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Article

Designing a Multi-Epitope Vaccine Against Small Ruminant Lentiviruses induced Maedi Visna and Caprine Arthritis Encephalitis Among Sheep and Goats

Muhammad Zeeshan Shabbir ^{1,*†}, Rumesa Ghazanfar ^{1,†}, Zainab Nagari ^{1,†},
Muhammad Atiq Ur Rehman ^{2,†} and Chandra Sourav ³

¹ BioMind Research Institute, MindSet Development Foundation, Islamabad, Pakistan.

² Advanced Radiation Technology Institute (ARTI), Korean Atomic Energy Research Institute, Jeongeup, 56212, Republic of Korea.

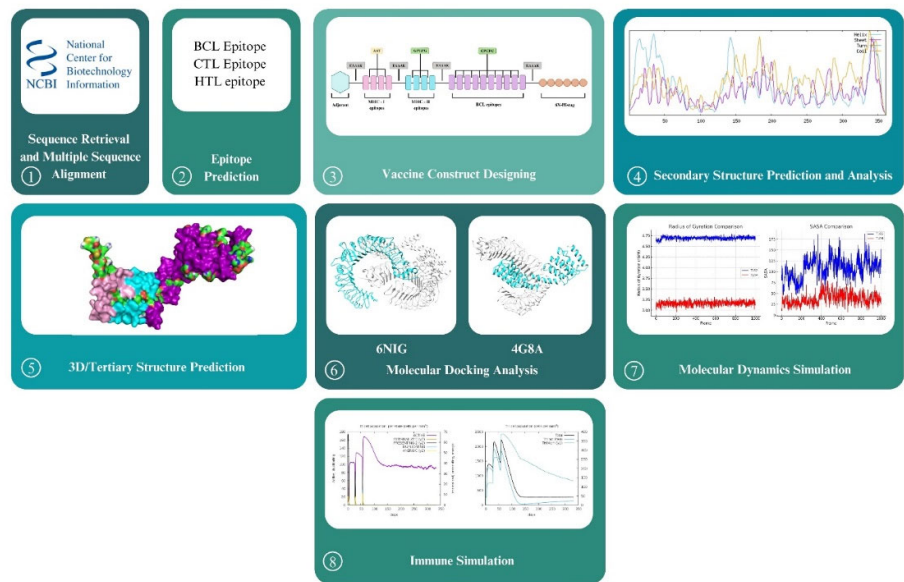
³ Department of Electronics and Information Engineering, Jeonbuk National University, Jeonju, 54896, South Korea.

* Correspondence: muhammadzeeshanawan6@gmail.com

† Muhammad Zeeshan Shabbir, Rumesa Ghazanfar, Zainab Nagari, Muhammad Atiq Ur Rehman should be considered as joint first authors.

Abstract

Small Ruminant Lentiviruses are one of the common viral agents among livestock animals mainly sheep and goats and are responsible for fatal diseases such as Maedi-Visna and Caprine Arthritis Encephalitis, hence resulting in compromised livestock outcomes. Developing a safe and effective vaccine against these viruses, that can provide protection in broad range of host species has been a great challenge, hence we employed different bioinformatic tools to design a novel multi-epitope vaccine construct based on the envelope gene of the virus. After predicting the secondary and tertiary structure of the vaccine construct, the structural accuracy was further enhanced by refinement and the stability/binding affinity of the vaccine construct was validated by using cutting-edge computational tools such as molecular docking and molecular dynamic simulation. Furthermore, the immune system activation potential was validated via immune simulation studies followed by the codon optimization and in-silico cloning. The vaccine construct showed good levels of immunogenicity and antigenicity without any allergic/toxic effects. The study revealed that the multi-epitope vaccine shows significant potency to combat SRLVs among goats and sheep simultaneously.



Graphical Abstract: Summarizing the Reverse Vaccinology based Vaccine Design against SRLVs induced MV and CAE. 1. First the amino acid sequences encoding the envelope gene of the most common SRLV isolates were retrieved from the NCBI and multiple sequence alignment was performed to generate a consensus sequence. 2. Furthermore, different webserver were utilized to predict the BCL, CTL and HTL epitopes that were immunogenic, non-toxic and non-allergenic. 3. The vaccine construct was generated by joining adjuvant, BCL, CTL and HTL together with the help of linker molecules. 4. SOPMA was utilized to predict the secondary structure of the construct and different secondary structure components were analyzed along with solubility analysis of the generated construct 5. The tertiary structure of the vaccine construct was predicted via the I-TASSER protein prediction server and was further refined via Galaxy Refine tool (further verified via ProCheck server). 6. The interaction between the vaccine construct and the two TLR molecules (TLR-2 and TLR-4) was analyzed with Molecular Docking Analysis via ClusPro. 7. Furthermore, the stability of the docking complexes was verified with the Molecular Dynamic Simulations. 8. The immune stimulating potential of the vaccine construct was evaluated using the C-ImmSim server. All the above steps ensured that the generated vaccine candidate offers broader coverage and protection, while being immunogenic, non-toxic and non-allergenic.

Keywords: Reverse vaccinology; Small Ruminant Lentiviruses; molecular docking; molecular dynamics simulation

1. Introduction

Maedi-Visna (MV) is a common viral infection mainly prevalent among sheep and it progresses slowly throughout the life cycle leading to the development of severe pneumonia along with neurological aberrations such as tremors, weakness and paralysis etc. (Mosa and Zenad 2020). The infected sheep also experience chronic indurative mastitis (Blacklaws and diseases 2012). Another common disease associated with the MV is Caprine Arthritis Encephalitis (CAE), however in most cases the host organism is goat. CAE infected goats exhibit a range of symptoms such as young goats have incomplete muscles development leading to ‘encephalitis’ where the infected goat experiences paresis, dizziness, seizures, difficulty in coordination ultimately leading to paralysis (Moroz, Czopowicz et al. 2022). While the older goats usually have ‘arthritis’, exhibition of enlarged and painful limbs. Both MV and CAE affected sheep and goats experience sudden weight loss, lower milk production along with poor fur coat development (ENACHE, BARAITAREANU et al. 2017).

Two common routes of MV and CAE transmission are: respiratory/oral (inhalation of the infective agents) and colostrum milk (Rahman, Ahmed et al. 2022).

The viral agents responsible for the incidence of MV and CAE are Maedi-Visna Virus (MVV) and Caprine Arthritis Encephalitis Virus (CAEV) respectively (Blacklaws, Berriatua et al. 2004) and it has been recently revealed that both these species are a viral continuum and can infect goat and sheeps without any specie barriers and are collectively called Small Ruminant Lentiviruses (SRLVs) (Minardi da Cruz, Singh et al. 2013). The genomic size of these SRLVs is around 8.4-9.2 kb (Santry, de Jong et al. 2013, Arcangeli, Torricelli et al. 2022). The viral genome encodes both structural and non-structural components. Majorly three structural genes: gag, pol and env make up the viral capsid, however the regulatory functions are performed by tat, vif and ref genes (Querat, Audoly et al. 1990, Pépin, Vitu et al. 1998, Gomez-Lucia, Barquero et al. 2018, Olech, Valas et al. 2018). Based on the genetic sequence information (gag and pol genes) from different viral SRLV isolates, these SRLVs have been divided into four genotypes namely A-C, E. Genotype-A includes the MVV whereas the CEAV isolates are included in the Genotype B, C and E (Molaei, Bazzucchi et al. 2020, Davaasuren, Molaei et al. 2024).

Timely diagnosis of SRLVs is a crucial parameter while designing any preventive measurement or treatment as the viral agent stays dormant in the host organism for a long period without the appearance of any notable clinical symptoms and hence remains undetected (Michiels, Van Mael et al. 2018). Various preventive/eradication measures have been employed to limit the spread of viral diseases among ungulates however, vaccination stands out as the most reliable treatment. Likewise, SRLV based vaccine development has also been targeted in the past (See Table 1), however currently, there is no commercially available vaccine against SRLVs that could provide protection against MVV and CAEV continuum simultaneously. Hence the aim of the study is to utilize the advancements in the field of bioinformatics, reverse vaccinology and protein structure predictions to develop a vaccine construct that could simultaneously provide protection against MV and CAE induced by SRLVs in diverse goats/sheep breeds, while using the coding sequence of the envelope gene of the virus: mainly involved in viral attachment and epitope presentation, so that the resulting vaccine construct is capable to boost sufficient immunity and can provide protection against diverse serotypes of the viral SRLV agents.

Table 1. Major Vaccine Candidates Against MV and CAE.

TYPE VACCINE	OF VIRAL AGENT	TARGETED SPECIE	TARGETED PARAMETERS	STUDY	OUTCOMES	REFERENCES
Attenuated molecular clone of Maedi Visna (MVV) (2005)	Maedi- Visna Virus (MVV) virus	Sheep	Four sheep infected attenuated molecular clone of maedi visna virus (MVV) (LV1-1KS1); while comparing them with control group (n=4)	were with the ELISA test indicating partial protection.	Low viral titer was detected in the ELISA test	(Pétursson, Matthíasdóttir et al. 2005)

DNA based vaccine on the envelope gene (2005)	Maedi-Visna Virus (MVV)	Sheep	pcDNA plasmid (pcDNA-env) encoding the env glycoproteins of MVV, boosted with combined pcDNA-env and pCR3.1-IFN-γ plasmid inoculations were introduced in both test and control groups	Early protective effect was observed in the vaccinated sheeps that restricted the virus replication however it disappeared after two years.	(González, Reina et al. 2005)
DNA vaccine containing the Maedi-Visna virus (MVV) gag gene (2007)	Maedi-Visna Virus (MVV)	Sheep	Sheep were immunized eight times over a period of 2.5 years with the Maedi-Visna (MVV) gag gene, VR1012-gag-CTE and pcDNA3.1-gag-CTE.	No positive outcome was observed. In fact, the vaccine may have made them more susceptible to the virus.	(Torsteinsdóttir, Carlsdóttir et al. 2007)
Use of B7 costimulatory molecules as adjuvants in a prime-boost vaccination against Visna/Maedi ovine lentivirus (2009)	Visna/Maedi ovine lentivirus	Sheep (Llewyn sheep breed	Sheep were primed with particle-mediated epidermal bombardment (PMED) using plasmids containing the VMV gag and env genes.	Enhanced early cellular immune response.	(De Andres, Reina et al. 2009)
In silico discovery of epitopes of gag and env proteins for the development of a multi-epitope vaccine candidate	Maedi-Visna	Sheep	Retrieved the DNA sequence for gag and env gene of MVV KV1514 isolate from NCBI, and a multi-epitope vaccine was designed based on the 19 epitopes.	Gag protein was detected to be more conserved and a strong affinity was shown between the final multi-epitope vaccine and TLR-2/4.	(Koçkaya, Can et al. 2023)

The table depicts the various types of vaccine candidates that have been reported over the years against MVV and CAEV infections. The type of vaccine candidate is shown in the first column, whereas the targeted viral agent and specie are mentioned in the second and third column. Furthermore, the parameters chosen for the specific study and the outcomes are depicted in the fourth and fifth column respectively. Moreover, the reference articles of the study are mentioned in the sixth column.

Protein Sequence Retrieval

The protein sequences (amino acid sequences) of the envelope gene of the prevalent SRLV serotypes namely A1(NCBI Accession No. M60609), A1(NCBI Accession No. OL436272), A2(NCBI Accession No. AY101611), B1(NCBI Accession No.436260) and B2(NCBI Accession No. OL436262) were retrieved from the protein server of the National Center of Biotechnology Information (NCBI) (for details see Table 2) in the FASTA format and were subjected to multiple sequence alignment aided by Clustal Omega (Sievers and Higgins 2014). The conserved regions were further analyzed using JalView: a tool commonly used to analyze the multiple sequence alignment results and the assigned conservation scores were used to generate a consensus sequence that served as a representative of the SRLVs envelope gene sequence (Martin, Procter et al. 2020). The consensus sequence was also verified with the BioEdit software to ensure the accuracy of the conservation analysis (Hall, Biosciences et al. 2011). A flow chart describing the methodology of the study can be seen in the Supplementary Figure 1.

Table 2. Reference Isolates for the Generation of In-silico Based Vaccine Candidate Against MV and CAE.

Serotype	NCBI Accession Number	Region	Gene Type	Accession Links
A1	M60609	Iceland	Envelope	https://www.ncbi.nlm.nih.gov/nucleotide/M60609
A1	OL436272	Poland	Envelope	https://www.ncbi.nlm.nih.gov/nucleotide/OL436272
A2	AY101611	USA	Envelope	https://www.ncbi.nlm.nih.gov/nucleotide/AY101611
B1	OL436260	Poland	Envelope	https://www.ncbi.nlm.nih.gov/nucleotide/OL436260
B2	OL436262	Poland	Envelope	https://www.ncbi.nlm.nih.gov/nucleotide/OL436262

This first column shows the serotype of the selected isolates whereas its accession number is given in the second column. The third column contains the information about the region and followed by its targeted gene type in fourth column. All the information is retrieved through NCBI for which the link is provided in the last column.

BCL Epitope Prediction

Since antibodies play a crucial role in stimulating the specific immunity, identifying the BCL epitopes is an essential part of designing a reserve vaccinology-based vaccine. BCL epitopes are generally classified into two types: conformational and linear. Various publicly accessible tools including the ABCPRED; predicts all the possible BCL epitopes on the basis of respective antigenicity scores (Saha, Raghava et al. 2006), BCEPRED; predicts the flexibility of the predicted BCL epitopes (Saha and Raghava 2004) and BEPIPRED; predicts the possible linear epitopes, were utilized for linear BCL epitopes prediction (Jespersen, Peters et al. 2017). Furthermore, the antigenicity of the selected epitopes was again validated by using VaxiJen 2.0 by keeping default parameters and a threshold value of >0.4 (Doytchinova and Flower 2007). All the peptide sequences that had lower antigenicity scores were discarded. Determination of the allergenicity and toxicity of the short-listed BCL epitopes was done by using AllerTOP: used to determine the allergen BCL epitopes (Dimitrov, Bangov et al. 2014) and ToxinPred: used for the determination of toxicity for the predicted BCL epitopes respectively (Sharma, Naorem et al. 2022). Both of the above tools were used with default parameters in order to find the potential linear BCL epitopes which have the ability to generate a potential and a robust immune response.

CTL and HTL Based Epitopes Prediction

Prediction of CD8+T Cell Epitopes

A comprehensive computational tool-based approach was employed to predict the potential CD8+ T cell epitopes, hence a combination of ProPred-I (Singh and Raghava 2001), EpiJen (Doytchinova, Guan et al. 2006), IEDB MHC-I Binding Prediction tools based on the NetCpan EL 4.1 updated versions was utilized. IEDB MHC-I Binding Prediction is based on the machine learning approaches and has been updated to NNAlign_MA to encompass the multiallelic dataset (Trolle, Metushi et al. 2015). The available alleles for BoLA on IEDB database (see Table 4) were utilized to match them with the query SRLV envelope sequence. Based on percentile score and other parameters, the predicted epitopes were further validated by AllerTOP, VaxiJen 2.0 and ToxinPred respectively to select those CTL epitopes that were antigenic, immunogenic, non-toxic and non-allergenic.

Prediction of CD4+T Cell Epitopes

The HTL epitopes were predicted by utilizing the publicly available NetMHCIIpan 2.1 tool which has been efficiently trained by making use of the artificial neural networks (ANN) and includes the Bovine leukocytes alleles of the MHC-II (Yang, Wei et al. 2024). The predicted epitopes were selected on the basis of their binding affinities (IC50: less than 50nm), percentile rank (<0.5) and prediction scores (>0.9) respectively. Furthermore, the NET-CTL 1.2 based tools like VaxiJen 2.0 and EpiJen were used to check the antigenicity of the predicted epitopes. Whereas the commonly used tools like ToxinPred and AllerTOP were used to determine the toxicity and allergenicity of the predicted epitopes.

Prediction of Discontinuous Epitopes

Discontinuous epitopes are of significant importance as they help us to predict the antigen residues that might go to interact strongly with the antibody of the host organism. Hence, ElliPro webserver was used to make the discontinuous epitopes predictions using default parameters (Ponomarenko, Bui et al. 2008).

Design of the SRLV Based Vaccine

All the selected BCL (ten), CTL (four) and HTL (four) epitopes were fused together to design the SRLV based vaccine candidate. All these epitopes were highly conserved, highly antigenic, non-toxic and non-allergenic. At the N-terminal end of the construct, a TLR2 agonist adjuvant namely Pam3CSK4 was used to enhance the immune response and it was connected with the first CTL epitope by using the "EAAAK" linker that provided flexibility between the adjuvant and the multiepitopic regions. Further, CTL epitopes were linked together via the "AAY" linkers whereas they were joined further to the HTL epitopes using the "EAAAK" linker molecule. Special linker sequences such as the "GPGPG" were used for linking all the HTL epitopes, they were further joined with the BCL epitopes that were linked together by the "GPGPG" linkers. Furthermore, the "EAAAK" linker was used to connect the rest of vaccine construct with the Histidine 6x-Tag at the N-terminal of the vaccine construct to assist the purification/recovery of the vaccine construct after expression.

Physiochemical Properties

The physiochemical properties of the generated vaccine candidate were determined by using the Expasy ProtParam online server and as a result various properties of the vaccine construct such as molecular weight, molecular formula, isoelectric point (PI), instability index (II), estimated half-life, number of atoms and Grand average of hydropathicity (GRAVY) were calculated (Gasteiger, Hoogland et al. 2005). Furthermore, the solubility of the vaccine candidate was also evaluated using SolPro to ensure its viability inside the living cell (Magnan, Randall et al. 2009).

Secondary Structure Prediction

The secondary structure of the vaccine construct was predicted by using the SOPMA online server with default parameters and the percentage of different structural parameters such as alpha helix (H), beta sheets (E), random coils (C) and beta-turns was estimated (Geourjon and Deleage 1995). Additionally, the protein structure of the vaccine was also analyzed on a neural network tool PSIPRED to reveal different type of chain elements and types of amino acid (McGuffin, Bryson et al. 2000).

Tertiary Structure Prediction

The tertiary structure of the vaccine construct was predicted by using the widely utilized protein prediction server called I-TASSER (Zhang 2008). It mainly relies on the multiple threading approach

to predict the 3-D structures of the proteins by using the iterative assembly tools. The linear sequence of the vaccine construct was uploaded on the I-TASSER server to get the tertiary structure of the protein. All the predicted models were analyzed by using the ProCheck server (Laskowski, MacArthur et al. 1993). Based on the Ramachandran plots and other outcomes, the best model was chosen and further refinement of the chosen model was performed using the Galaxy Refine server (Heo, Park et al. 2013). The refined models were again analyzed using the ProCheck server, and the best model was chosen for further studies on the basis of residues in the favored region, clash scores, Mol Probiy and Root Mean Square Deviation (RMSD) of atoms. In addition, the overall quality of the chosen model was also verified with a protein structure analysis ProSa webserver (Wiederstein and Sippl 2007).

Molecular Docking Analysis of the SRLV Based Vaccine Construct with TLR-2 and TLR-4 Receptors

The interactions between the potential SRLV based vaccine construct and the most common transmembrane pattern recognition receptors such as TLR-2 and TLR-4 were predicted at the protein-protein level by using the ClusPro webserver (Kozakov, Hall et al. 2017). ClusPro works around three algorithms involving combination of rigid-body docking, energy-based scoring and clustering. It employs the use of FFT based technique; a modified PIPER algorithm, exploring billions of protein interacting/docking orientations based on the energy calculations of Van der Waals, electrostatic and desolvation forces. Post-docking, the server then ranks them based on multiple scoring schemes be it balanced, electrostatic favored or hydrophobic favored followed by grouping the similar ones based on their iRMSD (interface Root Mean Square Deviation). The largest cluster score indicates the most probable docked site. For this purpose, the structures of the human TLR-2 complex (PDB ID:6NIG) and TLR-4 complex (PDB ID: 4G8A) were retrieved from the Protein Data Bank and their extracellular domains were utilized to check their possible interactions with the vaccine construct.

The output of ClusPro webserver was analyzed and the best models showing the interaction between extracellular domains of the ligands and receptor molecule were studied further based on their highest cluster scores and lowest energy values. Large cluster scores indicate the appearance of many similar confirmations, hence increasing the confidence to choose it as the best docked pose and lowest energy values suggests a strong and a favorable interaction between the two proteins. Hence, together a higher cluster score and low energy confirmation works best together and the best docking complexes for each ligand molecule were selected.

Furthermore, energy and stability linked properties of the docked complexes were analyzed by using PRODIGY (Xue, Rodrigues et al. 2016). PRODIGY gives a valuable output regarding the docked models binding affinity (delta G) and interfacial properties like interface area, interfacial contacts and interacting residues along with the dissociation constant (K_d). Furthermore, PDBePISA was used to evaluate if our docked model is biologically meaningful. It also identified the interfaces between chains and evaluates interaction energies (Krissinel and Henrick 2007). The chosen docked complex structures were also visualized manually by using Chimera (Pettersen, Goddard et al. 2004) and PyMol (Seeliger and de Groot 2010). Furthermore, the properties of the complex such as the number of chains, no. of bonds, interacting residues etc. were also identified by confirmed by PDBSum (Laskowski, Jabłońska et al. 2018).

Molecular Dynamic Simulations

After completing the docking studies, the vaccine construct containing the TLRs was subjected to additional examination for stability within the binding pocket. The Schrodinger Maestro software package was utilized to perform a 100 ns molecular dynamics simulation of the complexes (Kosloff and Kosloff 1983). The vaccine construct containing the TLRs complex was prepared using the Protein Preparation tool available in the SCHRODINGER suite. The OPLS4 force field and TIP3P water model were utilized under constant temperature and pressure conditions, with a periodic boundary condition implemented via the System Builder tool. Equilibrium simulations were conducted at a temperature of 300 K and a pressure of 1 bar, utilizing 100 ns for both NVT and NPT ensembles. The

molecular dynamics simulations were performed under NPT conditions for a period of 100 ns to analyse the complex’s RMSD, RMSF, Rg, and SASA.

Immune Simulation Studies

The natural immune stimulating potential of the generated vaccine construct was tested using the C-ImmSim server: a machine learning based tool that visualizes the host humoral immune system against the administered vaccine construct (Rapin, Lund et al. 2011). LPS-free injection with a random seed of 12.643 was chosen whereas the simulation volume was kept at 50. Furthermore, the number of simulation steps were kept at 1000 with intervals of 1, 84 and 168.

Codon Optimization and In-Silico Cloning

Efficient expression of the vaccine construct in a suitable prokaryotic organism such as E. coli is essential to ensure the large-scale production of the protein product. To ensure this, a combination of tools was utilized. First, the codon optimization was carried out using the JCat Server (Grote, Hiller et al. 2005) which ensured the best expression of the construct along with the prediction of the GC content percentage and CAI scores. To facilitate the proper insertion of the linear construct into the cloning vector, restriction sites were added for *XhoI* and *BamHI* restriction enzymes at the N and C terminals of the vaccine sequence respectively. The construct was later cloned into the pET28a (+) cloning vector by using the SnapGene tool (<https://www.snapgene.com/>).

3. Results

Sequence Retrieval

The amino acid sequences coding for the envelope protein of the selected SRLV isolates responsible for the incidence of MVV and CAEV were successfully retrieved from NCBI and multiple sequence alignment was done to visualize the conserved residues by JalView and BioEdit software. As a result, a conserved consensus sequence based on the envelope gene sequence of the most prevalent SRLV isolates was generated.

Linear BCL Epitopes Prediction

Linear BCL epitopes were successfully predicted through the use of highly interactive and authentic servers such as ABCPRED, BCEPRED, BEPIPRED respectively. Furthermore, VaxiJen 2.0 was utilized for the prediction of immunogenicity of epitopes whereas their allergenicity and toxicity was validated via AllerTop and ToxinPred respectively. As a result, ten BCL epitopes (15-mer) were selected. Furthermore, the antigenicity scores of the selected epitopes ranged 0.51 to 1.26 whereas the toxicity predicting SVM scores ranged -0.57 to -1.40. The outcomes of the linear BCL epitopes prediction are listed in the Table 3.

Table 3. List of selected BCL Epitopes for the Generation of In-silico Based Vaccine Candidate Against MV and CAE.

Sequence	Sequence Length	Score	AllerTop	VexiJen	ToxinPred	SVM Score	Molecular Weight
YMEAQGENRRSRRNLQ	16	0.80	Non-Allergen	Antigen (0.6151)	Non-Toxin	-1.04	1880.28
RVKAKYSCESNLGGLD	16	0.77	Non-Allergen	Antigen (1.1465)	Non-Toxin	-0.59	1625.09
DLLGTERGEEKQKQVNV	16	0.89	Non-Allergen	Antigen (0.7215)	Non-Toxin	-1.40	1716.11

TWKSMEPPLRETWGQV	16	0.80	Non-Allergen	Antigen (0.5101)	Non-Toxin	-0.80	1846.31
QQEQEKLLTEGKQKSW	16	0.79	Non-Allergen	Antigen (0.5243)	Non-Toxin	-1.29	1744.20
SKNRPSRVTWKSMEPP	16	0.70	Non-Allergen	Antigen (0.8433)	Non-Toxin	-0.99	1803.27
EGKQKSWVSIDLLGTE	16	0.60	Non-Allergen	Antigen (1.0230)	Non-Toxin	-1.01	1661.12
NESSKWTCAPRGKKGK	16	0.89	Non-Allergen	Antigen (0.9891)	Non-Toxin	-0.57	1649.06
NCSLPHINESSKWTC	16	0.70	Non-Allergen	Antigen (0.5145)	Non-Toxin	-0.57	1719.13
DRTGNGALGNVKNKNC	16	0.82	Non-Allergen	Antigen (1.2684)	Non-Toxin	0.73	1557.91

The table shows the selected BCL epitopes for vaccine development. The first column shows the sequence of the selected BCL epitope sequence whereas the sequence length and scores are listed in the second and third column respectively. Furthermore, the results of allergenicity analysis, antigenicity, toxicity are depicted in column 4-6 respectively. SMV score and molecular weight information is mentioned in the seventh and eighth column.

CTL Epitopes

CTL epitopes play a vital role in any vaccine construct preparation as they elicit cell mediated-immunity. At first, a total of twenty-one epitopes were predicted by the ProPred-I server out of which only four (9-mer) epitopes were filtered out to be further used in the vaccine construct. The selected epitopes had a minimal percentile rank ranging from 0.02 to 0.07, scores ranging from 0.76 to 0.94 and exhibited strong binding affinity (IC50 <50 nM) towards all 4 different BOLA alleles (BoLA-T2c, BoLA-HD6, BoLA-1:02301, BoLA-2:00801) and proved to be non-allergen, non-toxic and immunogenic. The results of the CTL epitope predictions are listed in the Table 4.

Table 4. List of selected CTL Epitopes for the Generation of In-silico Based Vaccine Candidate Against MV and CAE.

Allele	Epitope Sequence	Score	Percentile Rank	AllerTOP	VaxiJen	ToxinPred
BoLA-T2c	SMMHQQMLL	0.933478	0.04	Non-Allergen	Non-Immunogen (100%)	Non-Toxin
BoLA-HD6	RQQEQEKLL	0.945614	0.03	Non-Allergen	Immunogen (100%)	Non-Toxin
BoLA-1:02301	RQQEQEKLL	0.768478	0.07	Non-Allergen	Immunogen (100%)	Non-Toxin
BoLA-2:00801	YIAGGKQFW	0.859677	0.02	Non-Allergen	Immunogen (66%)	Non-Toxin

This first column shows the respective allele used to predict the CTL epitope sequence for the vaccine construct whereas the epitope sequence length, score and percentile length are listed in the second, third and fourth column respectively. Furthermore, the results of allergenicity analysis, antigenicity, toxicity are depicted in column 4-7 respectively.

HTL Epitopes

Being a crucial player in cellular and humoral based immunity in the host, a combination of tools was utilized for the successful prediction of the HTL epitopes. As a result, four HTL epitopes having highest binding affinity (IC50: <50nm), percentile rank (<0.5) and prediction scores (>0.9) were selected. Note that the Net Chop results showed the C-term of 3.0 and a threshold level of 0.5. Furthermore, it was also made sure that the selected epitopes were non-toxic and non-allergenic. The predicted HTL epitopes are listed in the Table 5.

Table 5. List of selected HTL Epitopes for the Generation of In-silico Based Vaccine Candidate Against MV and CAE.

Sequence	Leng th	Allele	Affini ty nM	Percent ile Rank	Bindi ng Level	AllerT OP	VaxiJen	Toxin Pred (SVM, Molecu lar Weight)
VTWKSMEPPLRE TWG	16	BoLA- DRB3*03 01	0.644	30.63	Strong Binder	Non- Allergen	Immuno gen (100%)	Non- Toxin (-0.89, 1817.31)
LWVIQMVLWGC LIWE	16	DRB1*01 02	4.41	7.00	Strong Binder	Non- Allergen	Immuno gen (66%)	Non- Toxin (-0.14, 1889.64)
VLWVIQMVLWG CLIW	16	DRB1*01 04	0.761	10.00	Strong Binder	Non- Allergen	Immuno gen (66%)	Non- Toxin (-0.30, 1859.62)
TWKSMEPPLRET WGQ	16	DRB1*01 20	0.648	29.46	Strong Binder	Non- Allergen	Immuno gen (66%)	Non- Toxin (-0.80, 1846.31)

The predicted HTL epitope sequence for the vaccine construct is shown in the first column whereas the epitope sequence length, respective allele, binding affinity and percentile rank are depicted in

the column and percentile length are listed in the second, third and fourth column respectively. Furthermore, the results of allergenicity analysis, antigenicity, toxicity are depicted in column 4-7 respectively.

Prediction of Discontinuous Epitopes

Discontinuous epitopes were predicted using the ElliPro webserver, the interacting amino acid residues were noted along with their antigenicity scores, the results of the discontinuous epitope prediction are listed in the Supplementary Table 1 and Supplementary Figure 2.

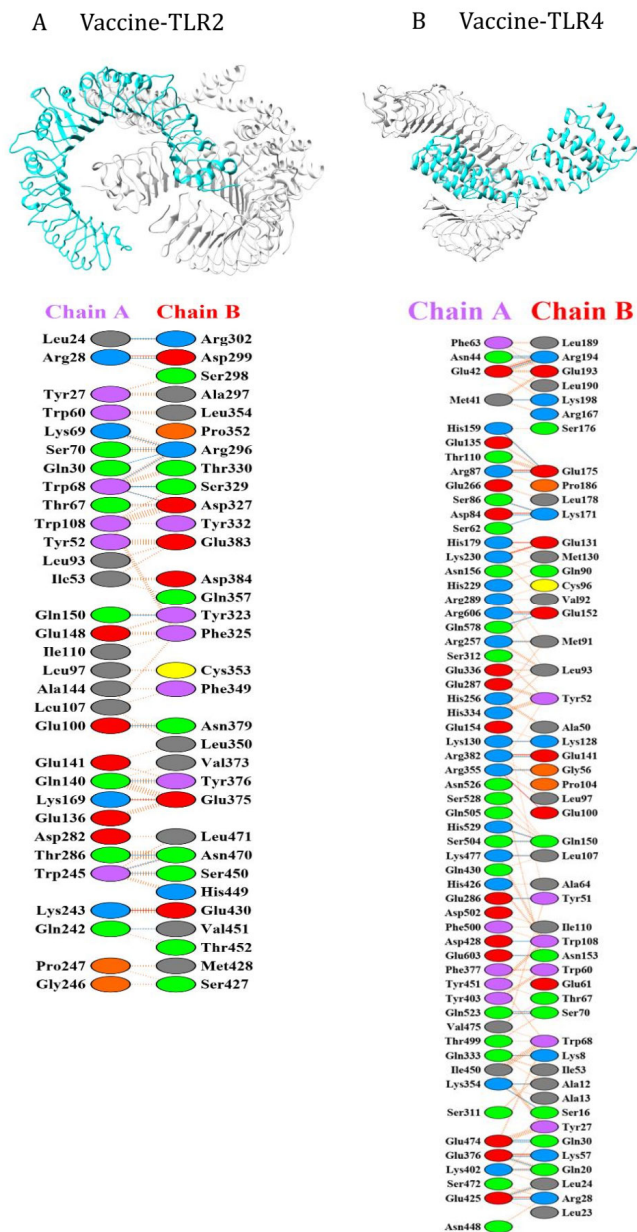


Figure 2. Molecular Docking Analysis for the SRLV Vaccine Construct. A. Illustrates the results of the molecular interaction/docking analysis between the SRLV vaccine construct and the TLR-2 receptor molecule (PDB ID:6NIG). The results were analyzed with PDBSum webserver. Here, the Chain A presents the vaccine construct whereas the Chain B stands for the receptor molecule while the different colored boxes show

interacting amino acid residues in the respective chains. Whereas, the different colored lines show different intermolecular forces such as hydrogen bonds and salt-bridges etc. **B.** Depicts the results of the molecular interaction/docking analysis between the SRLV vaccine construct and the TLR-4 receptor molecule (PDB ID:4G8A). The results were analyzed with PDBSum webserver. Here, the Chain A is for the receptor molecule whereas the Chain B represents the vaccine construct sequence. Different colored boxes show the interacting amino acid residues in the respective chains, whereas the different colored lines show different intermolecular forces such as hydrogen bonds and salt-bridges etc. The results collectively reveal the presence of a stronger molecular interaction between the generated SRLV vaccine construct and the TLR-4 receptor molecule as compared to the TLR-2 receptor.

Epitopes Selection Criteria

All the selected candidate epitopes possessed three desirable attributes: non-toxic, non-allergen and highly antigenic/immunogenic as confirmed by the results of the above bioinformatics tools such as ToxinPred, AllerTop and VexiJen. The tools utilized in the current study are mentioned in the Table 6.

Table 6. List of tools used in the study.

Tool Used	Link
Clustal Omega	https://www.ebi.ac.uk/jdispatcher/msa/clustalo
JalView	https://www.jalview.org/
BioEdit	https://bioedit.software.informer.com/7.2/
ABCPRED	https://webs.iitd.edu.in/raghava/abcpred/
BCEPRED	https://webs.iitd.edu.in/raghava/bcepred/bcepred_submission.html
BEPIPRED	https://services.healthtech.dtu.dk/services/BepiPred-2.0/
VAXIJEN 2.0	https://www.ddgpharmfac.net/vaxijen/VaxiJen/VaxiJen.html
AllerTOP	https://www.ddg-0pharmfac.net/allertop_test/
ToxinPred	https://webs.iitd.edu.in/raghava/toxinpred/
ProPred-I	http://crdd.osdd.net/raghava/propred1/
EpiJen	https://www.ddg-pharmfac.net/epijen/
ElliPro	http://tools.iedb.org/ellipro/
IEDB MHC-I Binding Prediction tools	http://tools.iedb.org/mhci/
NetMHCIIpan 2.1 server	https://services.healthtech.dtu.dk/services/NetMHCIIpan-2.1/
Expasy ProtParam	https://web.expasy.org/cgi-bin/protparam/protparam
SolPro	https://scratch.proteomics.ics.uci.edu/
SOPMA	https://npsa.lyon.inserm.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html
PSIPRED	https://bioinf.cs.ucl.ac.uk/psipred/
I-TASSER	https://zhanggroup.org/I-TASSER/
ProCheck server	https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/
Galaxy Refine server	https://bio.tools/galaxyrefine
ProSa webserver	https://prosa.services.came.sbg.ac.at/prosa.php
ClusPro	https://cluspro.bu.edu/
Protein Data Bank	https://www.rcsb.org/

PRODIGY	https://rascar.science.uu.nl/prodigy/
PDBePISA	https://www.ebi.ac.uk/pdbe/pisa/
PDBSum	https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/
Schrodinger software package	https://www.schrodinger.com/platform/products/maestro/
C-ImmSim server	https://wwwold.iac.rm.cnr.it/

The table describes the tools utilized in the current study. The first column depicts the name of the tool whereas the respective link is mentioned in the second column.

Design of the SRLV Based Vaccine

A multi-epitope vaccine construction involved the assembly of ten BCL epitopes linked together by GPGPG linkers. In the same manner four CTL and four HTL epitopes were linked by using the AAY and GPGPG linkers respectively. The adjuvant was kept at the N-terminal of the construct and it was joined with the CTL epitopes (MHC-I) molecules by using the EAAAK linkers, they were further connected with the HTL (MHC-II) epitopes (via the EAAAK linkers). Further, the HTL epitopes were linked with BCL epitopes by using the EAAAK linker. The C-terminal end of the vaccine construct had 6x His-Tag molecules to enable identification and purification of expressed portion of vaccine. The histidine tag was joined to the BCL epitopes by using the EAAA linker. As a result, a final construct with an amino acid length of 361 was generated. The one-code amino acid sequence and overall geometry of the final vaccine construct is shown in the Figure 1.

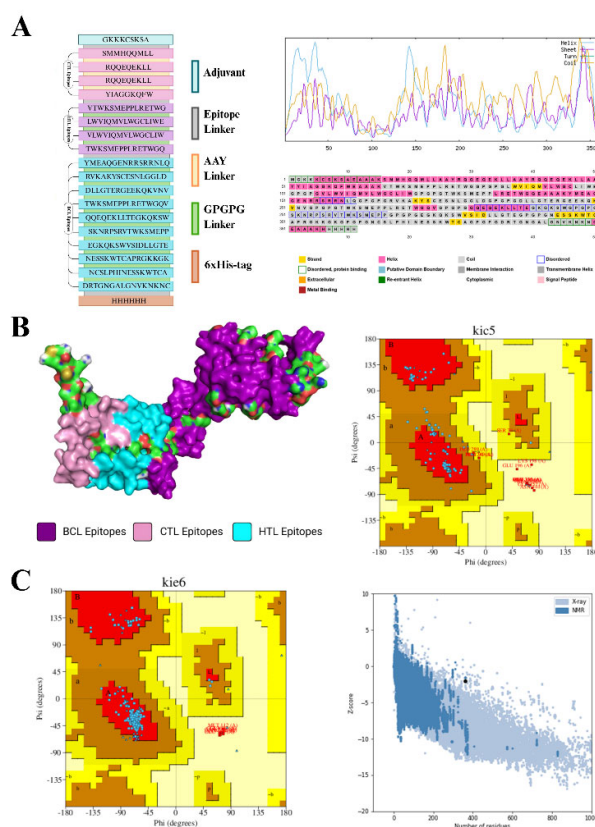


Figure 1. Schematic Diagram Illustrating the SRLV Based Vaccine Designing Strategy. **A.** Illustrates the geometry of the generated vaccine construct by joining adjuvant, BCL, CTL and HTL together with the help of linker molecules. All the components of the construct are represented in different colors. Furthermore, the results of SOPMA secondary structure analysis show the presence of different elements such as helix, turns and coils in the generated vaccine construct. Whereas the other elements of the structure are highlighted with different colored boxes. **B.** The tertiary structure of the protein construct is shown in different colors (highlighting different epitopes) when generated using the I-TASSER protein prediction server. The visualization of the prediction structure was aided by Chimera and PyMol. Furthermore, the quality of the predicted model was analyzed through the generation of the Ramachandran plot for the predicted model via the ProCheck webserver. **C.** Shows the Ramachandran plot generated via ProCheck server for the selected protein model (refined with Galaxy Refine tool) showing >90% of the residues in the allowed regions. The quality of the predicted model was also analyzed via the ProSa webserver via seeking help of the predicted Z-scores for each amino acid residue in the vaccine construct.

Physiochemical Properties

The physiochemical properties of the vaccine construct via ProtParam revealed that there are 5426 atoms in the vaccine construct with molecular formula $C_{1723}H_{2679}N_{505}O_{500}S_{19}$. The extinction coefficient for the construct measured in water is $108440\text{ M}^{-1}\text{ cm}^{-1}$. The estimated half life for the vaccine construct is 30 hours when measured in mammalian reticulocytes (in-vitro). The instability index (II) of 35.15 indicated that the protein construct is stable, further validated by the aliphatic index (53.02) and GRAVY (-0.840). SolPro analysis also showed that the generated vaccine candidate is soluble with a score of 0.978 upon overexpression. However, further details of the physiochemical properties of the vaccine construct are listed in the Table 7.

Table 7. Physiochemical Properties of the SRLV based Vaccine Construct.

Physiochemical Property	Result
No. of amino acids	361
Theoretical pI	9.38
Molecular Weight	39077.45
Total number of negatively charged residues (Asp + Glu)	33
Total number of positively charged residues (Arg + Lys)	47
Total no. of atoms	5426
Formula	$C_{1723}H_{2679}N_{505}O_{500}S_{19}$
Extinction coefficients	Ext. coefficient $108440\text{ M}^{-1}\text{ cm}^{-1}$ Abs 0.1% ($\approx 1\text{ g/l}$) 2.775, assuming all pairs of Cys residues form cystines
Estimated half-life	30 hours (mammalian reticulocytes, in vitro) >20 hours (yeast, in vivo) >10 hours (Escherichia coli, in vivo)
Instability Index	35.15 (Stable)
Aliphatic index	53.02

Grand average of hydropathicity -0.840
(GRAVY)

Secondary Structure Prediction

In order to have a complete understanding about the functional and structural properties of our vaccine construct, its secondary structure was predicted using SOPMA that uses homology bases and statistical methods. It revealed that there is an abundance of structure elements such as alpha-helix (26.04%) providing structural stability to the vaccine construct, along with turns, extended strands (18.28%) and loops hence allowing enough room for evolutionary changes and functional binding stuff. The structural composition not only gave insights about the potential antigenic regions rather its stability too. Supplementary Table 2 shows the results of the SOPMA analysis of the vaccine construct that predicted the distribution of the secondary structure. PSIPRED also validated the above fact that there exists different kind of secondary structure elements such as helix, chains and coils, along with hydrophobic, hydrophilic amino acid residues as illustrated in the Figure 1 (for more details see Supplementary Figure 3).

Tertiary Structure Prediction

The tertiary structure of the vaccine construct was predicted by the help of I-TASSER and the best model was chosen on the basis of different considerations, further it showed more than 90% of the residues lie in the favored region when analyzed through the Ramachandran plot. Hence, to further improve or enhance the efficiency/accuracy of the predicted model, the model was refined through the Galaxy Refine server and the model with the finest results (95% residues in the allowed region) was chosen for further studies and was viewed with the help of visualization tools such as Chimera and PyMol. The results of Galaxy Refine modelling are shown in the Supplementary Table 3 and the 3-D structure of the vaccine construct with Ramachandran plot for both unrefined and refined model are illustrated in the Figure 1. Furthermore, the selected model was also validated with the help of ProSa webserver that predicted the overall model quality Z score of -2.0 and the geometry of the 3-D structure was also validated via the ProCheck webserver (See Supplementary Figure 4).

Molecular Docking Analysis of the SRLV Based Vaccine Construct with TLR-2 and TLR-4 Receptors

Understanding the interactions between the proposed vaccine construct and the immune stimulators in the body holds great significance hence molecular docking analysis between the TLR-2 and TLR-4 receptor molecules was done using the ClusPro webserver. ClusPro generated a range of models that were individually visualized to observe the interacting residues between the ligands and the SRLV based vaccine construct. The best model was chosen on the basis of cluster size, energy values, interacting residues, location and type of bonding (Supplementary Table 4 and Supplementary Table 5). The analysis of the docking complex through PDBSum interaction analysis revealed some useful insights highlighting the major interacting residues and bond-types in the docked complex. The docked complex based on vaccine construct and the TLR-2 ligand (6NIG) revealed that there exists a good level of binding as evident by the presence of 16 hydrogen bonds, 3 salt bridges and 205 non-bonding contacts. Whereas, the TLR-4 (4G8A) based docked complex revealed that there are 10 salt bridges, 33 hydrogen bonds along with 340 non-bonded atoms, hence revealing a much stronger interaction between the suggested vaccine construct and the TLR-4 as compared to the TLR-2 molecule as shown in the Figure 2. The same was supported via the PRODIGY outcome which revealed that TLR-4 based ligand molecule has more stronger binding ability with vaccine construct as suggested by binding affinity of -22.5 kcal/mol compared to the -17.4 kcal/mol for the TLR-2 as it had more interfacial residues (30) as compared to 22 and more hydrogen bonds 31 versus 16. The disassociation constant also shows that 4G8A binds 10,000 times more tightly as compared to the 6NIG molecule. The results of the PRODIGY analysis are listed in the Table 8(a) and 8(b) respectively.

Table 8. (a): PRODIGY Analysis for the Docked Vaccine Construct with 6NIG Ligand.

Protein- Protein Complex	ΔG (kcal mol ⁻¹)	K^d (M) at °C	ICs charged- charged	ICs charge- polar	ICs charged- apolar	ICs polar- polar	ICs polar- apolar	ICs apolar- apolar	NIS charged	NIS apolar
6NIG_ Construct	-17.4	1.7e-13	22	21	50	16	33	39	27.14	33.27
The above table shows the outcome of the PRODIGY analysis for the docked vaccine construct for the TLR-2 based ligand 6NIG. Various affinity binding parameters are listed in the respective columns.										

Table 8. (b): PRODIGY Analysis for the Docked Vaccine Construct with 4G8A Ligand.

Protein- Protein Complex	ΔG (kcal mol ⁻¹)	K^d (M) at °C	ICs charged- charged	ICs charged- polar	ICs charged- apolar	ICs polar- polar	ICs polar- apolar	ICs apolar- apolar	NIS charged	NIS apolar
4G8A- Construct	-22.5	3e-17	30	38	96	13	31	20	23.69	37.85
The above table shows the outcome of the PRODIGY analysis for the docked vaccine construct for the TLR-4 based ligand 4G8A. Various affinity binding parameters are listed in the respective columns.										

MD Simulations

A molecular dynamics simulation was performed for 100 ns to analyze the dynamic motion and stability of the developed vaccine complex. The assessment of the docked complexes stability was conducted through the analysis of RMSD, RMSF, and Rg throughout the simulation duration. The RMSD graph for the TLR2-vaccine complex shows slight variations ranging from 2.76 nm to 5.91 nm, ultimately reaching stability after 40 ns. The TLR4-vaccine complex exhibited commendable stability ranging from 2.68 nm to 14.39 nm, reflecting only a slight variation after 25 ns. RMSF analysis was utilized to assess the flexibility and stability of the complexes. The TLR2-vaccine complex exhibited slight variations, with peak and lowest RMSF values recorded at 13.72 nm and 1.06 nm, respectively. In a similar manner, the TLR4-vaccine complex demonstrated maximum and minimum RMSF values of 3.96 nm and 0.72 nm, respectively. The analysis of Rg also assessed the structural equilibrium and protein compactness over the course of the simulation period. The simulation run revealed an average Rg value of 4.69 nm² for the vaccine-TLR2 complex, whereas the vaccine-TLR4 complex exhibited an average Rg value of 3.21 nm². Additionally, the SASA analysis indicated that vaccine-TLR2 and vaccine-TLR4 yielded average values of 1.13 nm² and 0.37 nm², respectively. The results of the MD simulation are listed in the Figure 3.

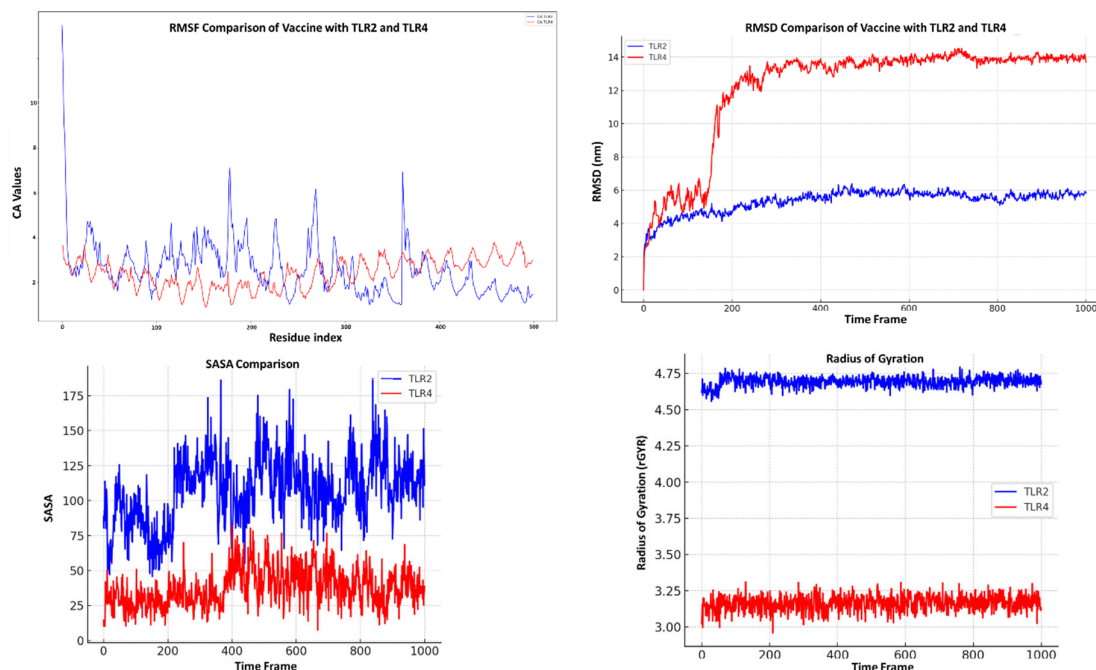


Figure 3. Molecular Dynamic Simulations for the SRLV vaccine construct. The figure shows the outcomes of the molecular dynamic simulations when carried out using the generated vaccine construct and two receptor molecules i.e. TLR-2 (6NIG) and TLR-4 (4G8A) using the Schrodinger Maestro software package. Different parameters such as CA, Radius of Gyration, SASA and RMSD were calculated and compared for both ligands with the construct molecule.

Immune Simulations

Both primary and secondary immune responses were elevated upon the administration of the vaccine candidate in the host body, as evident by a robust immune response as shown in the Figure 4. During the primary or humoral immune response, the IgM was the first antibody to be produced and peaked around 20-30 days, clearing out the antigen in around 50 days however the levels of IgM declined as soon as the response shifted towards secondary immune response components IgG (IgG1 and IgG2) indicating B cell and affinity maturation. IgG1 rose after IgM decline but persisted longer, whereas the IgG2 rose after IgG1 indicating its possible activation by the Th-1 factors indicating pathogen clearance. The persistent high levels of IgG antibodies reveal robust immune memory as shown in the Figure. Furthermore, the immune simulation studies also revealed the robust activation of the B cell as soon the pathogen gets detected in the body, whereas the presenting B cells rise later showing presentation of antigens to T-cells. During the transition phase, duplicating cells declined hence shifting to anergic cells activation to ensure that there occurs no over antibody production/excess immune activation. However, the B cell level persists showing efficient memory cells. Furthermore, it was also evident that there was efficient presentation of B cell epitopes to the T cell as the levels/concentration both the active state cytotoxic and helper T cells elevated gradually until 100 days post-injection (with good memory cells concentration) and the same was noted for both natural killer cells and the dendritic cells. The results of the immune simulations are illustrated in the Figure 4.

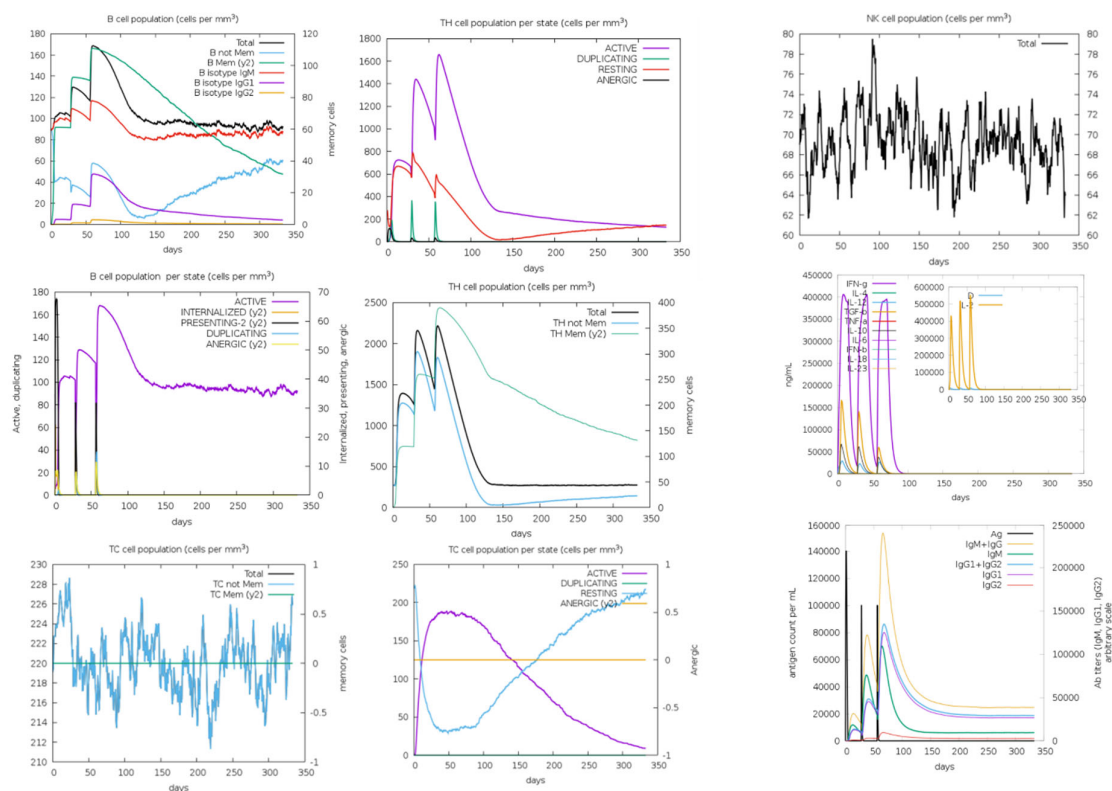


Figure 4. Immune Simulations for the SRLV vaccine construct. The figure shows the outcomes for the immune simulations when carried out using the generated vaccine construct via the online C-Imm Sim server. Different parameters such as B cell population, helper and cytotoxic T cell populations were determined along with concentration of memory cells.

SRLV Vaccine Molecular Cloning and Codon-Optimization

The vaccine construct was codon optimized using the JCat Codon Optimization server and the optimized codon had a length of 361 amino acids. The CAI value was measured at 1.0 whereas the GC percentage of the optimized construct was 54.85. Following that, after the addition of the *XhoI* and *BamHI* restriction enzymes sites at both N and C terminal of the construct respectively, it was successfully cloned into the pET28(a) + cloning vector using the SnapGene tool. The length of the generated vaccine construct-Pet 28a (+) clone was 6430 base pairs. The finally generated clone is shown in the Figure 5.

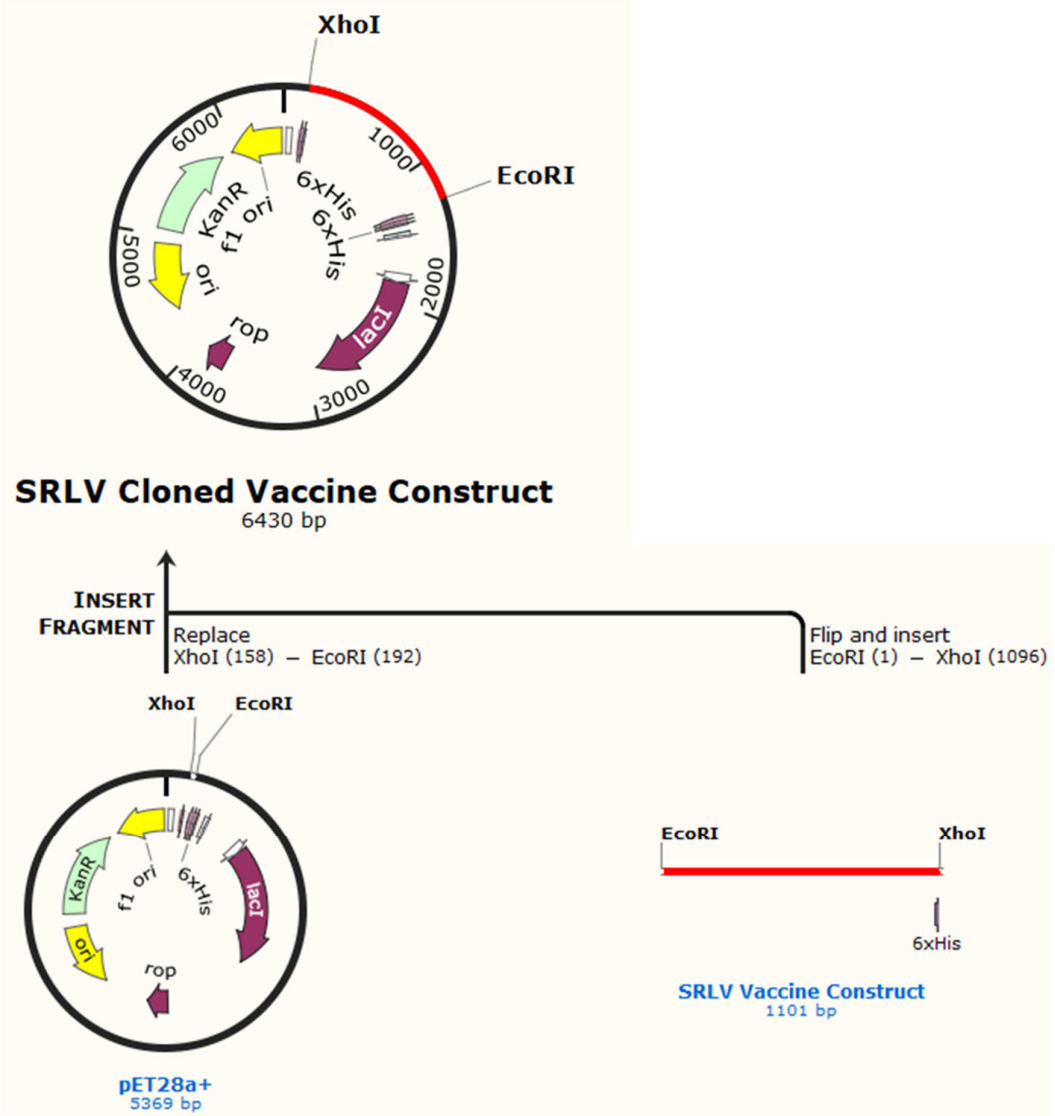


Figure 5. In-Silico Cloning and Codon Optimization for the SRLV vaccine construct. The figure shows the results of in-silico cloning, first the codon was optimized using the JCat Server, later the vaccine construct was cloned into the commercially available E. coli (Pet28a+) vector molecule by using the SNAPGene tool (clone is highlighted in red color). The generated clone had a length of 6430 base pairs.

4. Discussion

MV and CAE caused by SRLVs are among the most prevalent viral infections of livestock animals including goats and sheep. SRLVs possess longer incubation periods leading to identification ineffectiveness. Furthermore, they have a RNA genome that undergoes rapid mutation events hence limiting the efficacy of the available treatments. Vaccination stands out as the most effective treatment against viral diseases as it has the potential to stimulate the host cell's immune response and in turn it helps the body to defend itself against the invading foreign pathogens, however the traditional vaccine designing process is complex, tedious and expensive. Reverse Vaccinology is an advanced vaccine development process that utilizes cutting edge bioinformatics and computational biology tools to screen datasets from different proteomics, genomics, transcriptomics platforms to screen out those antigens that have the ability to stimulate hosts immune response at the cellular level hence making vaccine generation less complex, fast, more specific and an economical process. However, RV validates vaccine development computationally by validating

that the generated vaccine is immunogenic, non-allergenic and non-toxic. However, the validation of these parameters through experiments in wet-lab is essential for confirming the efficacy of the vaccine construct before going into clinical trials and hence the relevant experiments could be conducted in the future studies.

In this study, we have used the RV strategy to formulate a potential multi-epitope vaccine construct that could provide efficient protection against SRLVs in both goats and sheep simultaneously. Hence, the envelope gene involved in the viral host attachment was screened among five most prevalent SRLV isolates to find the conserved regions of envelope gene among different genotypes to ensure broader species protection with enhanced specificity. On the basis of the selected sequences, we first predicted the linear B cell epitopes on the basis of their higher antigenicity scores followed by the prediction of discontinuous B cell epitopes. Furthermore, CTL and HTL epitopes were predicted using specific alleles. It was made sure that all the selected epitopes were antigenic, non-toxic and non-allergenic as these epitopes had the ability to produce robust immune response in the host organisms. The final vaccine construct was generated by joining ten BCL, four CTL, four HTL epitopes linked together via different linker molecules. An adjuvant was added on the C-terminal to elicit sufficient immune response, whereas the 6x-Histidine Tag was added at the N-terminal to have ease during protein purification steps later on. The generated vaccine construct has a length of 361 amino acids and the vaccine showed good solubility while remaining antigenic, non-toxic and non-allergenic. The secondary structure prediction revealed the presence of secondary structure elements such as alpha helix (26.04%), extended strands (18.28%) and random coils (55.68%). Furthermore, the tertiary structure was generated via the I-TASSER and the results were further refined with the GalaxyRefine tool. Best refined model was chosen based on the outcomes of PROCHECK and ProSA server. The molecular docking analysis revealed that the generated vaccine construct had a strong interaction with the targeted TLR-4 receptor molecule (binding energy: -22.5 kcal/mol) as compared to the binding with the TLR-2 receptor (binding energy: -17.4 kcal/mol). This was also validated by the interaction analysis which revealed that there are 31 hydrogen bonds and 30 interacting residues between the TLR-4 receptor and vaccine construct whereas 16 hydrogen bonds and 22 interacting residues were present between TLR-2 receptor and the vaccine construct. The stability of the vaccine complex in the binding pockets and its motion was analyzed via the molecular dynamics simulation studies which revealed that both the TLR-2 and TLR-4 bounded vaccine complexes were stable however there exist more stability and better interactions for the TLR-4 vaccine construct complex as compared to the TLR-2 vaccine construct complex. Additionally, the immune simulating potential of the generated vaccine construct was also validated via the immune simulation studies which showed that the vaccine has the potential to activate the host cell immune system and there was enough concentration of B cell lymphocytes, which in turn activated the other immune cells such as cytotoxic and helper T cells along with natural killer cells. Meanwhile, good concentration levels for memory cells were noted ensuring long lasting immune effect. All this ensured that the vaccine has a potential to provide immunity against the SRLV strains among goats and sheep. Also, to facilitate the protein production in bacterial system, the optimization of codon was done through in-silico cloning via the SNAPGene tool and the optimized codon (361 amino acid) was successfully cloned in the commercially used vector. The length of the generated vaccine construct-Pet 28a (+) clone was 6430 base pairs. However, despite showing potentials of an effective vaccine, the above vaccine candidate must need to go under in-vitro validation experiments to ensure its safety and efficacy for the livestock.

5. Limitations

The current study utilized the highly conserved epitopes however different bioinformatic tools mainly focus on those epitopes that are easily expressed hence many other factors linked with underlying immunity may get ignored. Furthermore, the linkers used in the study may need some further validation as they may lead to the instability of the vaccine construct and pose issues with the safety construct.

6. Conclusions

SRLVs the main responsible agent behind MV and CAE incidence among goats and sheep, leading to loss in milk and meat production globally. A novel vaccine candidate was developed to combat diverse SRLV strains by using bioinformatic and immunoinformatic approaches. The vaccine was generated by combining ten BCL epitopes, four CTL and four HTL epitopes joined to each other by respective linkers. Furthermore, an adjuvant Pam3CSK4 and histidine tag was added to generate a more robust immune response and purified product respectively. The vaccine showed good antigenicity while being non-cytotoxic and non-allergenic. Furthermore, the vaccine showcased stable and high-affinity interaction when docked/simulated with TLR-2 and TLR-4 receptors. From above findings, the generated vaccine serves as a potential candidate against the SRLV caused MV and CEC. However, these findings need further support via in-vitro and animal experiments to further elucidate the safety and the efficacy of the vaccine candidate.

Author Contributions: Muhammad Zeeshan Shabbir (MZS): Research idea, study design, data collection, interpretation, write-up, coordination and data management. Rumesa Ghazanfar (RG): data screening, epitopes selection and interpretation, figure designing, in-silico technical work related to molecular docking. Zainab Nagari (ZN): epitope selection, writeup, figures designing and in-silico technical work related to protein structure determination. Muhammad Atiq Ur Rehman (MAR): study design, write-up, data management and immune simulation studies. Chandra Sourav (CS): Molecular Dynamic Simulation, Writeup.

Acknowledgments: The authors have nothing to report.

Ethics Statement/Consent Information: This study was entirely in-silico based while using the publicly available data and did not involve any animal or human participation. Therefore, ethical approval and consent to participate were not required

Conflicts of Interest: The authors declare no conflicts of interest.

Data Availability Statement: The data that support the findings of this study are available on request.

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