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Chimeric vaccine design against the epidemic Langya Henipavirus using immunoinformatics and validation via immune simulation approaches

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ABSTRACT

In July 2022, a new virus called Langya virus (LayV) was discovered in China in patients who had a fever. This virus is a type of Henipavirus (HNV) and is considered a potential threat as it could spread from animals to humans. It causes respiratory disease with symptoms including fever, coughing, and fatigue and is closely linked to two other henipaviruses that are known to infect humans, namely *Hendra* and *Nipah* viruses. These viruses may cause fatal respiratory illnesses. Investigators believe that the *LayV* is spread by shrews, and may have infected humans directly or via an intermediary species. Thus, the use of vaccines or immunizations against *LayV* is an alternate strategy for disease prevention. In this study, we employed various immunoinformatics methods to predict B cell, HTL and T cell epitopes from the *LayV* proteome in order to find the most promising candidate for a *LayV* vaccine. The most potent epitopes that are immunogenic and non-allergenic were joined with each other through suitable linkers. Human *β-defensin 2* was employed as an adjuvant to increase the immunogenicity of the vaccine construct. The final sequence of a multi-epitope vaccine construct was modelled for docking with TLRs. Concisely, our results suggest that the docked complexes of vaccine-TLRs seemed to be stable. Additionally, *in silico* cloning was done using *E. coli* as the host in order to validate the expression of our designed vaccine construct. The GC content of 54.39% and CAI value of 0.94 revealed that the vaccine component expresses efficiently in the host. This study presents the novel vaccine construct for *LayV* which will be essential for further experimental validations to confirm the immunogenicity and safety of the proposed vaccine structure, and eventually to treat *HNV*-related diseases.

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1. Introduction

Nearly two years after the discovery of the deadly coronavirus, another highly virulent zoonotic virus known as the *Langya Henipavirus* was discovered in the two eastern Chinese provinces during the third wave or post-COVID-19 outbreak. The most recent reports on infection-related febrile fever in humans concern several cases. Concisely, 35 people have been found to carry the *LayV*, but no cases outside of China have garnered global notice [1,2]. Eastern China's Shandong and Henan provinces have seen the majority of the affected individuals, and from 2018 to 2022, 35 patients with *LayV* and a recent history of animal contact were evaluated. A patient's throat swab sample was used to identify the virus, which was then isolated as a phylogenetically unique *HNV* after being subjected to metagenomics analysis [3].

The majority of the symptoms described were moderate, such as fever, tiredness, coughing, appetite loss, muscle pains, nausea, and headaches, however, a smaller number of patients suffering with potentially more difficulties, such as pneumonia and severe abnormalities in liver and kidney function. The World Health Organization (WHO) estimates that this virus family is particularly dangerous, with a death rate of 40%–75%, which is substantially greater than the mortality rate for COVID-19 [4,5].

LayV belongs to the *Paramyxoviridae* family, genus *Henipavirus*. Whereas, the *Hendra* and *Nipah* viruses, which cause deadly illnesses in people, are also members of this genus. Both these zoonotic viruses are contagious and provide a constant risk to humans and livestock, however, *LayV* has not been properly investigated ye [6,7]. The *Nipah* and *Hendra* are classified as biosafety category 4 (BSL-4). As a result, until the actual threat of *LayV* is determined, it should be handled as biosafety BSL-4 facilities [4,5].

Effective vaccination is required to boost the human body's immune system against infection. However, no vaccine on the market can treat *HNV* infection. The immune system makes use of B and T cells to identify the pathogen's antigenic component and to activate the body's defenses. B cells create antibodies against infections, while cytotoxic T cells directly destroy infected cells [8]. Antigen-presenting cells commonly display MHC epitopes. The body's immunity is boosted by vaccination and immunization so that it can combat pathogens and respond effectively to them [9]. A large collection of information on the pathogens is sequentially accessible which can be used to identify a promising protein for vaccines via computational methods called subtractive proteomics [10,11]. A protein must meet several requirements in order to be used in the production of a vaccine, including being a membrane protein, being highly virulent and antigenic, and not being similar to humans [12]. This approach has been widely used by various studies to design potent vaccine candidates for different infectious agents [13,14].

The current research aims to develop multi-epitope vaccines (MEVC) for the treatment of *LayV* using various immunoinformatic approaches. A computationally constructed vaccine is more efficacious, cost-effective, and thermodynamically stable than a traditional vaccination. This research aimed to develop a multi-epitope vaccine for *LayV* to boost the body's defenses against this infection. The epitopes chosen for the construction of the vaccine were non-allergic, non-overlapping, and highly immunogenic. Furthermore, the developed vaccine 3D immune stimulation, and physiochemical properties were studied and protein structure was docked with TLR3, TLR7, and TLR8. Finally, assessments were then completed to verify vaccine efficacy and, stability.

2. Methodology

2.1. Retrieval, antigenicity, and immunogenicity of LayV HNV proteome

LayV proteome was obtained from the *Zhang, X.-A* et al. study upon request. The sequence of each *LayV* protein was then subjected to subtractive proteomics to identify a high antigenic and non-allergenic epitope for the development of the vaccine. The proteome of HNV consists of nine proteins: including Nucleocapsid protein, P phosphoprotein, V protein, W protein, C protein, Matrix protein, Fusion protein, Attachment glycoprotein, and Polymerase [1,15]. Concisely, to predict the antigenicity of each protein, we uploaded all proteins to the VaxiJen server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), with default value 0.4 [16]. Furthermore, AlgPred an online server (http://www.imtech.res.in/raghava/algpred/) was used to predict the allergenicity of all *LayV* proteins [17].

2.2. Epitopes prioritization of the LayV proteome

The *LayV* proteome was analyzed to select the most promising epitopes i.e. highly antigenic and non-allergenic for the potential multiepitope vaccine. At first, we used the web-server NetCTL1.2 (https://services.healthtech.dtu.dk/service.php NetCTL-1.2) with a default value of 0.75 [18] to predict the cytotoxic T lymphocytes (CTL) epitopes. Furthermore, the helper T cell epitopes were identified via the IEDB (The Immune Epitope Database) server (http://tools.iedb.org/mhcii/), which use seven sets of HLAs (Human Leukocyte Antigens) alleles for epitope prediction [19]. Concisely, The IC50 value is used by the abovementioned online web server to characterize the HTL (Helper T Lymphocyte), while epitopes with IC50 values < 50 nM are regarded as excellent binders. The sequences of the chosen proteins were then subjected to the ABCPred online web-server (http://crdd.osdd.net/raghava/abcpred/) to predict the B-cell epitopes (with a threshold score of 0.8) which are crucial for producing the protective host antibody responses [20,21]. The predicted epitopes were then finally evaluated for antigenic and non-allergenic properties via VaxiJen and AlgPred servers to shortlist them for the development of a multi-epitope vaccine, and the most antigenic and non-allergenic epitopes were chosen for vaccine development.

2.3. Vaccine design and prediction of physiochemical properties

Different antigenic linkers were employed to combine the promising qualities of chosen HTL, CTL, and B cell epitopes. Concisely, the final vaccine design is made up of adjuvant, HTL CTL, HTL, and B cells joined by AAY, GPGPG, and KK linkers, respectively. The adjuvant Human β-Defensin 2 (HBD2) is used at the vaccine's *N*-terminus to increase immune responses and stabilize the vaccine construct. HBD2 has been reported to stimulate pro-inflammatory anti-viral immune response and also induce stronger anti-tumor activity by promoting the maturation of DC [22]. Moreover, in an anti-viral response, the higher production of IFN-β, IFN-γ, MxA, PKR, RNaseL, NOD2, TNF-α, IL-1β, and IL-6 has been reported which makes it a perfect choice for the attenuation of antiviral activity of the designed vaccine candidate. Following that, the resulting structure of generated vaccine was assessed for allergenic and antigenic properties using online internet webservers such as VaxiJen [16] and AllerTop v.20 (https://www.ddg-pharmfac.net/AllerTOP/) [23]. Furthermore, ProtParam an online tool (https://web.expasy.org/protparam/) [24] was used to measure the physiochemical properties including, molecular weight, theoretical pI (isoelectric point), GRAVY, aliphatic index, instability index, grand average of hydropathicity, and both *in-vivo* and *in-vitro* half-life of the constructed vaccine.

2.4. Structure prediction and validation

The constructed vaccine sequence was first submitted to PSIPRED [25] for protein secondary structure prediction, PSIPRED is an online secondary protein prediction server with high accuracy, furthermore, while, the vaccine's tertiary structure was predicted by using the amino acid sequence. Concisely, sequence was upload to Robetta web-server (http://robetta.bakerlab.org/) [26], which predicts the tertiary structure by using comparative modeling approaches. Furthermore, Robetta server predicted five models for constructed vaccine model, and we chose the accurately predicted protein structure based on the protein structure properties. The chosen model was then validated using the two online web servers. Concisely, the quality of the protein structure was examined using the RAMPAGE servers (http://mordred.bioc.cam.ac.uk/rapper/rampage.php) [27], and ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php) [28]. Based on quality ratings, these servers assess the protein structure. There are no errors in the structure if the result falls within the experimentally established range.

2.5. TLRs vaccine docking, molecular modeling, and MM/GBSA (molecular mechanics generalized born and surface area) analysis

TLRs are essential inflammatory pathway regulators that play critical roles in modulating immunogenicity against infections [29]. TLRs identify pathogen-associated molecular patterns (PAMPs), causing gene expression to alter the intracellular cascades. The host's innate immune system recognizes the invaders and responds appropriately via TLR recognition. TLRs have a vital role in identifying different components of viruses, such as nucleic acids and envelope glycoproteins, which trigger a number of cascades that include the creation of IFN-I, inflammatory cytokines, and chemokines. Furthermore, when TLRs stimulate dendritic cell maturation, adaptive immune responses are triggered. Thus, considering the interaction between TLRs and the designed vaccine is essential to forecast the immune response [30,31]. The HDOCK server (http://hdock.phys.hust.edu.cn/) provides integrated tools which are used for accurate and fast protein-protein docking [32]. We utilized the HDOCK server to assess the binding of a newly designed vaccine derived from *LayV* proteome to the human toll-like receptors (TLRs) i.e. TLR3, TLR7, and TLR8. To find protein-protein interaction, the HDOCK server employs a hybrid algorithm that integrates template-based and template-free techniques. This server is distinguished from others by its ability to accept the amino acid sequence as an input file. In addition, we utilized the web server HawDock (http://cadd.zju.edu.cn/hawkdock/) to determine the binding free energies of the vaccine-TLR complex via MM/GBSA approach [33]. The selection of a particular TLR is based on its role in targeting the IRF3 pathways, nuclear translocation, and blockage of dsRNA during the infection [1,31].

2.6. Human immunological modeling and in silico cloning

Codon optimization is essential for the K12 strain of *Escherichia coli* (*E. coli*) to express vaccines optimally. Herein, the nucleotide sequence of designed vaccine construct was reverse-translated and optimized using the VectorBuilder codon optimization tool (https://en.vectorbuilder.com/tool/codon-optimization) [22]. The VectorBuilder codon optimization tool evaluated the vaccine's high expression based on the CAI (Codon adaptation index) score and GC (guanine-cytosine) content percentage. Moreover, we used the SnapGene tool (https://www.snapgene.com/), to cone the multi-epitope vaccine sequence into the pET vector for in-vivo expression as described previously [34]. Finally, we investigated the capability of the designed vaccine to stimulate an immunological response against the highly antigenic *LayV MEVC* (Multi epitopes vaccine construct) via the *C*-ImmSim web-server (http://www.cbs.dtu.dk/services/CImmSim-10.1/) [35]. The abovementioned server applied the PSSM model to determine how the immune system's different components-cytokines, antibodies, interferons, helper T cells 1 and 2- responded to the administered vaccinations.

3. Results and discussions

3.1. LayV proteome's antigenicity and allergenicity

The immune responses are essential for the elimination and neutralization of invasive pathogens [36]. When infections are subsequently encountered, the immune response creates memory immune cells that distinguish the target and retain antibodies of that

particular pathogen, which are the foundation for the formation of vaccines [37]. To create a multi-epitope vaccine against the *LayV*, we employed computational modeling and integrated immunological informatics methods.

The *LayV* whole proteome was obtained from the recently conducted *Zhang, X.-A* et al. study on *Henipavirus* [1]. *LayV's* whole proteome comprises eight distinct proteins that were assessed for allergenicity and antigenicity (nucleocapsid protein, P phosphoprotein, V protein, W protein, C protein, matrix protein, fusion protein, attachment glycoprotein, and polymerase).

Following antigenic and allergenic assessments, all proteins were confirmed to be antigenic with an antigenic score less than 0.4 as well as non-allergenic. The results showed that all proteins were antigenic and non-allergenic. Furthermore, all proteins were then screened for possible B cell, cytotoxic T cell, and helper T cell epitopes in order to develop the multi-epitope vaccine against *LayV*. Table 1 represents the antigenicity and allergenicity of each protein of *LayV*.

3.2. Immunogenic CTL, HTL, and B-cell epitope prediction

The whole *LayV* proteome was employed in this work to map B and T cell epitopes based on antigenic and non-allergenic properties. Dendritic cells and B cell monocytes exhibit MHC-II proteins on their surfaces, while all nucleated cells express MHC-I glycoproteins [38,39]. The proteins on the nucleated cell surface are imported when the cytotoxic T cells are presented with the epitopes, triggering an immediate immune response against invading pathogens. The previous studies suggest that Cytotoxic T lymphocytes (CTLs) is significant because it amplifies the immune response to infections. In order to anticipate the immunogenic CTL epitopes, we submitted each protein separately. Except for the C protein, a total of 170 CTL epitopes were chosen based on the combined score predicted by the NetCTL server. Only 41 of the 170 CTL epitopes that were tested by Allertop for allergenicity and were confirmed to be non-allergenic. Furthermore, based on the combined score and antigenicity score, only one epitope was chosen for each protein. While for the V protein and P protein, we selected the same epitope (GTEIINLTI) because this epitope was highly antigenic and non-allergenic for both proteins. The details of all the epitopes are provided in Supplemental Tables S3–S5. The stimulation of B cells to create antibodies as well as the activation of CTL to eliminate infected cells is a key component of the human immune system [39]. Helper T cells, a crucial component of acquired or adaptive immunity, stimulate both of these immune system pathways in humans [40]. After activation of antigen-presenting cells, the precursor of HTL evolves into a particular type of HTL needed for the development of certain immune responses [41]. Because HTL plays a crucial role in the immune response, we subjected each protein separately to the IEDB server to predict the helper T cell epitopes that would induce the immunological response. Concisely, IEDB predicted 271 total epitopes for all proteins, however only 81 were chosen for further study based on a positive score. These 81 epitopes were then screened using the adjusted rank score, and the top 8 epitopes for each protein were selected, including the common P protein and W protein epitope (APAFQMNPNAKEYYP). Table 2 and Supplemental Tables S5–S7 provide further information. The B cell is one of the immunogenic factors for the activation of humoral immunity and long-term immunity [42]. The selection of B cells epitopes with the highest score is vital for the construction of an efficient multi-epitope vaccine. Concisely, for the multi-epitope vaccine design, epitopes having a length of 16-mer and a score higher than 0.51 (default) were selected. The selected epitopes were then screened for antigenic and non-allergenic properties, and the one epitope with the highest antigenic score for each protein was chosen for the final *LayV* vaccine construct. Concisely, The ABCPred server predicted 567 epitopes for all proteins, of which 266 were chosen based on a score greater than 0.8.

Moreover, based on the antigenicity and allergenicity analyses, 77 epitopes were selected from among them. Furthermore, based on the high antigenicity score and non-allergenic feathers, one epitope was chosen for each protein. Whereas one epitope (TRPEELSLEERNGTIS) was common in V protein, W protein, and C protein therefore we only selected this epitope for these three proteins for further analysis. More detail is given in Table 2 and Supplementary Tables S9–S11. While Fig. 1 represents the step-by-step analysis conducted in this study.

3.3. Construction of MEVC for LayV

Multi-peptide vaccines that have been created computationally are a good alternative since they can be produced fast, are affordable, induce particular antibody responses, and decrease the anaphylaxis risk produced by antigens. The proposed epitopes vaccines are very moderately immunogenic, thus to boost immunogenicity, the nontoxic HBD-2 (human beta defensin-2) adjuvant was connected to the N terminal of the vaccine [43]. The adjuvant increases the capacity of greater immune responses to antigens.

Table 1
Antigenicity and allergenicity of Langya Henipavirus proteins.

Protein name	Antigenicity Score	VaxiJen Prediction	Algpred Prediction
Nucleocapsid protein	0.48	ANTIGENIC	NON-ALLERGEN
P phosphoprotein	0.46	ANTIGENIC	NON-ALLERGEN
V protein	0.50	ANTIGENIC	NON-ALLERGEN
W protein	0.50	ANTIGENIC	NON-ALLERGEN
C protein	0.42	ANTIGENIC	NON-ALLERGEN
Matrix protein	0.51	ANTIGENIC	NON-ALLERGEN
Fusion protein	0.43	ANTIGENIC	NON-ALLERGEN
Attachment glycoprotein	0.54	ANTIGENIC	NON-ALLERGEN
Polymerase	0.45	ANTIGENIC	NON-ALLERGEN

Table 2
Highly antigenic and non-allergenic epitopes are predicted for the whole Henipavirus proteome for the vaccine.

Protein Name	Pos	Peptide	Score	Antigenicity Score	Allertop	epitopes
attachment glycoprotein	573	EEIWCIAVTEGRKQKE	0.88	1.50	NON-ALLERGEN	BC
C protein	27	LGCQQQKTEPQHGSCS	0.92	1.09	NON-ALLERGEN	BC
fusion protein	193	SVGIKLTQYYSEILTA	0.94	0.81	NON-ALLERGEN	BC
nucleocapsid protein	348	NRSYLEPIYFKLGQNA	0.9	0.95	NON-ALLERGEN	BC
P phosphoprotein	61	TRPEELSLEERNGTIS	0.88	1.67	NON-ALLERGEN	BC
polymerase	516	PYEIIDYVLSGKYKTD	0.96	0.74	NON-ALLERGEN	BC
V protein	61	TRPEELSLEERNGTIS	0.88	1.69	NON-ALLERGEN	BC
W protein	61	TRPEELSLEERNGTIS	0.88	1.69	NON-ALLERGEN	BC
attachment glycoprotein	174	DTTIKPVEY	1.93	1.40	NON-ALLERGEN	CTL
fusion protein	288	VQELMPISY	1.12	1.15	NON-ALLERGEN	CTL
nucleocapsid protein	102	ITDISEFDH	0.92	0.72	NON-ALLERGEN	CTL
P phosphoprotein	259	GTEIINLTI	1.32	1.08	NON-ALLERGEN	CTL
polymerase	3	FSDVSISDI	1.48	1.81	NON-ALLERGEN	CTL
V protein	259	GTEIINLTI	1.32	1.08	NON-ALLERGEN	CTL
W protein	319	ESDITIFDL	0.96	0.96	NON-ALLERGEN	CTL
attachment glycoprotein	557	NYYSITSATISCFMY	0.49	0.99	NON-ALLERGEN	HTL
C protein	144	LRLIRLMCPAYSRAV	0.25	0.62	NON-ALLERGEN	HTL
fusion protein	471	KSEEFLKGINPSIIT	0.99	0.46	NON-ALLERGEN	HTL
P phosphoprotein	184	APAFQMNPNAKEYYP	0.44	0.81	NON-ALLERGEN	HTL
polymerase	1381	TNLIFQQVMLLGLSA	0.74	0.70	NON-ALLERGEN	HTL
V protein	182	KTAPAFQMNPNAKEY	0.8	0.93	NON-ALLERGEN	HTL
W protein	184	APAFQMNPNAKEYYP	0.44	0.81	NON-ALLERGEN	HTL

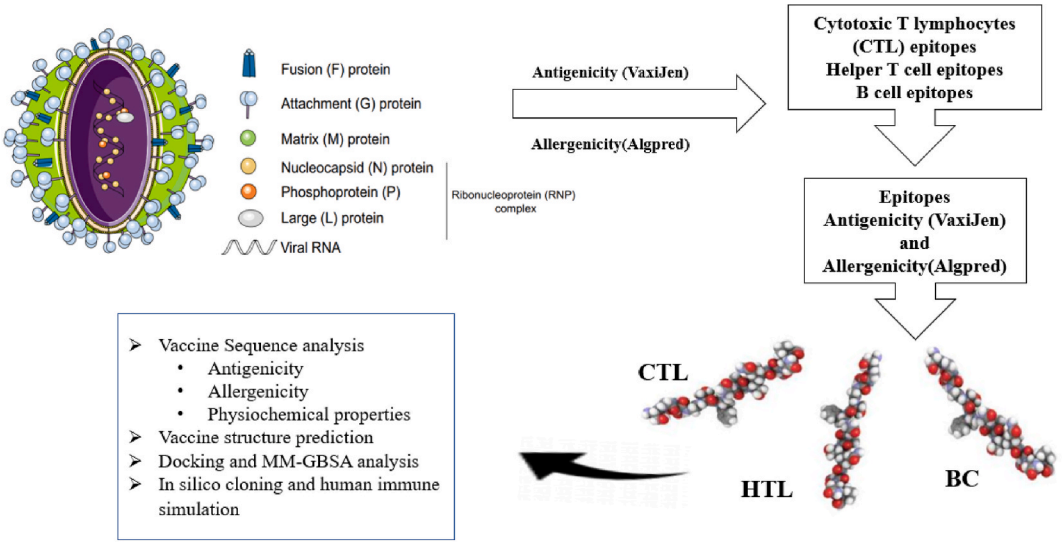

Fig. 1. Represents a step-by-step analysis of the construction Langya Henipavirus vaccine, the virus proteome was retrieved from the previous study conducted by *Zhang, X.-A* et al. All proteins were checked for antigenicity and allergenicity. CTLs, HTLs, and BCs epitopes were predicted and highly antigenic and non-allergenic epitopes were selected, the adjuvant HBD2 was used to boost up the vaccine efficiency, and the linker AAY, GPGP, and KK were used to join the CTL, HTL, and BC epitopes respectively. After the construction of the vaccine sequence, biochemical properties, protein structure prediction, docking and *in silico* cloning, and human immune simulation were checked.

Therefore, a multi-epitope vaccine design with HDB-2 adjuvant was constructed by combining the particular CTL, HTL, and B cell epitopes with the help of a number of linkers, such as EAAK, GPGPG, AAY, and KK [9]. Concisely, all of the selected CTL epitopes were joined by using the AAY linkers, and the adjuvant to the CTL epitopes were connected together using the EAAK linker. The GPGPG and KK linkers, respectively, were used to connect each of the selected HTL and B cell epitopes as shown in Fig. 2A. The aforementioned linkers have two characteristics: first, they avoid folding by keeping the epitopes apart, and second, they aid in the epitopes' ability to manifest an effective immune response. To guarantee that no reaction would occur in the experimental environment, the antigenic and allergenic properties of constructed vaccine sequence was tested by using VaxiJen and Allertop server. The results showed that our constructed vaccine (including 379 amino acids) was highly antigenic with an antigenicity score of 0.782 and non-allergenic. This suggests that our designed vaccine could induce immunological responses without resulting in an allergic reaction, and it can be considered for future studies. Additionally, the constructed vaccine sequence was subjected to Robetta online 3D protein modeling server. The Robetta server constructed five protein 3D models for the constructed vaccine, and the best model was selected after further

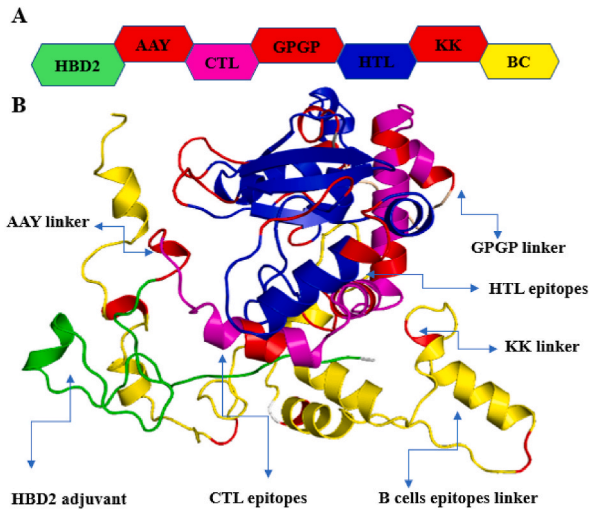

Fig. 2. Represents the secondary and tertiary structure, A represents the best-constructed protein model by Robetta, green color residues represent adjuvant, all red residues represent linkers, magenta color residues represent CTL epitopes, blue color residues represent HLTs, and yellow color residues represent BC epitopes. B represents the tertiary structure of constructed vaccine. The yellow color represents B cell epitopes, red represents GPGPG linkers, pink represents CTL epitopes, blue represents HTL epitopes and green represents HBD2 adjuvant.

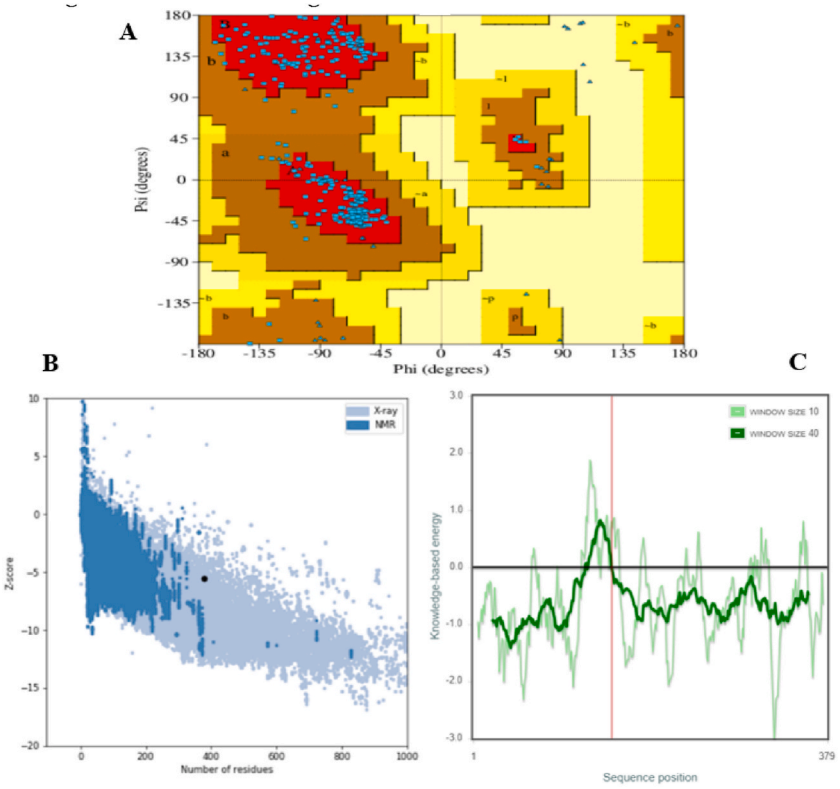

Fig. 3. Structure validation of proposed vaccine tertiary structure. A) The Ramachandran plot analysis results demonstrate that 93.8% of the residues are in the most favored regions, while 6.2% are in the allowed regions of the constructed models. The Ramachandran plot analysis was applied to the Robetta-generated models, and the model with the highest proportion of residues in the favorable zone and the lowest percentage in the outlier region was chosen as the best. Additionally, ProSA-web was used to examine the quality and any flaws in the top models. ProSA-web results from **B** and **C** revealed that the predicted structure is correct and could be used for further research. A z-score of −5.54 was ProSA-web was reported.

verification. The final selected protein structure of the vaccine construct is shown in Fig. 2B while Fig. 3 demonstrates the structure validity of the 3D protein structure predicted for the vaccine construct.

3.4. Validation of MEVC 3D structure

The 3D vaccine model was predicted by the Robetta online server utilizing a comparative modeling approach. The best model for the constructed vaccine was chosen among the 5 created models using the Ramachandran plot analysis and ProSA-web (Fig. 3A–C). Concisely, The Ramachandran plot analysis was applied to the Robetta-generated models, and the model with the highest proportion of residues in the favorable zone and the lowest percentage in the outlier region was chosen as the best (Fig. 3A). Additionally, ProSA-web was used to examine the quality and any flaws in the top models. The input models' Z-scores were determined to be −5.54. (Fig. 3B). The aforementioned Z-scores are within the acceptable range for protein structures of comparable size. As a result, the chosen model can be prepared for further study (Fig. 3C), including molecular docking and simulations.

3.5. Physicochemical properties of the MEVC

The physicochemical characteristics of the *LayV* vaccine model were estimated by using the ProtParam web server. The determined molecular weight was 41,303 Da. While had a pI (isoelectric point) of 9.11. The pI values of our design protein structure reveal that it is acidic in nature, suggesting that the proposed vaccine model is appropriate for further analysis. The *in vivo* half-life of the vaccine model was more than 20 h. The proposed vaccine model was found to be stable, with an instability rating of 33.66. Whereas, the GRAVY and aliphatic indexes were determined to be −0.379 and 71.13, respectively. The protein's hydrophilic nature, shown by its low GRAVY score, suggests that it will interact with adjacent water molecules more smoothly [11].

3.6. Molecular docking of MEVC with TLR-3, TLR-7, and TLR-8

The HDOCK server was used to study the interaction between the designed vaccines and TLR-3, TLR-7, and TLR-8. The MEVC-TLR3/TLR7/TLR3 complexes docking scores were −355.18 kcal/mol, −309.14 kcal/mol, and −322.20 kcal/mol.

These results confirm the MEVC's strong binding to TLR3, TLR7, and TLR8. Furthermore, we calculated the binding free energies for the MEVC-TLR complex by MM-GBSA analysis was performed. Table 3 MM/BGSA results represent the bind-free energy of MEVC-TLR complexes. While Fig. 4 represents the best docking pose of MEVC-TLRs complexes predicted by HDOCK MM-GBSA analysis. Fig. 4A show the best docking pose of MEVC-TLR3 along with the interacting residues, while Fig. 4B depict the best docking pose and interaction between MEVC-TLR7 while Fig. 4C represent the best docking result for MEVC-TLR8 complex.

3.7. Immune simulation of the proposed vaccines

To ensure the maximum possible production of the protein in *E. coli* (strain K12), the VectorBuilder codon optimization algorithm was utilized. The sequence of optimized codons was 1140 nucleotides long. The optimized nucleotide sequence's codon adaptation index (CAI) was 0.94 and its average GC content was 54.39%, which specified that the vaccine protein can be successfully synthesized in *E. coli*. The ideal range for GC content should be 30%–70% [11]. By incorporating the modified codon sequences into the pET vector, the restriction clone was ultimately created as shown in Fig. 5. Finally, the designed vaccine's effectiveness to elicit an immune response was evaluated during the *in silico* immunological simulation. The immune system's response was dramatically boosted after one dosage of MEVC injection, and the anti-toxicity of elicited antibodies was much strengthened. Antigen titers persisted higher 5–10 days after the first dose of injection of the constructed vaccine before being neutralized. A substantial antibody response induction was found for our antigenic vaccine. Furthermore, the constructed vaccine had the highest levels of IgM and IgG antibody titers, followed by IgM titers as shown in Fig. 6A. Moreover, the vaccine was examined for the production of different IFN (interferon), IL (interleukins), TNF (Tumor Necrosis Factor), and TGF (transforming growth factor) (transforming growth factor). Among the other elements, the IFN-g response was the strongest, peaking at >600,000 within the first 15–20 days. Fig. 6B depicts the concentrations of interleukins and cytokines generated by the injection of the proposed vaccine.

3.7.1. Study limitations

This study does not include the *in vitro* and *in vivo* validation analysis of MEVC however, the standard immunoinformatic approaches were adopted to construct the vaccine design for *LayV*, and the constructed model was validated via physiochemical properties as well as structure and functional properties. Further experimental validations need to confirm the immunogenicity and

Table 3
Docking score of constructed vaccine with the receptors.

Complexes	Respecter ligand docking results			MM/GBSA results				
	Docking Score	Confidence Score	Ligand rmsd (Å)	VDW	ELE	GB	SA	TOTAL
MEVC/LTR-3	−355.18	0.9838	165	−169.05	−621.89	784.61	−21.26	−27.58
MEVC/LTR-7	−309.14	0.9602	62.97	−151.96	306.68	−179.8	−20.76	−45.84
MEVC/LTR-8	−322.2	0.9691	47.74	−160.05	−664.79	801.11	−20.85	−44.58

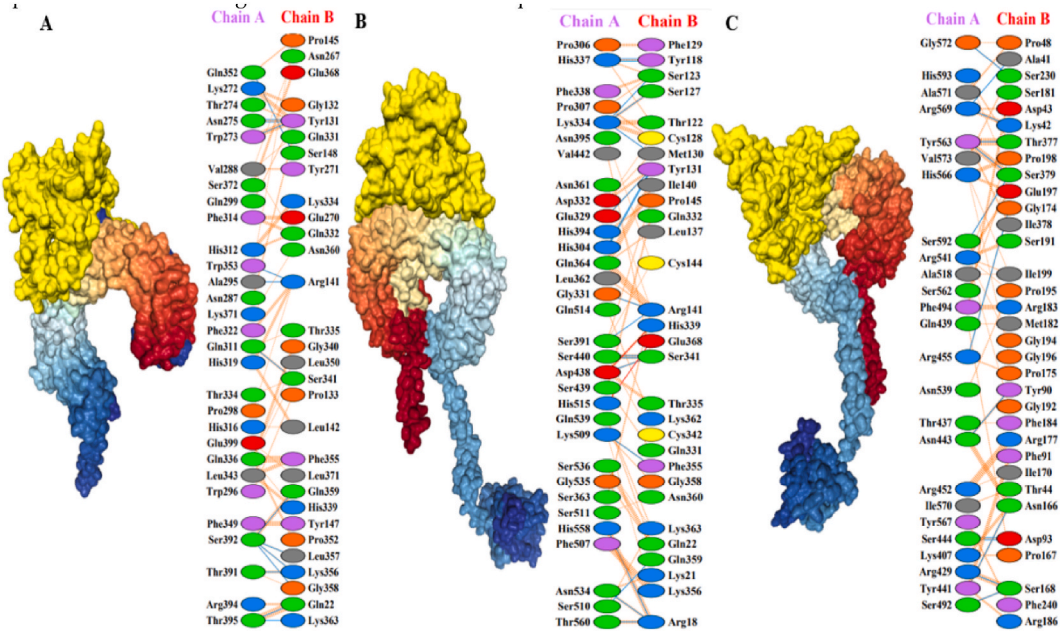

Fig. 4. The top docking complexes of the constructed vaccine and TLR-3, TLR-7, and TLR-8 were predicted by HDOCK. **(A)** Represent the docking complex of MEVC with TLR3, **(B)** represent the docking complex of TLR7 with MEVC and **(C)** represents the docking complex of TLR8 with MEVC.

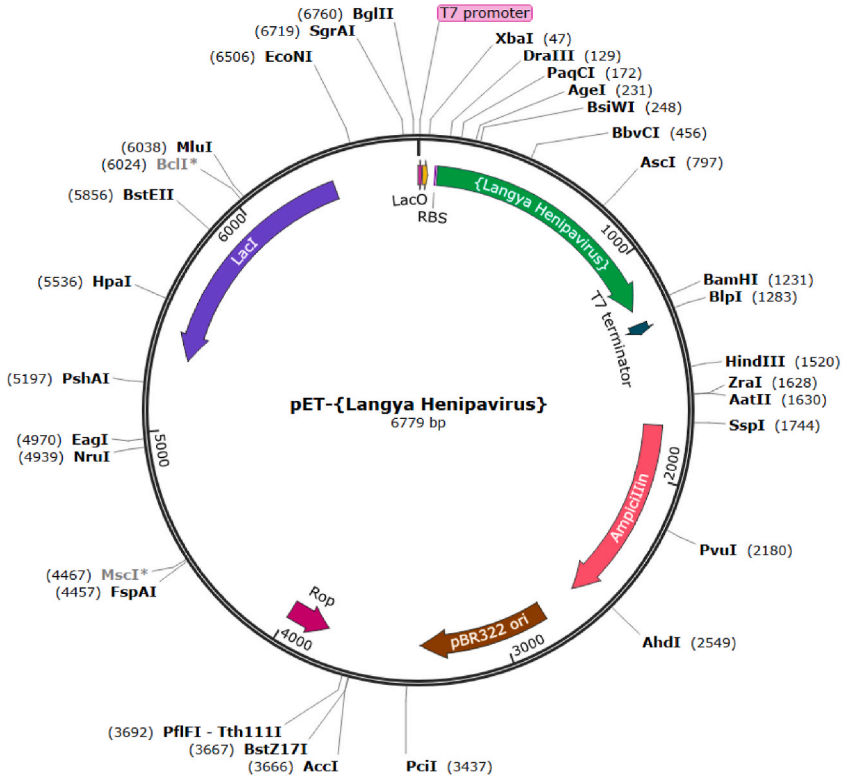

Fig. 5. Represents the constructer vector for the Langya Henipavirus. The final constructed vaccine was *in silico* restricted and cloned into the pET expression vector, with the vaccine insert represented by the green section and the vector by the black circle.

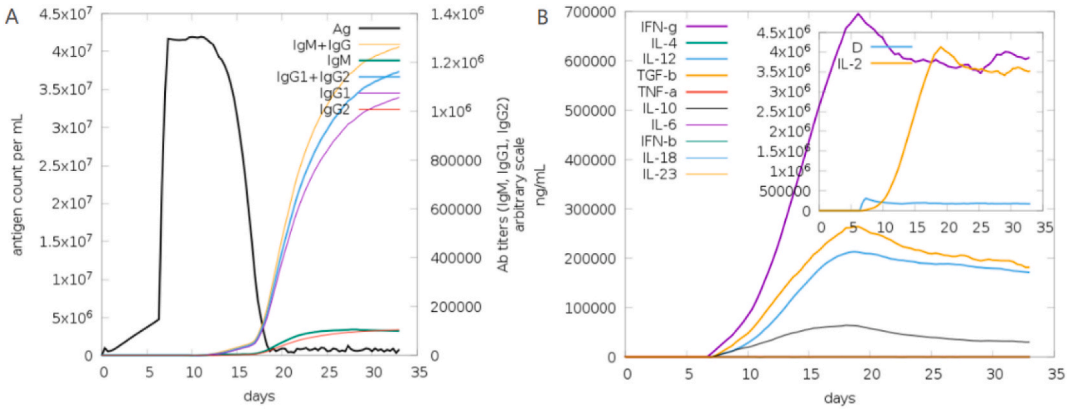

Fig. 6. Immune simulation results of the constructed protein, **(A)** represents the immunoglobulins and immune-complex results after one dose of injection, while **(B)** represents the cytokines and interleukins concentrations.

safety of the proposed vaccine structure, and eventually to treat HNV-related diseases.

4. Conclusion

We intended to create a multi-epitope-based vaccine for *LayV* using immunoinformatics techniques in this research. This scientific study starts with the retrieval of the whole viral proteome, followed by the prediction of immunogenic B-cell and T-cell epitopes for immunity induction. To improve the immunogenicity of effective epitopes, predicted epitopes were linked with appropriate linkers and adjuvants. Furthermore, to assess the binding affinity and stability of TLRs and vaccine complexes, allergenicity, antigenicity, physiochemical characteristics, and molecular docking were done. Finally, to confirm the vaccine construct's stability and efficient expression, *in silico* cloning was performed. This study employs a variety of techniques to build a whole proteome vaccine model based on multi-epitopes; the suggested vaccine must be confirmed experimentally to assure experimental validity. This research will assist in infection prevention by developing an efficient immune memory against Langya Henipavirus virus infections.

Author contribution statement

Aamir Fahira: Conceived and designed the experiments; Performed the experiments.

Qiangzhen Yang: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Rana Sherdil Amin: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Uzma Arshad: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Muhammad Idrees Khan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ali Alamdar Syed Shah: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Abdul Rahman Alshammari: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zhou Wang: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Liaqat Ali: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Yongyong Shi: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17376.

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