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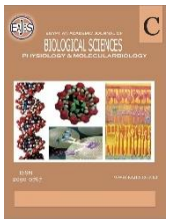

Building Resilient Immunity Against Powassan Virus: The Necessity of A Comprehensive Immunoinformatic Approach and Coordinated T and B Cell Responses

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ABSTRACT

Background: Powassan virus (POWV), a vector-borne pathogen primarily impacting humans as incidental hosts, has recently surfaced as a significant worldwide health concern, carrying the potential for severe clinical consequences or enduring neurological sequelae. Currently, there are no approved vaccines for POWV. The key to developing effective vaccines lies in identifying protective factors. Despite significant outbreaks and the absence of antiviral treatments, progress in creating an epitope-based vaccine for POWV has been slow. **Aim of the work:** Our aim was to employ various immunoinformatics and docking simulation methods to design an epitope-based vaccine capable of triggering a robust immune response and predict inhibitors that could potentially target therapeutic sites effectively. **Methodology:** At first, the complete POWV proteome was obtained from a database and scrutinized to identify the protein with the highest immunogenicity. The structural attributes of the designated protein were thoroughly explored. Subsequently, the chosen protein underwent testing to assess its capacity to stimulate both humoral and cell-mediated immunity through T and B cells. **Results:** The leading B cell and T cell epitopes were pinpointed as a peptide segment encompassing 7 amino acids from position 353 to 360, represented by the sequence TLAGPRSKY. This peptide exhibited the potential to interact with up to 19 different HLAs, offering extensive population coverage that ranged from 62.53% to 86.97%. To confirm the binding interaction within the HLA binding cleft, we conducted in-silico docking techniques. Furthermore, we assessed the allergenicity of these epitopes. In our post-therapeutic approach, we predicted the three-dimensional structure, conducted validation and verification processes, and subsequently performed molecular docking studies to identify potential drug-binding sites and suitable therapeutic inhibitors for the target protein. **Conclusion:** Nevertheless, this computational approach to epitope-based peptide vaccine design and target site prediction against POWV represents a pioneering advancement in Powassan virus research. It's important to note that the outcomes will require validation through in vitro and in vivo trials.

INTRODUCTION

Powassan virus (POWV), an infectious pathogen with potentially lethal consequences for humans, falls under the Flaviviridae family and belongs to the genus Flavivirus (Nelson *et al.*, 2022). Currently, POWV exhibits a divergence into two distinct genetic lineages: POWV (lineage 1) and deer tick virus (DTV) (lineage II) (Nelson *et al.*, 2022).

Discriminating between these two lineages necessitates genetic sequencing as the sole means of differentiation. In North America, POWV stands as the sole tickborne flavivirus of recognition, posing a threat by occasionally triggering incidents of encephalitis, meningoencephalitis, aseptic meningitis, or noninvasive infections in individuals who have suffered tick bites from POWV-carrying vectors (Hermance & Thangamani, 2017). Both POWV encephalitic and non-neuroinvasive infections are categorized as nationally notifiable illnesses in the United States. Furthermore, it is noteworthy that POWV is among the 24 non-enteric zoonotic diseases in Canada that are accorded a high risk of emergence, a concern that has garnered prioritization from public health authorities (Adam-Poupart *et al.*, 2021). The initial documented case of human infection with POWV dates back to 1958 in Ontario, Canada, and to this day, the virus remains active in various regions spanning Canada, the United States, and Russia (Campbell & Krause, 2020). While instances of neuroinvasive illness resulting from POWV infection remain relatively rare, there has been a discernible increase in prevalence, particularly in the United States, during recent years, notably between 2016 and 2019 (Stone & Pinto, 2023). Neurological manifestations such as encephalitis, meningitis, and meningoencephalitis have been reported as outcomes of infection. Distressingly, almost half of patients afflicted with neuroinvasive illness experience enduring neurological sequelae, and approximately 10% of cases prove to be fatal (Krett *et al.*, 2022). Regrettably, there are currently no licensed vaccines or antiviral therapies available for the treatment of POWV infection.

Powassan virus much like other tick-borne flaviviruses (TBFVs), is classified as a positive-sense RNA virus enveloped within a lipid bilayer. Within the POWV family, there exist two distinct lineages, lineage I and lineage II, commonly known as the deer tick virus (Kemenesi & Banyai, 2018). Notably, these lineages share an astonishing 96% amino acid sequence identity in their envelope (E) glycoprotein, rendering them serologically and clinically indistinguishable. It's worth highlighting that POWV lineage I (POWV-I) and lineage II (POWV-II) are transmitted by different tick species (Lange *et al.*, 2023). POWV-I is transmitted by Ixodes cookei, whereas POWV-II is vectored by Ixodes scapularis. The rise in tick-borne diseases is believed to be attributable to factors such as residential expansion into forested regions and global climate changes, which result in prolonged tick seasons. Indeed, there has been a noticeable expansion in the geographic range of Ixodes scapularis in the United States in recent decades (Jaenson *et al.*, 2012). The glycoprotein envelope (E) of flaviviruses forms a homodimer and consists of three distinct domains, namely EDI, EDII, and EDIII. EDI encompasses the N-terminus of the E protein and assumes the form of an eight-stranded β-barrel structure, serving as a pivotal molecular hinge (Zhang *et al.*, 2017). On the other hand, EDII functions both as a dimerization domain and contains a fusion loop peptide. This fusion loop is instrumental in facilitating pH-dependent membrane fusion, particularly within the late endosome during viral entry. Lastly, EDIII takes on the characteristics of an Ig-like domain and plays a critical role in enhancing interactions with host cells. It undergoes significant structural changes necessary for the fusion of the viral membrane with host membranes, and notably, it serves as a target for various neutralizing antibodies against flaviviruses (Zhang *et al.*, 2021). Recent efforts in the development of a POWV vaccine have centered on utilizing the pre-membrane (prM) and envelop (E) proteins of POWV as the antigen. These endeavors have encompassed the exploration of various platforms, including mRNA, DNA, and Virus-Like Particles (VLPs) (Malonis *et al.*, 2022). These platforms have demonstrated their ability to induce neutralizing and protective antibody responses in mice. However, it's essential to acknowledge that the prM and E proteins of POWV encompass numerous antigenic sites, of which only a fraction are

targets for functional antibodies capable of inhibiting POWV infection effectively (VanBlargan *et al.,* 2021). Furthermore, it is recognized that certain conserved epitopes within EDII and prM can elicit broadly reactive antibodies that, while not strongly neutralizing, can potentially contribute to a phenomenon known as antibody-dependent enhancement (ADE) in other flaviviruses. Though the role of ADE in POWV remains uncertain, a promising strategy could involve the development of a vaccine focused solely on crucial, functional epitopes necessary to elicit a protective antibody response. Such an approach could offer distinct advantages in terms of vaccine efficacy and safety. Exploration of EDIII-based subunit vaccines has been conducted for flaviviruses like Dengue and Zika virus (Kubinski *et al*., 2020). However, the immunogenicity of the Powassan virus, EDIII has yet to be characterized. It's worth noting that despite their promise, recombinant subunit vaccines can sometimes elicit relatively weak or moderate immune responses. To enhance the effectiveness of subunit vaccines, a developing technique involves the presentation of viral antigens on self-assembling protein nanoparticles. This innovative approach aims to improve the immunogenicity of the vaccine by enhancing the antigen's presentation and immune system recognition, potentially leading to a more robust and protective immune response (Brisse *et al.,* 2020).

Indeed, the organized and multivalent presentation of viral structural proteins can effectively mimic a viral particle, thereby eliciting a more potent immune response. This strategy capitalizes on the principle that presenting multiple copies of antigens in a structured manner enhances the immune system's recognition and response. Furthermore, multivalent antigen display plays a pivotal role in improving the efficiency of cross-linking B cell receptors, a critical step in the activation of B cells and subsequent antibody production (Li *et al.,* 2019). This approach has been successfully employed in combatting various infections, including HIV, influenza, SARS-CoV-2, and other pathogens, demonstrating its versatility and efficacy in vaccine development and immunization strategies (Brisse *et al.,* 2020). Extensive efforts in the field have resulted in the generation of several neutralizing monoclonal antibodies (mAbs) against POWV (EDIII). These mAbs were produced by immunizing mice with an mRNA-based vaccine expressing POWV prM/E, a strategy that has proven effective in eliciting an immune response (VanBlargan *et al.,* 2021). Notably, the identified epitopes on the EDII and EDIII regions have been recognized as major targets of protective neutralizing POWV mAbs. Among these mAbs, POWV-80, a murine monoclonal antibody, has demonstrated cross-neutralizing properties. It targets a complex epitope located within the lateral ridge/C-C' loop of the EDIII region. This epitope comprises the N-terminus, C-C', and FG loops of EDIII. However, it's important to note that despite these findings, only a limited number of POWV mAbs that specifically target the EDIII have been identified. Further research is needed to pinpoint the specific key epitope(s) within the EDIII region that is targeted by neutralizing mAbs, shedding light on potential avenues for vaccine development and therapeutic interventions (Malonis *et al.,* 2022). As documented in previously published literature, phylogenomic investigations have revealed a striking evolutionary similarity of more than 80% between deer tick virus (DTV) and POWV (Robich *et al.,* 2019). The comprehensive analysis of their genetic makeup, characterized by an 11kb single-stranded, positive-sense RNA genome encoding seven nonstructural proteins and three structural proteins-capsid (C) protein, pre-membrane (preM) protein, and envelope (E) glycoprotein has been the focus of molecular epidemiology studies (Gaibani *et al.,* 2013). Despite the complete sequencing of their genomes, the enigma surrounding how these viruses initiate infection and persist within their host organisms has driven the quest for innovative vaccination strategies. Consequently, this study adopted an in-silico approach for the identification and

characterization of epitopes. This approach holds promise as it provides a solid foundation for future researchers to explore and potentially develop vaccines against the Powassan virus. The in-silico methodology offers a valuable platform for the advancement of research in the development of potential Powassan virus vaccines.

Close to a decade ago, researchers made significant strides by identifying B-cell and T-cell epitopes, including HLA class-I CD8+ T-cells and HLA class-II CD4+ T-cells, in their pursuit of unraveling the underlying viral pathogenesis of Powassan virus (Ranasinghe et al., 2016). To address the challenge posed by the considerable polymorphisms observed within diverse population groups, a population coverage analysis was carried out. This comprehensive analysis was essential for mitigating the impact of extreme genetic variations and represents a crucial step toward the development of a novel vaccine against Powassan virus. The flavivirus nonstructural 1 (NS1) protein boasts an intriguing structure-function relationship due to its glycosylation. It takes on various structural configurations to fulfill diverse intracellular and extracellular roles, including involvement in the replication complex, aiding in virus assembly, and countering the complement system's defensive actions. Moreover, NS1 plays a pivotal role in fostering protective immunity through antibody-mediated cellular cytotoxicity, and antibodies targeting NS1 can offer passive protection against virus challenges in animal models (Carpio & Barrett, 2021).[20]. Historically, NS1 has been employed as a diagnostic marker for flavivirus infections because of its ability to fix complement and specificity. The nonstructural 1 protein of flaviviruses possesses an atypical structure-function relationship due to its glycosylation and ability to adopt diverse conformations, enabling it to perform various intracellular and extracellular functions. These functions encompass participation in the replication complex, facilitation of virus assembly, and antagonism of the host complement system. Historically, NS1 has served as a valuable diagnostic marker for flavivirus infections due to its capacity to fix complement and its specificity. Given its role in disease pathogenesis and its capacity to induce a robust humoral immune response following infection, NS1 represents a promising candidate for inclusion in flavivirus vaccine development efforts. Given its significant role in disease pathogenesis and the robust humoral immune response it triggers following infection, NS1 emerges as a promising candidate for inclusion in potential flavivirus vaccines (Rastogi *et al.*, 2016). Such inclusion could enhance vaccine efficacy by targeting a crucial player in the virus's lifecycle and pathogenicity. In our investigation, we conducted an in-depth analysis of the nonstructural 1 (NS1) protein of Powassan virus. Our primary aim was to identify potential immunogenic regions within NS1, with the goal of predicting a promising candidate for vaccine development. Additionally, we performed a comprehensive genome-wide search to pinpoint the most suitable drug target site within the virus's genetic makeup. Subsequently, we employed computational methods to simulate the inhibition of this target site using a predicted inhibitor molecule. This study was undertaken with the overarching objective of laying the groundwork for future laboratory-based efforts aimed at developing a comprehensive therapeutic and preventive strategy against Powassan virus infection. By combining computational and analytical approaches, we sought to contribute valuable insights and potential avenues for combating this viral pathogen effectively.

MATERIALS AND METHODS

Study Design:

The research methodology applied in the development of the Powassan virus strategy is succinctly illustrated in Figure 1 below. The diagram provides an overview of a vaccination approach targeting the non-structural protein of Powassan virus (NS1) for vaccine formulation. All experiments were executed on a personal computer operating the Windows XP Professional Edition. The study also relied on internet resources and a

spectrum of online and offline tools. Vaccines designed to identify distinctive B and T-cell epitopic peptides extracted from the NS1 were developed using bioinformatics methodologies. In this investigation, in silico analysis was employed to identify the unique B-cell and T-cell epitope proteins of NS1 that hold the highest antigenic significance for Powassan virus subtypes. Selection criteria encompassed considerations of antigenicity, stability, and peptide length for specific B and T-cell epitopes within the NS1 protein. The comprehensive viral proteome of NS1 strains was obtained from the NCBI database. Diverse immunological frameworks of conserved NS1 sequences were evaluated using a variety of databases and bioinformatics tools. Amino acid sequences were retrieved from the NCBI database. The antigenic nature of the NS1 sequence was ascertained utilizing the VaxiJen server and the Kolaskar and Tongaonkar technique. Subsequent to the assessment of numerous B and T-cell epitopes, the vaccine efficacy of one efficient peptide from each B and T-cell epitope, based on their antigenic determinants, was evaluated. B-cell epitope prediction servers were employed for the examination of the NS1, conserved non-structural protein, leading to the identification of two identical B-cell epitopes. Detailed analysis of amino acid sequences for B and T-cell epitopes was conducted using ABCPred and the Immune Epitope Database.

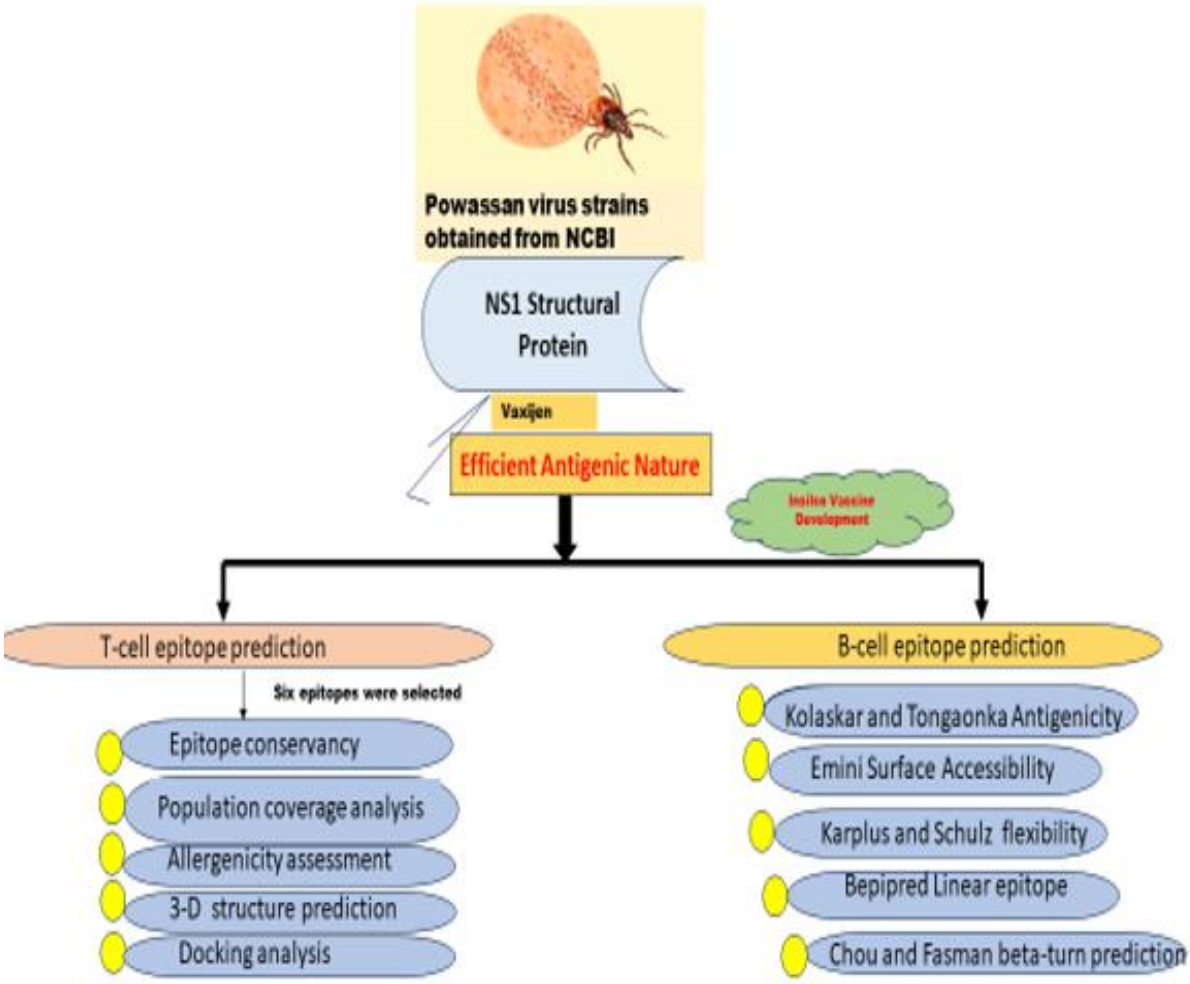

Fig. 1: A graphical depiction illustrating the methodologies employed in the formulation of a vaccine targeting the non-structural protein NS1.

Retrieval of The Sequence:

The amino acid sequences of the Powassan virus NS1 protein 1 (Non-Structural protein 1) were acquired in FASTA

format from the UniProt Knowledge Base (UniProtKB) database. UniProtKB serves as a comprehensive repository for protein sequences and associated annotations, functioning as a central hub for gathering functional insights into proteins while maintaining precision, uniformity, and extensive annotation. Subsequently, these sequences underwent scrutiny for antigenic properties, solvent exposure, surface accessibility, flexibility, and the presence of MHC class I binding sites (The UniProt Consortium, 2014).

Identifying The Protein With The Most Pronounced Antigenic Properties:

The next step involved submitting the proteins to the VaxiJen v2.0 Server, using default parameters, to predict potent antigens and subunit vaccines and pinpoint the protein with the highest antigenic potential. A virus was designated as the target organism, and a standard sequence format was provided for analysis. Subsequently, all the antigenic proteins, along with their respective scores, were filtered using Excel. For in-depth exploration, a single antigenic protein with the highest antigenicity values was selected.

Analysis Of Both Primary And Secondary Protein Structures:

The selected protein's physiological and chemical properties were analyzed using both the ProtParam tool from the Expasy server (Wilkins *et al.,* 1999 2005) and a self-optimized prediction method with alignment (SOPMA) (Geourjon & Deleage, 1995). The current settings in ProtParam were used to assess multiple protein characteristics. These characteristics encompass the protein's theoretical isoelectric point (pI), molecular weight, amino acid composition, grand average hydropathicity (GRAVY), estimated half-life, extinction coefficient, instability index, and aliphatic index. Additionally, SOPMA provided predictions for properties such as solvent accessibility, transmembrane helices, globular regions, bend regions, random coil regions, and coiled-coil regions, offering a comprehensive insight into the protein's structural and functional attributes.

Pre-therapy Procedures For The Development Of A Powassan Virus Vaccine (T cell epitope identification):

In this regard, NetCTL-1.2, a web-based system specifically designed for predicting human CTL epitopes within any given protein, was employed (Larsen *et al*., 2007). It generated an overall score by considering factors such as proteasomal cleavage, TAP transport efficiency, and MHC class I affinity predictions. For our current study, we set the threshold value for epitope identification at 0.5, ensuring a balance between sensitivity (0.89) and specificity (0.94). Furthermore, another resource tool from the Immune Epitope Database (IEDB) was employed to predict several critical parameters for each selected peptide. This included the proteasomal cleavage score, TAP score (Transporter Associated with Antigen Processing), processing score, and MHC-1 binding score (Zhang *et al.,* 2006). These scores were calculated using the SMM (Stabilized Matrix Method) algorithm, providing valuable insights into the antigenic potential of each peptide.

Prediction of Epitope Conservancy:

The IEDB analysis resource was employed to predict the conservancy of specific epitopes. Conservancy, in this context, refers to the percentage of protein sequences where the epitope is present at or above a specified level of identity. The epitope conservation calculator was executed as an online Java application, providing insights into the extent to which these epitopes are conserved across different protein sequences (Soria-Guerra *et al.,* 2015).

Calculation of Population Coverage:

The IEDB population coverage tool was utilized to compute the population coverage for each epitope (Fleri *et al.,* 2017). This tool relied on MHC binding and/or T cell restriction data to estimate population coverage through an online interface. Its primary objective is to ascertain the percentage of individuals expected to mount a response to a specific set of epitopes, taking into account their known MHC restrictions. For each specific population coverage assessment, the tool provided three key pieces

of information: Anticipated population coverage, Population recognition of HLA combinations and recognition of 90% of the population (PC90). To perform these calculations, all epitopes and their associated MHC-I molecules were inputted into the tool, and the population coverage metrics were subsequently determined.

Assessment of Allergenic Potential:

To predict the allergenicity of the epitope intended for vaccine development, we employed the online tool known as AllerHunter. This service evaluates allergenicity by merging the allergenicity assessment guidelines established by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) with the use of support vector machines (SVM) in pairwise sequence similarity analysis. AllerHunter is proficient in discerning both allergenic and non-allergenic components with remarkable specificity, rendering it an invaluable resource for forecasting allergen cross-reactivity (Muh *et al.*, 2009).

Creation of the Three-Dimensional (3D) Epitope Structure:

For conducting docking simulation studies, we introduced the highly conserved TLAGPRSKY epitope into the PEP-FOLD server. PEP-FOLD is a de novo approach specifically engineered to predict peptide structures based on amino acid sequences (Thevenet *et al.*, 2012). Its methodology relies on utilizing a structural alphabet (SA) that characterizes the structural conformations of four consecutive amino acid residues. This approach associates the predicted sequence of SA letters with both a greedy algorithm and a coarse-grained force field. As a result, the PEP-FOLD server-generated five proposed 3D structures. Subsequently, we selected the most suitable model from these structures for the purpose of analyzing its interactions with HLAs.

Docking Simulation Study:

Molecular docking holds a pivotal position in the field of computational drug design. Its primary function is to forecast the favored spatial arrangement of a ligand when interacting with the binding site of a receptor molecule. The intensity of this interaction between the ligand and the receptor is quantified using the experimentally determined inhibition constant, denoted as Kd. A docking study was conducted to validate the binding interaction between HLA molecules and the epitope we identified, using AutoDock Vina. The binding energy of the receptor-ligand interaction can be measured by Eq. 1:

$$DG_{bind} = DG_{complex} - (DG_{ligand} - DG_{receptor})$$

This relationship between DG and Kd is shown by Eq. 2

$$DG_{bind} = -RT \ln K_{eq} = - RT \ln K_d$$

Molecular docking was conducted to assess the binding affinity of chosen ligands with a target protein, aiming to identify the most promising inhibitors. During the docking procedure, specific parameters were configured as follows: dummy atoms were employed to represent the docking site, the placement phase utilized the Triangle Matcher algorithm, receptor refinement was carried out in a rigid manner, both initial and final scoring was conducted using the London DG scoring function, and a total of 20 poses were retained for each compound to investigate ligand interactions with the selected residues within the active site. To discern the most suitable conformation or pose of the inhibitor within the active site of the target protein, the S-score or docking score was employed as a key criterion (Joe *et al.*, 2022).

Forecasting the B-cell Epitope:

We employed IEDB's B-cell epitope prediction algorithms to forecast linear B-cell epitopes within the provided highly immunogenic protein sequence. The key factors considered for B-cell epitope prediction encompassed flexibility, antigenicity, surface accessibility, hydrophilicity, and linear epitope properties. Our analysis incorporated the utilization of various algorithms, including Karplus and Schulz for flexibility prediction, Kolaskar and Tongaonkar for antigenicity assessment, Emini for surface accessibility estimation, Parker for hydrophilicity prediction, and

Bepipred for linear epitope prediction, all sourced from the IEDB analysis resource **(**Singh *et al.,* 2021). Additionally, Chou and Fasman's beta turn prediction tool was applied, guided by wet lab experiments that highlighted the presence of antigenic elements within beta turn regions of the protein.

RESULTS

Distinguishing the Most Antigenic Protein:

The exploration of the non-structural protein of the POWV virus resulted in 985 findings. These proteins were subsequently assessed by the VaxiJen server, which assigned an overall score to each protein sequence, reflecting its potential to trigger an immune response. Out of all the proteins examined, the one with the accession number NP_775517.1 garnered the top score of 0.4916 in the VaxiJen analysis. This particular protein, known as non-structural protein 1 (NS1), consists of 353 amino acids. In light of these findings, this protein has been selected for further investigation in the ongoing study.

Determining the Primary and Secondary Protein Structures:

The function of a protein is closely linked to its structural attributes. The ProtParam service is employed to scrutinize the protein sequence and compute various parameters that offer insights into the protein's stability and function. Additionally, a protein's secondary structural properties can reveal significant functional information. According to the ProtParam analysis, the protein exhibits an Instability Index (II) of 43.94, an Aliphatic Index of 73.12, and a negative GRAVY (grand average hydropathy) score of 0.351. Furthermore, SOPMA was employed to assess the protein's secondary structural characteristics, revealing that random coils constitute 51.84% of the protein's structure. Alpha helices account for 22.66%, while extended strands make up 20.11% of the protein's composition. Additionally, Beta turns were found to constitute 5.38% of the total structure. Tables 1 and 2 display the parameters obtained from both tools, while Fig. 2 depicts the secondary structure plot.

Table 1: Diverse physicochemical characteristics of the Powassan virus non-structural (NS1) protein.

Parameter	Value
Molecular Weight	39266.71
Ext. coefficient	76150
Abs 0.1% (=1 g/l) 1.939, assuming all pairs of Cys residues form cystines	75400
Ext. coefficient	
Abs 0.1% (=1 g/l) 1.920, assuming all Cys residues are reduced	6.29
Theoretical pI	45
Total number of negatively charged residues (Asp + Glu)	42
Total number of positively charged residues (Arg + Lys)	43.94
The instability index (II) is computed to be	
Aliphatic index	73.12
Grand average of hydropathicity (GRAVY)	-0.351

Table 2: Analyzing the secondary structure of Powassan virus non-structural (NS1) protein using SOPMA.

Secondary structure	Percentage
Alpha helix (Hh)	22.66%
3_{10} helix (Gg)	0.00%
Pi helix (Ii)	0.00%
Beta bridge (Bb)	0.00%
Extended strand (Ee)	20.11%
Beta turn (Tt)	5.38%
Bend region (Ss)	0.00%
Random coil (Cc)	51.84%
Ambiguous states (?)	0.00%
Other states	0.00%

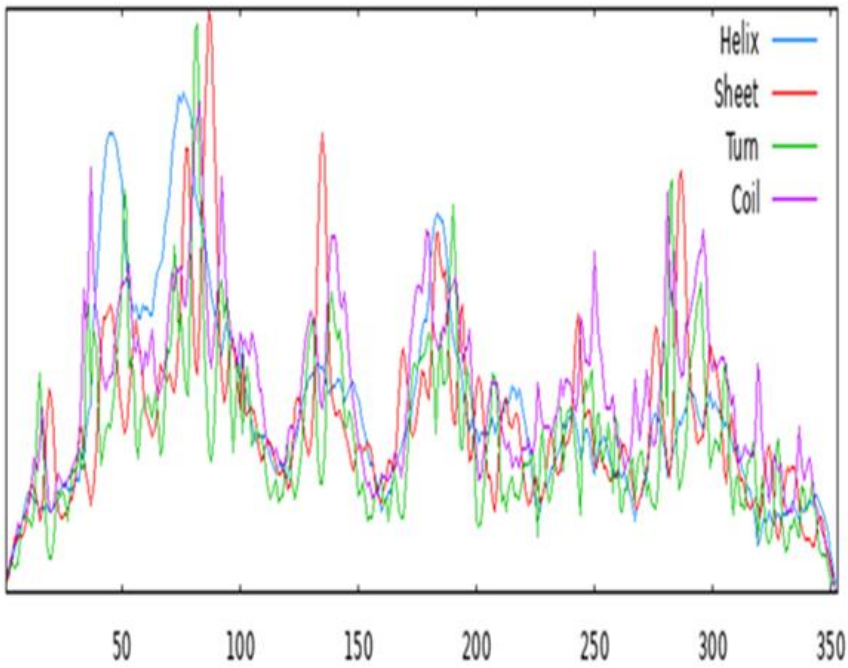

Fig. 2: The secondary structure plot for the envelope protein of the Powassan virus is represented as follows: helices are denoted in blue, extended strands are shown in red, and beta turns are depicted in green

T-cell Epitope Prediction:

The NetCTL server was employed to predict T-cell epitopes within the current protein. Utilizing a neural network architecture, the server detects potential overlapping epitopes along the provided protein sequence and computes a cumulative score by forecasting peptide-MHC class 1 binding, encompassing all MHC class 1 supertypes, proteasomal C-terminal cleavage, and TAP transport efficiency. The initial six epitopes, namely EVSEWYDGY, STAPELNLV, HTDQSMWMS, LTDLRNCTW, TLAGPRSKY, and GTDCWYAME, were selected from the generated results based on their highest cumulative scores. According to the IEDB MHC class 1 binding prediction tool, the previously identified epitopes were found to be recognized by a variety of MHC class 1 molecules. This program employs the stabilized matrix method (SMM) to compute the HLA binding affinity of epitopes in IC50 nM units. The affinity of these epitopes for

MHC-I molecules is inversely proportional to the IC50 value. In the current analysis, we specifically selected MHC-I molecules with associated IC50 values less than 200 nM (IC50 < 200), ensuring that the specified epitopes displayed a higher affinity for these MHC-1 molecules (as listed in Table 3**).** The IEDB's MHC-I processing efficiency tool calculates a comprehensive score for each epitope, taking into account the efficiency of proteasomal cleavage, TAP transport, and MHC-I binding. Proteasomal enzymes break down proteins into smaller peptides, which are then recognized by MHC class 1 molecules and form complexes with them. These peptide-MHC class 1 complexes are subsequently transported to the endoplasmic reticulum via transport-associated proteins (TAP) before being presented on the cell's plasma membrane to T-cells. A higher aggregate score for the peptides indicates that they are processed more effectively for presentation, a crucial step in generating an efficient immune response. These are the results obtained from the IEDB MHC-1 binding analysis and processing tools (Table 3). A more robust immune response is contingent on the successful recognition of epitopes by HLA molecules with substantial affinity. Therefore, a peptide that can be recognized by a greater number of HLA alleles holds the greatest potential to trigger a potent immune response. Among the five epitopes under investigation, one particular epitope has demonstrated interaction with a higher number of HLA alleles compared to the others. The 9-mer epitope YYYELYPTM exhibited affinity for a remarkable 19 MHC-I molecules, including HLA-B15:02, HLA-A30:02, HLA-A29:02, HLA-A03:01, HLA-A23:01, HLA-A24:02, HLA-A26:01, HLA-A30:02, HLA-A02:01, HLA-A03:01, HLA-A25:01, HLA-A11:01, HLA-B40:01, HLA-B27:05, HLA-B46:01, HLA-A68:01, HLA-A29:02, HLA-B48:01, and HLA-B*15:02.

Table 3: The top six T-cell epitopes, along with their associated MHC-1 alleles, cumulative processing score, and epitope conservation outcome.

Epitope	Interacting MHC-1 allele with an affinity, 200 (total score of proteasome score, TAP score, MHC score, processing score and MHC-1 binding)	Epitope conservancy analysis result
EVSEWYDGY	HLA-A*26:01 HLA-A*25:01 HLA-B*35:01 HLA-A*01:01 HLA-A*68:01 HLA-A*29:02	28.65%
STAPELNLV	HLA-A*68:02 HLA-A*02:06 HLA-A*02:01	53.24%
HTDQSMWMS	HLA-A*01:01 HLA-A*02:06 HLA-A*68:02 HLA-A*30:01 HLA-A*30:02 HLA-A*11:01 HLA-A*25:01 HLA-A*02:01	53.76%
LTDLRNCTW	HLA-A*01:01 HLA-A*32:01 HLA-B*53:01 HLA-B*14:02 HLA-B*18:01 HLA-B*35:01 HLA-B*08:01 HLA-A*02:06 HLA-A*30:01	42.91%
TLAGPRSKY Non allergen	HLA-B*15:02 HLA-A*30:02 HLA-A*29:02 HLA-A*03:01 HLA-A*23:01 HLA-A*24:02 HLA-A*26:01 HLA-A*30:02 HLA-A*02:01 HLA-A*03:01 HLA-A*25:01 HLA-A*11:01 HLA-B*40:01 HLA-B*27:05 HLA-B*46:01 HLA-A*68:01 HLA-A*29:02 HLA-B*48:01 HLA-B*15:02	67.37%
GTDCWYAME	HLA-A*01:01 HLA-A*02:06 HLA-B*35:01 HLA-A*68:01	65.21%

Epitope Conservancy Prediction:

Given that conserved epitopes can enhance the effectiveness of immunization, we anticipate an enhanced level of epitope conservation. Specifically, the epitopes EVSEWYDGY, STAPELNLV, HTDQSMWMS, TDLRNCTW, TLAGPRSKY, and GTDCWYAME demonstrated epitope conservancy analysis rates of 28.65%, 53.24%, 53.76%, 42.91%, 67.37%, and 65.21%, correspondingly (Table 3).

Prediction of Population Coverage:

The optimal Major Histocompatibility Complex Class I (MHC-I) binding peptides corresponding to individual epitopes were determined and subsequently subjected to a population coverage analysis. Population coverage analysis involves the computation of the proportion of individuals residing within a particular geographical area who are potentially capable of mounting an immune response against the queried epitopes. Utilizing the Population Coverage Analysis tool provided by the Immune Epitope Database (IEDB), it was ascertained that the four epitopes achieved the highest population coverage, with an impressive 86.97% of the population in North East Asia and 88.7% in Europe being potentially responsive to these epitopes. The epitopes exhibited a population coverage of 64.86% in America while North America achieved a cumulative population coverage of 77.56%. A population coverage of 62.53% was observed in East Africa, along with 72.79% in North Africa and 69.77% in West Africa respectively. In the context of emerging Powassan virus-affected regions, specifically along with North East Asia, Southeast Asia, South West Asia, South Asia and East Asia, the epitopes displayed population coverage rates of 86.97%, 71.17%, 77.37%, 69.65% and 65.8% respectively. Furthermore, cumulative population coverage of and 88.7% was observed for Europe (Figure 3).

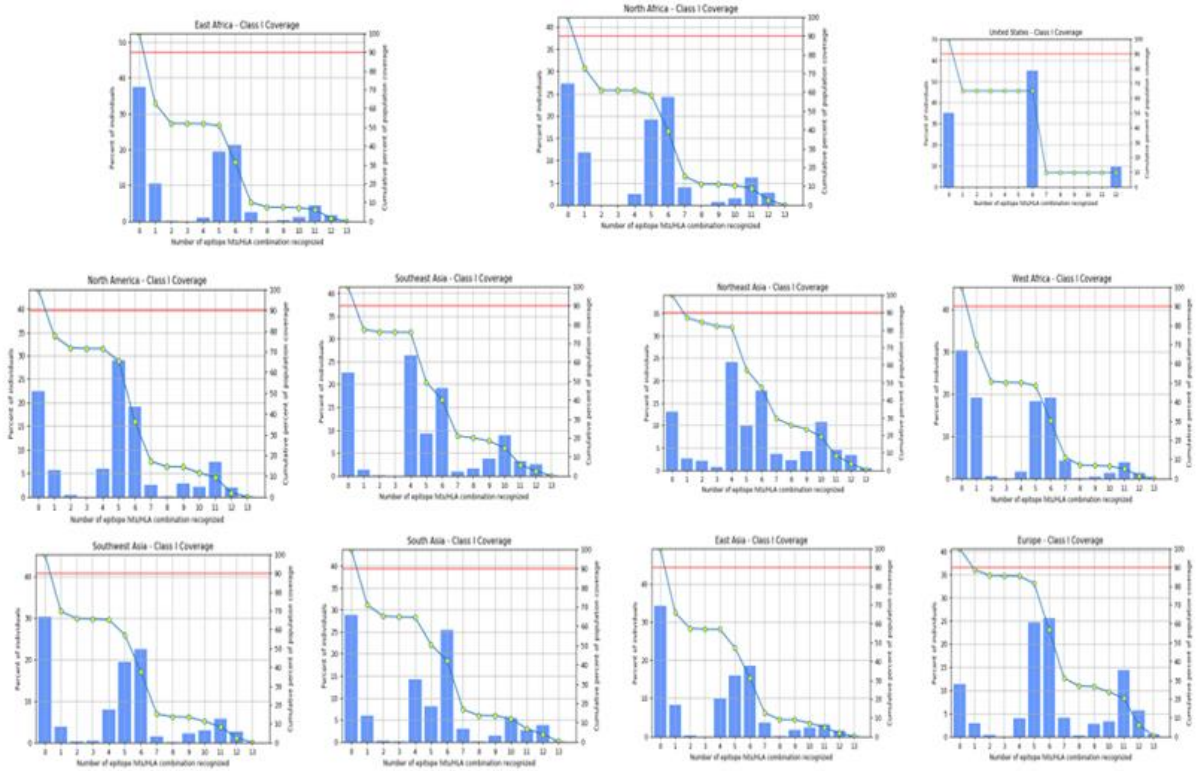

Fig. 3: Population coverage was assessed using MHC-I restriction data, focusing on various regions affected by POWV. These regions were chosen for the purpose of evaluating the population coverage potential of the suggested epitopes.

Allergenicity Assessment:

The examined sequence does not meet the criteria delineated by the FAO/WHO assessment framework for predicting cross-reactive allergens. Therefore, according to the FAO/WHO evaluation scheme, the scrutinized sequence is designated as a non-allergen. Moreover, within the group of five epitopes, the TLAGPRSKY epitope is also identified as a non-allergen. Notably, the AllerHunter prediction algorithm concurs with this classification, assigning a non-allergenic status to the analysed sequence with a score of 0.04. This prediction demonstrates a noteworthy sensitivity and moderate specificity of ≥85%.

Selection of T Cell Epitope:

Among the five initially selected epitopes, 'TLAGPRSKY' emerged as a more favourable candidate for vaccine development when taking into account its comprehensive epitope conservancy, population coverage, and its strong binding affinity for the highest number of HLA molecules.

B-cell Epitope Prediction:

B cell epitopes must possess specific attributes crucial for their effective recognition by B cells. These essential characteristics encompass hydrophilicity, surface accessibility, and prediction of beta-turn structures. To identify potential B cell epitopes in the query protein, various web-based tools within the IEDB were employed for scanning. One of these tools, the Kolaskar and Tongaonkar antigenicity prediction tool, assessed the protein for B cell epitopes by analysing the physicochemical properties of the amino acids and their prevalence in known B cell epitopes. The tool yielded results, as shown in Table 4 and Figure 4, predicting an average antigenic propensity value of 1.025 for the protein, with a maximum value of 1.216 and a minimum of 1.0. The tool was set with a threshold value of 0.880 to identify antigenically potent regions, and it pinpointed a 9-mer epitope from amino acid positions 260 to 340. This particular region exhibited favourable B cell epitope characteristics.

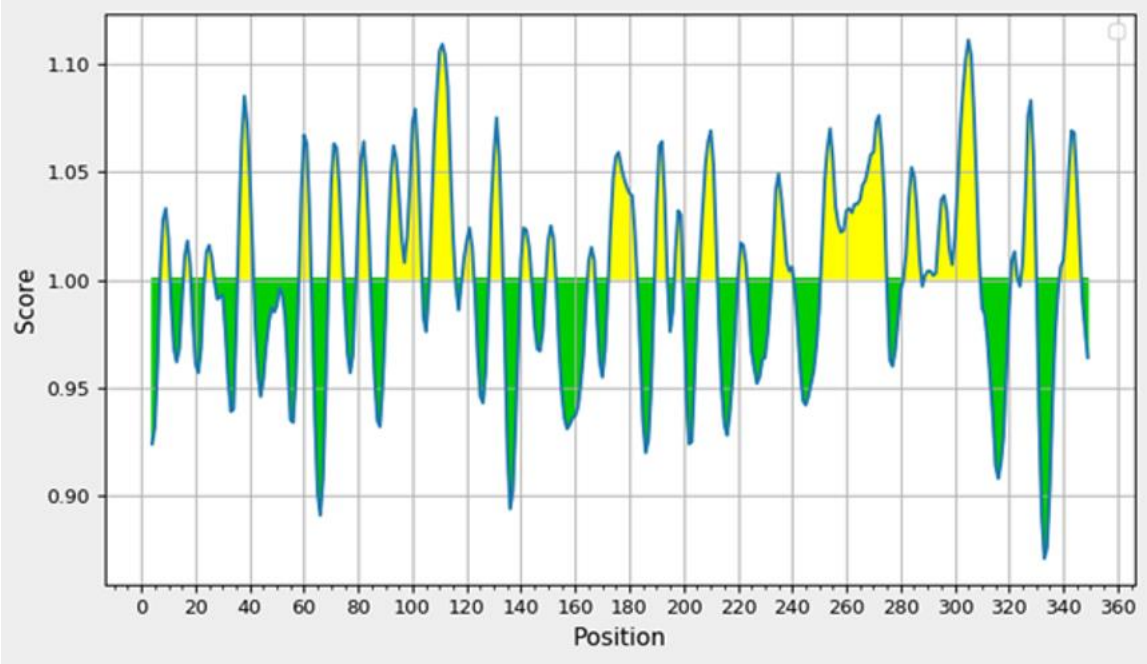

Fig. 4: The Kolaskar and Tongaonkar antigenicity results affirm the antigenic nature of the NS1 protein, with a threshold value of 0.90. The residues located within the yellow regions exhibit antigenic properties.

Table 4 : Kolaskar and Tongaonkar antigenicity analysis.

No.	Start	End	Peptide	Length
1	16	24	GEGLVVWKE	9
2	31	36	GYAYHP	6
3	41	47	TLAQALR	7
4	52	61	RGVCGVVPQN	10
5	75	81	LNLVLSE	7
6	86	92	LTIVVDK	7
7	125	130	LWSVPD	6
8	142	149	VGECPLYR	8
9	154	163	VFTVAEFGVG	10
10	165	171	RTKVFLD	7
11	214	220	IHELILT	7
12	239	250	LDSHLFLPVTLA	12
13	274	281	PLRVVRDH	8
14	311	325	PEWCCRACELPPVTF	15
15	337	346	IRPVHSQGGL	10

Surface accessibility of B cell epitopes is essential because hydrophilic regions are typically exposed on the surface, making them more likely to trigger a B cell immune response. To assess this, we employed the Emini surface accessibility prediction and Parker hydrophilicity prediction tools. The results of these analyses are presented in Table 5, and visual representations generated by both tools can be found in Figures 5 and 6, respectively.

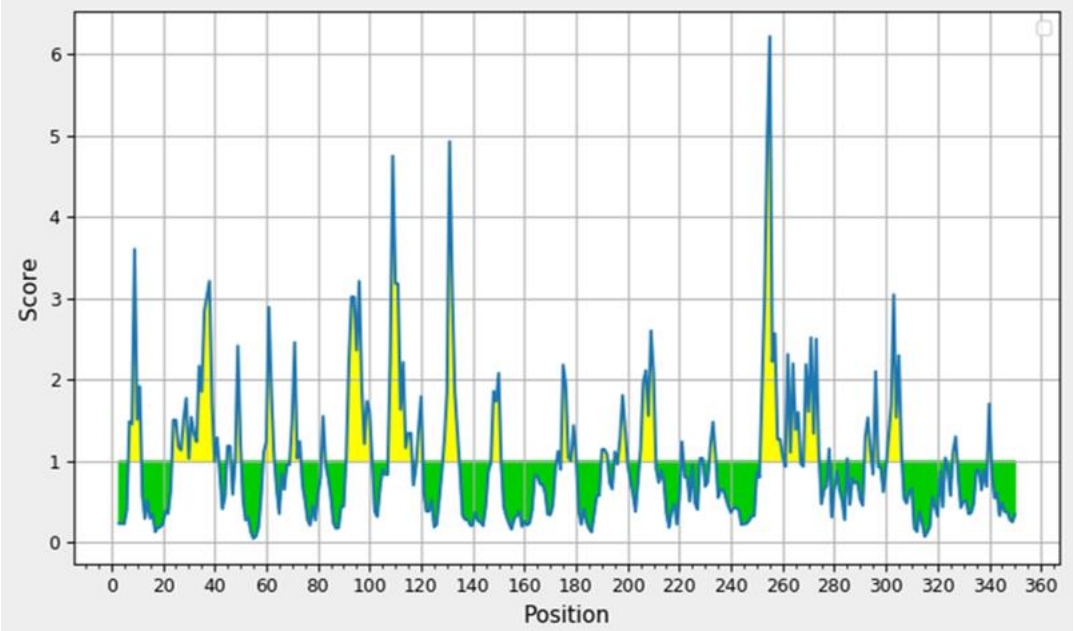

Fig. 5: The Emini surface accessibility analysis confirms that the NS1 structural protein stands out as the most antigenic protein. In the accompanying graph, the x-axis represents the sequence position, while the y-axis represents the surface probability. A threshold value of 1.000 has been applied, with antigenic regions displayed in yellow for those positions that exceed this threshold.

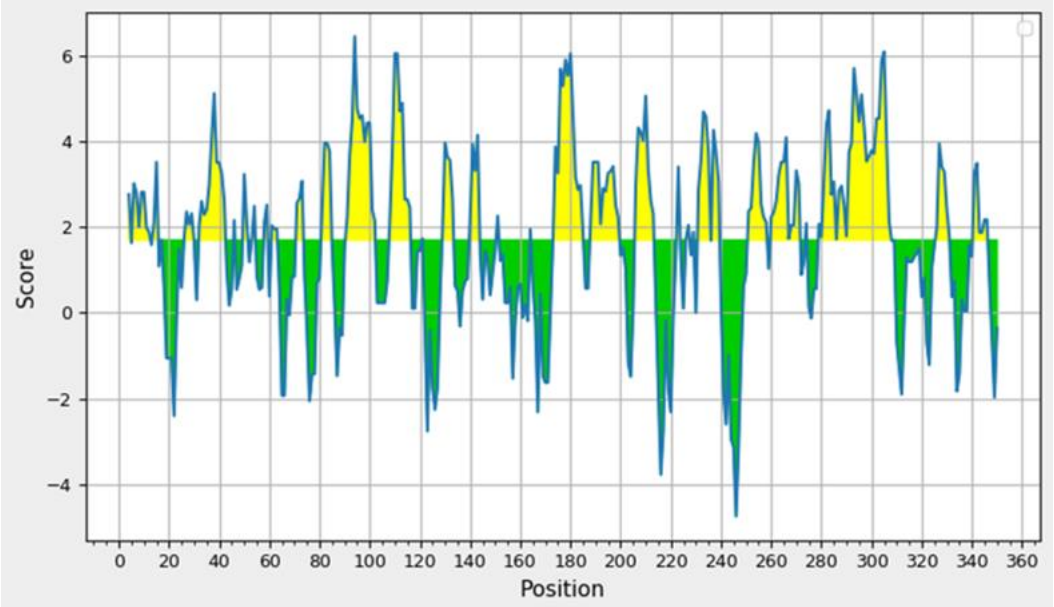

Fig. 6: The Parker hydrophilicity prediction analysis confirms that the NS1 structural protein stands out as the most antigenic protein. In the accompanying graph, the x-axis represents the sequence position, while the y-axis represents the surface probability. A threshold value of 1.694 has been applied, with antigenic regions displayed in yellow for those positions that exceed this threshold.

Table 5. Emini surface accessibility analysis.

No.	Start	End	Peptide	Length
1	24	39	EVSEWYDGYAYHPESP	16
2	91	101	DKTDPADYRGG	11
3	108	116	KTGKESKVS	9
4	129	134	PDSPRR	6
5	175	180	EASKEC	6
6	205	210	SFRNDT	6
7	252	260	PRSKYNRIP	9
8	269	274	PWDQTP	6
9	301	306	RSTTES	6

Beta turns within a protein typically exhibit characteristics of surface accessibility and hydrophilicity. To pinpoint the beta-turn regions within the query protein, we conducted a Chou and Fasman beta-turn prediction. The results of this analysis have identified a consistent predicted beta turn region spanning from position 260 to 353. Beta turns are known to play a substantial role in inducing antigenicity, making this discovery particularly relevant. Experimental data has revealed that the portion of a peptide interacting with an antibody often exhibits a degree of flexibility. Utilizing the Karplus Schulz flexibility prediction tool, we have identified flexible regions within the query protein. Notably, the analysis highlights the region spanning from position 342 to 353 as the most favourable in terms of flexibility. A visual representation of these results is available in Figures 7 and 8.

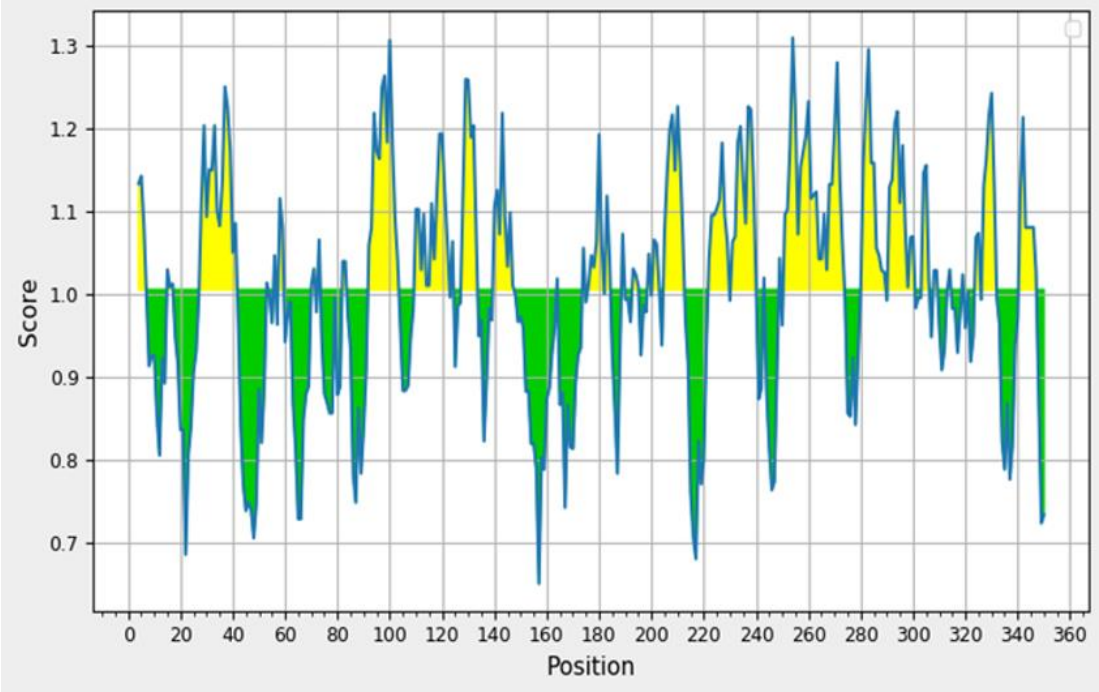

Fig. 7: Chou and Fasman beta-turn prediction confirms that the NS1 structural protein stands out as the most antigenic protein. In the accompanying graph, the x-axis represents the sequence position, while the y-axis represents the surface probability. A threshold value of 1.006 has been applied. The regions having beta turns in the protein are shown in yellow color, above the threshold value.

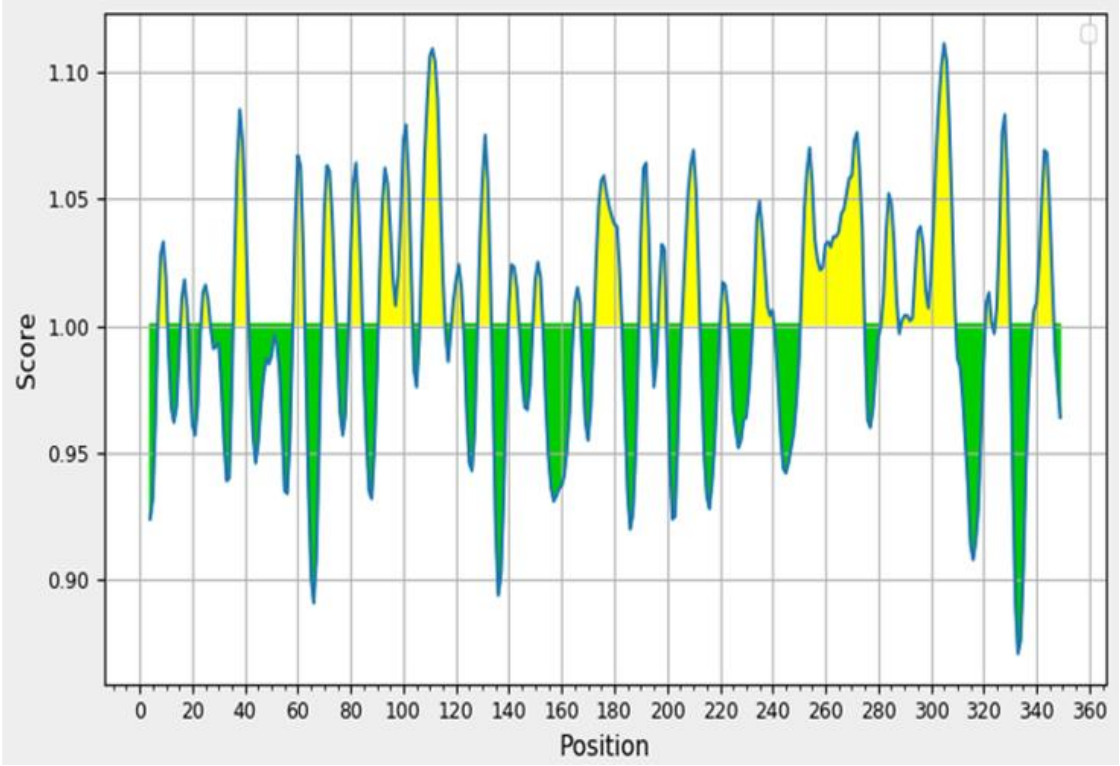

Fig. 8: Karplus and Schulz flexibility prediction was performed on the antigenic protein. In the generated graph, the x-axis corresponds to the position, while the y-axis displays the flexibility score. A threshold value of 1.001 has been applied, and the flexible regions of the protein, which exceed this threshold, are depicted in yellow.

Bepipred is a machine learning process that relies on the Hidden Markov model. It serves as a valuable tool for identifying Linear B cell epitopes. The rationale behind its utilization is to address the limitation of single-scale amino acid propensity profiles, which may not consistently predict antigenic epitopes accurately. By employing Bepipred, researchers aim to achieve improved results in epitope prediction, surpassing the performance of receiver operating characteristic (ROC) plots. The Bepipred predicted epitopes for the protein are detailed in Table 6, and a visual representation of these epitopes can be found in Figure 9. Following a comprehensive analysis of data obtained from various B cell epitope prediction tools, it has been determined that the region spanning from amino acids 340 to 353 is the most promising region for eliciting a B cell immune response.

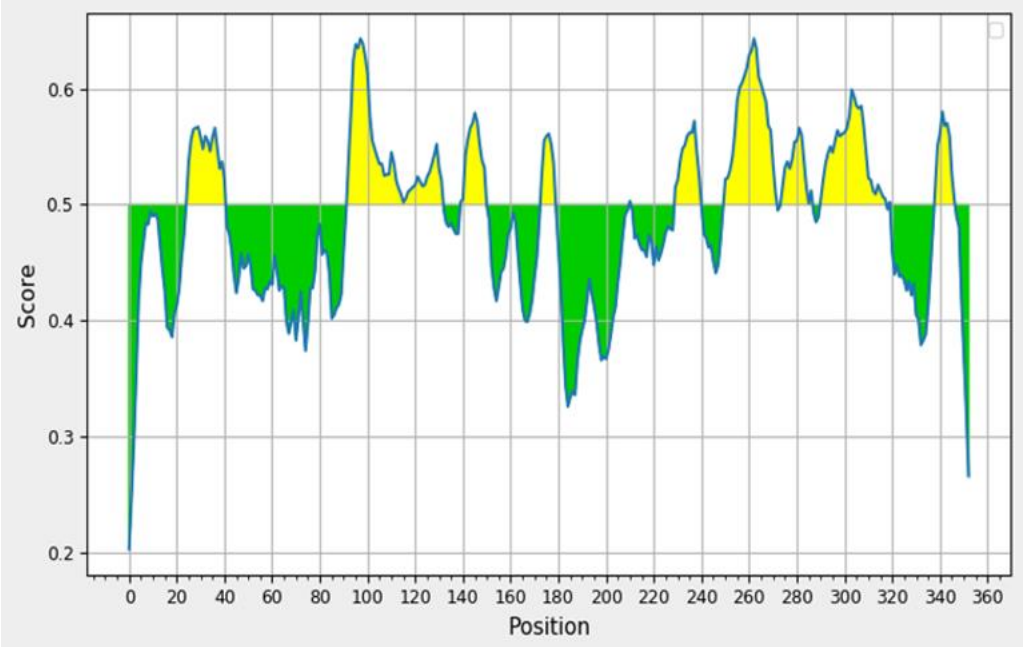

Fig. 9: The Bepipred linear epitope prediction was conducted on the most antigenic protein. In the accompanying graph, the x-axis corresponds to the position, while the y-axis represents the prediction score. A threshold value of 0.500 has been applied, with regions containing beta turns highlighted in yellow. The highest peak region on the graph indicates the most potent B-cell epitope.

Table 6. Bepipred Linear Epitope Prediction**.**

No.	Start	End	Peptide	Length
1	25	41	VSEWYDGYAYHPESPDT	17
2	93	132	TDPADYRGGTPMVLKKTGKESKVSWKSWGKSILWSVPDSP	40
3	140	150	DGVGECPLYRR	11
4	174	179	GEASKE	6
5	211	211	G	1
6	230	240	SHTIDNDGVLD	11
7	251	272	GPRSKYNRIPGYSEQVRGPWDQ	22
8	275	287	LRVVRDHCPGTSV	13
9	291	318	SHCDKRGASVRSTTESGKIIPEWCCRAC	28
10	320	320	L	1
11	339	347	PVHSQGGLV	9

Docking Simulation Study:

The binding free energy assessment of the epitope 'TLAGPRSKY' was conducted using the MM/PBSA method. The MM-PBSA calculations for the protein-ligand complex were carried out based on the final 10-ns trajectories utilizing the g_mmpbsa tool integrated into the GROMACS software. Snapshots of the HLA-DRB1*0408-TLAGPRSKY complex, obtained from molecular dynamics (MD) simulation trajectories, were extracted between 6000 ps and 8000 ps at intervals of 15 ps for MM/PBSA calculations. Similarly, coordinates of the HLA-DRB1*0408-TLAGPRSKY complex were taken from the simulation time between 4000 ps and 6000 ps, also at intervals of 15 ps. Among these snapshots, it was observed that only the 'TLAGPRSKY' epitope properly occupied the receptor and exhibited the lowest binding energy of -6.5 kcal/. The smaller binding energy signifies stronger binding affinity and suggests suitability for vaccine development. Furthermore, to validate the quality and reliability of the 'TLAGPRSKY' epitope, Ramachandran plot analysis was employed, and the PDB sum generates tool was utilized. The refined model exhibited that the majority of the protein's residues (>90%) occupy the most favored regions of the Ramachandran plot, signifying improved structural quality (Fig. 10 and Table 7). Therefore, the 'TLAGPRSKY' epitope was deemed an efficient candidate for vaccine development.

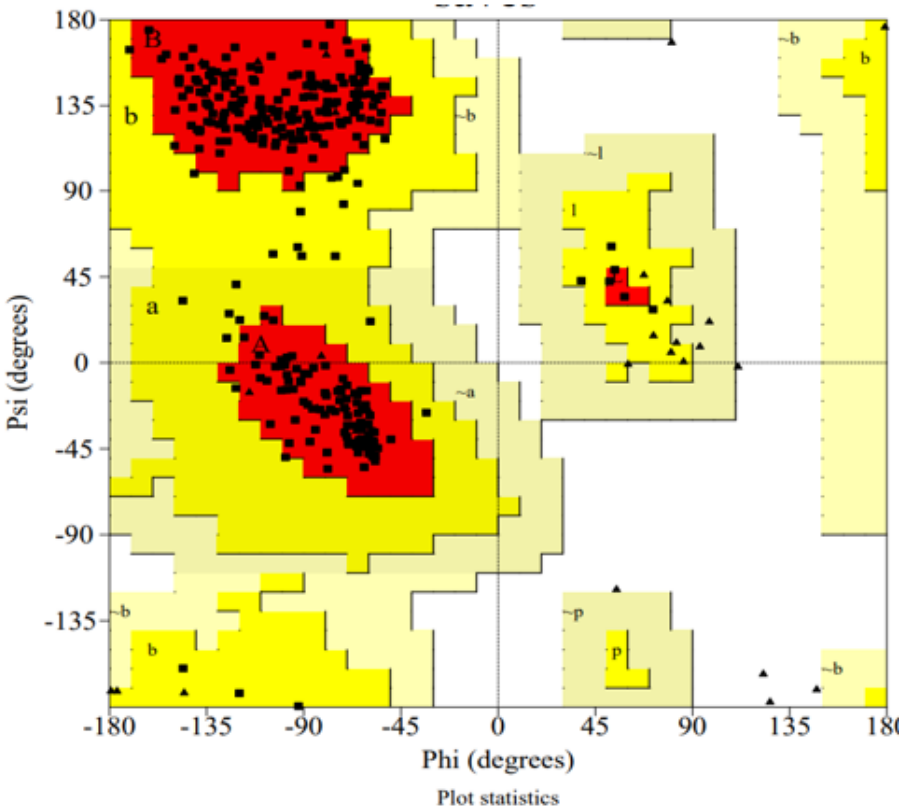

Fig. 10: The provided text succinctly describes a Ramachandran Plot associated with the predicted model of the NS1 protein 1 from the Powassan virus. In this plot, distinct color-coded regions signify various structural conformations: the red area represents the favored conformational space, the yellow region indicates allowed conformations, the light yellow region signifies generously allowed conformations, and the white region represents disallowed conformations. It's important to note that the torsion angles Phi (Φ) and Psi (Ψ) are pivotal determinants of the protein's overall structural orientation, as emphasized within the plot.

Table 7. Ramachandran plot of NS1 (Non-Structural protein) from Powassan virus**.**

Ramachandran Plot Statistics	POWV NS1 Protein	
	Residues	%
Residues in the most favored regions [A,B,L]	358	74.58
Residues in the additional allowed regions [a,b,l,p]	35	7.29
Residues in the generously allowed regions [a,b,l,p]	2	0.41
Residues in the disallowed regions [xx]	1	0.20
Number of non-glycine and non-proline residues	480	100
Number of end residues (excl. Gly and Pro)	2	-
Number of glycine residues	24	-
Number of proline residues	27	-
Total number of residues	533	-

Model Building And Refinement:

The three-dimensional structure of the Powassan virus non-structural protein NS1 was predicted using the Phyre2 server. In molecular biology, the tertiary structure of a protein elucidates its molecular functions and interactions. However, it's important to note that homology modeling tools often introduce some local distortions into the models they generate. To address this issue, the model produced by MODELLER was further refined using ModRefiner, aiming to achieve a more accurate stereochemical representation. Subsequent analyses were conducted using this refined model. A visualization of the 3D model generated by Swiss-PDB is presented in Figure 11 for reference.

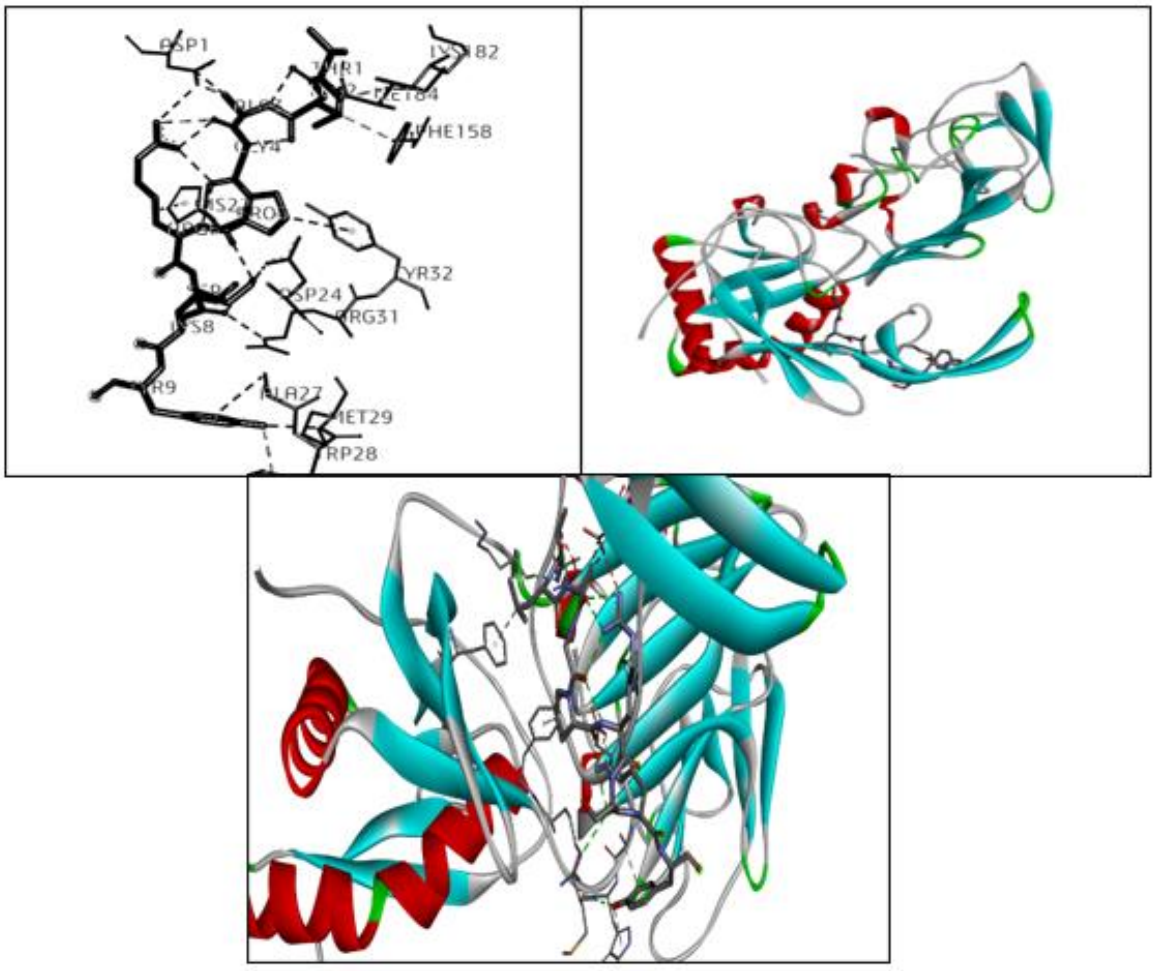

Fig. 11: 3D Model of Structured Vaccine.

Analysis of Molecular Docking and Simulation:

In the course of this study, a molecular dynamics simulation was conducted to assess the stability of a predicted epitope, TLAGPRSKY, when forming a protein complex. Several key structural metrics were evaluated using the GROMACS 5.0 package. Notably, the analysis included the Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and Residual Index (Ri). The RMSD value, reflecting the overall deviation of the complex from its reference structure, was computed at 0.49 nm. The RMSF score, which characterizes the local structural fluctuations within the complex, was found to be 0.12 nm. These results collectively indicate a high level of stability in the formed complex, suggesting that the predicted epitope TLAGPRSKY forms a stable interaction with the associated protein (Fig. 12).

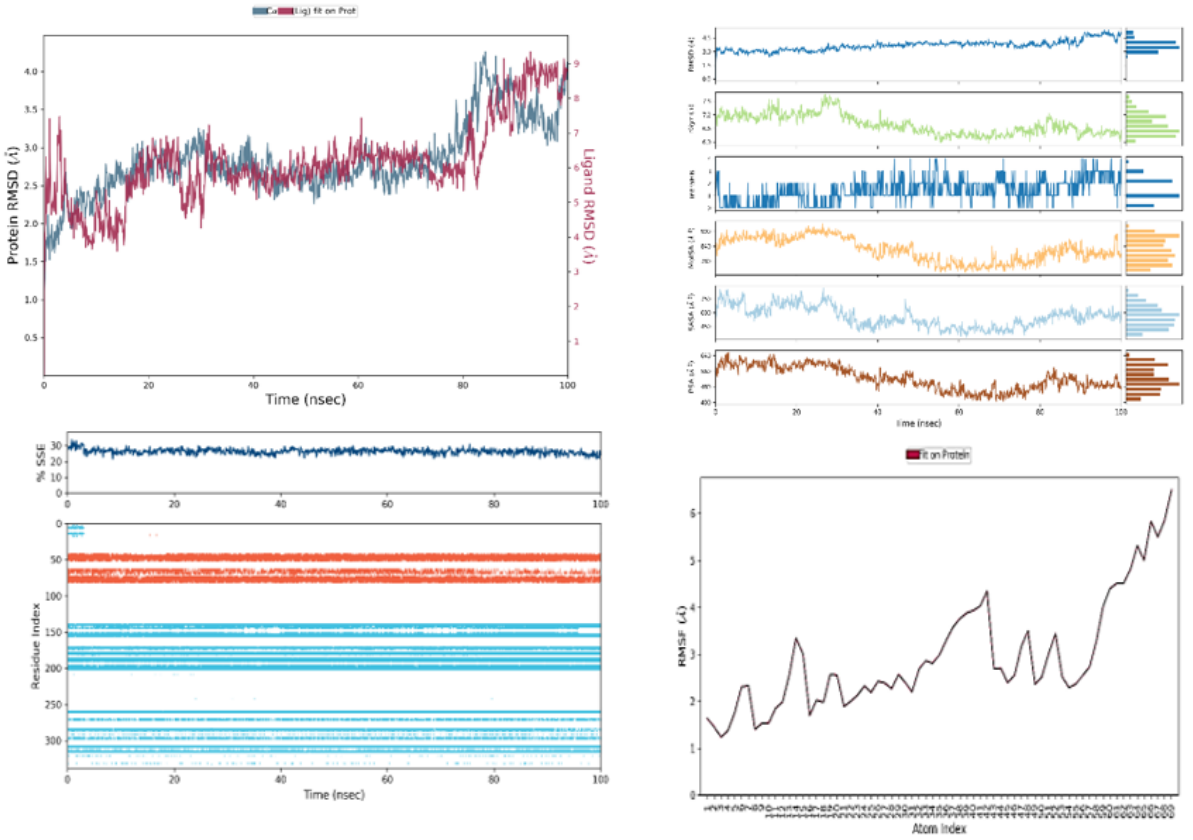

Fig. 12: Molecular dynamic simulation results of constructed vaccine. The RMSD plot (A) The Rg (radius of gyration) plot. (B). Residue index (C) and RMSF profile of docked complexes (D)**.**

DISCUSSION

In the current era, diseases constitute a widespread global challenge. Given the rising occurrence of newly emerging viruses affecting human communities, the rapid development of vaccines has gained paramount importance for efficiently countering the growing prevalence of viral threats (Trovato *et al.*, 2023). In recent times, the emergence of next-generation sequencing and the progression of genomics and proteomics technologies have triggered a significant paradigm shift in the domain of computational immunology (Trovato *et al.*, 2023). Owing to the unprecedented abundance of accessible data, modern immunoinformatics tools are experiencing rapid development and deployment. These tools enable a proactive approach to vaccine development by harnessing an improved

understanding of the immune responses of the human body to a wide range of organisms. In a starkly contrasting scenario, minimal progress has been achieved in the realm of Powassan virus research and intervention. The genome of the POWV consists of an approximately 11-kilobase (kb) positive-sense RNA sequence. Within this genome, there are three structural proteins: capsid (C), pre-membrane (prM), and envelope (E), as well as seven non-structural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (Baker & Shi, 2020). Additionally, the genome encodes both 5' and 3' untranslated regions (UTRs). This positive-sense RNA is translated as a single, elongated polyprotein. Subsequently, it undergoes cleavage by both viral and host proteases, leading to the formation of ten distinct proteins necessary for the viral life cycle. The entry process of the POWV involves a series of orchestrated events. It commences with the virus binding to one of several possible host receptors, subsequently initiating receptor-mediated endocytosis (Van Leur *et al*., 2021). In the acidic milieu of the endosome, the virion undergoes a process of uncoating, which enables the release of its genetic material into the cytoplasm through mechanisms that facilitate endosomal escape. Following a successful entry into the cytoplasm, the translation of viral components initiates, culminating in the synthesis of new genomic RNA and the processing of polyproteins. Following this, progeny virions can undergo assembly within the endoplasmic reticulum and subsequently mature as they pass through the Golgi apparatus. Ultimately, they undergo fusion with host membranes and are released. It is important to highlight that the maturation status of these progeny virions can exert a considerable impact, not only on their ability to infect but also on the host's antibody responses. This phenomenon arises due to conformational alterations that govern the accessibility of antigenic sites on the envelope protein, which serves as the primary determinant of Powassan virus antigenicity. Consequently, there has been a noticeable increase in research endeavors, primarily centered on the development of a Powassan virus vaccine (Kapoor & Zash, 2023). However, as of the present moment, no tangible progress has been made in this pursuit. In our current study, our efforts have been channeled toward the identification of significant immunogenic epitopes situated within Powassan viral proteins, utilizing advanced immunoinformatics tools. Additionally, our study aims to formulate predictions for the design of a potential vaccine specifically targeting the Powassan virus envelope protein NS1. Traditional vaccination strategies have historically relied on the utilization of complete pathogens, either in a live attenuated or inactivated form. However, these conventional vaccine approaches have introduced significant safety concerns (Brisse *et al.,* 2020). This stems from the possibility that the pathogens employed for immunization may reactivate and induce infections in recipients. Furthermore, the genetic diversity of pathogen strains across various geographical regions can lead to a reduction in vaccine efficacy, rendering them less effective for specific populations or in distinct locales. Indeed, novel vaccine approaches such as DNA vaccines and epitope-based vaccines offer promising solutions to address the challenges associated with traditional vaccination methods. These innovative vaccine modalities have the potential to surmount these barriers by generating highly effective, specific, robust, and enduring immune responses while minimizing structural complexity and eliminating undesirable side effects (Pardi *et al.,* 2018).

DNA vaccines, for instance, harness the genetic material of the pathogen to stimulate a targeted immune response without the risk of causing infection (Xu *et al.,* 2020). In contrast, epitope-based vaccines concentrate on distinct immunogenic segments of the pathogen, optimizing the immune response against the most crucial components while minimizing the incorporation of extraneous antigens (Hajissa et al., 2019). Epitopes play a pivotal role in the creation of efficacious vaccines targeting

infectious pathogens. These segments located on the surface of antigens interact with antibodies, B-cell and T-cell receptors, serving as the specific portions of a pathogen that the immune system recognizes and combats. By pinpointing and choosing the most potent epitopes, it becomes possible to design vaccines that elicit a robust immune response against the pathogen while minimizing any potential harm to the host (Palma, 2023).) These advances represent significant steps forward in vaccine development, holding the promise of enhancing both safety and efficacy in immunization efforts (Xu *et al.,* 2020). Furthermore, in addition to DNA vaccines and epitope-based vaccines, the application of neutralizing peptide-based vaccine design has proven successful through in silico approaches. These methods have been effectively employed in the development of vaccines against a range of viruses, including influenza virus (Nuwarda *et al.,* 2021) monkeypox virus (Lozano& Muller, 2023), human coronaviruses (Shahcheraghi *et al.,* 2021) and hepatitis C virus (Guo et al., 2018). These groundbreaking studies have played a pivotal role in advancing the field of immunoinformatics, establishing its importance in the domain of vaccine development and offering innovative strategies for combating viral diseases. In the realm of vaccine design, while the majority of epitope-based vaccines predominantly stimulate B-cell epitopes, the development of T-cell epitope-based vaccines is motivated by the robust CD8+ T-cell mediated immune response exhibited by the host organism when faced with infected T cells (De Groot *et al*., 2009). Additionally, the phenomenon of antigenic drift can compromise the effectiveness of humoral immunity and the memory response to antigens, allowing pathogens to evade the immune system (van de Sandt *et al.,* 2012). T-cell-based vaccines, however, do not face this limitation. In fact, a multi-epitope vaccine, which combines potent B-cell epitopes with T-cell epitopes, holds great promise as it can elicit both cell-mediated and humoral immune responses (van de Sandt *et al.,* 2012). A T-cell epitope is deemed strong and potent when it exhibits a high degree of conservation among the sequenced POWV NS1 proteins stored in the database. In the context of the present study, the calculated conservation rate for the predicted T-cell epitope "TLAGPRSKY" is notably high, with a level of 67.37% conservation. Among the six potential epitopes selected through the NetCTL T-cell epitope analysis, "TLAGPRSKY" stands out as the most conserved. In the context of epitope-based vaccine development, a highly conserved epitope holds the promise of providing broader protection against various strains of the virus. This is particularly crucial for RNA viruses like POWV, which have a higher propensity for mutation due to the absence of proofreading activity in their RNA polymerase. Therefore, an ideal vaccine candidate epitope should be derived from a portion of the protein that exhibits substantial conservancy, thereby ensuring an effective and long-lasting immunization response. The selected epitope "TLAGPRSKY" has received further endorsement as the result from the MHC-I and epitope interaction tool indicates that this epitope interacts with an impressive number of HLA alleles. Specifically, it has been found that "TLAGPRSKY" demonstrates interactions with a total of 19 HLA-A and HLA-B alleles. The broad spectrum of interactions observed between this epitope and various Human Leukocyte Antigen (HLA) alleles enhances its candidacy for vaccine development, suggesting that it may confer a more extensive range of immune responses across individuals with diverse HLA profiles. The specific and high-affinity binding demonstrated by the "TLAGPRSKY" epitope to a wide array of HLA alleles is highly advantageous. This is particularly crucial because the efficacy of an epitope-based vaccine is critically dependent on the accuracy and strength of the interaction between the epitope and HLA alleles. Such precise interactions ensure the efficient activation of the immune system, leading to a robust and targeted immune response. In

essence, the capacity of the "TLAGPRSKY" epitope to efficiently bind with multiple HLA alleles underscores its potential as a valuable component of an epitope-based vaccine, capable of effectively engaging the immune systems of a diverse population. The specific and high-affinity binding observed with the "TLAGPRSKY" epitope is of paramount importance, as it plays a pivotal role in determining the effectiveness of an epitope-based vaccine. To further assess the potential impact, an analysis was conducted to determine the population coverage of those HLA alleles to which "TLAGPRSKY" demonstrated affinity. This analysis serves as a critical step in assessing the potential of the vaccine to confer comprehensive protection across diverse populations, thereby playing a pivotal role in optimizing the vaccine's efficacy and global reach. The highest population coverage was observed in Europe, where the tick-borne encephalitis virus (TBEV) is a prevalent pathogen. Notably, in the Western Hemisphere, specifically in Canada, the Powassan virus stands as the sole tick-borne flavivirus with endemicity. In the United States, a cumulative population coverage of 77.56% was documented. In the context of emerging regions affected by the Powassan virus, namely North East Asia, South East Asia, South West Asia, South Asia, and East Asia, the epitopes exhibited population coverage rates of 86.97%, 71.17%, 77.37%, 69.65%, and 65.8%, respectively. Furthermore, it is noteworthy that the epitope has been classified as non-allergenic, a pivotal attribute for any vaccine. These findings strongly suggest that the vaccine holds the potential to be highly effective for a vast population spanning a geographically diverse area. The epitope underwent a docking process and was subsequently subjected to comparative analysis against a control to evaluate its docking efficiency with a specific Human Leukocyte Antigen (HLA) allele, specifically HLA-B*3501. The results of this analysis demonstrate that the epitope can bind with similar efficiency when compared to the control utilized as a reference in the study. This computational assessment reaffirms the epitope's robust affinity for Major Histocompatibility Complex Class I (MHC-I) molecules, further substantiating its potential as a promising novel vaccine candidate.

Powassan virus's non-structural protein NS1 was subjected to exploration for B cell epitopes, given that B cell epitopes can elicit both primary and secondary immune responses. A comprehensive analysis of the protein was conducted using various tools available from the Immune Epitope Database (IEDB), focusing on critical attributes of B cell epitopes (Stone & Pinto, 2023). Upon cross-referencing all the data, a specific region spanning from amino acid positions 260 to 340 emerged as a robustly predicted B cell epitope according to the Bepipred tool. This particular region is characterized by the presence of beta turns and flexibility. It is noteworthy for its surface accessibility and relatively higher hydrophilicity compared to other regions, thereby establishing its antigenic potential. Among the predicted epitopes, the 9-mer sequence "PVHSQGGLV" stands out as the most promising B cell epitope, as indicated by the predictive results.

Given the chronic nature of POWV infection, which can persist for months to years and often leads to severe encephalitis (inflammation of the brain) and meningitis (inflammation of the membranes that surround the brain and spinal cord) there is a pressing need for a therapeutic solution capable of alleviating or completely eradicating these long-lasting symptoms (Scroggs *et al.*, 2023). Additionally, a universal drug is imperative for comprehensive protection against POWV infection. In instances where vaccine efficacy is diminished due to viral mutations, this pharmaceutical agent could serve as a supplementary measure to expedite the alleviation of symptoms. Furthermore, in situations where a sudden outbreak of Powassan virus occurs in an area lacking vaccination coverage, the feasibility of vaccination may be limited, necessitating the reliance on post-therapeutic interventions or

pharmaceuticals as the primary means of addressing the outbreak until vaccination becomes a viable option. Within the scope of this current investigation, our emphasis lies in the development of pre-therapeutic vaccines, which can serve as a model for conducting in vitro and in vivo studies to evaluate their efficacy as potential vaccine candidates.

According to the VaxiJen analysis, the examination of the non-structural protein NS1 of the POWV virus has unveiled its potential as a promising target for drug development. Valuable insights have been acquired through both primary and secondary analyses of this protein, utilizing ProtParam and SOPMA tools. The significant prevalence of coiled regions observed in the results generated by SOPMA implies a heightened level of conservation and structural stability within the protein (Kader et al., 2022). In our current investigation, we have successfully identified a potent T-cell epitope in conjunction with a B-cell epitope. These epitopes possess substantial potential for the formulation of a multi-peptide vaccine capable of eliciting a comprehensive immune response against the POWV virus. Although this study predominantly relies on computational algorithms, it is imperative to validate all criteria in vitro to assess the immunogenicity and confirm the presence of these epitopes within the protein. To complement this in silico analysis, both in vivo and in vitro experiments are imperative. In this context, the utilization of binding chip assays may prove to be a valuable tool for ascertaining binding affinity.

Conclusion:

Given that the current study focused on identifying epitope-binding HLA alleles through computational tools, it's possible that there are additional HLA alleles capable of recognizing these epitopes. Moreover, the predicted epitopes may not exhibit the same binding affinity in experimental settings as they do in computational predictions. The POWV non-structural protein NS1 plays a distinctive role in viral maturation, multiplication, and infection, rendering it an intriguing target for drug development. However, extensive laboratory-based techniques are necessary to validate the findings of this study. Nevertheless, these findings can serve as foundational data for future research in this field.

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REFERENCES

Adam-Poupart, A., Drapeau, L. M., Bekal, S., Germain, G., Irace-Cima, A., Sassine, M. P., Simon, A., Soto, J., Thivierge, K., & Tissot, F. (2021). Occupations at risk of contracting zoonoses of public health significance in Québec. *Canada communicable disease report = Releve des maladies transmissibles au Canada*, *47*(1), 47–58.

Baker, C., & Shi, P. Y. (2020). Construction of Stable Reporter Flaviviruses and Their Applications. *Viruses*, *12*(10), 1082. https://doi.org/10.3390/v12101082

Brisse, M., Vrba, S. M., Kirk, N., Liang, Y., & Ly, H. (2020). Emerging Concepts and Technologies in Vaccine Development. *Frontiers in immunology*, *11*, 583077. https://doi.org/10.3389/fimmu.2020.583077

Campbell, O., & Krause, P. J. (2020). The emergence of human Powassan virus infection in North America. *Ticks and tick-borne diseases*, *11*(6), 101540.

Carpio, K. L., & Barrett, A. D. T. (2021). Flavivirus NS1 and Its Potential in Vaccine Development. *Vaccines*, , *9*(6), 622. https://doi.org/10.3390/vaccines9060622

De Groot, A. S., Moise, L., McMurry, J. A., & Martin, W. (2008). Epitope-Based Immunome-Derived Vaccines: A Strategy for Improved Design and Safety. *Clinical Applications of Immunomics*, *2*, 39–69.

Fleri, W., Paul, S., Dhanda, S. K., Mahajan, S.,

Xu, X., Peters, B., & Sette, A. (2017). The Immune Epitope Database and Analysis Resource in Epitope Discovery and Synthetic Vaccine Design. *Frontiers in immunology*, *8*, 278. https://doi.org/10.3389/ fimmu. 2017.00278

Gaibani, P., Cavrini, F., Gould, E. A., Rossini, G., Pierro, A., Landini, M. P., & Sambri, V. (2013). Comparative genomic and phylogenetic analysis of the first Usutu virus isolate from a human patient presenting with neurological symptoms. *PloS one*, *8*(5), e64761. https://doi.org/10. 1371/journal.pone.0064761

Geourjon, C., & Deléage, G. (1995). SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *Computer applications in the biosciences:CABIOS*, *11*(6), 681–684. https://doi.org/10.1093/ bioinformatics/11.6.681

Guo, X., Zhong, J. Y., & Li, J. W. (2018). Hepatitis C Virus Infection and Vaccine Development. *Journal of clinical and experimental hepatology*, *8*(2), 195–204.

Hajissa, K., Zakaria, R., Suppian, R., & Mohamed, Z. (2019). Epitope-based vaccine as a universal vaccination strategy against *Toxoplasma gondii* infection: A mini-review. *Journal of advanced veterinary and animal research*, *6*(2), 174–182. https ://doi.org/10.5455/javar.2019.f329

Hermance, M.E., **&** Thangamani, S. (2017). Powassan Virus: An Emerging Arbovirus of Public Health Concern in North America. *Vector borne and zoonotic diseases (Larchmont, N.Y.)*, *17*(7), 453–462.

Jaenson, T. G., Jaenson, D. G., Eisen, L., Petersson, E., & Lindgren, E. (2012). Changes in the geographical distribution and abundance of the tick Ixodes ricinus during the past 30 years in Sweden. *Parasites & vectors*, *5*, 8. https://doi.org/10.1186/1756-3305-5-8

Joe, S., Salam, A. A. A., Neogi, U., N, N. B., & Mudgal, P. P. (2022). Antiviral drug research for Japanese encephalitis: an updated review. *Pharmacological reports : PR*, *74*(2), 273–296. https://doi.org/10.1007/s 43440-022-00355-2

Kader, M. A., Ahammed, A., Khan, M. S., Ashik, S. A. A., Islam, M. S., & Hossain, M. U. (2022). Hypothetical protein predicted to be tumor suppressor: a protein functional analysis. *Genomics & informatics*, *20*(1), e6.

Kapoor, A. K., & Zash, R. (2023). Powassan Virus. In *StatPearls*. StatPearls Publishing.

Kemenesi, G., & Bányai, K. (2018). Tick-Borne Flaviviruses, with a Focus on Powassan Virus. *Clinical microbiology reviews*, *32*(1), e00106 -17.

Krett, J. D., Beckham, J. D., Tyler, K. L., Piquet, A. L., Chauhan, L., Wallace, C. J., Pastula, D. M., & Kapadia, R. K. (2022). Neurology of Acute Viral Infections. *The Neurohospitalist* , *12*(4), 632–646.

Kubinski, M., Beicht, J., Gerlach, T., Volz, A., Sutter, G., & Rimmelzwaan, G. F. (2020). Tick-Borne Encephalitis Virus: A Quest for Better Vaccines against a Virus on the Rise. *Vaccines*, *8*(3), 451. https://doi. org/10.3390/vaccines8030451

Lange, R. E., Dupuis Ii, A. P., Prusinski, M. A., Maffei, J. G., Koetzner, C. A., Ngo, K., Backenson, B., Oliver, J., Vogels, C. B. F., Grubaugh, N. D., Kramer, L. D., & Ciota, A. T. (2023). Identification and characterization of novel lineage 1 Powassan virus strains in New York State. *Emerging microbes & infections*, *12*(1), 2155585.

Larsen, M. V., Lundegaard, C., Lamberth, K., Buus, S., Lund, O., & Nielsen, M. (2007). Large-scale validation of methods for cytotoxic T-lymphocyte

epitope prediction. *BMC bioinformatics*, *8*, 424. https://doi.org/10.1186/1471-2105-8-424

Larsen, M. V., Lundegaard, C., Lamberth, K., Buus, S., Lund, O., & Nielsen, M. (2007). Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. *BMC bioinformatics*, *8*, 424. https://doi.org/10.1186/1471-2105-8-424

Li, J., Yin, W., Jing, Y., Kang, D., Yang, L., Cheng, J., Yu, Z., Peng, Z., Li, X., Wen, Y., Sun, X., Ren, B., & Liu, C. (2019). The Coordination Between B Cell Receptor Signaling and the Actin Cytoskeleton During B Cell Activation. *Frontiers in immunology*, *9*, 3096. https://doi.org/10.3389/fimmu.2018.03096

Lozano, J. M., & Muller, S. (2023). Monkeypox: potential vaccine development strategies. *Trends in pharmacological sciences*, *44*(1), 15–19.

Malonis, R. J., Georgiev, G. I., Haslwanter, D., VanBlargan, L. A., Fallon, G., Vergnolle, O., Cahill, S. M., Harris, R., Cowburn, D., Chandran, K., Diamond, M. S., & Lai, J. R. (2022). A Powassan virus domain III nanoparticle immunogen elicits neutralizing and protective antibodies in mice. *PLoS pathogens*, *18*(6), e1010573. https://doi.org/10.1371/journal.ppat.1010573

Muh, H. C., Tong, J. C., & Tammi, M. T. (2009). AllerHunter: a SVM-pairwise system for assessment of allergenicity and allergic cross-reactivity in proteins. *PloS one*, *4*(6), e5861. https://doi.org/10.1371/journal.pone.0005861

Nelson, J., Ochoa, L., Villareal, P., Dunn, T., Wu, P., Vargas, G., **&** Freiberg, A.N. (2022). Powassan Virus Induces Structural Changes in Human Neuronal Cells In Vitro and Murine Neurons In Vivo. *Pathogens*, 11(10):1218

Nuwarda, R. F., Alharbi, A. A., & Kayser, V. (2021). An Overview of Influenza Viruses and Vaccines. *Vaccines*, *9*(9), 1032.

Palma M. (2023). Epitopes and Mimotopes Identification Using Phage Display for Vaccine Development against Infectious Pathogens. *Vaccines*, 11(7), 1176. https://doi.org/10.3390/vaccines11071176

Pardi, N., Hogan, M. J., Porter, F. W., & Weissman, D. (2018). mRNA vaccines - a new era in vaccinology. *Nature reviews. Drug discovery*, *17*(4), 261–279.

Ranasinghe, S., Lamothe, P. A., Soghoian, D. Z., Kazer, S. W., Cole, M. B., Shalek, A. K., Yosef, N., Jones, R. B., Donaghey, F., Nwonu, C., Jani, P., Clayton, G. M., Crawford, F., White, J., Montoya, A., Power, K., Allen, T. M., Streeck, H., Kaufmann, D. E., Picker, L. J., … Walker, B. D. (2016). Antiviral $CD8^+$ T Cells Restricted by Human Leukocyte Antigen Class II Exist during Natural HIV Infection and Exhibit Clonal Expansion. *Immunity*, *45*(4), 917–930. https://doi.org/10.1016/j.immuni.2016.09.015

Rastogi, M., Sharma, N., & Singh, S. K. (2016). Flavivirus NS1: a multifaceted enigmatic viral protein. *Virology journal*, *13*, 131. https://doi.org/10.1186/s12985-016-0590-7

Robich, R. M., Cosenza, D. S., Elias, S. P., Henderson, E. F., Lubelczyk, C. B., Welch, M., & Smith, R. P. (2019). Prevalence and Genetic Characterization of Deer Tick Virus (Powassan Virus, Lineage II) in *Ixodes scapularis* Ticks Collected in Maine. *The American journal of tropical medicine and hygiene*, *101*(2), 467–471. https://doi.org/10.4269/ajtmh.19-0281

Scroggs, S. L. P., Offerdahl, D. K., Stewart, P. E., Shaia, C., Griffin, A. J., & Bloom, M. E. (2023). Of Murines and Humans: Modeling Persistent

Powassan Disease in C57BL/6 Mice. *mBio, 14*(2), e0360622.

Shahcheraghi, S. H., Ayatollahi, J., Aljabali, A. A., Shastri, M. D., Shukla, S. D., Chellappan, D. K., Jha, N. K., Anand, K., Katari, N. K., Mehta, M., Satija, S., Dureja, H., Mishra, V., Almutary, A. G., Alnuqaydan, A. M., Charbe, N., Prasher, P., Gupta, G., Dua, K., Lotfi, M., … Tambuwala, M. M. (2021). An overview of vaccine development for COVID-19. *Therapeutic delivery*, *12*(3), 235–244.

Singh, J., Malik, D., & Raina, A. (2021). Immuno-informatics approach for B-cell and T-cell epitope based peptide vaccine design against novel COVID-19 virus. *Vaccine*, *39*(7), 1087–1095. https://doi.org/10.1016/j.vaccine.2021.01.011

Soria-Guerra, R. E., Nieto-Gomez, R., Govea-Alonso, D. O., & Rosales-Mendoza, S. (2015). An overview of bioinformatics tools for epitope prediction: implications on vaccine development. *Journal of biomedical informatics*, *53*, 405–414. https://doi.org/10.1016/j.jbi.2014.11.003

Stone, E. T., & Pinto, A. K. (2023). T Cells in Tick-Borne Flavivirus Encephalitis: A Review of Current Paradigms in Protection and Disease Pathology. *Viruses*, *15*(4), 958.

Stone, E. T., & Pinto, A. K. (2023). T Cells in Tick-Borne Flavivirus Encephalitis: A Review of Current Paradigms in Protection and Disease Pathology. *Viruses*, *15*(4), 958.

Thevenet, P., Shen, Y., Maupetit, J., Guyon, F., Derreumaux, P., & Tufféry, P. (2012). PEP-FOLD: an updated de novo structure prediction server for both linear and disulfide bonded cyclic peptides. *Nucleic acids research*, *40*(Web Server issue), W288–W293. https://doi.org/10.1093/nar/gks419

Trovato, M., Sartorius, R., D'Apice, L., Manco, R., & De Berardinis, P. (2020). Viral Emerging Diseases: Challenges in Developing Vaccination Strategies. *Frontiers in immunology*, *11*, 2130. https://doi.org/10.3389/fimmu.2020.02130

UniProt Consortium (2013). Update on activities at the Universal Protein Resource (UniProt) in 2013. *Nucleic acids research*, *41*(Database issue), D43–D47. https://doi.org/10.1093/nar/gks1068

van de Sandt, C. E., Kreijtz, J. H., & Rimmelzwaan, G. F. (2012). Evasion of influenza A viruses from innate and adaptive immune responses. *Viruses*, *4*(9), 1438–1476.

Van Leur, S. W., Heunis, T., Munnur, D., & Sanyal, S. (2021). Pathogenesis and virulence of flavivirus infections. *Virulence*, *12*(1), 2814–2838. https://doi.org/10.1080/21505594.2021.1996059

VanBlargan, L. A., Errico, J. M., Kafai, N. M., Burgomaster, K. E., Jethva, P. N., Broeckel, R. M., Meade-White, K., Nelson, C. A., Himansu, S., Wang, D., Handley, S. A., Gross, M. L., Best, S. M., Pierson, T. C., Fremont, D. H., & Diamond, M. S. (2021). Broadly neutralizing monoclonal antibodies protect against multiple tick-borne flaviviruses. *The Journal of experimental medicine*, *218*(5), e20210174. https://doi.org/10.1084/jem.20210174

Wilkins, M. R., Gasteiger, E., Bairoch, A., Sanchez, J. C., Williams, K. L., Appel, R. D., & Hochstrasser, D. F. (1999). Protein identification and analysis tools in the ExPASy server. *Methods in molecular biology (Clifton, N.J.)*, *112*, 531–552. https://doi.org/10.1385/1-59259-584-7:531.

Xu, Z., Patel, A., Tursi, N. J., Zhu, X., Muthumani, K., Kulp, D. W., & Weiner, D. B. (2020). Harnessing Recent Advances in Synthetic DNA and Electroporation Technologies for Rapid Vaccine Development Against COVID-19 and Other Emerging Infectious Diseases. *Frontiers in medical technology*, *2*, 571030.

Zhang, X., Jia, R., Shen, H., Wang, M., Yin, Z., & Cheng, A. (2017). Structures and Functions of the Envelope Glycoprotein in Flavivirus Infections. *Viruses*, *9*(11), 338. https://doi.org/10.3390/v9110338

Zhang, X., Zhang, Y., Jia, R., Wang, M., Yin, Z., & Cheng, A. (2021). Structure and function of capsid protein in flavivirus infection and its applications in the development of vaccines and therapeutics. *Veterinary research*, , *52*(1), 98. https://doi.org/10.1186/s13567-021-00966-2