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Designing and Modelling of Trivalent Chimeric Vaccine for Capripoxvirus: Lumpy Skin Disease, Sheep Pox and Goat Pox by Immunoinformatics Approach

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Abstract

Capripoxvirus belongs to poxviridae family and causes three economically important diseases in ruminants namely, lumpy skin disease (LSD) in cattle, sheeppox in sheep, and goatpox in goats. Albeit non-zoonotic in nature, they have potential to cause high economic loss among the farmers. *Capripoxvirus* members share common structural proteins and can rise cross-immunity among them. The present study aimed to design a recombinant chimeric-vaccine from immunogenic proteins of these three members to protect all the host species by using immunoinformatics analysis. The palmitoylated EEV (Extracellular Enveloped Virion) membrane glycoprotein of LSD virus, SPPV-ORF 117 of sheeppox virus, B5R (EEV host range protein) of goatpox virus, and a common protein to all the members, P32, were the major immunodominant proteins used in the present chimeric vaccine construction. Several computational tools were applied to define the most immunogenic regions and different possible adjuvants and universal T-helper agonists were linked to the new construct. The designed vaccine construct was examined for physicochemical properties, immunogenicity, 3D model, Docking analysis and Molecular Dynamics simulations, by using reliable software. After evaluation of the results, the final designed vaccine is expected to have potential in stimulating the humoral response in addition to the cellular responses with acceptable stability.

Introduction

The genus *Capripoxvirus* (CaPV) belongs to the *Poxviridae* family, comprise three important species namely – lumpy skin disease virus (LSDV) causing lumpy skin disease (LSD) in cattle and buffaloes only, sheeppox virus (SPPV), and goatpox virus (GTPV), causing sheep pox (SPP) in sheep and goat pox (GTP) in goats respectively (Tuppurainen et al. 2017). Amongst them, LSD is categorized as a notifiable disease by the Office International des Epizooties (OIE, 2016). It is one of the non-zoonotic and vector-borne diseases, and is economically important disease of the Bovine species, mainly affecting cattle (both *Bos indicus* and *Bos taurus*) and buffalo (*Bubalus bubalis*). Among them, *Bos taurus* is most susceptible in comparison to indigenous breeds (Chervyakova et al. 2016; Gupta et al. 2020; Kumar et al. 2021).

Mechanical transmission is the prime route of disease transmission, mainly through vectors (Sanz-Bernardo et al. 2020). In most of the endemic countries like Egypt, Ethiopia, and Sub-Sahara Africa, with the onset of rainy and summer seasons there is a spike in the disease spread, which is corresponding to the peak activity of the vectors (Abdallah, El Damaty, and Kotb 2018; Carn 1993). This disease is of economic importance in cattle due to many factors that covers fever, suppressed milk production, secondary mastitis, abortion, fertility loss, lameness, severe emaciation, and extensive damage to hides. As a result, this will affect meat, milk, and overall production of the animal. Furthermore, it is associated with high morbidity (3 to 85%) and a mortality rate of 3 to 5% (Abutarbush et al. 2015; Gupta et al. 2020).

Vaccinating the animals, culling of infected and the exposed animals, adopting scientific carcass disposal techniques, regular cleaning, and disinfection of the animal shelters, and proper insect control are the major measures to contain the outbreaks of LSD, Sheep pox, and Goat pox (Tuppurainen et al. 2017). Cross-immunity has been observed among the member species of the genus *Capripoxvirus* (Kitching 1983; Tuppurainen and Oura 2012). According to OIE, 2016, the economic losses due to lumpy skin disease can be reduced by administration of live LSD vaccines in endemic areas. In this regard, four live-attenuated *capripoxvirus* strains have been used as vaccines for the control and prevention of LSD. The homologous live-attenuated strain (Neethling strain) confers immunity that is lasting up to 3 years. However, heterologous live attenuated strain virus vaccine (Sheep and/or goat pox vaccine) may induce local, sometimes severe reactions. Therefore, heterologous live attenuated vaccine is not recommended in goat and sheep pox free regions as they may become a source of infection in the susceptible host species (Gupta et al. 2020). Presently, there are no new generation recombinant vaccines are available for the commercial use against Capripox virus (OIE, 2016, 2019).

There are reports of incomplete protection and adverse reactions after vaccination of live vaccines in cattle in Middle East and the Horn of Africa region. A recent study has confirmed the common Kenyan sheep and goat pox vaccine virus

(KSGP) O-240 to be LSDV, but not SPPV (Tuppurainen et al. 2014) and vaccinated animals may end up in clinical form of the disease if the virus in the vaccine is of partially attenuated. In addition, it's been identified that Kedong and Isiolo goatpox strains, which are capable of infecting goats, cattle, and sheep, for their potential to use them as candidates for broad-spectrum vaccine against all the capripox viral diseases (Kitching 2003; Tuppurainen et al. 2014). Hence, targeting the main immunogenic proteins of all three members of *capripoxvirus* for producing a recombinant and a safe polyvalent vaccine is the prime objective of this article.

The palmitoylated EEV membrane glycoprotein of LSDV is similar to the Vaccinia virus strain Copenhagen F13L protein. This F13L is the most abundant protein among EEV membrane proteins and, have a molecular weight of ~ 37kDa with a palmitoylated component (Grosenbach, Ulaeto, and Hruby 1997; Kushwaha et al. 2019). The gene for F13L is homologous to the B2L gene of Orf Virus and is potentially immunogenic and, it was observed that ORFV B2L can elicit neutralizing antibodies against LSD in mice (Kushwaha et al. 2019; Yogisharadhya et al. 2017; Zhao et al. 2011). Four viral proteins from sheeppox virus -SPPV-ORF 060, SPPV-ORF 095, SPPV-ORF 117, and SPPV-ORF 122, are the orthologs of immunodominant proteins L1, A4, A27, and A33 of Vaccinia virus (VACV), respectively (Chervyakova et al. 2016; Kushwaha et al. 2019).

The B5R protein of the vaccinia virus is reported to induce the neutralizing antibodies in infected animals (Hooper et al. 2000; Hooper, Custer, and Thompson 2003; Law and Smith 2001) and the antisera produced against B5R protein is efficient in neutralizing EEV (Benhnia et al. 2009). The immunogenicity of GTPV homolog of B5R protein has been evaluated along with other immunogenic proteins (Kushwaha et al. 2019; Zheng et al. 2009). Among IMV (intracellular mature virion) proteins, P32, which is a homolog of the VACV H3L, is one of the most immunodominant proteins (~ 35kDa), located on the surface of IMV of all poxvirus members (Kushwaha et al. 2019; Lin et al. 2000). High titers of neutralizing antibodies were reported to be induced by the recombinant H3L protein in immunized mice (Bhanot et al. 2009; Davies et al. 2005). Therefore, the combination of four proteins viz. palmitoylated EEV membrane glycoprotein and P32 of LSDV, SPPV-ORF 117 (EEV glycoprotein) of SPPV, and B5R (EEV host range protein) of GTPV were selected for designing the trivalent chimeric vaccine and its molecular modeling process.

Materials And Methods

Sequence Retrieval

The amino acid sequence of all the four proteins *i.e.* palmitoylated EEV membrane glycoprotein (AOE47604.1) and putative IMV envelope protein/P32 (AOE47650.1) of LSDV, EEV glycoprotein (AVI09752.1) of SPPV, and EEV host range protein/B5R protein (ABS72326.1) from GTV were retrieved from NCBI in FASTA format. (https://www.ncbi.nlm.nih.gov/)

MHC-I Binding Epitope Prediction

A length of 9-mer peptides of the target proteins with bovine MHC-I alleles (6 alleles- BoLA-D18.4, BoLA-JSP.1, BoLA-HD6, BoLA-T2a, BoLA-T2b, BoLA-T2C) were identified by two servers (NetMHC 4.0 and IEDB). The "NetMHC" 4.0 (http://www.cbs.dtu.dk/services/NetMHC/) is an artificial neural network-based server to align the subjected sequences which allow insertions and deletions during the alignment. The threshold level set for strong and weak binders were rank 0.5% and 2%, respectively. The predictions were done for all 6 alleles mentioned above.

Prediction of CTL Epitopes and TCR-Peptide/Peptide-MHC Interfaces

The "CTLPred" (http://www.imtech.res.in/raghava/ctlpred/index.html) server benefits from precise machine learning techniques like support vector machine (SVM) and ANN as single algorithms or in combination. The prediction was done using the consensus method with default sensitivity and specificity.

MHC-II Binding Epitope prediction

The prediction of MHC-II binding epitopes was performed by using four online servers: “NetMHCIIpan” 3.1, “RANKPEP”, “TepiTool”, and “IEDB”. Three bovine MHC alleles [Alleles - BoLA-DRB3 (DRB3_0101, DRB3_0217, DRB3_0303)] were selected for the analysis of peptide binding ability to MHC class II epitopes.

The “NetMHCIIpan 3.1” (http://www.cbs.dtu.dk/services/NetMHCIIpan/) was used with BoLA-DRB3 (DRB3_0303, DRB3_0217, DRB3_0101) bovine alleles and it is a useful method for quantitative prediction of peptide binding to bovine MHC class II molecules. This method is based on the contact between target protein directly with the MHC class II binding cleft which is very crucial to identify CD4+T-cell antigens.

“RANKPEP” (http://imed.med.ucm.es/Tools/rankpep.html) predicts MHC II binding epitopes which are based on their Position Specific Scoring Matrices (PSSMs) ranking for all possible peptides. However, the prediction was done using only one allele-HLA-DR52 (DRB3*02). All other settings are defined as default. All the six alleles of bovine species BoLA-T2a, BoLA-T2b, BoLA-T2C, BoLA-D18.4, BoLA-HD6 and BoLA-JSP.1, were evaluated using the “TepiTool” (http://tools.iedb.org/tepitool/) server. This server applies variety of algorithms to predict MHC II epitopes within the protein sequence. Only14-mer peptide predictions were selected.

“IEDB”(http://tools.iedb.org/mhcii/) server was used find MHC-II binding epitopes with “IEDB recommended 2.22” approach with a length of 15-mer epitopes. Two MHC alleles- H2-IAb and H2-IAd were chosen from mouse origin for prediction.

Linear B-Cell Epitope Prediction

“BCPREDS”, “LBtope” and “ABCpred” were used to predict B-cell epitopes. 20-mer epitopes of each protein were evaluated by “LBtope” (http://crdd.osdd.net/raghava/lbtope/) using the LBtope_Fixed_non_redundant method (non-redundant dataset).

Three approaches- AAP, fixed-length BCPred, or flexible-length method (FBCPred), are offered by the “BCPREDS” (http://ailab.ist.psu.edu/bcpred/predict.html) server and fixed-length “BCPred” was selected for this study.

“ABCpred”(https://webs.iiitd.edu.in/raghava/abcpred/) is the first server tool that is based on the recurrent neural network which uses using fixed-length patterns. Prediction parameters such as a threshold level and peptide length were set to be 0.51 and 16-mers.

Selection of the Epitope Regions

The results from all the above tools were combined to identify the highly overlapping regions with preference for the selection of only those epitopes that can induce multiple immune responses [Th1 (MHC-I), Th2 (MHC-II), CTL, and B-cell responses]. All selected epitopes were then analyzed by various online tools to choose the most appropriate epitopic regions for our vaccine construct.

Engineering the Vaccine and Evaluation of Physicochemical Properties of the Designed Construct

The favorable epitopes from all the four proteins were selected in consonance with the results of all the above-mentioned software and combined them end to end with a combination that yielded maximum antigenicity and low allergenicity, and a TLR agonist adjuvant and two universal T-helper peptides were finally linked to build the final construct.

“Solpro” (http://scratch.proteomics.ics.uci.edu) server was used to determine the solubility of the vaccine peptide in *Escherichia coli* (*E. coli*) and yeast cells. The molecular weight (MW), amino acid composition, aliphatic index, theoretical

pI, instability index, and grand average of hydropathicity (GRAVY) were determined by using the ProtParam tool (http://web.expasy.org/protparam/).

Allergenicity Prediction

Two important servers were being used to define allergenicity; The “AllergenFP” v.1.0 (http://ddg-pharmfac.net/AllergenFP/) works with an accuracy of 88% which recognizes allergens and non-allergens by depending on physicochemical properties of the peptide/protein.

The “Algpred” (http://www.imtech.res.in/raghava/algpred/) was another software used which employs numerous approaches, *viz*., IgEpitope, blast, mast, and SVM, to predict allergenic proteins with higher accuracy.

Antigenicity Prediction

The two reliable servers to estimate the antigenicity of a protein/peptide are “VaxiJen” (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) v2.0 and the “ANTIGENpro”. The ANTIGENpro (http://scratch.proteomics.ics.uci.edu) web-server works by multiple representations of the given protein sequence and giving antigenicity score. VaxiJen v2.0 server was used with threshold=0.4; its accuracy varies between 70 and 89% and is based on the chemical properties of query proteins amino acid sequences to define its antigenicity.

Prediction of Tertiary Structure and Homology Modeling

“Robetta” (https://robetta.bakerlab.org/submit.php) is a reliable protein structure prediction server developed by the Baker lab, University of Washington. This server provides four main options for structure prediction; 1) Deep learning-based method (TrRosetta), 2) Rosetta Comparative Modeling (RosettaCM), 3) Rosetta Ab Initio (RosettaAB), and 4) A fully automated pipeline, that first predicts protein domains as independent folding units and models each domain unit with (2) or (3), and then finally assembles each domain into form full chain models. This server predicts the five most possible models for any given protein sequence and the best can be selected based on the error estimate graph.

Refinement of 3D Modeled Protein Structure

Two programs, “GalaxyRefine” (http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE) and “3Drefine” (http://sysbio.rnet.missouri.edu/3Drefine) were applied to refine the best 3D model obtained from “RosettaAB” server.

The “3Drefine” employs an iterative optimization-based hydrogen bonding network along with minimization of the atomic-level energy on an optimized model using composite physics and the knowledge-based force fields. The “GalaxyRefine” server can refine a protein model through mild and aggressive relaxation methods.

Validation of Refined 3D Models

The validation of all refined 3D models was performed by the “ProSA-web” (https://prosa.services.came.sbg.ac.at/prosa.php) (Protein Structure Analysis), “QMEAN“(https://swissmodel.expasy.org/qmean/) and “ERRAT” (http://services.mbi.ucla.edu/ERRAT).

“ProSA-web” server calculates interaction energy of every protein residue to define if it can achieve certain energy criteria. “ERRAT” uses a method called empirical atom-based approach to substantiate the predicted protein structures by evaluation between the statistics of non-bonded atom-atom interactions in the query protein sequence and the database of high-resolution crystallographic structures. “QMEAN” gives local estimates of predicted model quality which is derived from the QMEAN scoring function as a per-residue plot and as well as global score from the collection of high-resolution PDB structures (Z-score).

Conformational B-Cell Epitope Prediction

The “DiscoTope 2.0” (http://www.cbs.dtu.dk/services/DiscoTope) server was used to identify B-cell epitopes from the predicted 3D structure of the protein. “DiscoTope” applies two approaches to find B-cell epitopes, 1) Contact numbers deduced from surface accessibility of the protein and 2) Novel epitope propensity amino acid score. A selected threshold was −3.7 with the specificity and sensitivity of 0.75 and 0.47, respectively.

Molecular Docking of the Designed Vaccine Construct with TLRs

The 3D structures of TLR molecules were developed by homology modeling using “Robetta” server and horse TLR9 (PDB ID–3wpb), mouse TLR3 (PDB ID –3cig), and human TLR8 (PDB I –6ty5) as templates for bovine, ovine and caprine TLRs respectively.

For protein-protein docking of vaccine and the TLR molecules such as bovine TLR9, ovine TLR3, and caprine TLR8 as the receptors, we used “ClusPro protein-protein docking” server (https://cluspro.org/home.php). It is a molecular docking algorithm that works by rotating the ligand with 70,000 rotations and for each rotation, it translates the ligand in x, y, z with respect to the receptor on a grid and chooses the 1000 rotation/translation combinations which are having the lowest score.

Molecular Dynamics Simulation

The stability assessment of the vaccine-TLR complexes was performed in a simulated biological environment in water box under alternating boundary conditions at 10ns using “VMD” and “NAMD” software. First, the vaccine-bovine TLR complex was edited to generate individual pdb and psf files of corresponding ligand and receptor using CHARMM force field, and later the files were merged in VMD tool(Soteras Gutiérrez et al. 2016; William Humphrey, Andrew Dalke, and Klaus Schulten 1996). The complex was then embedded in a 12Å sized water box. NAMD software was used to simulate the docked complex(Phillips et al. 2020). Initially, 50000 energy minimization steps were performed then the system was subsequently heated from 0 K to 310 K. Further, NPT analysis was performed followed by 10 ns molecular dynamics (MD) simulation analysis with a time step of 2 femtoseconds (fs) using Langevin dynamics algorithm. The same method was followed for the vaccine-caprine TLR complex. Here we used Particle Mesh Ewald (PME) to create the periodic boundary conditions. The trajectory analysis of the simulation and the RMSD analysis was executed using the VMD tool.

Codon Optimization

"Reverse Translate - Bioinformatics" (http://www.bioinformatics.org/sms2/rev_trans.html) server was used for reverse translation of the final vaccine construct. "Optimizer" (http://genomes.urv.es/OPTIMIZER/) was used for Codon optimization and to assess the properties of the optimized DNA sequence in the host like Codon Adaptation Index (CAI), GC content of the sequence, and Codon.

"Reverse Translate" receives a protein sequence as an input and makes use of a standard codon usage table to develop a DNA coding sequence which is most likely non-degenerate coding sequence. The default standard codon usage table was developed using all the *E. coli* coding sequences in GenBank and was retrieved from Codon Usage Database.

Results

Sequence Retrieval and Analysis

All the four protein sequences were obtained from the virus strains that are occurring in India and neighboring countries: LSD- South African NI2490/KSGP strain (KX683219.1), Sheep pox virus- Romanian strain (MG000157.1), and Goat pox virus- Indian strain (MN072620.1).

Results of MHC-I, CTL Epitopes, MHC-II, and B-Cell Epitope Predictions

The summary of the results of NetMHC 4.0, IEDB and CTLPred of MHC-I, NetMHCIIpan 3.1, TepiTool, RANKPEP and IEDB of MHC-II and, LBtope, BCPREDS and ABCpred of B-Cell epitope predictions are represented in **Table1** and **Table S1-3 (Supplementary tables*)**

Selection of Epitope Segments

All predicted epitopes from each protein were compared to determine overlapped regions that could be used to construct the polyvalent vaccine. Results are depicted in **Table 2**. Three highly antigenic epitopes from each of the four proteins were selected and combined to form four separate immunogenic peptides, which were then combined end to end by all possible combinations to get a sequence of high antigenicity with low allergenicity.

Evaluation of the Selected Epitopic Segments

The selected epitopes were subjected for evaluation separately by AllergenFP, Algpred, VaxiJen v2.0, and ANTIGENpro servers for their allergenicity and antigenicity.

The sequence of the final combination of all four protein epitope region after evaluation for its antigenicity and allergenicity is

SLITITIILAFFCIKISIMTSMVSLITMNDWISDYLDGTWGEDGNVLFKEKNQYFDKILQINNVNYNK
KTEYNIGSNVTFFCGNNTRGSCKPGFVLIGTKYSVCGINSSWIFTLSAYVIRLSSAIKIINLHNTKYLS
KKRANWMAHRFPDFSYYVSHPLVSFHECFDEIISQAKKNINIASFCCIEYVKVKIGGDNDPGVLLGGIYSTYAPLALDLQRRFETFKAL.

Vaccine Engineering and its Allergenicity and Antigenicity

After evaluation one combination was selected with a length of 226 amino acids to be used in the vaccine construct. Besides, one TLR agonist adjuvant derived from short TLR4 agonist peptide (RS09) and, two universal T-helper (TpD and PADRE) were linked to the construct. Short linker sequences (GGS) were used to link all the segments together. The combinations were evaluated for their antigenicity, allergenicity, and solubility, and results are summarised in **Table 3.**

The AllergenFP and Algpred predictions showed that the protein construct was not an allergen. The antigenicity prediction was done by the “VaxiJen v2.0” and “ANTIGENpro” servers and showed that this construct was probably an antigen. The final vaccine construct is illustrated in Fig. 1.

Physicochemical Properties of the Vaccine

The physicochemical properties of the final designed vaccine construct were evaluated by “ProtParam” and other servers. Results are summarized in **Table 4**. The construct was stable and have an isoelectric point (pI) of 9.21 and a molecular weight of around 31.7 kDa. The probability of solubility was predicted by “Solpro” and the results revealed that the protein was insoluble upon overexpression.

Tertiary Structure Prediction and Refinement

“Robetta” server suggested 5 models, among which the best model (model 5) was selected based on the error estimate graph (Fig. 2). The obtained 3D structural models were then refined by “GalaxyRefine” and “3D-Refine” servers; each server suggested five refined models.

Validation of the Refined 3D Structures

All refined structures were examined by 3 programs: the “QMEAN”, “ProSA-web” and “ERRAT”. The results of the final vaccine construct are summarized in **Table S4**. Comparison of the resulting models showed that refined model no. 5 by “3DRefine” was the best structure for the vaccine construct. The ERRAT graph for refined model 5 by 3DRefine is shown in Fig. 3. The final refined 3D model is illustrated in Fig. 4a and 4b.

Conformational B-Cell Epitopes Prediction

DiscoTope 2.0 server identified 44 conformational B-cell epitopes within the vaccine construct with a threshold score of −3.70. Results are depicted in **Table S5**.

Molecular docking and dynamics simulation:

The protein-protein docking was conducted using the “ClusPro” server which resulted in 29 interaction models for individual TLR. The best models with weighted scores for the lowest energy were selected and shown in Fig. 5a and 5b.

The complex’s stability was evaluated through the root mean square deviation (RMSD) of the backbone of docked complex versus simulation time (Fig.6a and 6b) and root mean square fluctuation (RMSF) of all side-chain residues of docked complex versus residue number in the sequence (Fig.6c and 6d). RMSD developed plots showed the range of 0.5 to 3.0 Å and 0.15 to 3.4 Å values for the vaccine-bovine TLR and vaccine-caprine TLR complexes respectively which confirmed the stability of docked complex during simulations. RMSD values steadily increased from 0 to 2.5ns in the first simulation and 0 to 4ns in the second one, and thereafter reached a stable state up to 10ns. The average RMSD values of the complex were found to be 3Å in both cases. RMSF of amino acid side chains was noted to assess the stability of the ligand-receptor interaction. RMSF plot fluctuations were minimum for maximum residues, thus indicating uninterrupted interaction between ligands and receptors.

Codon Optimization

Reverse translation of the designed vaccine construct was performed by the "Reverse Translate - Bioinformatics" server and “Optimizer" was used for Codon optimization and evaluation of the properties of the optimized DNA sequence in the host (*E .coli*). The final Codon Adaptation Index (CAI) was 1.00 which is completely ideal for expression in the desired organism. The percentage of GC content was 49.3%; the ideal percentage ranges between 30% and 70%. These results shown that the optimized DNA sequence is appropriate for cloning and expression (Fig. 7).

Discussion

CaPVs causes a highly contagious disease in cattle, goats, and sheep and its endemicity in many countries causes significant economic losses (Chervyakova et al. 2016; Kushwaha et al. 2019). The outbreak of Capripox viral disease can be prevented and eradicated by vaccination, culling of the infected and exposed animals from the herd, proper methods of disposal of carcasses, regular cleaning and hygienic maintenance of animal sheds, disinfection of the animal premises, and proper insect control measures (Tuppurainen et al. 2017). According to OIE, lumpy skin disease in endemic areas can be controlled by administering live LSD vaccines. For instance, the homologous live attenuated strain vaccine (Neethling strain) induces the immunity which lasts for 3 years, whereas heterologous live attenuated virus strain vaccine (Sheep and/or goat pox vaccine) reported to produce local, sometimes severe reactions. Hence, this live vaccine is not advised in nations that are free from goat and sheep pox because the live vaccines can become a source of infection in the susceptible goat and sheep populations (Gupta et al. 2020). There are no new generation recombinant capripox viral vaccines that are commercially available (OIE 2016, 2019).

There are reports of cross-immunity occur among the member species of *Capripoxvirus* (Kitching 1983; Tuppurainen and Oura 2012) and hence, a single vaccine developed against LSD, SPP, and GTP would be an ideal solution and, is

technically and economically feasible (Gupta et al. 2020; Kitching 2003).

Based on all the above facts and considering the safety and immunogenicity, the recombinant subunit vaccine could be the best solution for this problem. Many bioinformatics tools have been benefited the scientific field during the last decades. *i.e.* designing and modeling of epitope vaccine or peptide subunit vaccine for overcoming the difficulties of controlling and preventing the infectious diseases (Dehghani et al. 2017; Dehghani, Hashempour, and Hasanshahi 2020; Negahdaripour et al. 2017). A variety of advanced bioinformatics computational tools have been applied considering various criteria for the construction of peptide vaccine in this study. The major immunogenic regions from the Palmytilated EEV membrane glycoprotein and P32 (putative IMV envelope protein) of LSDV, SPPV-ORF 117 (EEV glycoprotein) of SPPV, and B5R protein (EEV host range protein) of GTPV were selected by combining the most possible immunogenic epitopes based on MHC-I, MHC-II, B-cell, and CTL based epitope prediction servers. To enhance both efficiency and potential of the predicted epitope vaccine, an adjuvant is added to the vaccine construct for improving its overall immunogenicity (Borhani, Bamdad, and Hashempour 2017).

A TLR agonist (RS09), one among proposed adjuvants, was selected due to its proven properties that can be used in the approved vaccines for inducing T-cell proliferation and antibody responses (Borhani et al. 2017; Duthie et al. 2011; Jiang et al. 2016; Kasturi et al. 2011). The RS09 (APPHALS) adjuvant can bind and induce TLR4, TLR7, and TLR9, and stimulate and NF-κB activation (Shanmugam et al. 2012). The other adjuvants that were included in this proposed vaccine construct are peptide - Pan DR Epitope (PADRE) (Rosa et al. 2004) and a universal CD4 memory T-cell recall chimeric peptide (TpD: ILMQYIKANSKFIGIPMGLPQSIALSSMVAQ), as they both able to induce T helper response in the host animal (Fraser et al. 2014). Free rotation must be allowed for the epitopes and adjuvants for enhanced independent recognition by the immune receptors, thus a short flexible linker (GGS) sequence was added between the above two segments.

The results revealed that the proposed new vaccine was non-soluble upon overexpression, stable, immunogen, non-allergen, hydrophobic with pI: 9.2. Yeast is the best host for this protein expression which can support both post-translational modification in protein and high stability. This suggests that the construct could be a novel and economic vaccine candidate as well as appropriate for production, storage, and consumption. The final vaccine sequence was optimized to achieve a higher level of expression in *E. coli*. The CAI and GC content were determined and the results manifested that the optimized sequence had a proper condition to be expressed in *E. coli* host. The expressed product of this trivalent epitope vaccine in the host will be a non-soluble protein that may accumulate as inclusion bodies (IBs).

Even though IBs are interpreted as undesirable by-products in recombinant protein production it is ignored since multiple advantages of IBs have been suggested in the case of bacteria, viz., IBs are nearly pure polypeptides and they act as source of ready-to-use protein. Furthermore, there are many methods are available to reach IBs which are folded and get them as functional protein (Ramon, Señorale, and Marin 2014).

The tertiary structure for the final designed vaccine was constructed by 'Robetta' server; and ProSA, QMEAN, and ERRAT results showed the 3D model was improved remarkably through refinement steps and its final structure was used in the Docking process. Molecular docking analysis showed the lowest energy value between the designed vaccine and different TLR that indicated the great ability of the new vaccine to bind to TLRs and induce the immune responses. Here TLRs were selected based on the involvement in the pathogen, i.e. as TLR9, TLR3, TLR7, and TLR8 are mainly involved in viral infections (Fisher et al. 2011; Werling, Piercy, and Coffey 2006; West, Koblansky, and Ghosh 2006). The Molecular Dynamics Simulation data showed stable RMSD and RMSF values, which indicates the complex is stable in the physiological state.

The present study can provide all necessary data to start experimental research on the novel vaccine construct as the authors employed around 30 online software including “IEDB”, “CTLPred”, “Solpro”, “Robetta”, and “ProtParam” to design and evaluate the novel trivalent chimeric *capripox* vaccine. After careful analysis of diverse adjuvants, the ultimate choices

were added to guarantee the induction of both humoral and the cellular immune response to protect cattle, goat, and sheep against the *capripoxvirus* infection. The suggested vaccine was intensely and meticulously evaluated *in silico* to determine its physical and immunological features for robust activation of both arms of the immune system.

There are several studies conducted using the same strategy to design an efficient multi-epitope vaccine for the prevention of various diseases, such as, against the infection of *Helicobacter pylori*, *Plasmodium falciparum*, Zika virus, and even against prostate cancer (Maharaj et al. 2021; Nezafat et al. 2017; Patra et al. 2020; Shahid et al. 2020). Also, many scientists designed epitope-based subunit vaccines against the present global issue, the Coronavirus Disease-2019 (COVID-19), a pandemic disease caused by SARS Coronavirus-2 (SARS-CoV-2) (Bhattacharya et al. 2020; Rakib et al. 2020; Sarkar et al. 2020).

Conclusion

To the best of our knowledge, this investigation was the first study to design a prophylactic chimeric vaccine against LSD, sheeppox, and goatpox infections, based on reliable epitopic predictions by using several softwares. In this study, three regions with highly immunogenic epitope overlap from each selected protein were used to construct the vaccine and approved adjuvants were added to induce adaptive and innate immunity efficiently to get a better immune response. The designed model was studied for its interaction with TLR molecules, MD simulation, and all other physiochemical properties including allergenicity. Therefore, from the present study it can be concluded that this chimeric trivalent vaccine could be an economic and effective strategy. Further experiments needs to be conducted to ascertain and support the findings of present study.

Abbreviations

ANN - Artificial Neural Network

CAI - Codon Adaptation Index

CaPV - *Capripoxvirus*

COVID-19 - Coronavirus Disease-2019

CTL - Cytotoxic T lymphocytes

EEV - Extracellular Enveloped Virion

GRAVY - Grand Average of Hydropathicity

GTP - Goat Pox

GTPV - Goatpox Virus

IBs - Inclusion Bodies

IMV - Intracellular Mature Virion

kDa - Kilo Daltons

KSGP - Kenyan sheep and goat pox vaccine virus

LSD - Lumpy Skin Disease

LSDV - Lumpy Skin Disease Virus

MD – Molecular Dynamics

MHC-I - Major Histocompatibility Complex class-I

MHC-II - Major Histocompatibility Complex class-II

MW - Molecular Weight

NCBI - National Center for Biotechnology Information

OIE - Office International des Epizooties

PADRE - Pan DR Epitope

PDB - Protein Data Bank

PME - Particle Mesh Ewald

RMSD - Root-Mean-Square Deviation

RMSF - Root Mean Square Fluctuation

SPP - Sheep Pox

SPPV - Sheeppox Virus

SVM - Support Vector Machine

TLR - Toll-Like Receptor

VACV - Vaccinia Virus

Declarations

Data availability statement

The data that supports the findings of this study are available in the manuscript and supplementary material of this article with complete transparency.

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Conflicts of interest

Author Pashupathi M declares that he has no conflict of interest. Author Anbazhagan S declares that he has no conflict of interest. Author Barkathullah N declares that he has no conflict of interest. Author Swagatika Priyadarsini declares that she has no conflict of interest. Author Snehasmita Panda declares that she has no conflict of interest. Author Nikhil K.C declares that he has no conflict of interest. Author Ajay Kumar declares that he has no conflict of interest.

Author Contributions

Pashupathi M - Conceptualization, Methodology, Resources, Software, Validation, Visualization, Writing-original draft; Anbazhagan S and Barkathullah N - Conceptualization, Visualization, Resources, Writing-review & editing; Swagatika Priyadarsini and Snehasmita Panda - Methodology, Resources, Software; Nikhil K.C and Ajay Kumar - Validation, Proof-reading, Resources; All authors reviewed the manuscript.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Tables

Table 1 The epitopic predictions of LSDV: palmitoylated EEV membrane glycoprotein by various servers

MHC I		CTL	MHC II				Linear B-cell epitope		
NetMHCI	IEDB	CTLpred	NetMHCII	IEDB	RANKPEP	TepiTool	Lbtope	ABCpred	Bcpreds
163-171	285-293	174-182	201-215	282-296	191-199	52-65	32-51	106-121	102-121
43-51	210-218	163-171	200-214	284-298	210-218		35-54	354-369	101-120
149-157	43-51	237-245	199-213	283-297	350-358		34-53	331-346	98-117
129-137	237-245	264-272	202-216	285-299	34-42		38-57	249-264	99-118
50-58	149-157	344-352	198-212	281-295	60-68		33-52	104-119	103-122
224-232	328-336	252-260	340-354	258-272	123-131		108-127	55-70	104-123
320-328	285-293	254-262	339-353	257-271	44-52		39-58	52-67	100-119
210-218	163-171	333-341	95-109	141-155	170-178		36-55	159-174	106-125
50-58	335-343	322-330	341-355	256-270			37-56	147-162	97-116
289-297	233-241	335-343	190-204	286-300			178-197	244-259	105-124

Table 2 The final selected epitopes of all four immunogenic proteins

Protein origin	Epitopic regions			Final peptides
LSDV:palmytilated EEV gp	149-171	35-56	102-120	HECFDEIISQAKKNINIASFCCIEYVKVKIGGDNDPG VLLGGIYSTYAPLALDLQRRFETFKAL
SPPV:EEV gp	49-63	60-71	150-175	SLITITIILAFFCIKISIMTSMVSLITMNDWISDYLDGT WGEDGNVLFKEKNQ
GTPV:B5R	177-188	36-58	117-139	YFDKILQINNVNYNKKTEYNIGSNVTFFCGNNTRGSC KPGFVLIGTKYSVCGINSSWI
LSDV: P32	261-280	181-198	236-248	FTLSAYVIRLSSAIKIINLHNTKYLSKKRANWMAHRFP DFSYYVSHPLVSF

Table 3 The results of different software to evaluate combined epitopes

	Algpred		AllergenFP v.1.0		VaxiJen		AntigenPro	Solubility	
Adjuvant linked seq	**IgE**	**MAST**		**Score**		**Score**	**Score**		**Score**
1	N	N	N	0.83	A	0.6265	0.363042	INS	0.800022
2	**N**	**N**	**N**	**0.83**	**A**	**0.627**	**0.356389**	**INS**	**0.800916**
3	N	N	N	0.82	A	0.6328	0.380842	INS	0.811106
Without adjuvant	N	N	N	0.8	A	0.6705	0.233273	INS	0.8528

1- RS09 –linker-PADRE- linker- Epitope – linker – TpD

2- RS09 –linker - Epitope – linker - PADRE- linker – TpD

3- RS09 - linker- Epitope – linker – TpD

And Without adjuvant final epitope.

(N-non allergen, A-antigen, INS-insoluble) (Bold – selected combination)

Table 4 All the physiochemical properties of the vaccine construct estimated by several servers

Physiochemical properties	
Predicted solubility Solpro	Insoluble (0.800916)
Molecular weight	31733.89 Da
No. of amino acids	287
Instability index	32.94/stable
Aliphatic index	96.2
Theoretical pI	9.21
Gravy	0.186
Total no. of negatively charged residues (Asp+Glu)	17
Total no. of positively charged residues (Arg+Lys)	27
The estimated half-life	4.4 hours (mammalian reticulocytes, in vitro), >20 hours (yeast, in vivo), >10 hours (Escherichia coli, in vivo).
Allergenicity	
AllergenFP v.1.0	Non allergen
Algpred	Non allergen
Antigenicity	
ANTIGENpro	Antigen
Vaxijen	Antigen
B cell epitope/DiscoTope	44/287

Figures

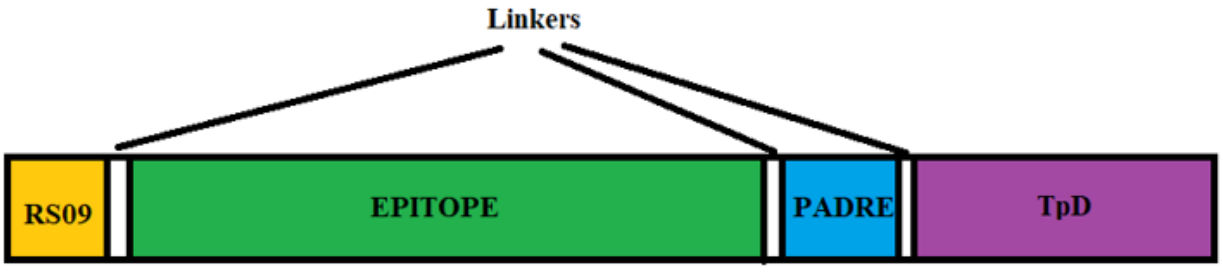

Figure 1

The final structure of the designed vaccine: final epitope and adjuvants: RS09 (TLR4 agonist), PADRE, and TpD, as the universal T-helper agonists. These segments are linked together by short linkers sequences GGS

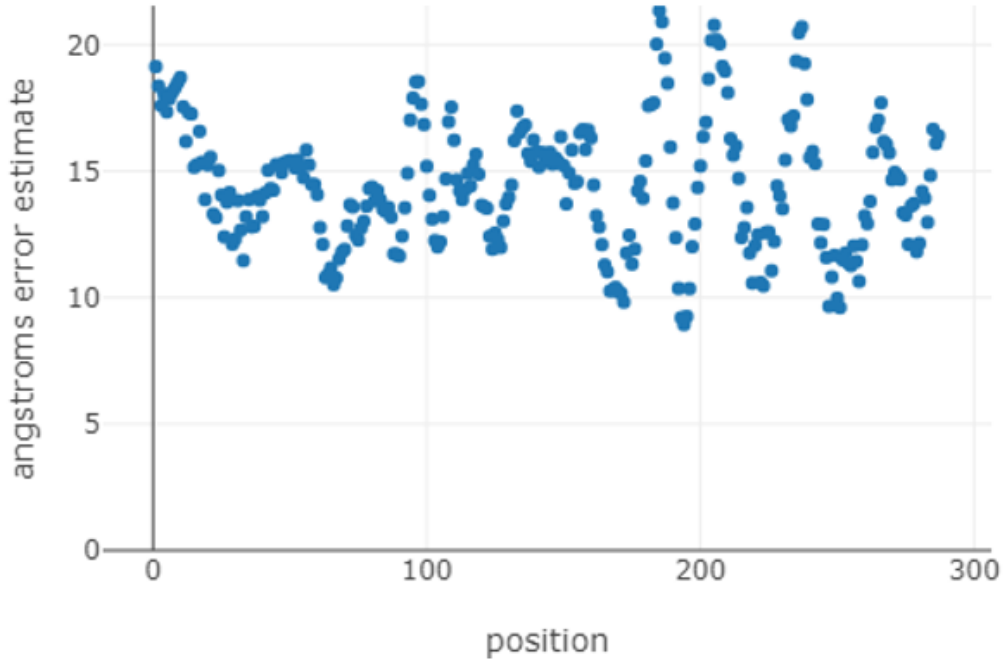

Figure 2

Error estimate graph of the “Model-5” predicted by “Robetta” server. The graph depicts probable errors against each amino acid position of the protein sequence

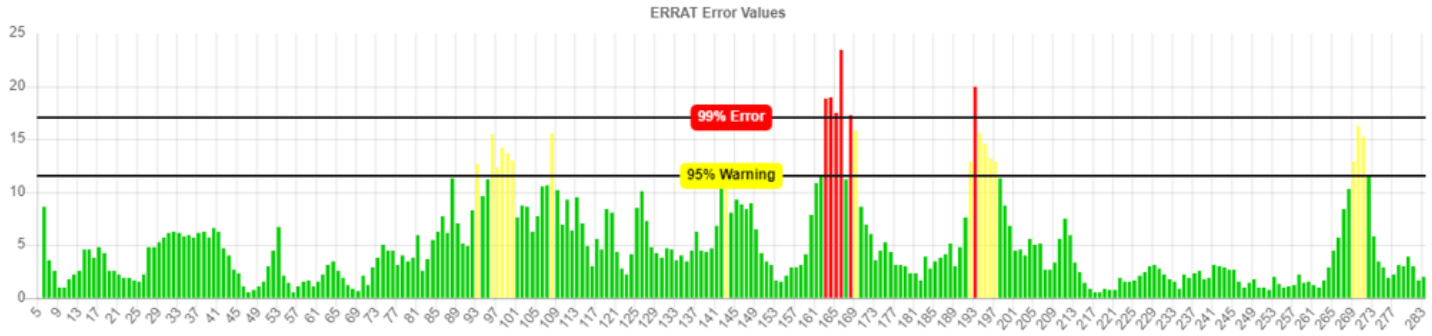

Figure 3

The ERRAT graph representing ‘ERRAT error values’ against each amino acids for refined model 5 by 3DRefine

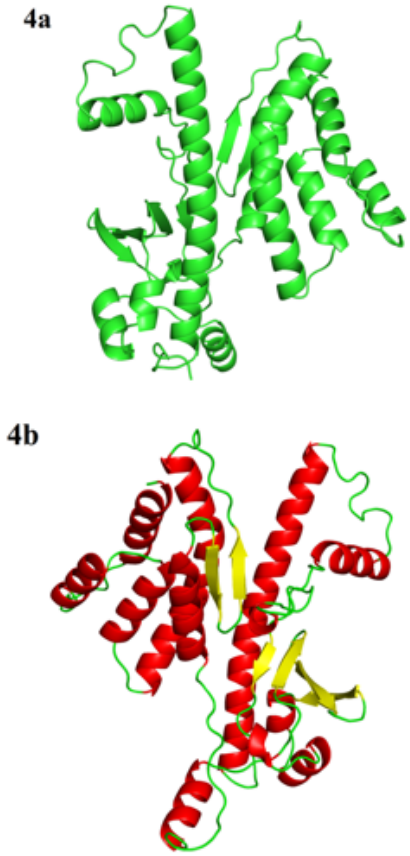

Figure 4

a and b. Final 3D structure of the vaccine predicted by “Robetta” and refined by two servers and finally evaluated by several tools

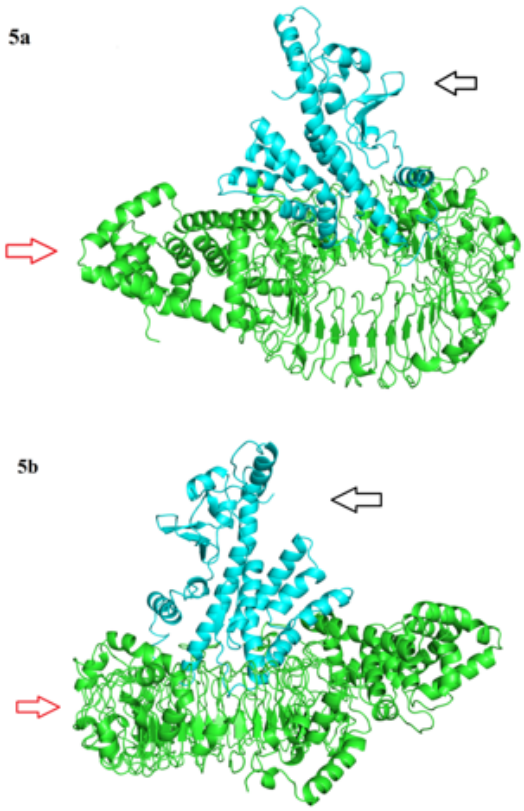

Figure 5

a and b: Molecular docking of the bovine TLR9 protein (red arrow) and the vaccine construct (black arrow)

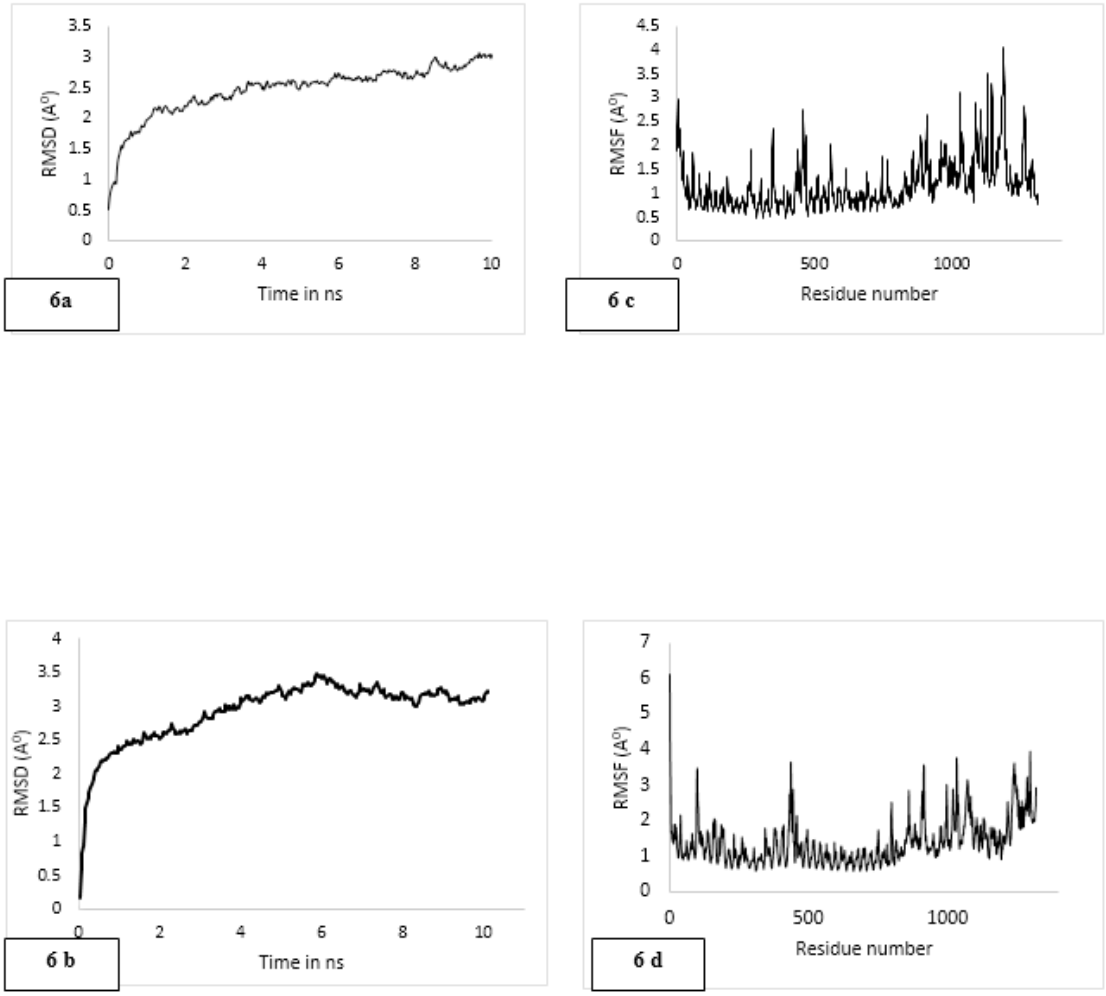

Figure 6

a and b represents RMSD plot and **Fig. c and d** represents RMSF plot for Bovine TLR9 - vaccine complex and Caprine TLR8 - vaccine complex, respectively

Type	Sequences	CAI	ENc	%GC	%AT
Query	ATGGCGCCGCCGCATGCGCTGAGCGGCGGCAGCAGCCTGATTACCATTACCATTATTCTG GCGTTTTTTTGCATTAAAATTAGCATTATGACCAGCATGGTGAGCCTGATTACCATGAAC GATTGGATTAGCGATTATCTGGATGGCACCTGGGGCGAAGATGGCAACGTGCTGTTTAAA GAAAAAAACCAGTATTTTGATAAAATTCTGCAGATTAACAACGTGAACTATAACAAAAAA ACCGAATATAACATTGGCAGCAACGTGACCTTTTTTTTGCGGCAACAACACCCGCGGCAGC TGCAAACCGGGCTTTGTGCTGATTGGCACCAAATATAGCGTGTGCGGCATTAACAGCAGC TGGATTTTTACCCTGAGCGCGTATGTGATTCGCCTGAGCAGCGCGATTAAAATTATTAAC CTGCATAACACCAAATATCTGAGCAAAAAACGCGCGAACTGGATGGCGCATCGCTTTCCG GATTTTAGCTATTATGTGAGCCATCCGCTGGTGAGCTTTCATGAATGCTTTGATGAAATT ATTAGCCAGGCGAAAAAAAACATTAACATTGCGAGCTTTTGCTGCATTGAATATGTGAAA GTGAAAATTGGCGGCGATAACGATCCGGGCGTGCTGCTGGGCGGCATTTATAGCACCTAT GCGCCGCTGGCGCTGGATCTGCAGCGCCGCTTTGAAACCTTTAAAGCGCTGGGCGGCAGC GCGAAATTTGTGGCGGCGTGGACCCTGAAAGCGGCGGCGGGCGGCAGCATTCTGATGCAG TATATTAAAGCGAACAGCAAATTTATTGGCATTCCGATGGGCCTGCCGCAGAGCATTGCG CTGAGCAGCCTGATGGTGGCGCAGTAA	0.776	20	48.6	51.4
Optimized	ATGGCGCCGCCGCACGCGCTGTCTGGTGGTTCTTCTCTGATCACCATCACCATCATCCTG GCGTTCTTCTGCATCAAAATCTCTATCATGACCTCTATGGTTTCTCTGATCACCATGAAC GACTGGATCTCTGACTACCTGGACGGTACCTGGGGTGAAGACGGTAACGTTCTGTTCAAA GAAAAAAACCAGTACTTCGACAAAATCCTGCAGATCAACAACGTTAACTACAACAAAAAA ACCGAATACAACATCGGTTCTAACGTTACCTTCTTCTGCGGTAACAACACCCGTGGTTCT TGCAAACCGGGTTTCGTTCTGATCGGTACCAAATACTCTGTTTGCGGTATCAACTCTTCT TGGATCTTCACCCTGTCTGCGTACGTTATCCGTCTGTCTTCTGCGATCAAAATCATCAAC CTGCACAACACCAAATACCTGTCTAAAAAACGTGCGAACTGGATGGCGCACCGTTTCCCG GACTTCTCTTACTACGTTTCTCACCCGCTGGTTTCTTTCCACGAATGCTTCGACGAAATC ATCTCTCAGGCGAAAAAAAACATCAACATCGCGTCTTTCTGCTGCATCGAATACGTTAAA GTTAAAATCGGTGGTGACAACGACCCGGGTGTTCTGCTGGGTGGTATCTACTCTACCTAC GCGCCGCTGGCGCTGGACCTGCAGCGTCGTTTCGAAACCTTCAAAGCGCTGGGTGGTTCT GCGAAATTCGTTGCGGCGTGGACCCTGAAAGCGGCGGCGGGTGGTTCTATCCTGATGCAG TACATCAAAGCGAACTCTAAATTCATCGGTATCCCGATGGGTCTGCCGCAGTCTATCGCG CTGTCTTCTCTGATGGTTGCGCAGTAA	1.000	22	49.3	50.7

Figure 7

The "**Optimizer**" server results showing two parameters with the codon-optimized sequence of the vaccine. The CAI of >0.8 is considered as good for expression in selected host, CAI of vaccine construct sequence was 1 and the average GC content was 49.3%.(ENc: Effective Number of Codons)

Supplementary Files

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- Graphicalabstract.tif
- SupplementarymaterialServerreferences.pdf
- Supplementarytables.pdf