of South and Southeast Asia, Mexico and Africa. Various species, such as swine, pigs, rabbits, wild boars and deer, are the sources of Genotype III and IV without producing illness and can sometimes infect people (Organization, 2017). Wild boars in Japan were the sources of Genotypes V and VI. Genotype VII was found in dromedaries in Middle Eastern nations, and Genotype VIII in Bactrian camels in China (Smith et al., 2014). HEV-VII and HEV-VIII, initially discovered in camels, have been shown to cross-transmit to nonhuman primates, with one instance of HEV-VII infection in humans (Lee et al., 2016). The distribution of HEVs varies with genotype. Drinking tainted water is the primary way HEV-I and HEV-II are spread through the fecal-oral pathway. The zoonotic foodborne transmission of HEV-III is linked to consuming raw or undercooked boar, deer, pig meat, and offal, especially liver. A cruise ship hepatitis E epidemic was linked to shellfish consumption. Consuming camel milk and meat has been linked to HEV-VII infection. It is usual for pregnant women to transmit HEV to their fetuses vertically.

Even while the death rate during epidemics is low between 0.2 and 5% pregnant women can have a mortality rate of up to 25%, which may be brought on by changed hormone levels and weakened immunity. In spite of the fact that HEV disease is believed to be self-restricting or asymptomatic in healthy individuals, it can cause serious illness with a high morbidity and fatality rate in patients who already have liver problems. Patients receiving organ transplants, immunosuppressive medications, and treatment for hematological malignancies may acquire chronic infections (Mazalovska & Kouokam, 2020). Human HEV virus virions are icosahedral particles spherical in shape. Its diameter ranges from 27–34 nm that are either non-enveloped (found in feces) or quasi-enveloped (found in blood or tissue culture). The capsid forms the viral shell and comprises capsomeres, homodimers of a single capsid protein. Three linear domains make up each capsid protein, generating unique structural elements: S (the continuous capsid), P1 (three-fold protrusions) and P2 (two-fold spikes) (Purdy et al., 2022). The genome of Hepatitis E virus is 7.5kb long. The whole genome is ordered in three partially overlapping Open Reading Frames (ORFs) (Debing et al., 2016). ORF1 encodes a non-structural polyprotein, ORF2 encodes the long capsid protein of 660-amino-acid (aa), and ORF3 encodes a small phosphorylated protein, its function is still unrevealed but may be involved in the release of virion from infected cells.

Because the production of either live attenuated or inactivated vaccine would be problematic due to the complexity and poor yield of viral culture, traditional procedures for creating a HEV vaccine have been ruled out. A more excellent knowledge of the HEV life cycle has resulted from adapting a few strains to cell culture, even though growing the virus has historically proved challenging (Shukla et al., 2012). Creating epitope-based vaccinations against various pathogenic pathogens has been made possible by recent advancements in computational epitope identification and silico analysis (Taherkhani & Farshadpour, 2015). Only ORF2 is helpful in developing a vaccine against HEV. To produce neutralizing antibodies, ORF2 produces capsid protein, which is immunogenic (Farshadpour et al., 2014; L. Wang & Zhuang, 2004). The HEV capsid protein's residues 271–286, 199–214, 463–478, 583–598, and 631–646 were found to be highly immunogenic by immunological analysis and sequencing (Shata et al., 2007).

VLPs, or virus-like particles, are formed by the amino acids 112 to 660 and trigger potent immune reactions (Shata et al., 2007). The primary B cell epitopes are found in the spike-forming amino acids 455–606 exposed on the virion surface (Li, Zhang, & Xia, 2015). The different forms of the ORF2

protein were prepared to create the vaccine candidates currently being developed to prevent HEV. Only the HEV 56 and 239 kDa vaccines were evaluated in human trials on clinical basis, out of all the developed vaccine candidates (Li, Zhang, & Xia, 2015). The HEV 239 vaccine made it to the phase III trial and was subsequently granted a license by the Chinese State Food and Drug Administration (SFDA) in 2011 (Zhu et al., 2010). The first preventative human vaccine against HEV infection is HEV 239, also known as Hecolin (Li, Zhang, & Xia, 2015). A protein's immunogenic region that is recognized by T cell receptors (T cell epitopes) or antibodies (B cell epitopes) and triggers humoral or cellular immune responses is known as an epitope (Bui et al., 2007). While T cell epitopes are groups of 8–11 amino acids or 12–25 amino acids that are presented to helper T lymphocytes (HTLs) or cytotoxic T lymphocytes (CTLs) by MHC class I or class II, respectively, B-cell epitopes are conformational (Patronov & Doytchinova, 2013). CTLs start the lysis of the cells that present the epitope, HTLs release cytokines that activate B cells and CTLs, and B cells neutralize pathogens by producing antibodies (Tan, Khan, & August, 2011; Testa & Philip, 2012).

In an experimental setting, epitopes are found by screening overlapping peptides derived from the antigen or by sequencing epitopes eluted from HLA-epitope complexes. Despite their considerable success, these two traditional approaches have certain technical drawbacks (Abdel-Daim, Abdelkhalek, & Hassan, 2015). The challenges and high cost of the experimental procedures address the need for new techniques to cut costs and time (Abdel-Daim, Abdelkhalek, & Hassan, 2015; Adel, 2012). Thus, computational methods were developed and presented to the scientific community to aid in the identification of the immunogenic epitopes (He & Zhu, 2015). The development of recombinant vaccines based on the ORF2 viral capsid protein VLPs has advanced significantly. Protein-based VLPs are self-assembling bionanoparticles that accurately resemble the native virions and reveal several epitopes on their surface.

METHODOLOGY

Retrieval of HEV's ORF2 Protein Sequence:

Retrieval of the Hepatitis E virus (HEV) Protein ORF2 sequence is accomplished using UniProtKB. The FASTA format yielded a 477-amino-acid sequence with the primary accession AOA896W9U2.

Physiochemical Analysis of Sequence:

Expasy Protparam was utilized to ascertain the physiochemical characteristics of a specific ORF2 protein sequence. Molecular weight, number of positive and negative charges, theoretical pI, aliphatic index, estimated half-life, instability index and grand average of hydrophobicity were the analysed physiochemical characteristics.

Construction of Secondary Structure of Protein sequence:

PSIPRED was opted to construct the secondary structure of the ORF2 protein sequence. This tool analysed the alpha helix, beta sheets, and coils of the ORF2 protein's secondary structure. The PSIPRED Cartoon's predicted confidence score and structural peaks increased confidence in the chosen sequence for vaccine design.

Prediction of Antigenicity & Allergenicity:

An online tool called VaxiJen2.0 was used to determine whether or not the chosen protein sequence was an antigen. After entering the sequence in the query box, a threshold value of 0.4 was used to select the virus as the target organism. The allergenicity of a chosen sequence was examined using AllerTOP v2.0, a prospective web server. Antigenicity and allergenicity are crucial factors when designing a vaccine because the sequence needs to have a high antigen score and not cause allergies.

Prediction of 3D structure of Protein:

trRosetta is a famous tool to build 3D structure of Protein. 3D structure is an important step in designing vaccine. FASTA sequence was pasted in query box to get the model, score, 2D & 1D structures of the protein.

Prediction of B-cell Epitopes:
A reliable tool called the Immune Epitope Database (IEDB) was selected to determine B cell epitopes. Linear epitopes were chosen to add linkers to them later. B cell epitopes were produced after pasting the sequence into the query box and choosing the "Bepipred Linear Epitope Prediction 2.0" method option. Then, using VaxiJen 2.0 and AllerTOP 2.0, respectively, their antigenicity and allergenicity were assessed.

Prediction of T cell Epitopes:
The Immune Epitope Database (IEDB) tool was utilized to predict T cell epitopes. MHC binding predictions were opted to recognize T cell epitopes. The ANN4.0 method was chosen for MHC-I Binding, and the sequence was pasted in FASTA format. This approach uses the ic50 value to predict epitopes. Frequent occurring alleles were selected, and "human" was chosen as the MHC source species. A sequence was submitted to obtain the results after all requirements were met.

Use of MS Excel for classification of Epitopes:
Microsoft Excel is an excellent tool for practical data analysis. The epitopes that were produced were saved in an Excel document. Various epitope characteristics were examined in neat rows and columns, including sequence number, lengths, peptide sequence, ic50 value, antigen scores, and starting and ending amino acid sequences. Another MS Excel file was created after choosing the most frequently occurring alleles. The antigenic and allergic characteristics of the chosen epitopes were examined by using the VaxiJen2.0 server and Allertop2.0, respectively.

For MHC-II Binding predictions:
IEDB Resource Analysis was utilized to obtain T cell MHC-II class epitopes. The "NN-align 2 3 (NetMHCII 2 3)" approach was chosen. The "human HLA-DR" alleles in the α and β chains were adopted independently. By default, allele length was set to fifteen. When all the requirements had been met the query was submitted. The binding epitopes of the MHC-II class were formatted and stored in an Excel file. The VaxiJen2.0 server was used to assess the antigenicity of the most frequently occurring epitopes, and the AllerTOP 2.0 server was used to determine their allergenicity.

Determination of Population Coverage:
The population coverage option was chosen after opening the IEDB Resource Analysis server. An interface appeared when the website was open. Five alleles were chosen, and coverage was examined globally. The class files for MHC-I and MHC-II were uploaded independently.

Binding of Linkers to MHC-I & MHC-II Epitopes:
EAAAK linker was used to join B cell epitopes at the start, GPGPG linker was used to link B cell epitopes and MHCI epitopes. MHC-I and MHC-II class epitopes were linked via AAY linker. Finally, a GST tag was used at the end.

Prediction of Antigenicity & Allergenicity:
The entire constructed sequence of vaccine was entered into the online antigen score prediction tool VaxiJen 2.0 server. Virus was chosen as the model organism. A sequence was submitted to obtain the results.
An online tool called AllerTOP 2.0 was used to determine whether or not the vaccine's sequence was allergenic. The sequence was submitted to determine its allergenicity. It is a crucial phase in the effective creation of a vaccine.

Prediction of Toxicity of constructed sequence of vaccine:
ToxinPred is an online tool for determining a sequence's toxicity. After pasting the sequence into the query box with all the values left at their default settings, the sequence was submitted to determine whether or not it was toxic.

Prediction of Solubility of constructed sequence of vaccine:
SoluProt is an online tool use to check the solubility of a vaccine. Sequence was subjected to query box and submitted to check the solubility.

Physiochemical properties of Vaccine:
ExPasy Protparam was used to examine the vaccine's physiochemical characteristics. The following factors were investigated: chemical formula, extinction coefficient, half-life, and stability index, and gravity value, total number of amino acids, molecular weight, and composition of amino acids.

Construction of Secondary structure of Vaccine:
The constructed structure of vaccine was replicated to forecast its secondary structure. The secondary structure was predicted using PSIPRED, and the sequence was entered in the query box. The job's name was written as "vaccine." An email address was provided to obtain the results. It takes a few seconds for this tool to display the results.

Construction of 3-Dimensional structure of Vaccine:
The 3-Dimensional structure of the vaccine was analysed through an online tool, SCRATCH protein predictor. The email address was entered, and the sequence was pasted into the box. The Tertiary Structure 3D Pro option was chosen, and the job was submitted. Results from this tool take at least half an hour to produce. The generated result was sent to the specified Gmail account.

Analysis of 3D Structure of Vaccine:
The PDB format contains the vaccine's final 3D structure. Standard computer files couldn't open it. Discovery Studio was installed to enable the file to be opened and analysed. A program called Discovery Studio works with files in PDB formats.

Refinement of 3D structure of Vaccine:
An online tool called Galaxy Refine was used to refine the vaccine's 3D structure. After uploading the downloaded vaccine model to this website, an upgraded version was acquired. A new window opened with the models and their scores.

Ramachandran Plot:
The Ramachandran plot was used to validate the improved and refined model further. It was used to determine the residual presence in favourable, less favourable & unfavourable regions. After uploading the chosen model, a Ramachandran plot was produced.

Docking Analysis of Vaccine:
A tool like Receptor (TLR2), 1o77, was taken from the RCSB PDB. One well-known resource for accessing an extensive database of protein 3D structures is the RCSB PDB. The PDB file was downloaded. ClusPro was chosen to dock. ClusPro is a web-based application for docking protein-ligand and protein-protein interactions. Ligand and receptor structures were uploaded to the ClusPro page. Various models were acquired.

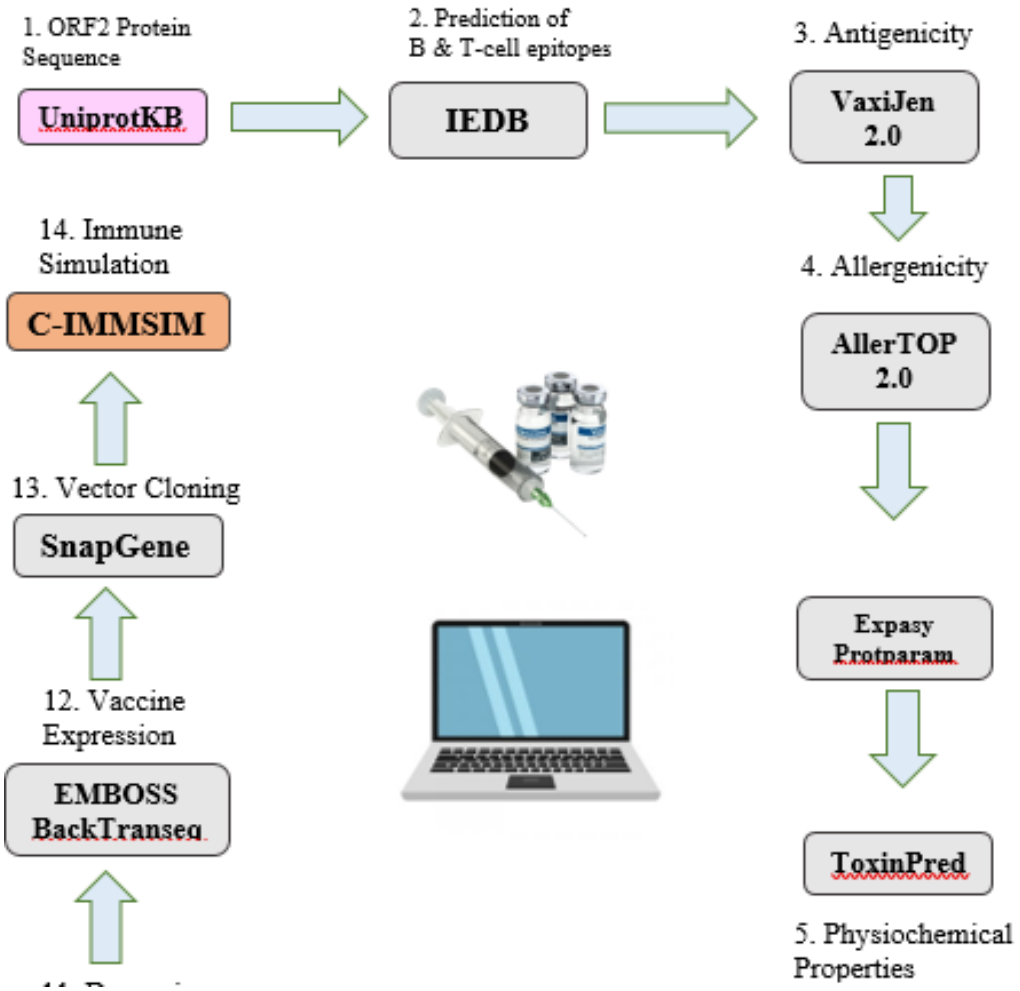
Molecular Dynamic Simulations of Vaccine:
ClusPro model 0 was downloaded from the Protein-Protein Docking analysis. An online tool for molecular dynamic vaccine simulation is called iMODs. The downloaded model that was chosen was uploaded and sent in. Analyses were conducted on the docked interaction, including B-factor/mobility, Atom index, Mode index, Eigenvalues, variance, and covariance.

Vaccine Expression Analysis of Vaccine:
The nucleotide sequence of vaccine was necessary for expression analysis. The protein sequence of vaccine was pasted in the query box on the EMBOSS BackTranseq website. An online tool called EMBOSS BackTranseq extracts nucleotide sequences from protein sequences. After the protein sequence was submitted, the nucleotide sequence was reverse translated. A copy of this sequence was made for additional study.

Codon Optimization of Nucleotide Sequence:
The nucleotide sequence was optimized, and the hindrance was decreased by the Java Codon Adaptation Tool. After submitting the duplicate sequence, the enhanced DNA sequence was acquired. The GC content has increased due to this improved sequence.

Cloning of vector & Gene of interest:
The last stage of the immune simulation was completed using SnapGene. By choosing New File in SnapGene, the enhanced DNA sequence was uploaded. DNA's linear structure was discovered. We downloaded the Pet-28b vector from the SnapGene online library. This vector was chosen for cloning because it has several restriction sites and high levels of inserted gene expression. The vector and gene of interest were carefully examined for cloning purposes. Both were examined, and sites that were compatible were found and replicated. The length of the vector was 5368 base pairs, whereas the fragment was 1179 base pairs.

Analysis of Expression of vaccine in Body:
To determine whether or not the vaccine would cause expression in the body, the C-IMMSIM tool was utilized. The vaccine's nucleotide sequence was submitted for this purpose, and the results were displayed as a graph. This graph provides comprehensive information on vaccine expression and immune functions.



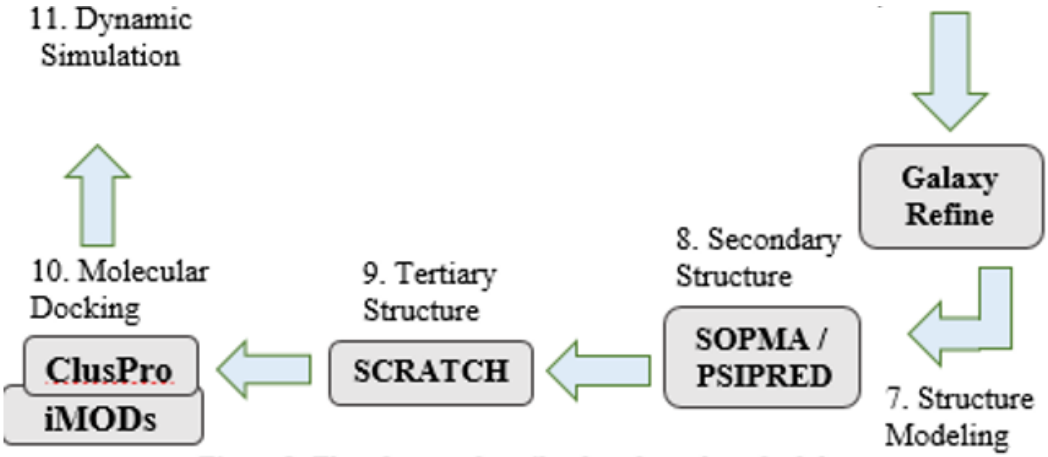


Figure 1: Flowchart to describe the adopted methodology

Results

Selection of targeted Protein & Characteristics:

A protein sequence of 477 amino acids focusing on ORF2 protein was chosen. This protein sequence showed a profound level of antigenicity with a worth of 0.6322 under threshold value of 0.4. The instability index of 33.56 (under 40) makes this protein stable. This protein grouping is Non allergic.

Table 1: Essential properties of selected protein sequence is described in this table.

Sequence Length	477 aa
Antigenicity Score	0.6322 (>0.5 is recommended)
Allergic Status	Non- Allergic
Instability Index	33. 56 (<40 is favourable)
Status	Stable Candidate

Prediction of B-Cell Epitopes:

Under a threshold of 0.500, IEDB resource analysis identified 19 B-cell epitopes in the uploaded protein sequence. This protein sequence scored 0.689 (>0.5), suitable for building a multi-epitope vaccine as shown in figure 2.

Prediction of T cell Epitopes:

MHC-I and MHC-II class epitopes were predicted in figure 3 and figure 4 respectively independently using IEDB Resource Analysis. Microsoft Excel was used to paste both epitope classes. The majority of the repetitive epitopes were separated into other files. Deep research was done on epitopes that were both non-allergenic and perhaps antigenic.

Prediction of Population Coverage Analysis:

In a separate MS Excel document, profiles of the most frequently occurring epitopes were created. IEDB Resource Analysis Population Coverage is where this material was uploaded. The epitopes of B cells and T cells were uploaded independently. Worldwide, the population coverage was examined. The epitopes of MHC-I and MHC-II classes showed the coverage of 78.13% and 70.55%, respectively. It boosts trust in epitopes for vaccine creation and displays the best coverage analysis globally.

Table 2: B-cell epitopes in ORF2 Proteins selected for vaccine construct.

No.	Start	End	Peptide	Length	Antigenicity
1	5	11	ASNYAQY	7	0.4343
2	22	27	PLVPNA	6	0.2923
3	39	55	PQTTTTPTSVDMNSITS	17	0.7474
4	66	70	IASEL	5	
5	72	98	IPSERLHYRNQGWRSVETSGVAEEEAT	27	0.7099
6	111	121	VNSYTNTPYTG	11	-0.1335
7	134	166	FRNLTPGNTNTRVSRYTSTARHRLRRGADGTAE	33	0.5686
8	182	190	TGTNGVGEV	9	0.1082
9	208	208	G	1	
10	211	219	TELISSAGG	9	-0.1206
11	228	234	VSANGEP	7	0.4439
12	243	261	ENAQQDKGIAIPH DIDLGD	19	1.09
13	267	288	QDYDNQHEQDRPTPSPAPSRPF	22	0.2333
14	304	319	AEYDQTTYGSSTNPMY	16	0.5003
15	334	341	QAVARSLD	8	0.5575
16	371	393	LSFWEAGTTKAGYPYNYNTTASD	23	0.6101
17	412	420	TSLGAGPVS	9	1.4363
18	432	458	ALAVLEDTIDYPARAHTFDDFCPECT	25	0.5801

Table 3: Selected Epitopes of MHC-I in ORF2 protein having antigen score greater than 0.4 & Non allergen.

Allele	Seq No	Start	End	Length	Peptide	ic50	Antigen Score
HLA-A*30:01	1	19	27	9	RYRPLVPNA	4	0.7326
HLA-A*31:01	1	12	21	10	RVIRATIRYR	5.02	0.7076
HLA-A*02:01	3	4	12	9	GLLDFALEL	5.27	1.2732
HLA-A*31:01	1	13	21	9	VIRATIRYR	6.62	1.2259
HLA-A*23:01	6	17	26	10	PMYVSDTVTF	10.77	0.568
HLA-A*30:01	1	19	27	9	RYRPLVPNA	4	0.7326

Table 4: Selected Epitopes of MHC-II in ORF2 protein having antigen score greater than 0.4 & Non allergen.

Allele	Seq No	Start	End	Length	Peptide	ic50	Antigen Score
HLA-DRB5*01:01	1	8	22	15	YAQYRVIRATIRYRP	2.2	0.6766
HLA-DRB5*01:01	1	9	23	15	AQYRVIRATIRYRPL	2.3	0.7314
HLA-DRB5*01:01	1	10	24	15	QYRVIRATIRYRPLV	2.5	0.6723
HLA-DRB3*01:01	6	14	28	15	STNPMYVSDTVTFVN	3.2	0.7788
HLA-DRB3*01:01	6	13	27	15	SSTNPMYVSDTVTFV	3.6	0.7044

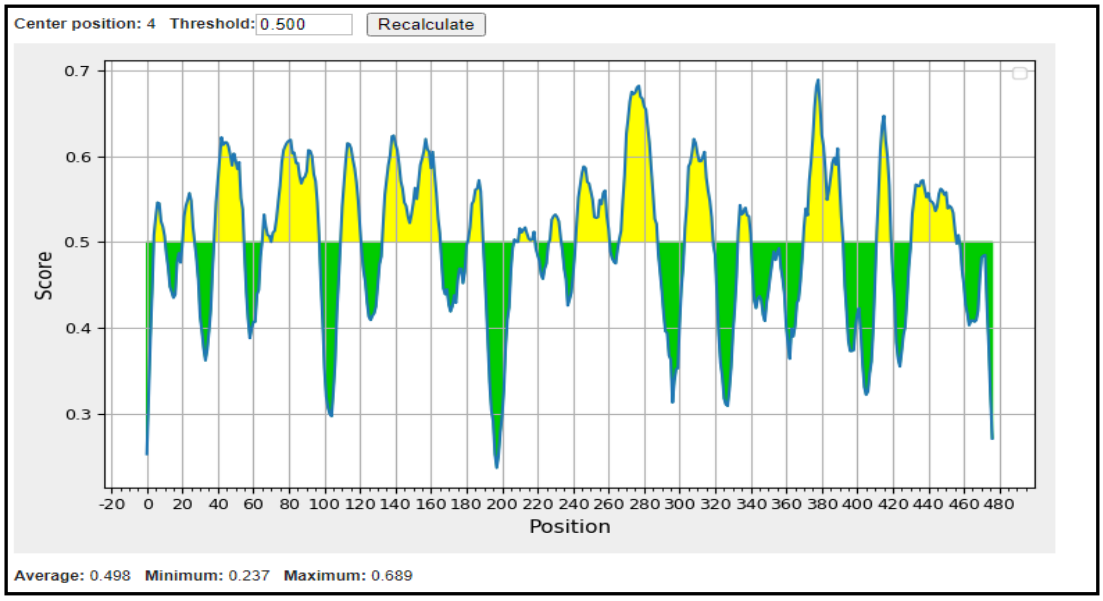


Figure 2: In this graph Yellow color above border line of 0.5 shows epitopes of B-cell as best candidates for vaccine development. Maximum score of selected epitopes is 0.698. (Note: score >0.5 is acceptable)

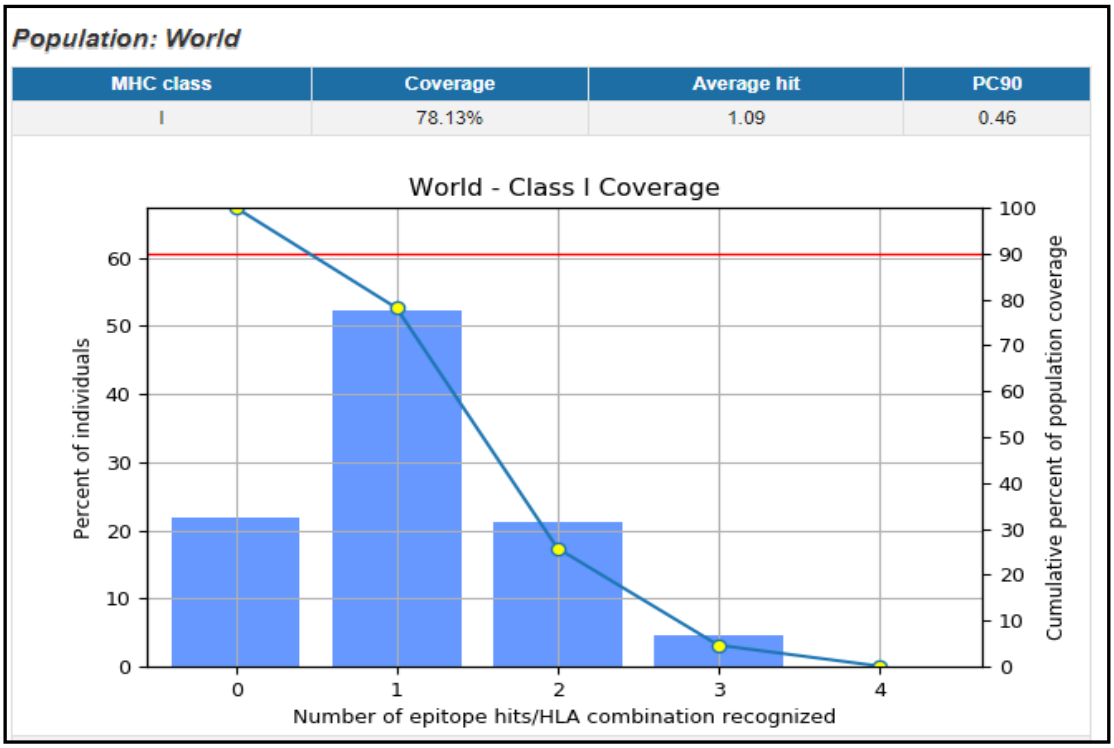


Figure 3: This graph shows the coverage of MHC class-1 epitopes covering 78.13% alleles all over the world.

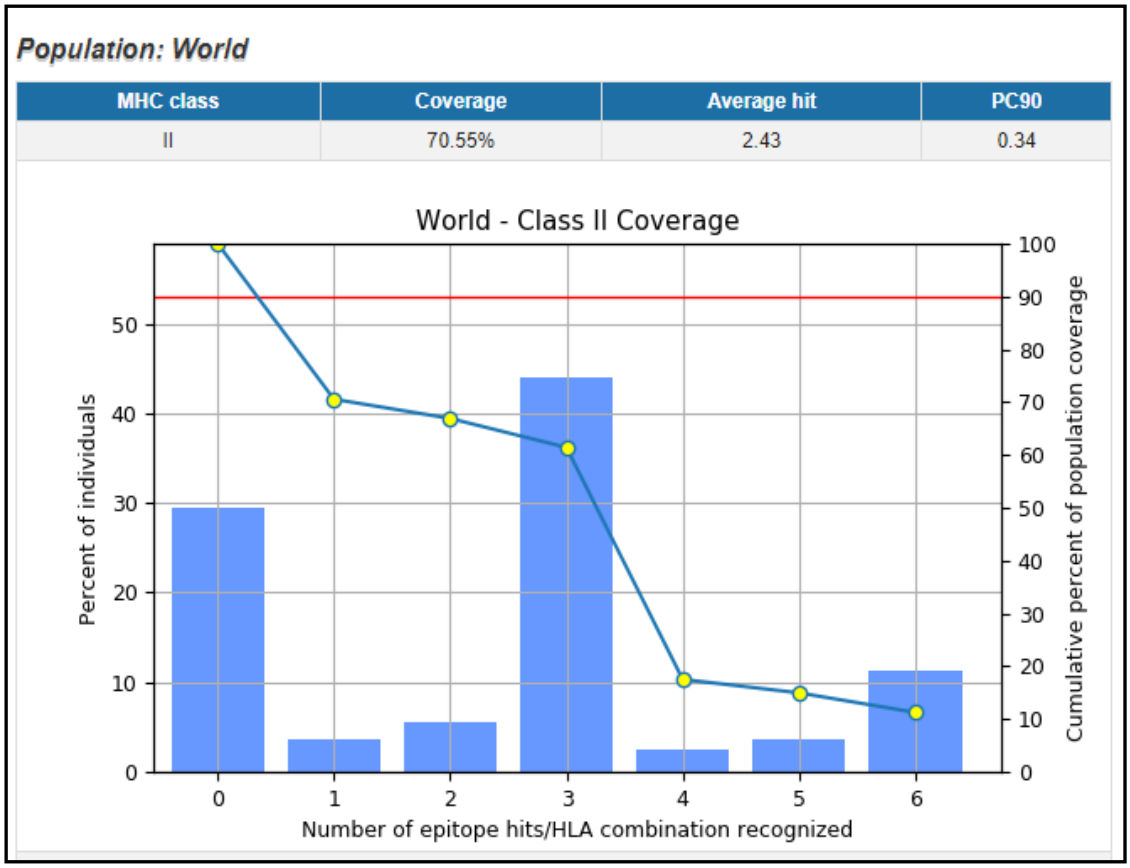


Figure 4: This graph shows the coverage of MHC class-1 epitopes covering 70.55% alleles all over the world.

Hepatitis E Vaccine construct:

The most repeated epitopes which were highly antigenic and non-allergic qualified for vaccine construction as shown in figure 5. The final sequence for vaccine constitutes 393 amino acids. The linkers EAAAK, GPGPG and AAY were used to join B cell epitopes and T cell epitopes. GST (Glutathione S-Transferase) Tag was used at the end of the sequence to enhance the solubility of ORF2 protein. Moreover this tag helps to increase the stability of ORF2 Protein during expression. The Final Constructed Sequence Of The Vaccine Is As Follows:
Eaaakasnyaqyplvpnapqttttptsvdmnsitsiaselipserlhyrnqgwrsvetsgvaeeeatvnsytntpytgfrnltpgntnt
rvsrytstarhrlrrgadgtaetgtngvgevgtelissaggvsangepenaqqdkgiaiphdidlgdqdydnqheqdrptpspapsrp
faeydqttygsstnpmyqavarsldlsfweagttkagypynynttasdtslgagpvsalavledtidyparahtfddfcpectgpgpg
ryrplvpnarviratiryrglldfalelviratiryrpmyvsdtvtfayyyaqyrviratiryrpaqyrviratiryrplqyrviratiryrplvstnp
myvsdtvtfvnsstnpmyvsdtvtfvgst

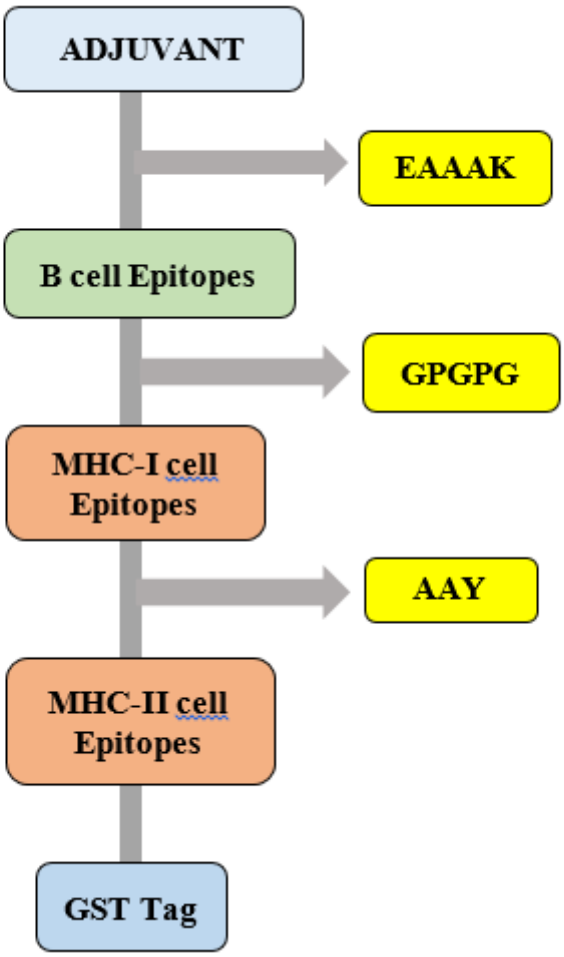


Figure 5: Schematic diagram of Vaccine Construct for Hepatitis E Virus

Physiochemical Properties of Vaccine:

The physiochemical properties of vaccine provide useful understandings of the characteristics of vaccine construct. The newly constructed vaccine has 393 amino acids weigh 43398.03 g/mol. The theoretical pI (Isoelectric point) 7.21 suggested that the vaccine was probably carrying net positive charge under normal physiological conditions. A higher value of an aliphatic index (measure of aliphatic amino acid such as valine, isoleucine and leucine) such as 66.56 suggested greater thermostability and hydrophobicity which contributes to the stability of protein. The estimated half-life value (1 hours (mammalian reticulocytes, >30 min in yeast and > 10 hours in Escherichia coli) provides

protein stability and denaturation rates in different environments. The instability index of 27.40 suggested that protein was stable as the value greater the 40 has considered unstable. If the value would be greater than 40 it would classy protein potentially unstable. The GRAVY value of – 0.529 suggested newly constructed vaccine hydrophilic. These characteristics were pivotal for the determination of stability, solubility and immunogenic physiology of vaccine construct. These properties guaranteed and enhanced the confidence that vaccine maintains its structural and physiological integrity.

Table 5: Physiochemical characteristics of constructed sequence of vaccine
Prediction of Antigenicity, Allergenicity & Toxicity of vaccine's construct:

Characteristics	Obtained Scores
Number of Amino Acids	393 aa
Molecular Weight	43398.03 g/mol
Isoelectric Point	7.21
Aliphatic Index	66.56
Instability Index	27.40 (<40 is favourable)
Grand average of Hydrophaticity	− 0.529

The antigen score of newly constructed vaccine showed that the nature of vaccine is antigenic with a value of 0.6127. Vaccine was non-allergic and non-toxic.

Prediction of solubility of vaccine construct:
The solubility score was 0.619 under a job id fd34269a27 which suggests a good solubility score above 0.5.

Table 6: This table shows the solubility details of vaccine's construct

Job ID: fd34269a27	
Sequences: Input Fasta	
Protein	Solubility Score
	0.619
Solubility score above 0.5 (towards green colour) indicates soluble expression, score Below 0.5 (towards red colour) indicates insoluble expression.	

Prediction of Secondary structure of vaccine
Among 393 amino acids, 83 amino acids composed alpha helix that was 21.12% of the total structure as shown in figure 6. No epitope was involved in beta turn which suggests that vaccine is linear. 239 amino acids were responsible for random coil. Random coil comprised 60.81% of total structure of newly constructed vaccine. It showed flexibility of vaccine which allows vaccine to interact with multiple immune cells and to adapt different environments. But on the same time α-helices and β-strands, can also hindrance in host immune response to recognise epitopes. But other factors such as structure, function, nature and relationship of epitopes are also involved in epitope recognition.

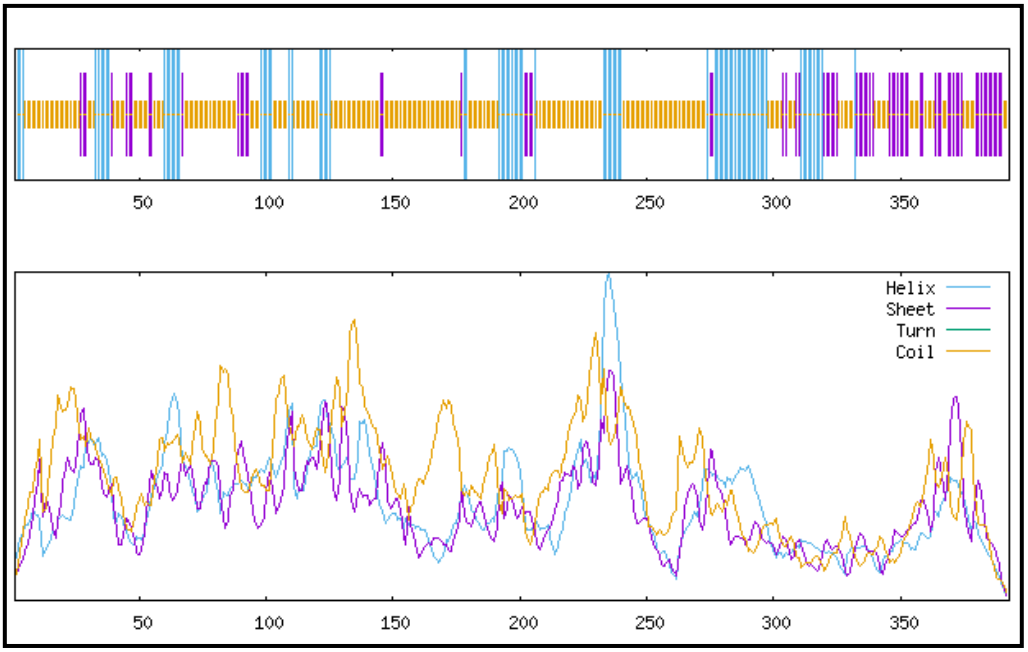


Figure 6: Secondary structure of Vaccine predicted by SOPMA server shows alpha Helix, beta turn, random coiling and Beta strands.

Prediction of 3-Dimensional structure of Vaccine:

SCRATCH protein predictor was used for the construction of three dimensional structure of protein as shown in figure 7, the final constructed vaccine structure was used as template. It predicted the arrangements of amino acids. This sequence of amino acid was further used to get structure of vaccine.

Refinement of 3D structure of Vaccine:

The refinement of three dimensional structure of protein was conducted using Galaxy Refine server as shown in figure 8. It predicted five models out of which Model 1 was selected for further analysis due to good stereochemical properties, side chain positioning and Rama favoured regions. Different parameters were evaluated such as GDT-HA (Global Distance Test-High Accuracy), RMSD (Root Mean Square Deviation), Molprobity, Clash score, poor rotamers and Rama favoured. These parameters were important to determine the accuracy and overall quality of the model.

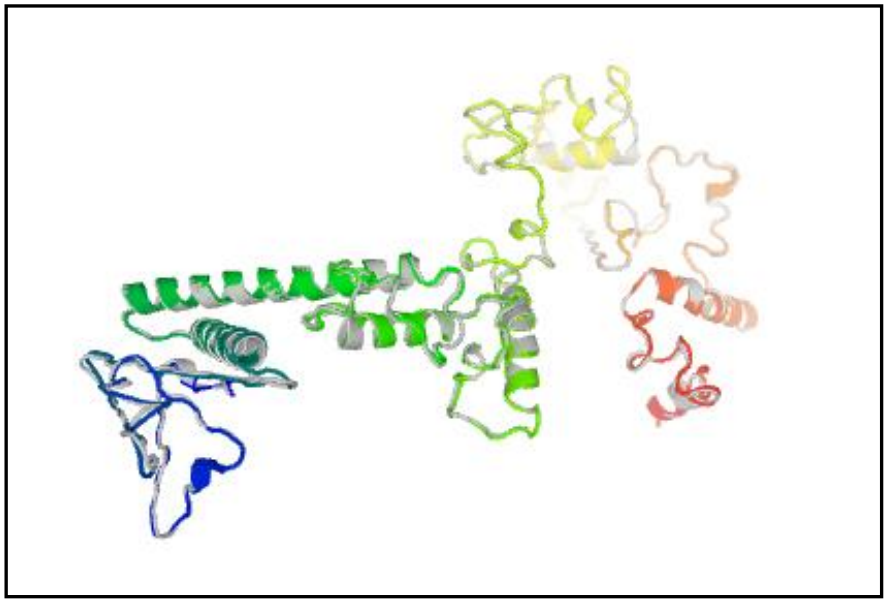


Figure 7: 3D structure of Vaccine

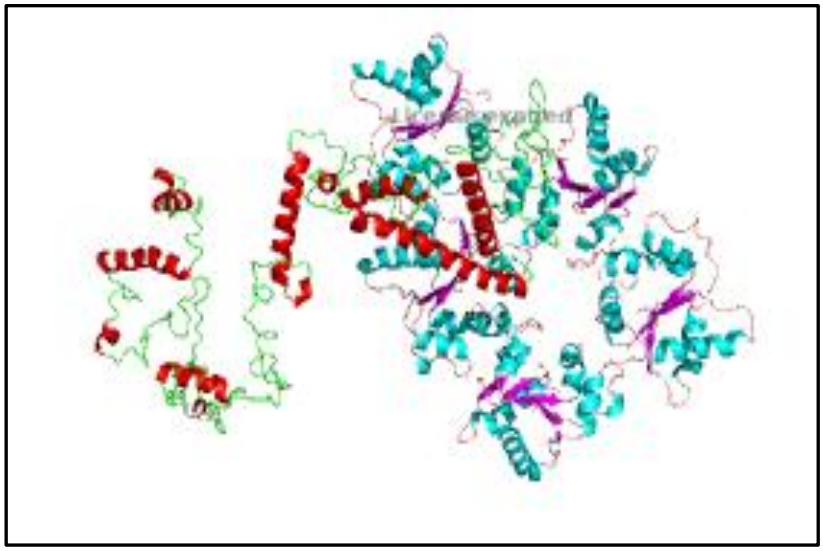
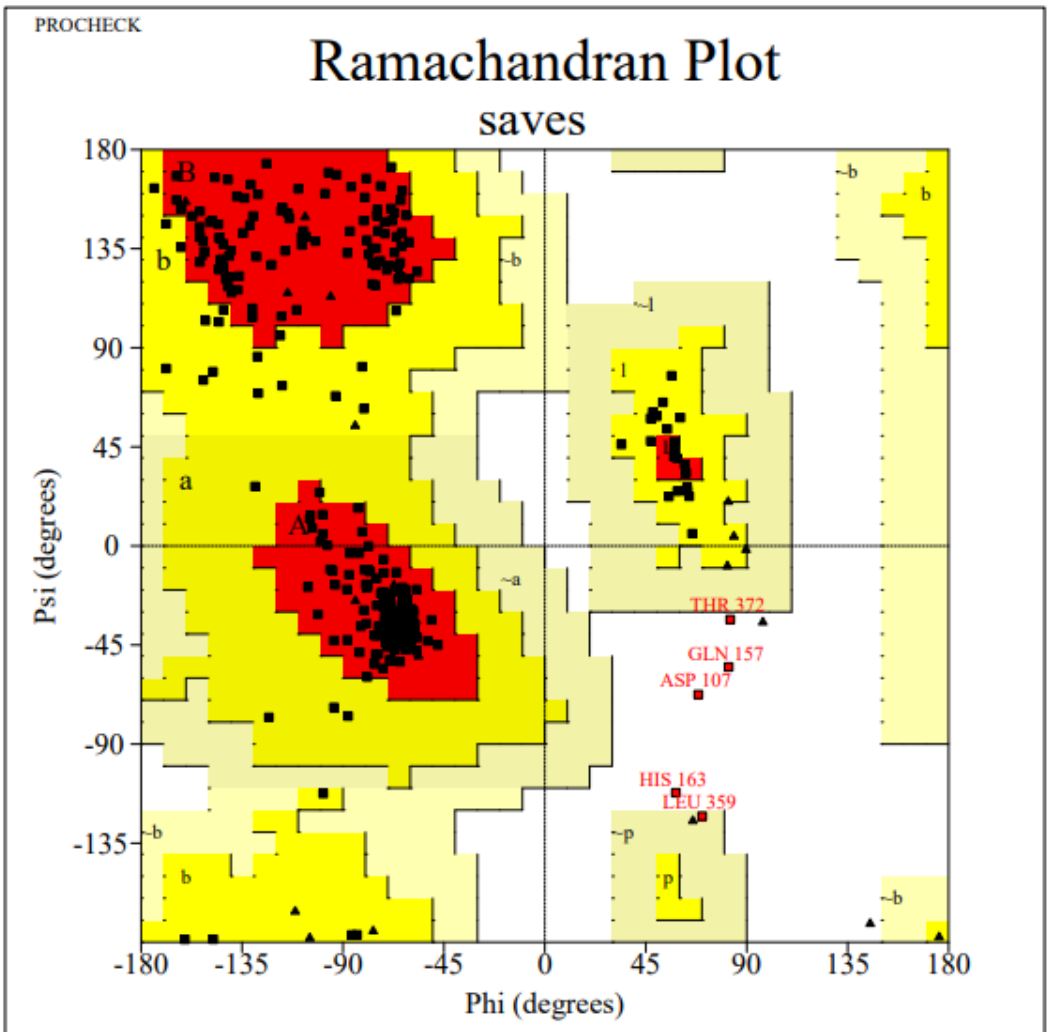


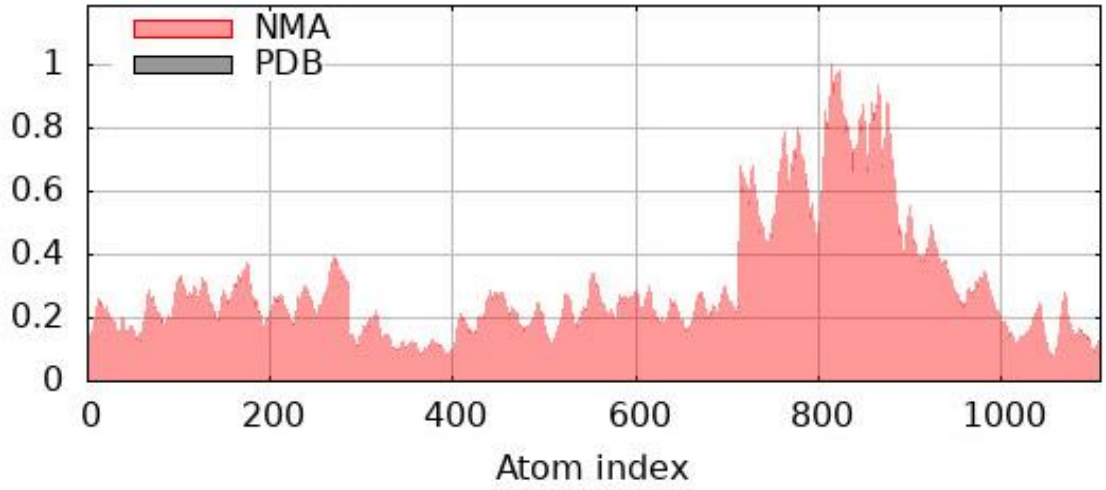
Figure 8: Refined structure of Vaccine

Ramachandran Plot:

To evaluate conformational stability and understand the favoured regions of protein structure, Ramachandran plot was used as shown in figure 9. To generate Ramachandran plot, Model 1 was verified by using PROCHECK server. This plot describes the residues in favoured, allowed, generously allowed and disallowed regions. 87.5% residues were in most favoured regions, 11.0% residues were in additional regions, 0.3% in generously allowed regions while only 1.2% residues were in the region that was not allowed. The good percentage of 87.5% indicated majority of the residues were located in favourable regions.


PROCHECK
Ramachandran Plot
saves
Psi (degrees)
Phi (degrees)
180
135
90
45
0
-45
-90
-135
-180
THR 372
GLN 157
ASP 107
HIS 163
LEU 359

Dynamic Simulation of Vaccine:



The iMODs server as used to carried out the dynamic simulation as shown in figure 10. Kept all the parameters constant, the docked PDB file was uploaded and results were displayed after few seconds. The results of dynamic simulation of vaccine receptor complex provides higher Eigen value which shows faster or global motions (figure 13). In protein structures, s stand for deviations from the average atomic locations (figure 11), which reflect mobility levels; large variances correspond to flexibility, while low variances correspond to rigidity, which can be evaluated using techniques such as PCA or NMA (figure 12). Higher deformability values indicate greater flexibility and adaptability to diverse conformations.

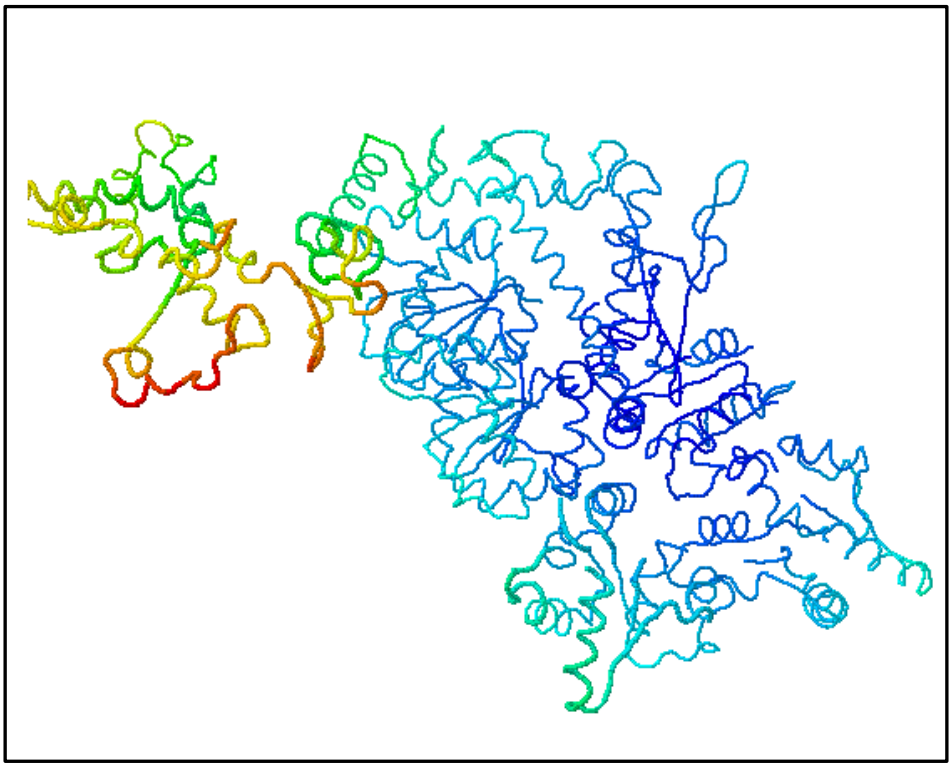


Figure 9: Ramachandran Plot shows the presence of residues (black dots) in favourable (Red) region, less favourable (Yellow) & non-favourable (White) region for the conformational stability of vaccine.

Deformability is a measure of a molecule's ability to change shape in response to external influences. Residues with higher residual index values have more mobility, while residues with lower values are more confined. The residual index evaluates each residue's mobility within a protein, providing insight into its relative flexibility. Higher values indicate greater mobility and flexibility (figure 14). The B-factor, also known as the temperature factor, is a measure of thermal atomic motion and is commonly represented physically by color-coded representations on protein structures (figure 15 and figure 16).

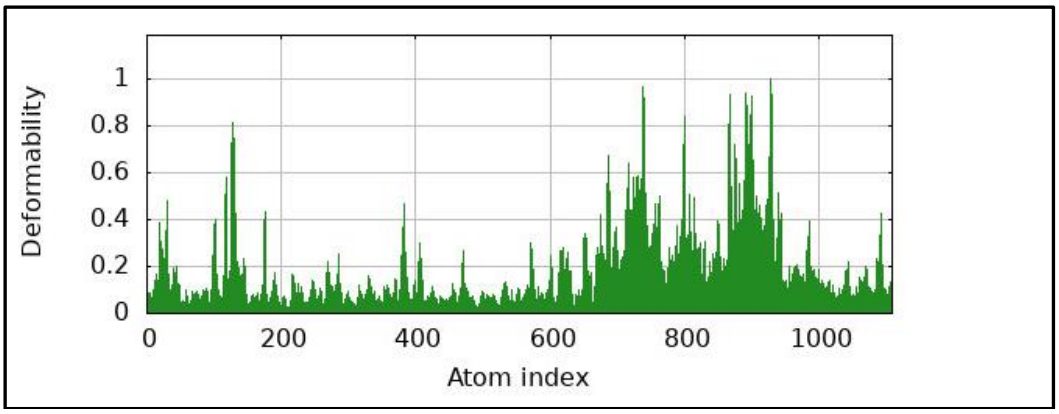


Figure 10: Simulated Structre of vaccine using iMODs

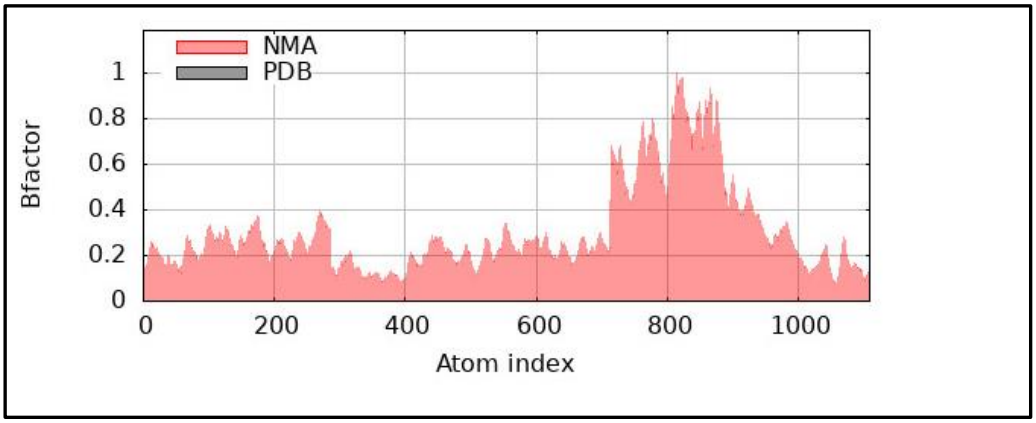


Figure 11: Atomic Index of Beta variance

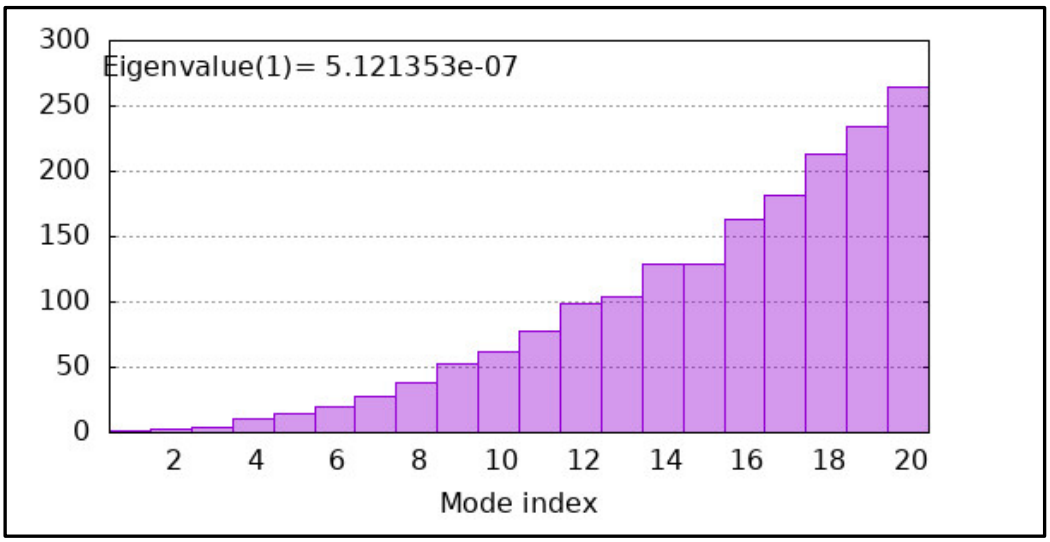


Figure 12: B-factor evaluated using NMA (Normal Mode Analysis & PDB (Protein Data Bank)

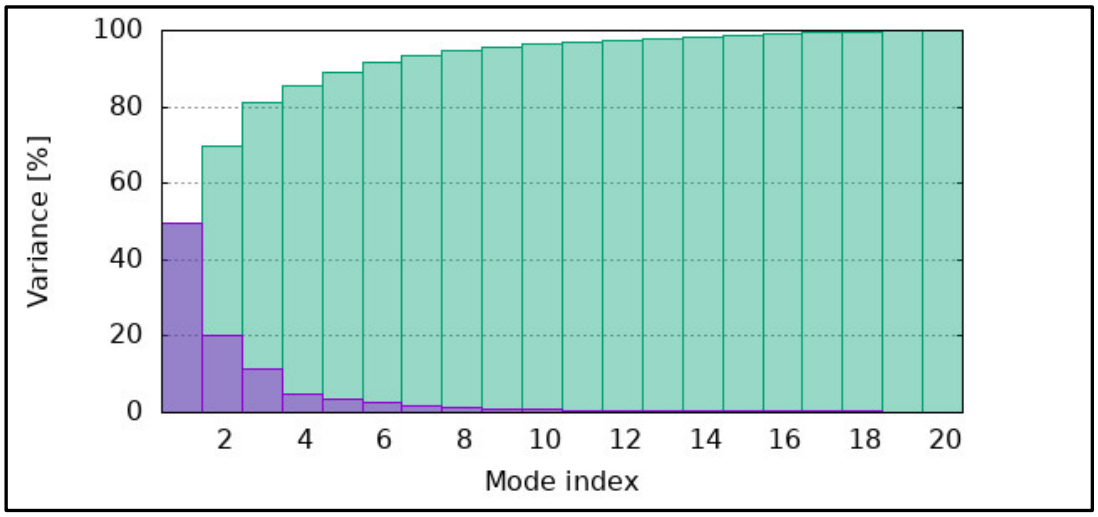


Figure 14: Relationship of Mode Index & Variance %. It represents the variability in immune responses & other outcomes associated with vaccine.

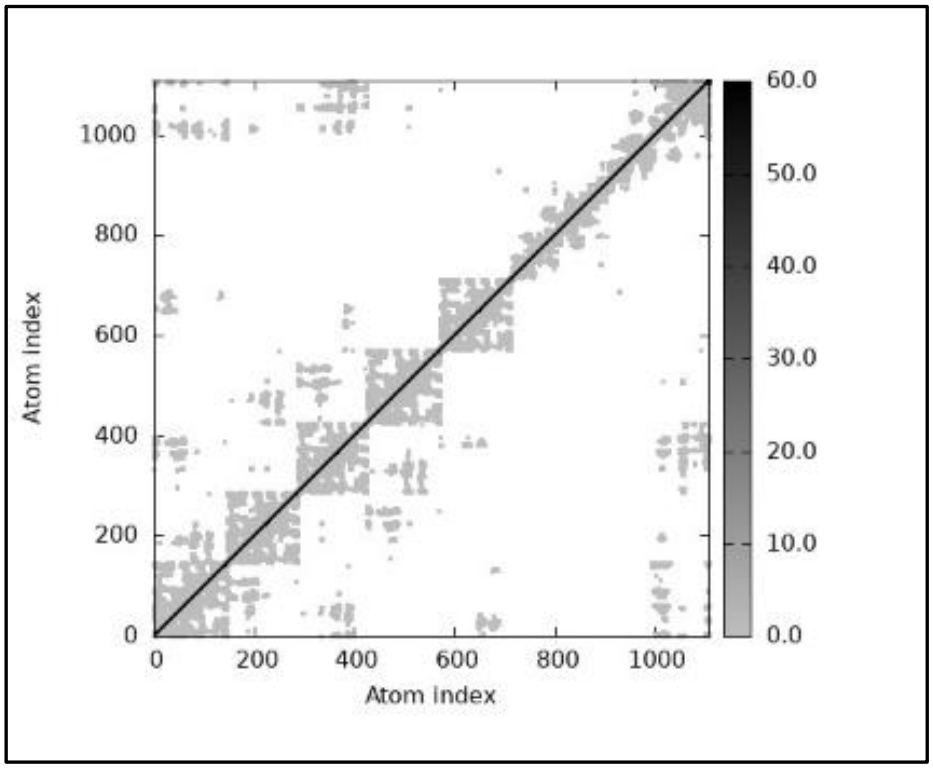


Figure 15: Elastic Network Model predicts the internal dynamics of protein. Gray dots indicates stiffer regions & lighter dots indicates flexible regions.

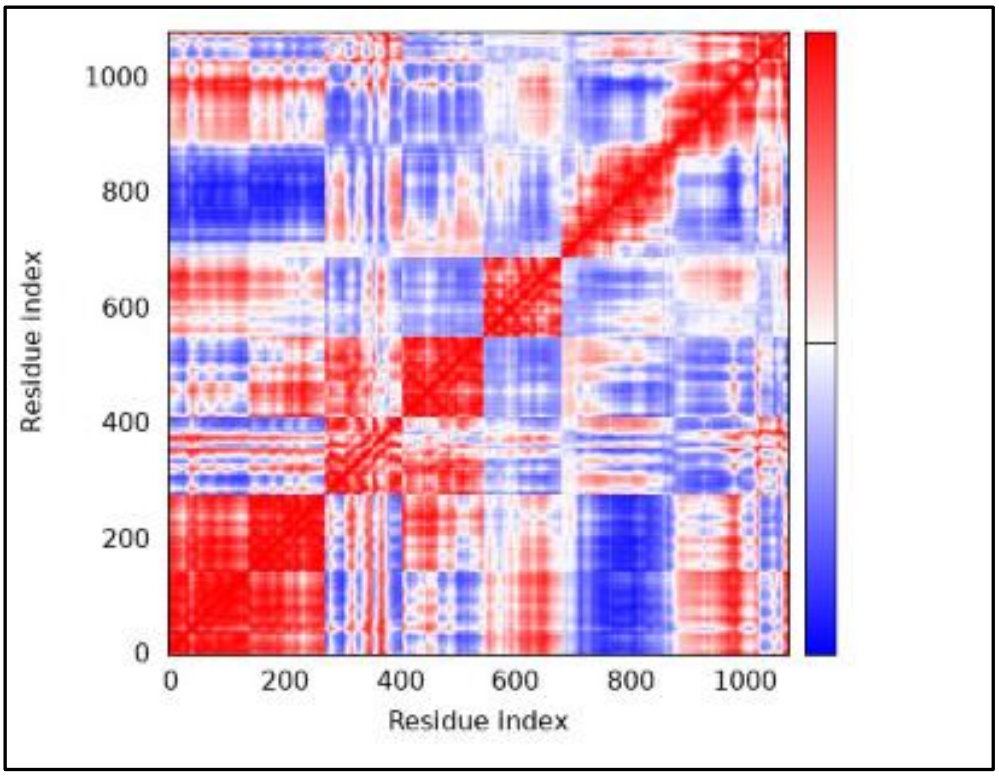


Figure 16: Covariance Map indicates coupling between pairs of residues; correlated (red), uncorrelated (white), anti-correlated (blue)

Nucleotide sequence of Vaccine Construct:
EMBOSS BackTranseq was used to reverse translate the protein sequence of vaccine into the nucleotide sequence for expression analysis of vaccine.

Optimization of Sequence:
Java Codon Optimization Tool, an online server was opted to optimize the nucleotide sequence and codon adaptation prediction. The optimized sequence has Codon Adaptation Index (CAI) value of 0.63 with Guanine-Cytosine content 67.5% as shown in figure 17.

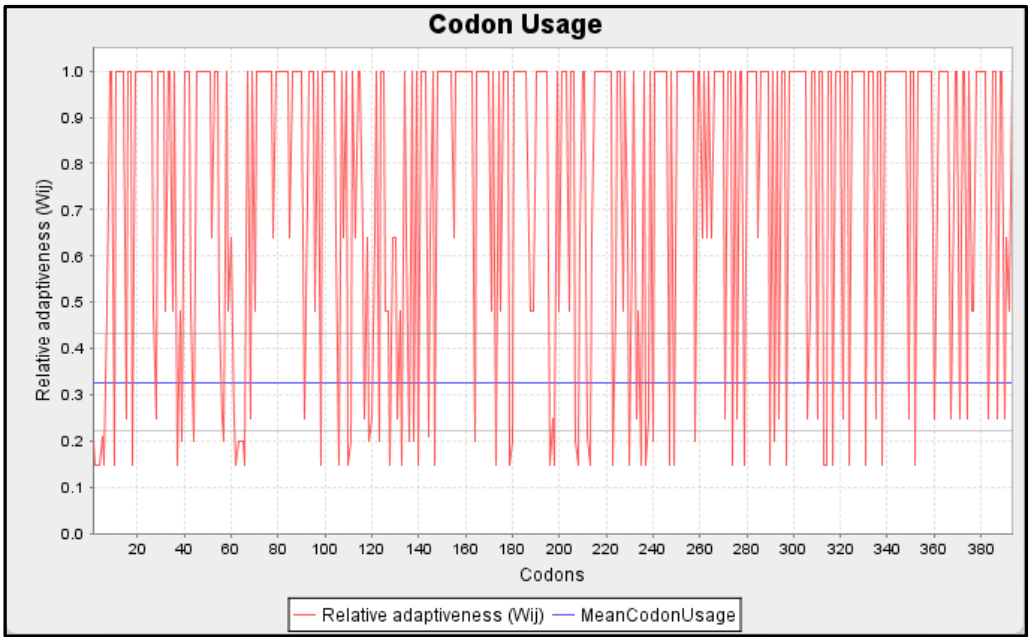


Figure 17: Codon optimization of designed vaccine shows sequence after adaptation

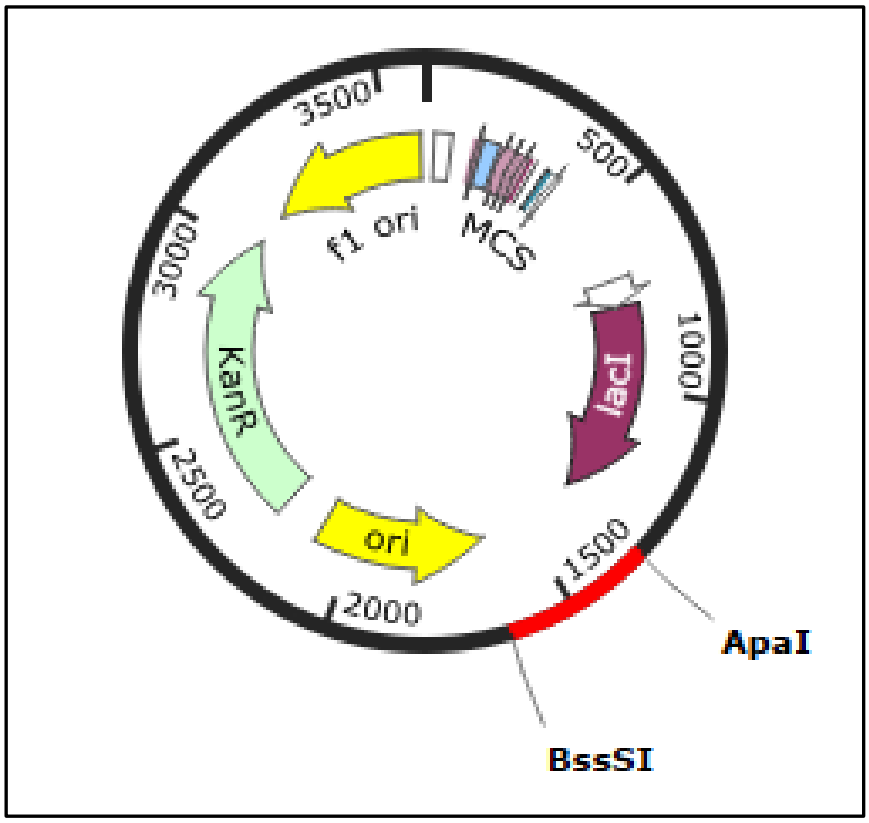


Figure 18: Insertion of codonSequence into vector; Cloned 3600 bp

In Silicon Cloning:
SnapGene software was used to insert an adapted codon sequence into
Pet-28b (+) vector (figure 18). The length of vector is 5368bp while the sequence was comprised of 1179bp. The final cloned vaccine was ready for expression analysis (figure 19).

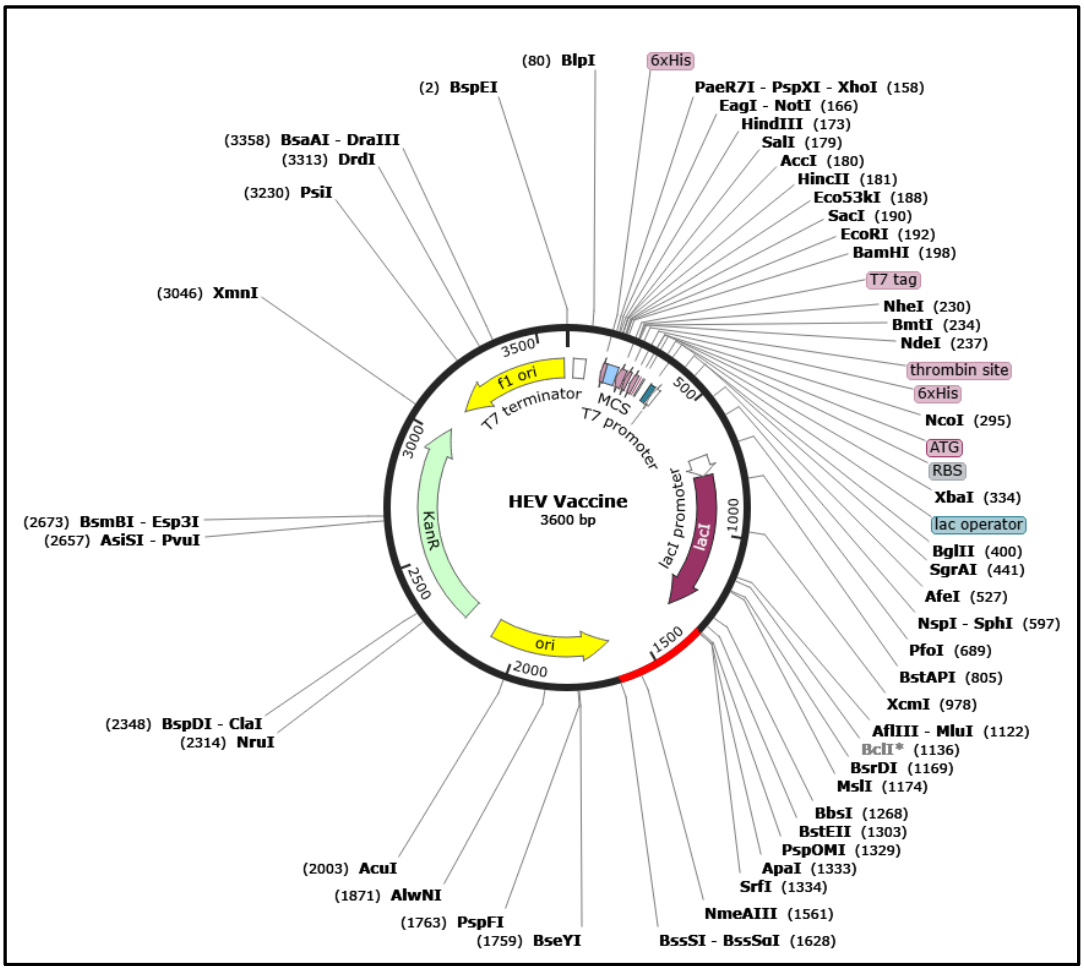


Figure 19: Detailed view of inserted codon sequence into vector

Analysis of Immune Simulation of Vaccine in Body:

The graph presents the relationship between the antigen count (in mL) and the antibody titers (IgM, IgG, and their subclasses) over a 35-day period (figure 20). Key variables include the antigen (Ag) and different immunoglobulin isotypes (IgM, IgG, IgG1, IgG2, etc.). The black line represents the antigen count in the body over time. The rapid increase in antigen count within the first few days suggests an effective immune response triggered by the vaccine. After reaching a peak, the antigen levels gradually decline, indicating clearance by the immune system. IgM Response: The green line (IgM) shows an initial surge, indicating an early immune response. In response to an infection or vaccination, IgM is the first antibody that was produced, signaling the body's immediate reaction. The blue line (IgG) rises sharply after IgM, demonstrating a switch to a more robust adaptive immune response. This is crucial for long-term immunity. The distinction between IgG1 and IgG2 is important, as these subclasses can indicate different immune pathways and responses. IgG1 (purple line) typically correlates with responses to protein antigens. IgG2 (cyan line) may indicate responses to polysaccharide antigens, suggesting a broad immune recognition.

The correlation between antigen levels and antibody responses highlighted the effective mechanism of the vaccine in stimulating an immune response. The decline in antigen levels with a concurrent rise in IgG suggests effective clearance and neutralization of the HEV by antibodies. After 4 days of simulation, the immunoglobulin M (IgM) and IgG were increased, the increased level of IgG2 was not observed (Figure 20). B memory (y2) and B isotype IgM levels were also increased (Figure 21). The level of TH memory (y2), TC & TH cells were also increased (Figure 22,23). The IFN-y level showed peak within 15 days of simulation (Figure 24). (Lu, Wu et al. 2024)

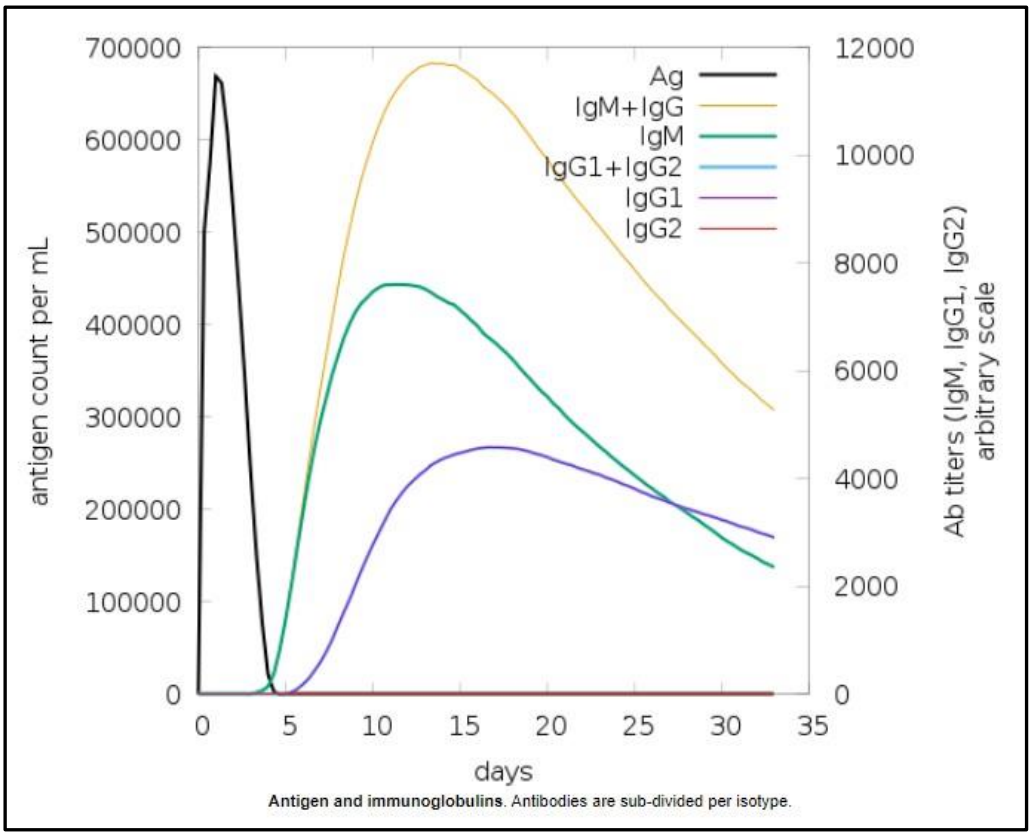


Figure 20: A graph shows immunization simulated with C-ImmSim; the action of vaccine in body in first 35 days; IgM & IgG (Yellow) clearly increase.

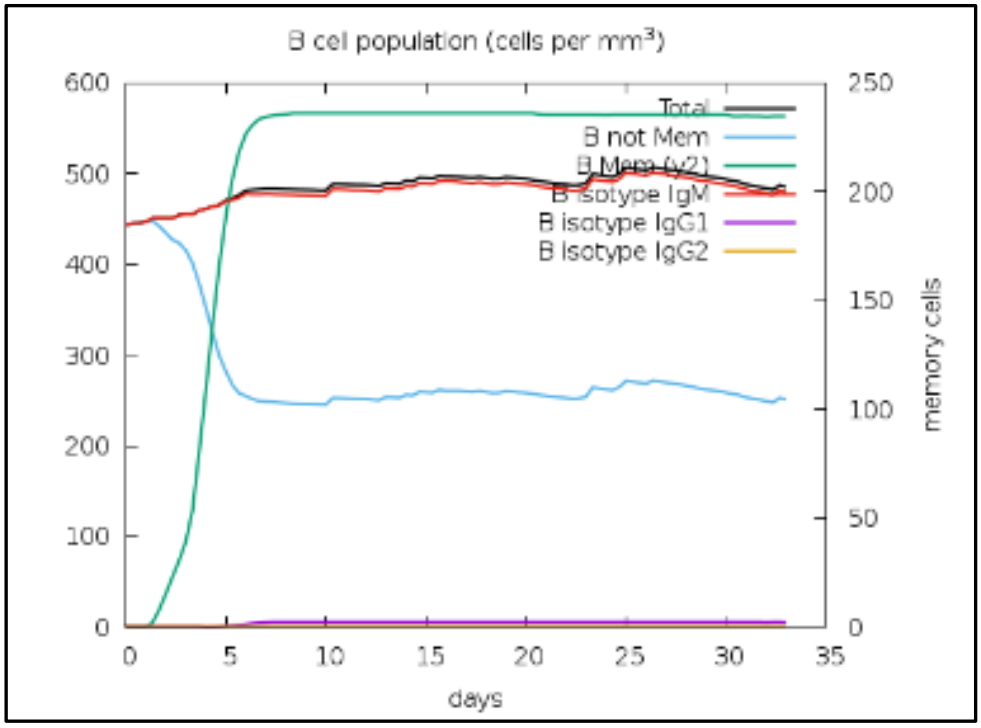


Figure 21: B-cell Population

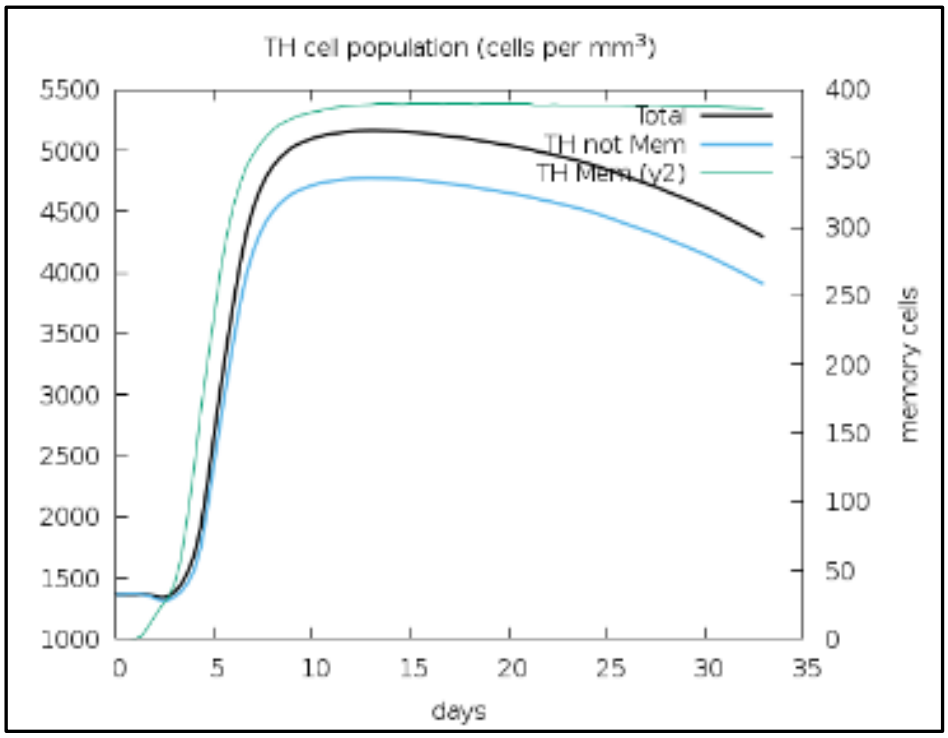


Figure 22: CD4 T-Lymphocytes

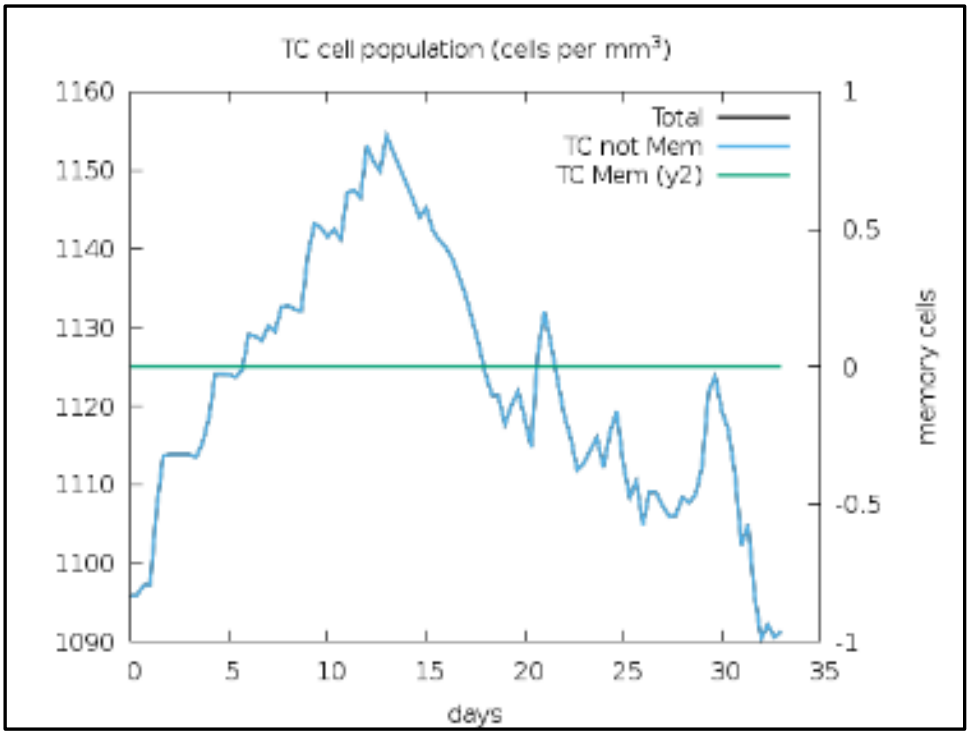


Figure 23: CD8 T-Cytotoxic Lymphocytes

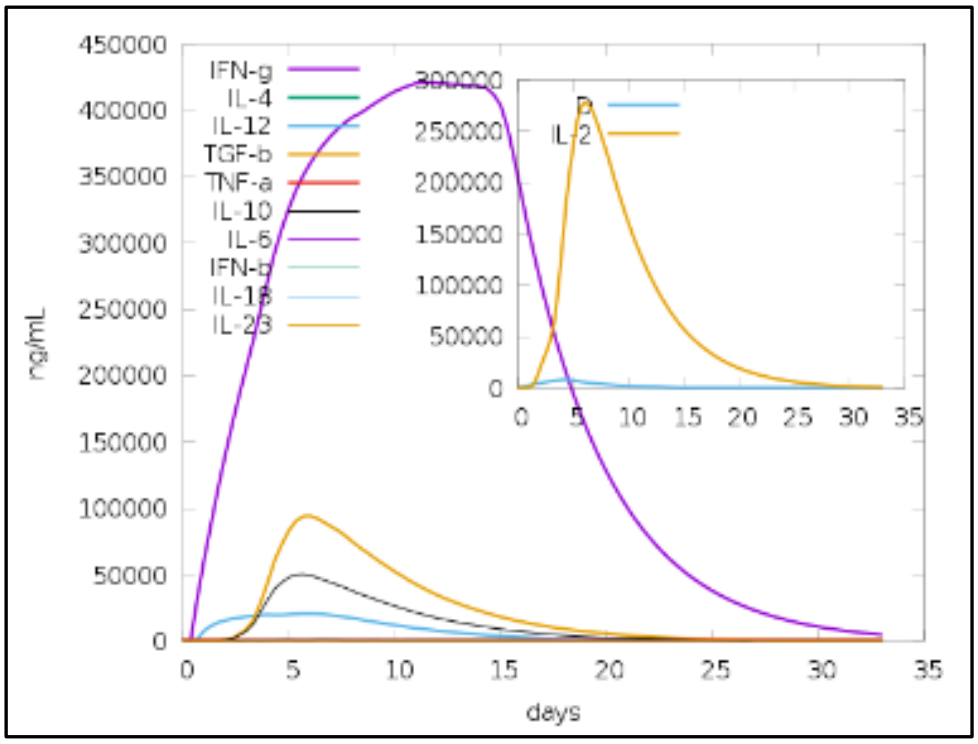


Figure 24: Concentration of Cytokines & Interleukins

Discussion

HEV is a foodborne virus that is spread by feces or oral contact and causes acute hepatitis in humans. It has emerged as a major global public health concern (Organization 2021). According to (Chaudhry, Verma and Koren 2015), pregnant women and those with serious liver disease are at a considerable risk of experiencing severe illness results due to HEV. Traditional vaccines raise several immune-related issues, such as the need for customized immunizations, the initiation of non-specific immune responses that can lead to vaccine allergy and autoimmunity, and pathogens with complex life cycles and antigenic variations.

With the prevalence of autoimmune hepatitis (Samad, Ahammad et al.) rising, recent research has shown that HEV may play a part in the development of autoimmune disorders (Wu, Huang et al. 2013). Furthermore, the protective effect following immunization was diminished in HEV-infected individuals with entire ORF2 antigens. It is crucial to further characterize ORF2 immunologically and its blood dynamics (Li, Huang et al. 2020). Immunologists have been compelled by these worries to develop a more effective HEV vaccine strategy that considers these difficulties. Immunoinformatic techniques can give functions to uncharacterized genes, which may be a more effective method for creating vaccines that protect immune compromised patients from certain pathogens.

Developing a vaccine against HEV is imperative to stop its spread and lessen its impact on the world. A vaccine can support public health initiatives and enhance general health by offering protection against HEV infection. Treatment for HEV is still unknown, but it is implicated in liver infection and extra hepatic indications. The most effective and efficient way to treat infections is through vaccination. Therefore, a vaccine to prevent HEV infection is necessary. Computational approaches to MEPV design are very economical, time-efficient, and innovative.

Compared to the traditional vaccine design approach, which is time-consuming and exhibits lower efficiency and efficacy than the epitope-based vaccine, immune informatics and computational research techniques are a better choice (Zhang 2018). The current study aims to build a computational vaccine model for HEV with a focus on in-silico methods. Acute self-limited hepatitis E is caused by the hepatitis E virus (HEV; previously known as enterically transmitted non-A, non-B hepatitis virus). This virus is thought to be endemic in many developing nations in Asia, Africa, and Latin America, with inadequate sanitary facilities (Purcell & Emerson, 2001a).

Water-borne epidemics characterize hepatitis E, while HEV is mainly roll out through the fecal-oral route. There are three ways that hepatitis E can manifest itself: sporadic infections, minor outbreaks, or

massive epidemics. There have also been isolated reports of HEV infection in non-endemic populations. Ikram, Alzahrani et al. (2023) predicted 9-mer T cell (MHC class I and class II) epitopes using 291 consensus sequences of chosen genotypes (1, 3, 4). Effective HEV vaccines should be able to stimulate human humoral and cellular immune responses. According to this study, a group of immunogenic epitopes may trigger immune responses from T and B cells. This could aid in creating a vaccine against HEV infection based on epitopes.

Our suggested approach for identifying immunogenic epitopes entails locating overlapping T cell, IFN-y, and B cell epitope regions from HEV's ORF2. The FASTA format yielded a 477 amino acid sequence with the primary accession AOA896W9U2. With a threshold value of 0.4 and a value of 0.6322, this protein sequence showed a high degree of antigenicity. This protein is categorized as stable with an instability index of 33.56 (less than 40). There are no allergies to this protein sequence. This protein sequence scored 0.689 (>0.5), suitable for building a multi-epitope vaccine. Worldwide, the population coverage was examined. The epitopes of MHC-I and MHC-II classes showed population coverage of 78.13% and 70.55%, respectively. The vaccine construct weigh about 43398.03 g/mol and consists of 393 amino acids.

According to the theoretical pI (isoelectric point) of 7.21, the vaccine will probably have a net positive charge under typical physiological circumstances. Under the job ID fd34269a27, the solubility score was 0.619, indicating a good solubility score above 0.5. Final vaccine construct constitute 393 amino acids has alpha helix, random coil & no beta turn which suggested it as linear vaccine structure. By checking its antigenicity & solubility scores which are 0.689 & 0.619 respectively, enhanced our confidence in vaccine construct. SCRATCH Protein Predictor was opted to establish three dimensional structure of vaccine.

The structure was refined by using an online tool Galaxy Refine. It improved vaccine's structure by enhancing scores, change orientation of bindings by placing them on narrow position in immunogenic regions. Multiple model was established and we selected Model 1 for further verification through Procheck Ramachandran Plot. Ramachandran plot showed 87.5% residues in most favoured regions while only 1.2% residues were in unfavourable regions. Simulation was conducted through iMODs which represented vaccine's flexibility & adaptability to diverse conformations.

The protein sequence of vaccine was reverse translated into nucleotide sequence by EMBOSS BackTranseq to check the expression of vaccine. This sequence was optimized by Java Codon Optimization Tool with GC content 67.5% & Adaptation Index value 0.63. Pet-28+ vector was selected for cloning. A cloned vaccine of 3600bp was set for expression analysis. The observed patterns in the graph that showed expression revealed that the vaccine is likely to elicit a strong immune response, characterized by both immediate (IgM) and long-term (IgG) antibody production. A balanced response with both IgG1 and IgG2 indicates a well-rounded immune activation, which is desirable for comprehensive protection. Since conventionally made vaccines are still undergoing trials, we suggest using immunoinformatic and reverse vaccinology to create a vaccine against HEV that is effective in order to save time. Our attempt demonstrates that the designed vaccine elicits a promising immune response, characterized by the production of both IgM and IgG antibodies, which are crucial for effective viral neutralization.

CONCLUSION

Hepatitis E virus is an imminent risk to pregnant & immunologically weak persons. Unfortunately no vaccine is currently available against HEV, universally. We have opted an immunoinformatic approach to design a vaccine against HEV by targeting ORF2 Protein which induced strong B & T Cell responses by simulated immunization. In this approach we conducted several analyses to evaluate the effectiveness of the suggested or designed vaccine, and the results were satisfactory. So, our attempt will hopefully be a good addition to already reported literature about in-silico approach for vaccine construction laying down a foundation for future studies in this field.

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