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A one-health approach to design an mRNA-based vaccine candidate against the lumpy skin disease virus as an alternative to live-attenuated vaccines

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Abstract. – OBJECTIVE: Recently, lumpy skin disease (LSD) has been spread over the Asian, European, and Middle Eastern regions making it a significant hazard to the chain of cattle production, milk production, and human milk consumption, requiring prompt attention. Lumpy skin disease virus has high morbidity and low fatality rates, but its infections have led to terrible economic and agricultural consequences. Although live-attenuated vaccines have been commercialized, farmers in different regions have not taken them well because of the allergic responses against the vaccines. The study aims to develop an mRNA-based vaccine candidate for LSDV, using immunoinformatic approaches to minimize allergenicity and homology while maximizing immunogenic potential.

MATERIALS AND METHODS: The study used extensive immunoinformatic approaches to shortlist five proteins from the LSDV genome that belong to the transmembrane region and are crucial in early viral interaction with host cells. The B-cell and T-cell-specific epitopes were chosen based on non-allergenicity, antigenicity, non-homology, surface accessibility, and lower IC50 inhibition values. The construct's stability, hydrophilicity, and antigenic potential were analyzed using the instability index, Grand Average of Hydropathicity (GRAVY) index, and antigenicity, respectively.

RESULTS: We selected a total of 34 epitopes, consisting of 12 B-cell-specific epitopes and 22 T-cell-specific epitopes. These epitopes were chosen based on their characteristics such as non-allergenicity, antigenicity, non-homology, surface accessibility, and lower IC50 inhibition values. Specifically, 11 epitopes were selected for Major Histocompatibility Complex-I, and another 11 epitopes were chosen for Major Histocompatibility Complex-II. The inclusion of the RS09 adjuvant enhanced the immunogenic potential of the vaccine. The instability index was found to be 38.60. Additionally, the GRAVY index, indicating hydrophilicity, was calculated as -0.151. Furthermore, the antigenicity value of 0.6073 confirmed its potential to elicit an immune response. Further supporting its immunogenic potential, strong immune stimulation was observed, with IgM+IgG titers reaching 6,000 (arbitrary units) and IFNg titers measuring 400,000 ng/mL. These results provide additional evidence of the vaccine's ability to stimulate a robust immune response.

CONCLUSIONS: The study results indicate that the developed mRNA-based vaccine candidate for LSDV has high immunogenic potential and could serve as an effective alternative to live-attenuated vaccines. Further experimental validations are required to test its efficacy. The study also highlights the potential of the One-Health approach to tackle non-zoonotic diseases that have significant consequences for the environment and humanity.

Key Words:
LSDV, Immunoinformatics, RS09-adjuvant, Zoonosis, One-Health, Cattle.

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Introduction

Lumpy skin disease virus (LSDV) is a dsDNA virus from the Capripoxvirus genus, Poxviridae family, and is around 150 kbp in size. Although there have been some speculative linkages to other Bovidae, including Oryx, LSDV has a very limited host range that restricts it to bovines. It spreads through arthropod vectors or direct animal-to-animal contact[1]. Even though significantly higher mortality rates have been seen[2] in some cases, such as in South Asia, where a death rate of up to 15% was found, lumpy skin disease (LSD) mortality rates are often lower than those noted for goat pox chick embryo-attenuated virus (GTPV) and Sheeppox virus (SPPV) belonging to the same family.

The cattle industry incurs significant economic losses due to LSDV, which reduces milk production, weight gain, and reproductive efficacy and causes severe skin lesions that reduce flesh quality[3]. This indicates that the increasing disease is alarming and deserves the attention of One-Health workers. Historically, LSDV has been confined to Africa; however, recent outbreaks in Asia and Europe have raised concerns about its global dissemination. The likelihood that LSD may spread from Africa to Australia and the Asia Pacific is rising due to its widespread worldwide[4]. China and Southeast Asia have been severely affected by the illness since 2019[5]. Furthermore, Vietnam, Thailand, and Malaysia officially confirmed the illness in 2021. It was formally announced by Indonesia, India, Sri Lanka, Pakistan, etc., in March 2022[5].

Viral lumpy skin disease spreads by ticks, animals such as flies and mosquitoes that feed on blood, or other animals that bite prey. There is no evidence of animals contracting the disease directly from another animal, even when in direct contact with sick buffaloes and cattles. However, skin lesions were observed following experimental infection in giant gazelles, goats, giraffes, sheep, and impalas, indicating that the virus may lead to infection or death in animals that have never been previously exposed to it[5]. LSD has a high morbidity but a low fatality rate. Fever, swollen lymph nodes, small nodules on the skin that cause severe anorexia, decreased milk supply, and infertility are the disease's hallmarks[6]. Overall, it impacts the economic worth of animals regarding meat and milk production, how well animals conceal, how strong their limbs are, and how well they reproduce, including if they have abortions and are infertile. Although the disease is native to African nations, it has recently been discovered[7] in uncharted territory.

Efforts to contain the spread of LSDV have proven difficult[4,8]. Vaccination is currently the most effective method of preventing LSDV outbreaks. Ring vaccinations are usually employed with quarantine, travel restrictions, and killing infected animals[9]. Live attenuated vaccines created from field isolates were the only immunizations commercially accessible up to this moment[10]. Nevertheless, the available vaccines have several drawbacks, including adverse effects like the Neeling disease and a limited scope of protection[11]. An adverse response, including local infection at the injection site, has also been recorded[12] after giving certain vaccines. Therefore, it is necessary to develop safe and effective vaccines against LSDV to limit its economic impact and prevent its spread.

The biggest perceived danger to the world's health and industrial livestock production continues to be virus-based infections. Due to vaccinations, two serious viral illnesses, smallpox, and rinderpest, have been eliminated globally in human and veterinary medicine[13]. Effective management methods for the main cattle viruses, such as the virus that causes lumpy skin disease, are lacking. The community spread of LSDV must be stopped, and multi-epitope or mRNA-based vaccinations significantly manage such diseases, as evident in recent studies[14-16]. The mRNA-based vaccines are predicted to be the most potent. Especially the minimal risk of high potency, insertional mutagenesis, quicker development cycles, and the possibility for cost-effective manufacturing of mRNA vaccines are other reasons they show significant promise[2].

Transmembrane proteins play a major part in the pathogenesis of LSDV through their regulation of viral structure and assembly, modulation of the immune system's response, and nucleotide biosynthesis[17]. Understanding the function and immunogenicity of LSDV transmembrane proteins can inform the creation of effective vaccines. Thus, this study utilizes the LSDV's transmembrane proteins as a potential vaccine candidate. These proteins contain pathogen-associated molecular patterns (PAMPs) identified by pathogen recognition receptors (PRRs) on host immune cells like macrophages, neutrophil monocytes, and dendritic cells engaged in

antigen uptake, sorting and presentation to the adaptive immune system for the provision of long-term host resistance[18].

The host range proteins with potential functions in regulation or obfuscation of the host immune system, in instrumentation or suppression of apoptotic host-cell death, and elements of cell and tissue tropism are a potential target, as protective immunity against these proteins will allow the host immune response to resist viral-mediated modulation[19]. The putative extracellular enveloped virus (EEV) host range protein plays a significant role in determining viral pathogenicity and host range[20]. Similarly, the Tyr/Ser protein phosphatase is essential for regulating cell growth, proliferation, differentiation, and transformation[21].

This study utilizes a reverse-vaccinology approach following the study design of Naveed et al[22] to computationally predict an mRNA-based vaccine construct against the lumpy skin disease virus. Reverse vaccinology is an approach that employs bioinformatics to design vaccines based on genomic and proteomic data analysis[23]. Several pathogens, including viruses, bacteria, and parasites, have been effectively treated with this method. The online IEDB resource is utilized here to predict adaptive immune response eliciting epitopes. Linkers and adjuvants used in our previous studies[15,22] were employed, and the construct was subjected to immune stimulation, molecular docking, and Molecular Dynamic (MD) simulation analyses. Allergenicity, toxicity, and antigenicity analyses of the epitopes and the vaccine candidate were performed at every step, ensuring that the construct was not allergenic to the host. If taken to experimentation, this construct will benefit the global and regional economies and the cattle industries worldwide as it is expected to immunize the hosts against LSDV and lumpy skin disease.

Materials and Methods

Protein Selection and Sequence Retrieval

The web server of UniProt, (www.uniprot.org/proteomes/UP000315615) was utilized to select potential candidates' proteins. The whole proteome of the lumpy skin disease virus was retrieved to select pathogenic proteins.

Epitopes Prediction

The IEDB server (tools.iedb.org/main/) was run to predict the epitopes of B-cells and T-cells for designing vaccine construct. The subcategory, termed Linear B-Cells Epitope Prediction Search Tool (http://tools.iedb.org/main/bcell/), was employed for the potential epitopes evaluation as done in the work of Biswas et al[13]. Similarly, for Major Histocompatibility Complex-I epitope prediction, Major Histocompatibility Complex-I Epitope Prediction Tool was utilized (http://tools.iedb.org/mhci/). MHC-II Epitope Prediction Tool (http://tools.iedb.org/mhcii/) was utilized to predict MHC- II epitopes from our selected proteins.

Evaluation of Immuno-Compatibility for the Epitopes

The Vaxijen Tool[24], (available at http://www.ddg-pharmfac.net/vaxijen), was run to predict the antigenic potential of the viral proteins. Similarly, a bioinformatics-based approach tool called AllerTop (available at: https://www.ddg-pharmfac.net/AllerTOP/) was run to check the allergenic capacity[25] and ToxinPred[26] (available at: http://crdd.osdd.net/raghava/toxinpred/) to evaluate the toxic epitopes. These tools assist in the design of an effective vaccine against the lumpy skin disease virus. The selection of epitopes was primarily based on antigenic, non-toxic, and non-allergenic analyses.

Host Homology

All the selected epitopes were compared to human and cattle genomes to evaluate their homology with the BLASTp web server (https://blast.ncbi.nlm.nih.gov/Blast.cgi McGinnis & Madden, 2004[27]) All epitopes with an E value greater than 0.05 were possible non-homologous peptides further used in the vaccine construction. The selection of non-homologous peptides was based on the fact that the host immune response will develop tolerance to its homologous peptides.

The Final Construct

The mRNA-based sequence of the candidate vaccine was predicted with the shortlisted epitopes against lumpy skin disease virus using the linkers glutamic acid-Alanine-Alanine-Alanine-Lysine (EAAAK), Glycine-Proline-Glycine-Proline-Glycine (GPGPG), Lysine-Lysine (KK), Alanine-Alanine-Tyrosine (AAY), and adjuvant RS09. The linkers and adjuvants helped in the alignment of the epitopes as well as inducing a strong immune response in the host cattle.

mRNA Vaccine Secondary Structure Prediction

The vaccine candidate's secondary structure was determined with the RNA fold tool (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) of Vienna RNA Package 2.0 (https://www.tbi.univie.ac.at/RNA/ Mathews et al[28]). The RNA fold program uses McCaskill's approach to calculate secondary structure. The minimum free energy (MFE) was also calculated using this approach.

Physiochemical Properties

To check whether the vaccine construct was antigenic and non-allergenic, the Vaxijen Tool and the Allertop Tool were run. For antigenicity evaluation, the threshold was adjusted to 0.5. The tool used to predict the physiochemical properties was Protparam (https://web.expasy.org/protparam/[29]). It determined the vaccine construct's solubility, stability, and hydrophilicity indices. Furthermore, the ProSA web tool (https://prosa.services.came.sbg.ac.at/prosa.phpp[30]) determined that the structure consisted of the construct.

Tertiary Structure Prediction and Evaluation

It was employed to check the structural stability of the vaccine design. For this purpose, two online tools, PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) and ROBETTA (https://robetta.bakerlab.org/) have been utilized to analyze the secondary and tertiary structure of the proteins, respectively. The predicted structural analysis of the proteins was validated using the Ramachandran Plot (https://saves.mbi.ucla.edu/[31,32]). The plot predicted that the vaccine structure was stable and had efficient torsional angles with maximum amino acids in the plot's allowed region.

Immune Simulation Interpretation

The web server C-IMMSIM[33-34] (available at https://kraken.iac.rm.cnr.it/C-IMMSIM/index.php?page=1) was run to interpret the immuno-compatibility details of the vaccine construct. Vaccines are typically administered in two to three doses for four weeks; hence we used three injections. The remaining settings were kept as such.

Molecular Docking Analysis

The construct's molecular interaction and immune stimulation potential were predicted through molecular docking. The ClusPro server (https://cluspro.bu.edu/[32]) was utilized to dock the construct with the bovine (*Bos taurus*) TLR-3 (UniProt ID: Q5TJ59) and TLR-4 (UniProt ID: Q9GL65), retrieved from UniProt (https://www.uniprot.org/[35]).

Molecular Dynamics Simulation

The next step was the docked complex validation through M.D. simulation analysis. It predicted the stability and mobility of the docked complex to validate the interaction for further studies. The server, iMODs (https://imods.iqfr.csic.es/ López-Blanco et al[36]), was accessed to predict the molecular dynamics interpretation of the docked complex.

Expression of the Construct

To successfully produce the construct in the *E. coli* cells, codon optimization was carried out. It was accomplished with the help of the Jcat Optimization tool (available at http://www.jcat.de/Result.jsp). It measures the efficiency with which mRNA expression translation is carried out[37] using the Codon Adaptation Index (CAI). The Guanine-Cytosine (GC). percentage of the final DNA sequence after optimization was also calculated. The final construct was then cloned in the modified expression vector, pBluescribe (NovoPro, Shanghai, China) using the same restriction sites in the construct and the vector. The expression analysis was performed with the Snapgene offline software (downloaded from https://www.snapgene.com/). The vector was chosen because of its utilization in a previous study by Namazi and Tafti[38] to clone the lumpy skin disease virus. Figure 1 illustrates the methodology utilized.

Results

Proteins Selection

Lumpy skin disease viral proteome was analyzed to narrow the potential vaccine candidates. A total of 156 proteins make up the lumpy skin disease virus proteome. They were screened using a variety of techniques. In the first phase, the subcellular localization of the proteome was screened using the CELLO service, which placed 83.3% of the proteome in the plasma membrane and 1% in the nucleus. Out of this 83.3%, the proteins highlighted in Table I were finalized based on their location and antigenicity profiles.

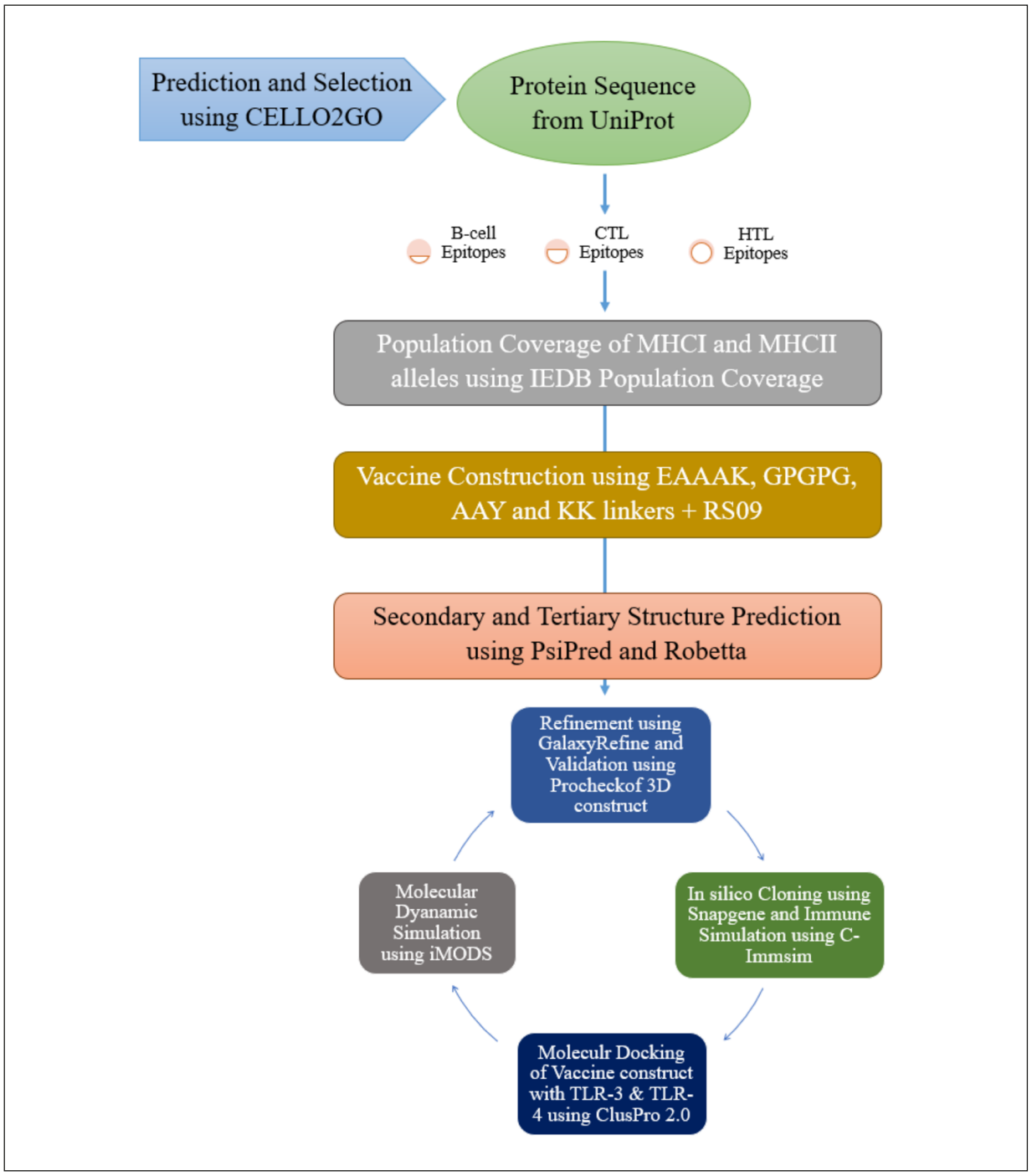

Figure 1. An overview of the process.

B-cell Epitopes Estimation

From six proteins, we chose twelve epitopes predicted by the Bepipred 2.0 algorithm of the linear epitopes tool in the IEDB database, as illustrated in Table II. The findings of the epitopes with non-toxic, antigenic, non-allergenic, and non-homologous (to eliminate vaccine design that may introduce tolerance) were finalized.

T-cell Epitopes Estimation

We finalized eleven epitopes of cytotoxic T lymphocyte (CTL) from the IEDB's MHC-I data-

Table I. Finalized protein candidates for the vaccine construct.

No.	Accession No.	Protein	Localization
1	A0A1C9HHB5	Tyr/Ser protein phosphatase	Nucleus/Plasma membrane
2	Q91T22	Putative viral membrane protein	Plasma membrane
3	A0A1C9HHG6	Putative DNA helicase transcriptional elongation factor	Plasma membrane
4	A0A1C9HHM7	G-protein coupled chemokine-like receptor	Plasma membrane
5	A0A1C9HHJ1	Putative EEV host range protein	Plasma membrane
6	Q9QCQ1	Major envelope protein	Plasma membrane

Table II. List of B-cell-specific epitope candidates.

Rank	Peptide	Antigenicity	Allergenicity
1	GWMVQKADKIDVSAQQ	0.93	Non-allergen
2	APTKMMRVTDYVYLGN	0.86	Non-allergen
3	KTVICLPNKMLASQWK	0.92	Non-allergen
4	ESVMSMYYEIDYKLYS	0.9	Non-allergen
5	TRLIKEPRTEINSLMP	0.96	Non-allergen
6	PSTYENTTTISNYTTA	0.86	Non-allergen
7	VHSTRANGEPRTEINS	0.91	Non-allergen
8	EYNIGSNVTFFCGNNT	0.88	Non-allergen
9	KVKIGGDNDPGVLLGS	0.93	Non-allergen
10	LDLQRRFETFKALNNN	0.87	Non-allergen
11	GAMIMAYLMSKRSKDI	0.9	Non-allergen
12	YFLYIYHSMREKRGAF	0.75	Non-allergen

base, listed in **Supplementary Table I.** As with the B-cell epitopes, the selection was finalized according to the antigenic, allergenic, non-toxic, and non-homologous findings. Eleven epitopes were selected for the helper T-lymphocyte (HTL) specificity (MHC-II-restricted epitopes) based on the same approach with the capacity to induce interleukins 4, 10, and interferon-gamma, as illustrated in **Supplementary Table II**.

Vaccine Construct

The vaccine construct shown in Figure 2A included linkers (EAAAK, AAY, K.K., and GPGPG) to link the epitopes. EAAAK linked the epitopes with the adjuvant, GPGPG was used as an inter-B-cell-epitopes linker, K.K. was used as an inter-MHCI-epitope-linker, and AAY was used to link the MHC-II restricted epitopes together. The RS09 adjuvant was used to increase the im-

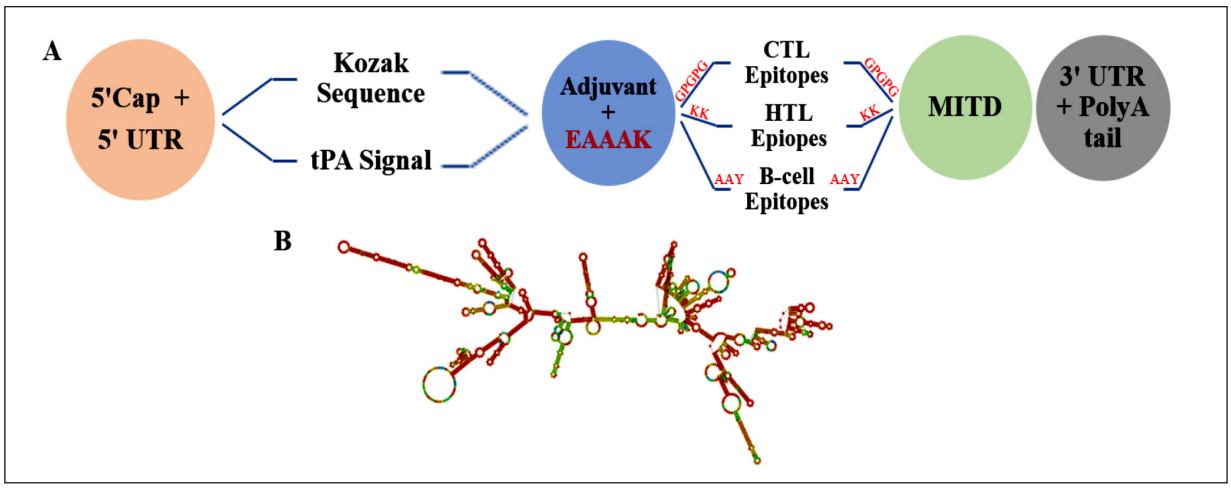

Figure 2. A, The proposed vaccine construct with the linkers in red. **B**, The mRNA secondary structure predicted with the RNA fold server.

munogenicity of the vaccine. A MITD sequence (a human immune receptor agonist), 5′ m7G cap sequences, a poly-A tail, the 5’ and 3’ UTRs, and a stop codon were employed to improve the stable translation potential of the vaccine.

mRNA Vaccine Secondary Structure Evaluation

The structure of the mRNA vaccine was inferred using the RNA fold web service and is depicted in Figure 2B. The structural free energies were also calculated on this site. As per the findings, the free energy of the mRNA vaccine was -779.51 kcal/mol, and the energy of the centroid structure was -706.22 kcal/mol. A stable mRNA structure was demonstrated in these results.

Evaluation of Physicochemical Properties

Per independent predictions, the vaccine was found to be antigenic, non-allergenic, non-toxic, and soluble. Moreover, the ExPasy ProtParam service was used to determine the physiochemical profile of the construct, illustrated in Table III. All the physiochemical properties of the vaccine construct predicted that the construct is thermally stable. The Grand Average of Hydropathicity (GRAVY) was found to be -0.419, indicating that the vaccine is hydrophilic.

Secondary and Tertiary Structures of the Vaccine Candidate

According to PSIPred, the secondary structure consists of alpha helices, as shown in Figures 3A-B. The tertiary structure of the construct is shown in Figure 3C. The stereochemical correctness of the structure verified using PROCHECK (European Bioinformatics Institute Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, United Kingdom) is depicted in the Ramachandran plot in Figure 3E. It demonstrates that 87.8% of residues were in the most preferred zones, 10% in the additionally allowed zone, and 1% in the generously allowed zones. The ProSA-web (Scheiblingkirchen, Niederösterreich, Austria) server anticipated a negative Z-score of -9.06 for the tertiary protein model, suggesting that it is consistent, as shown in Figure 3D.

Immune Simulation Response

The immune stimulation elucidated that the second and third responses outperformed the first. Immunoglobulin levels (indicated in Figure 4B) were high after antigen suppression, and IgM was found to be created in greater quantities than IgG. This rise indicated that immunological memory has developed due to antigen exposure. B-cell isotypes over an extended period demonstrate memory formation in the B-cell population. Furthermore, memory development also increased in CTL and HTL cells. Additionally, there was an increase in macrophage activity, although dendritic cell activity remained constant. The IFN- γ, and IL-2 levels also showed an escalation, as shown in Figure 4A.

Molecular Docking Analysis

ClusPro provided 15 models for docking vaccine construct and TLR-3 and ten models with TLR4. The first models (shown in **Supplementary Figure 1**) were selected for TLR-3 and TLR-4 docking, respectively. The energy for the TLR-3:vaccine docked complex (shown in **Supplementary Figure 1A**) was -1,338.9 kcal/mol, while the energy for the TLR-4:vaccine docked complex (shown in **Supplementary Figure 1B**)

Table III. Physicochemical properties, antigenicity, and allergenicity of the vaccine candidate.

Property	Measurement	Indication
Total Number of Amino Acids	597	Appropriate
Molecular Weight	65,981.30 kDa	Appropriate
Formula	C3012H4736N792O813S29	-
Theoretical pI	10.04	Basic
Total number of positively charged residues (Arg + Lys)	34	-
Total number of negatively charged residues (Asp + Glu)	88	-
Total Number of Atoms	9382	-
Instability index (II)	38.60	Stable
Aliphatic Index	77.32	Thermostable
Grand Average of Hydropathicity (GRAVY)	-0.151	Hydrophilic
Antigenicity	0.6073	Antigenic
Allergenicity	Non-allergenic	Non-allergenic
Toxicity	Non-toxic	Non-toxic

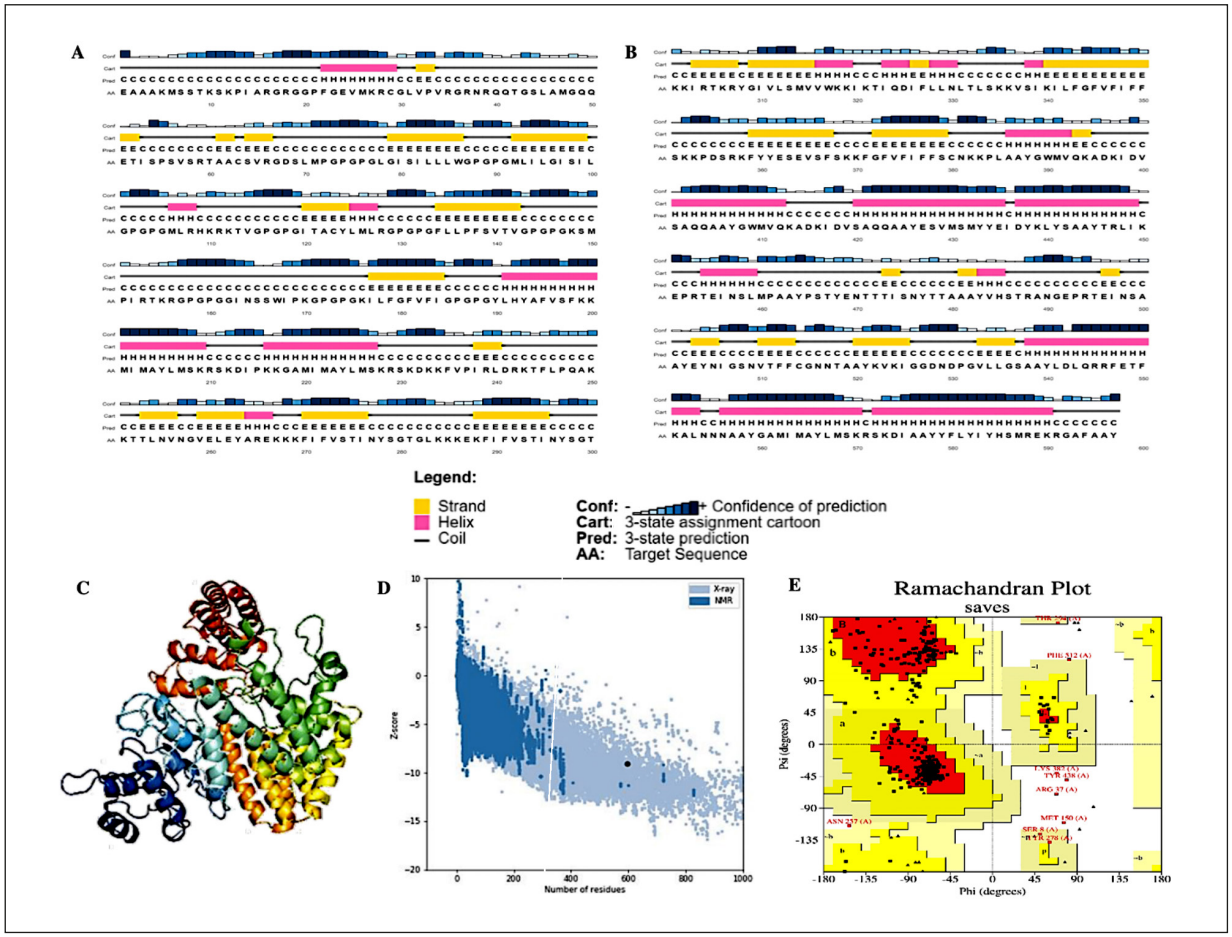

Figure 3. Secondary structure prediction and validation of the vaccine construct. **A-B**, Cartoon structure illustrated using Psipred. **C**, Tertiary structure predicted by ROBETTA. **D**, Z-score prediction using ProSA web. **E**, Ramachandran plot validating the secondary structure of the construct. The capital A, B, and L letters represent right-handed alpha-helices, beta-sheets, and left-handed alpha-helices, respectively, with significant confidence, whereas the small letters represent the same structures with lesser confidence.

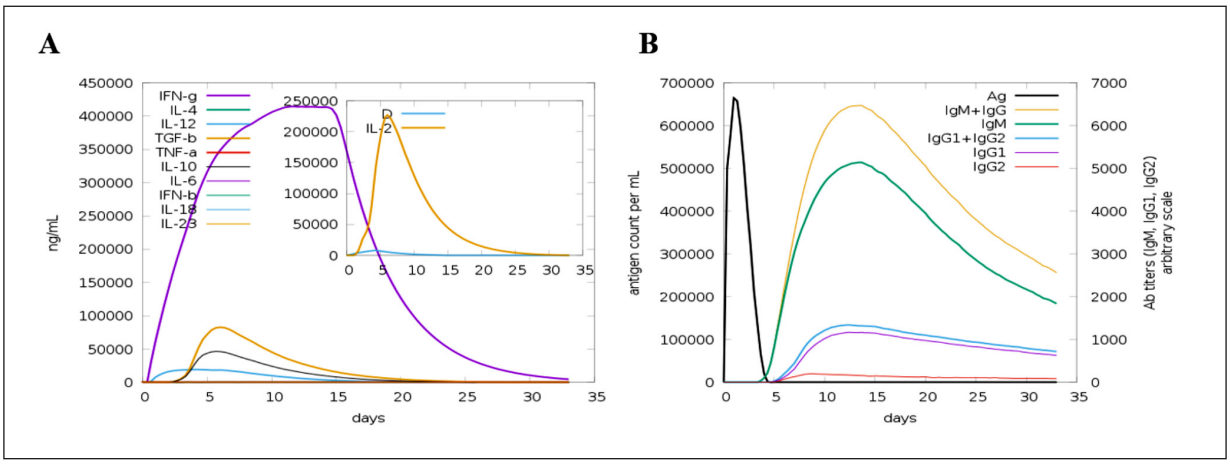

Figure 4. Immune response stimulated against vaccine construct using C-ImmSim server. **A**, The stimulation of interferons, interleukins, and tumor necrosis factors. **B**, The immunoglobulin production after antigen injection.

was -1,246.5 kcal/mol. These energy values indicate a substantial molecular interaction between the respective receptors and the vaccine.

Molecular Dynamics Simulation

The results are summarized in Figure 5. Peaks in both complexes' deformability graphs (Figures 5B and 5F) represented the deformable loci and indicated the amino acids with coiled forms. Normal mode analysis (NMA) is a computer method for analyzing the flexibility of the protein. The B-factor calculated for both the complexes (Figures 5A and 5E) showed that the thermal motion of the complex is relatively higher at the last amino acids, consistent with the deformability graphs. The covariance matrix depicted the relationships among amino acid duplets in the dynamical area (Figures 5C and 5G). The red part indicates the correlated residues, the white part shows anti-correlated residues and the blue part represents the non-correlated residues. Lastly, the elastic network map (Figures 5D and 5H) demonstrated the stiffness of each interacting atom in terms of dark and light grey dots. The dark grey dots indicated stiffer atomic interactions corresponding to lower deformability capacity.

Expression of the Construct

The mRNA vaccine construct had a length of 2,151 nucleotides. The ideal proportion of G.C. content should be 30-70% for successful vaccine expression inside the eukaryotic cell. This construct's average G.C. content percentage was 64.44%, with a CAI value of 0.95. **Supplementary Figure 2A** depicts the optimized construct. Successful cloning in the pBluescribe vector is shown in **Supplementary Figure 2B**. The HincII (Hinc from the organism *Haemophilus influenzae* and II denoting that it was the second restriction enzyme to be discovered from this species) restriction sites were used to linearize the construct and the vector producing identical cuts and simplifying final ligation. The map is shown in **Supplementary Figure 2C**.

Discussion

The lumpy skin disease virus (LSDV) is a highly host-specific virus that causes lumpy skin disease (LSD) in cattle and water buffalo. The disease is endemic in most Sub-Saharan African countries and can spread quickly across borders, causing significant economic losses. LSD has recently been proven[1] to be communicable to humans, and diagnosis is typically made through conventional or real-time PCR methods. The LSD has recently spread over the Asian, European, and Middle Eastern regions making it a significant hazard to the chain of cattle production that demands prompt and adequate responses[39]. There are no commercially available preventative solutions other than live attenuated vaccines, which have not proven effective. According to a

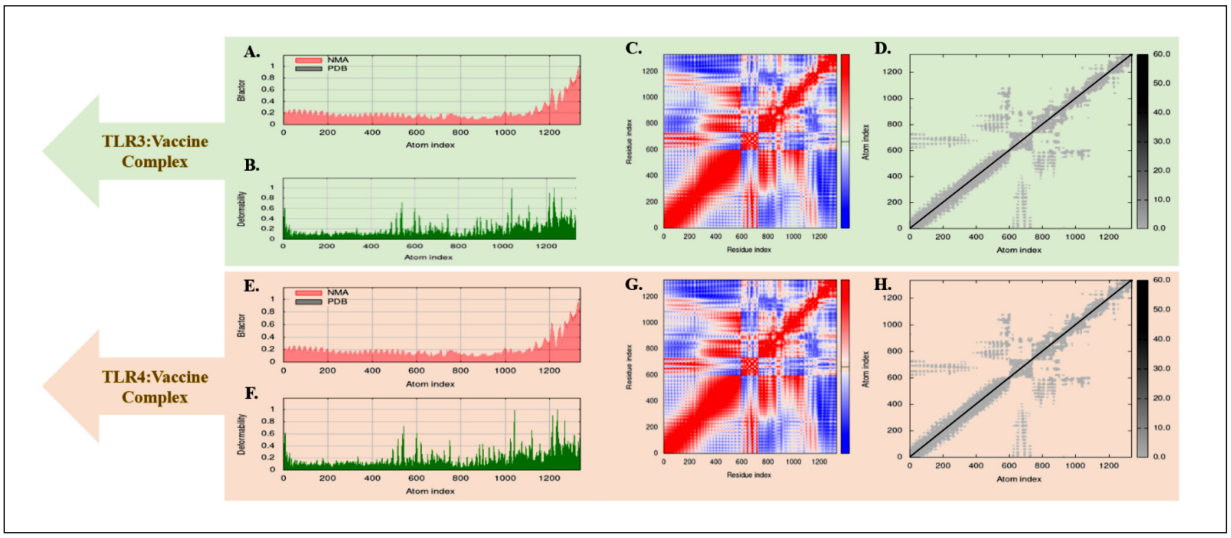

Figure 5. **A**, The B-factor graph for the TLR3: vaccine complex, **B**, The Deformability plot of the TLR3: vaccine complex. **C**, The covariance map of the TLR3: vaccine complex. **D**, The elastic network map of the TLR3: vaccine complex. **E**, The B-factor graph for the TLR4: vaccine complex. **F**, The Deformability plot of the TLR4: vaccine complex. **G**, The covariance map of the TLR4: vaccine complex. **H**, The elastic network map of the TLR4: vaccine complex.

recent study[40], the problem has led to significant economic reductions, including a 65% decrease in milk production and up to 6,300$ monetary loss for farmers.

The current study proposes an mRNA-based vaccine against the causative LSDV, considering the CDC's One Health approach (CDC, 2019)[41] and the significant damage this virus causes economically, environmentally, and agriculturally. The vaccine construct is proposed to be a competitive strategy compared to the already-existing live attenuated vaccines in terms of immunogenicity, safety, and potency. According to studies[4,42,43], live vaccines elicit a potent and durable protective immunity and have undoubtedly helped to manage the disease in many locations, but due to their adverse effects, farmers in affected areas have not endured them well.

Indeed, limited vaccination inflammation and a widespread moderate illness with skin infections have been linked[39] to live-but-attenuated vaccines. The possibility of insect transmission is also thought to exist when the vaccine variant is isolated from lesions in animals that have received the vaccination[44], and zoonosis has been reported[1,45] in different regions. Although the cause of this phenomenon is unknown, it is most likely the result of insufficient attenuation or the inclusion of non-homogenous particulates with varying levels of pathogenicity in the vaccination[11]. This study has faced both problems by taking small (continuous and discontinuous) peptide regions that are non-homologous to the host for an mRNA-based vaccine design nullifying any problem related to host-similarity or viral regeneration. To this effect, this study has distantly followed a review[23] that provides recommendations for researchers interested in using a current *in silico* vaccine creation approach, which may include an mRNA-based vaccine design that avoids worries about viral regeneration and host-similarity difficulties.

Moreover, all the peptides shortlisted for the vaccine candidate were checked for non-homology with the host cattle and the human genomes, an approach previously utilized to ensure the safety and enhanced immunogenicity of proposed vaccine candidates[46,47]. Although the immune response stimulation in this study suggests eliminating the vaccine candidate within five days of injection, it will not be toxic to the human host even if injected cattle's milk is harvested before the antigen's elimination, further validating the construct's stability and potential for commercial application. It also aligns with the One Health guidelines (CDC, 2019)[41] and minimizes any allergenicity or toxicity of the construct or the milk harvested from the treated host.

Most epitope prediction techniques are based on the recognition of MHC-restricted peptides. Finding peptides that can stimulate cytotoxic T cells is among the biggest hurdles in modern vaccine development. All MHC ligands do not need to function as T-cell epitopes[48]. Therefore, in addition to employing MHC binders, a more accurate prediction strategy for cytotoxic T lymphocyte (CTL) epitopes was utilized in this study. Support vector machines and artificial neural networks are used on recent CTL epitopes and non-epitope datasets to address these issues. We worked with modules based on these approaches validated in our previous studies[49-50]. The server of choice (IEDB) prediction accuracy is close to 90%[51], which is higher than that of other platforms like "CTLpred" (72%) and "RANKPEP" (60%)[52]. This indicates that IEDB is a reliable tool for predicting peptide binding, as Wang et al[53] suggested.

RS09 works as an *in-vivo* adjuvant in the final construct and is capable of binding to TLR-4 and promoting nuclear localization of NF- κB in macrophages. According to Shanmugam et al[54], RS09 can generate a strong antibody response compared to other peptides such as RS01. The vaccine candidate was predicted to elicit a stable immune response with the levels of IgM and IgG recorded up to 6,000 titers (antigen count per mL) after a month of injection. The interferons, interleukins, and lymphocyte memory cells were also strongly elicited, and the docking analyses with cattle TLRs demonstrated that the construct could stimulate the host's innate immune response. Most importantly, the proposed candidate was non-allergenic and non-toxic to the host, making it significantly improved over the live-attenuated vaccines associated with several localized allergic reactions, as discussed earlier[10,14]. Nonetheless, experimental analyses are required to validate the prediction. If followed, the candidate vaccine may end the spreading of the lumpy skin disease and be a one-stop answer for worldwide farmers' reservations.

Conclusions

The lumpy skin disease is an optimal example of how non-zoonotic diseases are as worthy of our efforts under the One-Health approach as

the zoonotic problems. Although the lumpy skin disease virus is presumed to be non-zoonotic, it is the source of many economic and environmental threats posed to humanity. The evidence of the virus and the lumpy skin disease spreading to human hosts has worsened the situation. To this end, this study has proposed an mRNA-based vaccine candidate to eliminate the virus in bovine hosts, irrespective of whether it spreads to humans. The design has been created to develop a long-lasting memory in the bovine host (cattle) without being allergenic or toxic. The epitopes selected for the construct were checked for homology with the cattle and human genomes, removing any doubt of host tolerance. The predicted antibody titers of up to 6,000 (arbitrary units), IFNg stimulated up to 400,000 ng/mL, hydrophilicity (GRAVY index of -0.151), and the stability of the construct makes it an improved and potent alternative to the live-attenuated vaccines if subjected to experimental validations.

Conflict of Interest
The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest. No conflict of interest was recorded. All the authors declare no conflict of interest.

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Authors' Contribution
Conceptualization, M.N, J.H, I.K.R and U.A.; methodology, T.A., A.A.K, and M.A; soft-ware, A.A.; validation, S.N.; formal analysis, M.N, J.H, I.K.R., U.A., T.A and A.A; investigation, M.N, J.H, I.K.R., U.A., T.A and A.A.S. resources, M.A, and T.L.N.; data curation, M.N.; writing-original draft preparation, U.A and F.H.; writing-review and editing, S.M and M.H.A; visualization, M.A and M.N.; supervision, M.N and T.A.; project administration, A.S.A.; funding acquisition, T.A.

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