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Original Research

Immunoinformatics approach to Rift Valley fever virus vaccine design in ruminants

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ABSTRACT

Introduction: Rift Valley fever (RVF) represents a significant public health challenge and economic burden due to its impact on livestock and potential to affect humans. Despite attempts to develop vaccines against the Rift Valley fever virus (RVFV), existing options are limited by concerns regarding the inability to differentiate between vaccinated and infected animals, vaccine-associated viremia, and the need for booster doses. This underscores the urgent need for a novel, effective, and safe vaccine, especially for use in ruminants, which this study seeks to address. **Methods:** Employing reverse vaccinology—a cutting-edge approach combining bioinformatics and reverse pharmacology—we aimed to develop a novel RVFV vaccine. We focused on the M-glycoprotein segment, identifying highly conserved and immunodominant epitopes in viral glycoprotein sequences from cattle, sheep, and goats in RVF-endemic regions of Africa. Predictions for B- and T-cell epitopes were made, followed by the design of an epitope-based vaccine incorporating ideal linkers and a *Bos taurus*-specific beta-defensin to enhance immunogenicity. The vaccine's secondary and tertiary structures were analyzed using SOPMA and AlphaFold2, respectively. **Results:** The vaccine candidate demonstrated promising physicochemical properties, with the M-glycoprotein sequences showing high antigenicity. Structural analysis revealed a composition of 31.55% alpha helices, 44.92% random coils, 5.35% beta turns, and 18.18% extended strands. Molecular docking with Toll-like receptors 7 and 8 indicated favorable molecular binding interactions, suggesting potential efficacy in stimulating an immune response. **Conclusion:** This study paves the way for the development of a novel, safe RVFV vaccine. While the results are promising, further translational research is necessary to confirm the vaccine's effectiveness in animals and its applicability for improving public health outcomes.

Key words: Arboviral diseases, Computational analysis, Molecular docking, Reverse vaccinology, Rift Valley fever virus, Vaccine development

INTRODUCTION

Rift Valley fever (RVF) is a viral disease most commonly seen in domesticated animals. People can develop RVF through contact with the blood, body fluids, or tissues of infected animals or through bites from infected mosquitoes. Rift Valley fever virus (RVFV) can cause severe disease in newborn ruminants such as sheep, goats, camels, and cattle, with up to a 100% fatality rate. Mosquitoes are the main infection vectors, although sandflies have also shown the capability to transmit the virus [1]. However, adults seem less susceptible. In ruminants, the virus causes RVF, an acute hemorrhagic fever, accompanied by abortion storms in pregnant females [2]. Abortion rates may be up to 100% in pregnant animals [3]. These dire statistics indicate the need for concerted efforts to develop preventive measures against RVFV's future resurgence.

The RVFV genome is tri-segmented, with large (L), medium (M), and small (S) segments. The L segment encodes the RNA polymerase (RNA-dependent L protein), the S segment encodes the nucleoprotein and the non-structural nucleoprotein (NSs), and the M segment encodes four proteins (two structural glycoproteins [Gn and Gc] and two non-structural glycoproteins [NSm and LGp]) [4].

RVFV is a vector-borne, zoonotic, negative-stranded, tri-segmented RNA virus that belongs to the genus *Phlebovirus* and family *Bunyaviridae*. Apart from affecting human and animal health, it has a negative socio-economic impact [5,6]. RVFV has been reported to be endemic to the Arabian Peninsula and Africa [1,7], although it was first identified in Kenya's Rift Valley in early 1930 [5] (hence its name). Kenya is reported to have experienced the deadliest epizoootic of RVFV before a major outbreak in South Africa between 1950

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and 1951, which led to about 500,000 abortions and 100,000 deaths among sheep; subsequent epizootics were observed in Zambia, Namibia, Mozambique, Sudan, Zimbabwe, and other East African countries [2,3]. RVFV outbreaks have been associated with periods of greater than average rainfall, although this cannot easily be predicted, thereby suggesting an interplay between the environment and human and animal health [3,8].

Conventional vaccines used extensively for controlling RVF include inactivated, live attenuated vaccines DNA vaccines, viral vector-based vaccines, viral replicon vaccines, and subunit vaccines, among others [9]. However, these vaccines lack important properties, such as the ability to differentiate infected from vaccinated animals. Live attenuated vaccines induce long-lasting immunity and are generally inexpensive to produce but are linked to an increase in the rate of abortion in pregnant animals and can have negative effects on internal organs, especially the liver, when the attenuated virus spreads inside hepatic cells, as in a naturally occurring RVFV infection [9]. Other vaccines also face certain difficulties, such as the inability to distinguish between infected and vaccinated animals, viremia, and the requirement for booster shots. Multi-epitope subunit vaccines incorporate predicted immunodominant epitope peptides from various immunogenic genes that have been identified through proteomic research [10]. Short immunogenic peptide fragments employed in multi-epitope vaccinations produce potent immune responses while considerably reducing the risk of allergic reactions in the host. Identifying the immunogenic epitopes obtained from viral glycoprotein or nucleocapsid sequences has greatly benefited the in silico design of peptide vaccines [11]. These epitopes are analyzed and approved using computational techniques based on their physicochemical characteristics, immunogenicity, and non-allergenicity. Subunit vaccines are cost-effective, non-toxic, and safer for animals than other RVFV vaccines. The aforementioned limitations of conventional vaccines and the untapped potential of multi-peptide-based vaccines have made this study necessary.

In this research, we use a computational design approach to develop an RVFV multi-epitope subunit vaccine, which incorporates several antigenic determinants that are conserved across different RVFV strains. The vaccine design is optimized for increased stability, immunogenicity, and manufacturability, using various adjuvants and carrier proteins and evaluated in different delivery systems. Overall, this manuscript describes a comprehensive and innovative approach to the development of an RVFV multi-epitope subunit vaccine, highlighting the potential of this approach for the development of potent vaccines against other viral diseases. Therefore, our objective in this study was to design a safe, non-allergenic, and non-toxic subunit vaccine that can elicit optimal immunity against RVF in ruminants.

METHODS

Retrieval of RVF Protein Sequences

The National Center for Biotechnology Information's (NCBI) immunoinformatics investigation yielded a total of 60 RVFV amino acid sequences, which included sequences from the L, M, and S genomic segments. These sequences came from nations like Kenya, Madagascar, South Africa, Rwanda, Namibia, Egypt, Zimbabwe, and Nigeria.

Antigenicity Prediction

The VaxiJen v2.0 server was used to determine the antigenicity of the RVF proteins[11]. Without using alignment, the VaxiJen server estimates a protein's antigenicity based on its physicochemical characteristics. The default threshold value was set at 0.4.

Membrane Topology of Selected Proteins

The trans-membrane topology of the protein sequences was assessed for their intra-cytoplasmic, extra-cytoplasmic, and transmembrane portions using the TMHMM server v2.0 [12]. TMHMM predicts transmembrane topology through a hidden Markov model. Proteins with large extra-cytoplasmic regions were considered for further analyses.

Multiple Sequence Alignment

The glycoprotein M sequences underwent repeated sequence alignment using the MEGA 11 program to show conserved regions across the different sequences [13]. The MUSCLE algorithm was specifically used to align the different protein sequences [14].

Prediction of Linear B-Cells

The ABCpred, SVMTriP, and BepiPred servers were used to predict the linear B-cell epitopes. For linear B-cell prediction, ABCpred employs a partly recurrent neural network (Jordan network) with a single hidden layer. The networks comprise 35 residues in a single hidden layer and a changeable optional window length [15]. SVMTriP is used to locate linear epitopes based on amino acid features such as secondary structure, flexibility, antigenicity, hydrophilicity, and

solvent accessibility to predict B-cells [16]. Using a hidden Markov model, BepiPred forecasts B-cell epitopes from antigen sequences [17].

Physicochemical Property Analysis

Analyzing the physicochemical characteristics of the chosen B-cells allowed for evaluating their suitability for vaccine development. ExPASy Protparam was used for this purpose [18]. Additionally, utilizing VaxiJen, AllerTop, and ToxinPred, these peptides were tested for their allergenicity, antigenicity, and toxicity, respectively [11,19,20].

Prediction of Cytotoxic T Lymphocytes (CTLs)

The glycoprotein's (M segment) CTL epitope was predicted using NetMHCII PAN 4.1 [21]. The server employs artificial neural networks (ANNs) to forecast peptide binding to MHC alleles. The network is taught to examine peptide ligands that have been eluted by mass spectrometry and have a high binding affinity (BA). The Bovine Leukocyte Antigen (BoLA) was chosen as the reference allele set in this study. The affinity of the glycoprotein peptides for the 20 BoLA alleles BoLA-JSP.1, BoLA-HD6, BoLA-T2c, BoLA-T2b, BoLA-T2a, BoLA-T7, BoLA-D18.4, BoLA-AW10, BoLA-T5, BoLA-1:00901, and BoLA-1:00902 was assessed. The chosen peptide has a length of nine amino acids.

Prediction of Helper T Lymphocytes (HTLs)

Strongly binding MHCII HTL epitopes were predicted using NetMHCII PAN 4.0 [21]. Based on machine learning techniques trained on binding affinity or mass spectrometry (eluted ligands) techniques, this tool forecasts the likelihood that a peptide will present an antigen. NETMHCII PAN 4.0 was used to predict epitopes [22]. The HTL epitopes were chosen to have a default length of 15 amino acids, and the binding affinity of overlapping glycoprotein peptides to the following alleles was discovered (the following DRB1 codes are available): DRB1_0101, DRB1_0102, DRB1_0103, DRB1_0104, DRB1_0105, DRB1_0106, DRB1_0107, DRB1_0108, DRB1_0109, DRB1_0110, DRB1_0111, DRB1_0112, DRB1_0113, DRB1_0115, and DRB1_0116.

Prediction of Interleukin-4- and Interleukin-10-Inducing Epitopes

HTLs, which produce cytokines like interleukins, are crucial in coordinating the immune response. Therefore, using IL4Pred and IL10Pred, the interleukin-4- and interleukin-10-producing capacity of a subset of strongly binding epitopes was evaluated [23,24].

Antigenicity, Toxicity, and Allergenicity Prediction

The toxicity, antigenicity, and allergenicity of strong CTL peptides and HTL epitopes that induce IL-4 and IL-10 were examined. The ToxinPred server, according to Gupta *et al.* [19], predicts peptide toxicity along with its physical properties, such as molecular weight, charge, hydrophobicity, and amphipathicity. The AllerTop v2.0 server reportedly uses machine learning methods including auto- and cross-variance transformation and amino acid E-descriptors and predicts the allergenicity of proteins and peptides, according to Dimitrov *et al.* [20]. Doytchinova *et al.* [11] claim that the categorization of antigens by VaxiJen v2.0 is solely based on the physicochemical characteristics of the proteins involved.

Epitope Conservation Determination

B-cells, HTLs, and CTLs were compared via multiple sequence alignment to determine whether they fall within conserved regions. For the final vaccine design, only completely conserved epitopes were considered. For the vaccine to be efficacious across a broad spectrum, highly conserved epitopes are required [25]. Furthermore, epitopes in the extracytoplasmic portion of the glycoprotein sequence were prioritized for selection.

Primary Construct Assembly

The chosen HTL, CTL, and B-cell epitopes served as the building blocks for the vaccine. To increase the final construct's immunogenicity, a stiff EAAAK linker was used to introduce a *Bos taurus*-specific beta defense adjuvant to the N-terminus of the vaccine. AAY linkers were used to connect additional CTL epitopes. The HTL and B-cell epitopes were joined together using GPGPG linkers. According to Kavoosi *et al.* [26], the glycine-rich linker GPGPG serves to increase solubility and promote free movement across adjacent domains. To boost the immunogenicity of the vaccine design using the EAAAK linker, a *Bos taurus*-specific beta defensin adjuvant was also inserted at the N-terminus [27].

The Proposed Vaccine's Allergenicity, Solubility, and Antigenicity

The allergenicity of the vaccine construct was assessed using the AllerTop v.2.0 server, while the antigenicity and solubility of the vaccine construct were as-

sessed using VaxiJen v2.0 and Protein Sol, respectively [11,20,28].

Analysis of the Vaccine Construct's Physicochemical Characteristics

Secondary and Tertiary Structure Prediction

The secondary structure was predicted using the SOPMA server. The server predicts protein secondary structure from amino acid sequences using the self-optimized prediction approach [29].

The AlphaFold server was used to predict the tertiary structure of the vaccine construct [30]. AlphaFold uses innovative neural networks in combination with the primary amino acid sequence and alignment sequence of homologs to predict the tertiary structure of a particular protein.

Conformational Epitope Prediction from 3D Vaccines

Thornton's approach and a residue clustering algorithm on the IEDB database are both used by the ElliPro server to predict conformational epitopes on the 3D structure. According to Ponomarenko *et al.* [31], ElliPro offers a framework for predicting tertiary structure models from amino sequences.

Refinement and Validation of the Tertiary Structure

The quality of the 3D structure of the chosen model, protein structure refinement, protein interaction prediction, GPCR applications, and water position prediction were all improved using the GalaxyWEB refine server [32]. By recreating and repackaging protein side chains using dynamic simulation techniques, this server improves 3D protein architectures, facilitating the production of proteins with appropriate structural relaxation.

Thereafter, structural analysis using Ramachandran and Z-score analysis was used to validate the vaccine's structure [33,34]. PROCHECK and ProSA-web were used. The Ramachandran plot, which displays the proportion of the amino acids present in the preferred, permitted, and forbidden zones, is produced by the PROCHECK server. ProSA-web performs mathematical analysis on the entire protein structure quality score and presents values and energy.

Codon Optimization and In Silico Cloning

To adapt the vaccine for expression in the *Escherichia coli* strain K-12 sub-strain MG1655 chosen as the expression host, codon optimization was performed using JCAT [35]. This is crucial for maximizing protein expression during the recombinant vaccine's cloning process. Using the SnapGene v5.1.7 program, the chimeric model was virtually cloned into an appropriate expression vector, *E. coli* K12 pET-28a (+) [36].

Molecular Docking

A computational procedure called molecular docking is used to predict the affinities of the ligand and receptor to create stable complexes. Using the HDOCK server, the final vaccine design was docked against toll-like receptor (TLR) 7 and 8 [37].

RESULTS

Antigenicity Prediction

Table 1 shows the antigenicity of selected proteins. The glycoprotein (M) sequences were seen to be the most antigenic of all the RVFV proteins**.**

Membrane Topology of the Selected Sequences

The development of subunit multi-epitope vaccines requires in-depth knowledge of transmembrane topology. To develop subunit multi-epitope vaccines, epitopes located in the extra-cytoplasmic segments are crucial for efficacy [38]. In this study, the TMHMM tool was used to analyze the topology of the different genome segments. The transmembrane topology of the selected proteins is presented in **Table 2**. A large part of the glycoproteins was in the extra-cytoplasmic portion, indicating their usability in designing a vaccine against RVF**V.**

Sequence Retrieval

A total of 60 RVFV M segment sequences from cattle, sheep, and goats were obtained from the NCBI. The sequences were obtained from a total of eight countries, including Kenya, Zimbabwe, South Africa, Egypt, Madagascar, and Namibia.

Multiple sequence alignment (MEGA)

The multiple sequence alignment showed large overlapping and conserved portions (**Figure 1**).

Prediction of Linear B-Cell Epitopes

Epitopes with between 10 and 20 amino acids that cut across at least two of the servers used (BepiPred, ABCpred, and SVMTriP) were selected. The physicochemical properties of the selected epitopes were then assessed for suitability in a vaccine construct. The results are presented in **Table 3**.

Table 1: Antigenicity properties of selected proteins

Genome Segment	Protein	Antigenicity
L	Polymerase	Non-antigen
M	Glycoprotein	Antigen
	Nucleocapsid	Non-antigen

Table 2: Transmembrane topology of selected protein highlighted in bold

Genome Segment	Protein	Protein Segment	Position
L	Polymerase	1-2091	Inside
M	Glycoprotein	1-16	Signal peptide
		17-584	Outside
		585-603	TMHelix
		604-675	Inside
		676-685	TMHelix
		686-1159	Outside
		1160-1178	TMHelix
		1179-1197	Inside
	Nonstructural	1-265	Inside
	Nucleocapsid	1-245	Inside

Table 3: Selected predicted linear B-cell-based epitopes

Peptide	Antigenicity	Allergen city
DSLREEEMPE	Antigenic	Non- allergic
VYLDKLDLKTEENLLPD	Antigenic	Non- allergic
SYWTGSFSPKCLSSRRCHLV	Antigenic	Non- allergic
SKLKTKMKGVCEVGVQALKK	Antigenic	Non- allergic

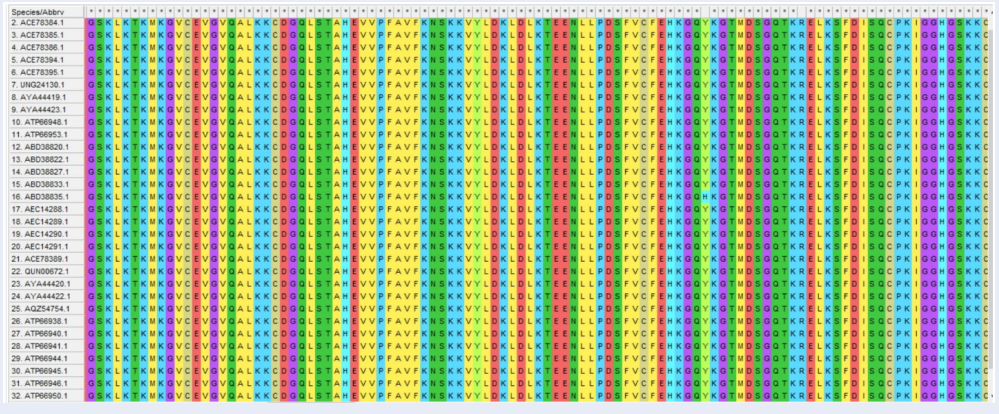

Figure 1: **Image from multiple sequence alignment showing large over-lapping and conserved portions.**

Table 4: Predicted CTL epitopes. Peptides selected for the final vaccine construct are highlighted in bold

Peptides	Antigenicity score	Instability status	Toxicity status	Allergenicity status
SLKKGSYPL	1.3734	Stable	Non- toxic	Non-allergic
KIKTVSSEL	1.4693	Stable	Non- toxic	Non-allergic
GNPCMKEKL	0.0854	Stable	Non- toxic	Non-allergic
TMAGIAMTV	1.9979	Stable	Non- toxic	Non-allergic
NCIDWVHKL	0.6161	Stable	Non- toxic	Non-allergic
RAPNLVSYK	0.1302	Stable	Non- toxic	Non-Allergic
RQMTGASLK	1.7603	Stable	Non- toxic	Non-allergic
EVVPFAVFK	0.4533	Stable	Non- toxic	Non-allergic
IQVSGVWKK	1.5642	Stable	Non- toxic	Non-allergic
SAHGNPCMK	0.9450	Stable	Non- toxic	Non-allergic
RVADNINQV	0.5002	Stable	Non- toxic	Non-allergic
VVFAEDPHL	1.4568	Stable	Non- toxic	Non-allergic
ITSTGTGTL	0.0479	Stable	Non- toxic	Non-allergic
LSAKPIQRV	0.1587	Stable	Non- toxic	Non-allergic
FKNSKKVYL	1.5282	Stable	Non- toxic	Non-allergic
LKIAPRKVL	0.6219	Stable	Non- toxic	Non-allergic
VQADLTLMF	1.8723	Stable	Non- toxic	Non-allergic
HKGQYKGTM	0.6591	Stable	Non- toxic	Non-allergic
TGFKISSAV	1.6721	Stable	Non- toxic	Non-allergic
TKDQCQILH	0.5748	Stable	Non- toxic	Non-allergic
VQADLTLM	1.8470	Stable	Non- toxic	Non-allergic
GDIGVHMA	0.2486	Stable	Non- toxic	Non-allergic
TKDQCQIL	0.4858	Stable	Non- toxic	Non-allergic
LEKGKFPL	1.6611	Stable	Non- toxic	Non-allergic
RETMAGIA	0.3986	Stable	Non- toxic	Non-allergic
LKIAPRKV	0.2515	Stable	Non- toxic	Non-allergic
RQMTGASL	1.5040	Stable	Non- toxic	Non-allergic

Table 5: Selected HTL epitopes

Peptides	Antigenicity status	Allergenicity status	Toxicity status	Stability status
QSAHYLNNDGKMASV	Antigenic	Non-allergic	Non-toxic	Stable
FLKIKTVSSELSCRE	Antigenic	Non-allergic	Non-toxic	Stable
VLKCLKIAPRKVLNP	Antigenic	Non-allergic	Non-toxic	Stable

Table 6: Conformational epitopes predicted from the vaccine's tertiary construct

No.	Predicted discontinuous epitope	Number of residues	Score
1.	A:V152, A:L153, A:N154, A:P155, A:G156, A:P157, A:G158, A:P159, A:G160, A:S161, A:K162, A:L163, A:T165, A:K166, A:K168	15	0.773
2.	A:K194, A:T195, A:E196, A:E197, A:N198, A:L199, A:L200, A:P201, A:D202	9	0.733
3.	A:D1, A:F2, A:A3, A:S4, A:C5, A:H6, A:T7, A:N8, A:G9, A:G10, A:I11, A:C12, A:L13, A:P14, A:N15, A:R16, A:C17, A:P18, A:G19, A:H20, A:G25, A:I26, A:C27, A:F28, A:R29, A:P30, A:R31, A:V32, A:K33, A:C34	30	0.711
4.	A:K180, A:G181, A:P182, A:G183, A:P184, A:G185, A:V186, A:Y187	8	0.697
5.	A:P117, A:G118, A:P119, A:G120, A:F121, A:E135, A:G136, A:P137, A:G138, A:P139, A:G140, A:V141, A:L142, A:K143, A:C144, A:L145, A:K146	17	0.669
6.	A:G81, A:T82, A:G83, A:T84, A:L85, A:A86, A:A87, A:Y88, A:R89, A:Q90, A:M91, A:T92, A:G93, A:A94, A:S95, A:K97, A:A98	17	0.639
7.	A:K125, A:T126, A:V127, A:S128, A:S129, A:E130	6	0.633

Table 7: Molecular docking for Toll-like receptors

Toll-like Receptor	Docking score	Confidence Score	Ligand rmsd (Å)
TLR-7	-278.26	0.9286	46.14
TLR-8	-324.28	0.9703	94.62

Prediction of CTLs

A total of 130 strongly binding CTL peptides were predicted for the selected BoLA alleles. These peptides were selected based on their binding score or ability. Strongly binding CTL peptides were examined for their stability index, toxicity, allergenicity, and antigenicity. **Table 4** lists the 27 epitopes from the glycoprotein that passed these tests. However, not all the 27 made the final construct.

HTL Prediction

A total of 97 peptides demonstrated strong binding for the selected MHCII alleles and were further assessed to induce interleukin-4 and interleukin-10 cytokines. After being evaluated for their physicochemical characteristics, epitopes that showed the potential to stimulate the production of the aforementioned cytokines were also tested for antigenicity, allergenicity, and toxicity. **Table 5** shows the HTL epitopes that scaled through the screening processes.

Primary Construct Assembly

The vaccine primary construct comprised a *Bos taurus*-specific beta defensin adjuvant, six CTL peptides, three HTL peptides, and two B-cell-based peptides. The adjuvant was attached to the first CTL peptide using the EAAAK linker and to subsequent CTL peptides using the AAY linker. The rest of the primary construct was linked using GPGPG linkers. Our final vaccine construct was a 202-amino-acid-long primary sequence (**Figure 2**).

Prediction of the Physicochemical Properties of the RVFV Vaccine

The antigenicity index for a chimeric model with 0.4 points was determined using VaxiJen v2.0 server output and found to be quite high (0.6926). The multiepitope protein construct was also anticipated to be dispersible upon overexpression using Protein Sol. The 202-amino-acid protein had a theoretical isoelectric point (Pl) of 9.44 and a relative molar mass (Mr) of 21.4 kDa as per the ExPasy Protparam web server. The sequence also had 26 cations (Arg + Lys) and 12 anions (Asp + Glu) residues. The assembled sequence

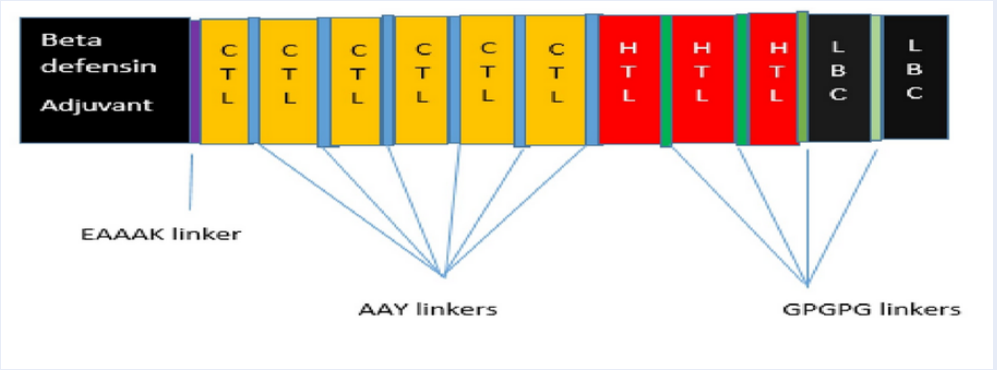

Figure 2: **Image showing vaccine primary construct comprising a Bos taurus-specific beta defensin adjuvant, six CTL peptides, three HTL peptides, and two B-cell-based peptides.**

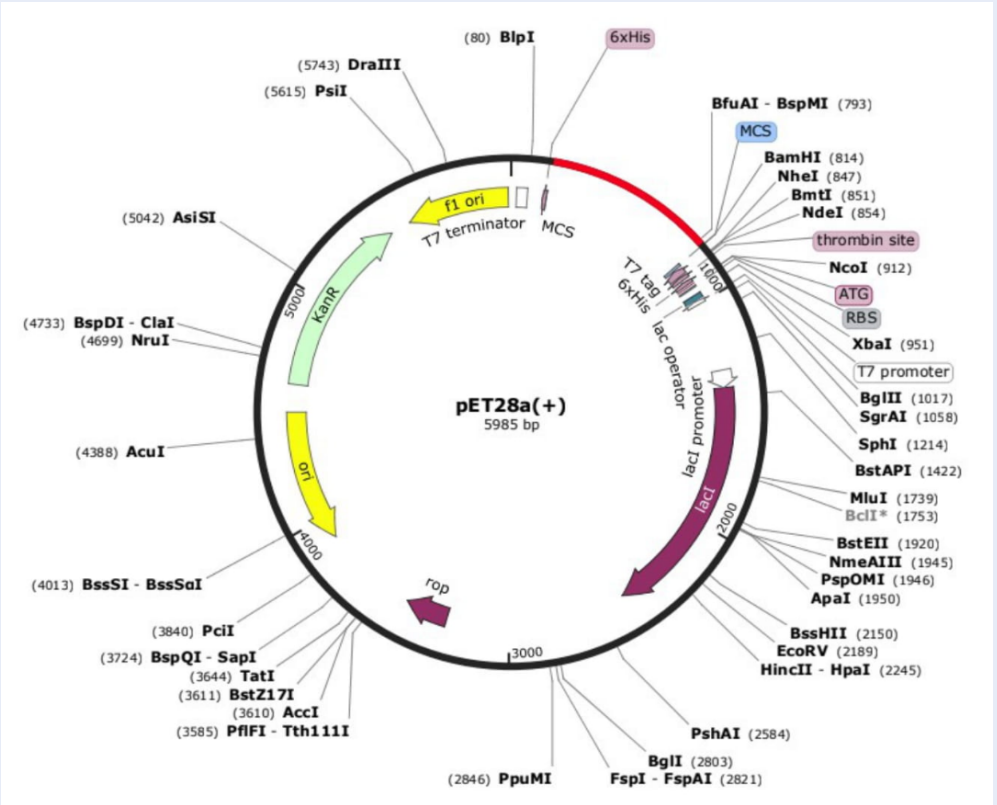

Figure 4: **Image showing *in* silico cloning of the vaccine in an *Escherichia coli* pET28a (+) vector, highlighting the vaccine is highlight in red.**

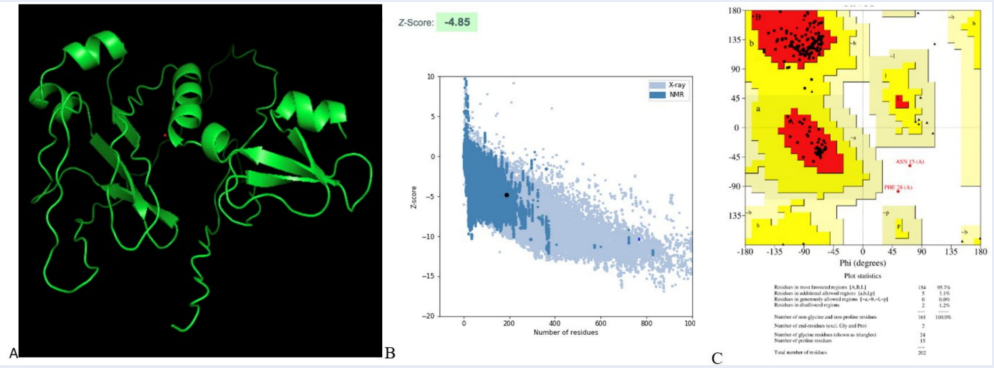

Figure 5: **Image showing refined tertiary structure (A), ProSA evaluation of refined tertiary structure (B), and Ramachandran evaluation of refined tertiary structure (C), with conformational epitopes with protrusion indexscores greater than 0.7 being shown in Figure 5 A-C**.

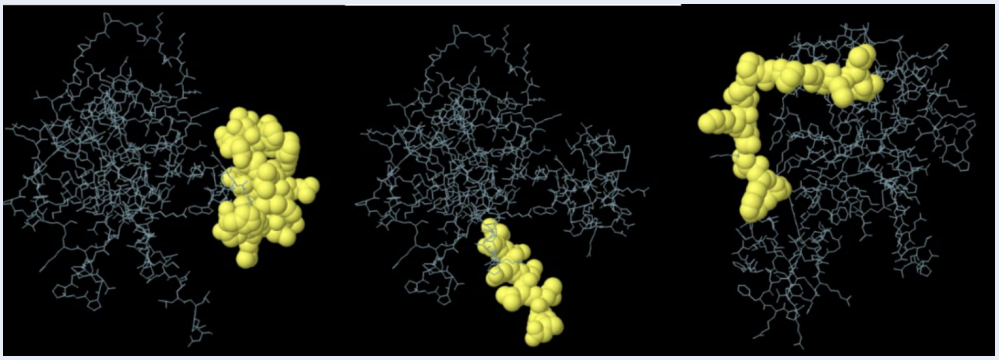

Figure 6: **3D image showing conformational epitopes, a framework for predicting tertiary structure models from amino sequences.**

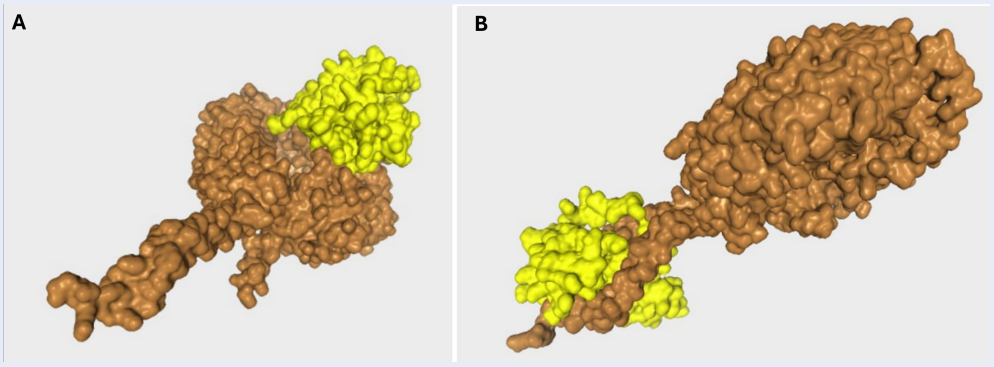

Figure 7: **Molecular docking of the construct and TLR-7 (A), TLR-8 (B)**. Ten models docking predicted between the vaccine construct and TLR7 and the rankings were based on the docking score, confidence score, and Ligand RMSD (Å)**.**

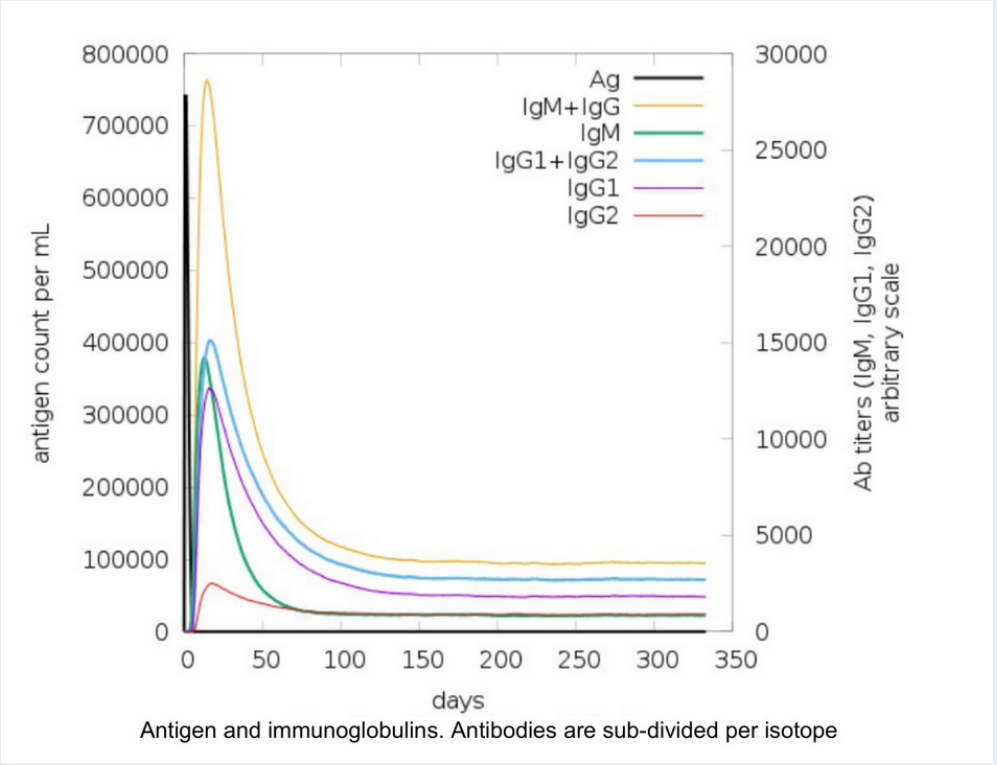

Figure 8: **Image showing immune response incentive, indicating the production of a strong and consistent main and secondary immune response**.

was also shown to be sturdy, with a stability index of 20.37. The multi-epitope had a DIVIDEND score of −0.121 along with an acyclic index of 81.19, which indicated that it was hydrophilic (**Figure 3**) and thermostable.

Codon Optimization

The JCAT server, reverse translation, and codon optimization (ECAI) optimizations for the adaptation of *E. coli* strain K-12 sub-strain MG1655 were performed[35]. The improved GC content after codon optimization was 53.3% at a Codon Adaptation Index (CAI) of 1.0. To successfully integrate the RVF construct into the *E. coli* expression system, a high GC content is a sign of stability. The restriction enzymes Xho1 and EcoR1 were utilized to clone the back-translated nucleotide into the *E. coli* expression system. Effective insertion of the segment into the pET28a (+) vector resulted in the production of a 6096 bp clone. The in silico cloning procedure of the vaccine in an *Escherichia coli* pET28a (+) vector is presented in **Figure 4**.

Prediction of Secondary and Tertiary Vaccine Structure

The SOPMA server was used to analyze the secondary structure of the intended protein, and the findings were as follows: alpha helix = 31.55%, random coil = 44.92%, beta turn = 5.35%, and extended strand = 18.18%. The tertiary structure is presented in **Figure 5**a.

Tertiary Structure Refinement and Validation

The PROCHECK assessment of the stoichiometric quality of the structure revealed the residue and overall structural computation. Ramachandran plot analysis indicated that 95.7 % of the protein remains were located in their preferred regions (**Figure 5** c). Furthermore, results from ProSA, which predicts implied oversights of a 3D model, protein, predicted a negative score of −4.85, which is a favorable indicator of model quality (**Figure 5** b).

Prediction of Discontinuous Epitopes

The ElliPro server predicted an aggregate of 91 conformational epitopes from 187 amino acid residues

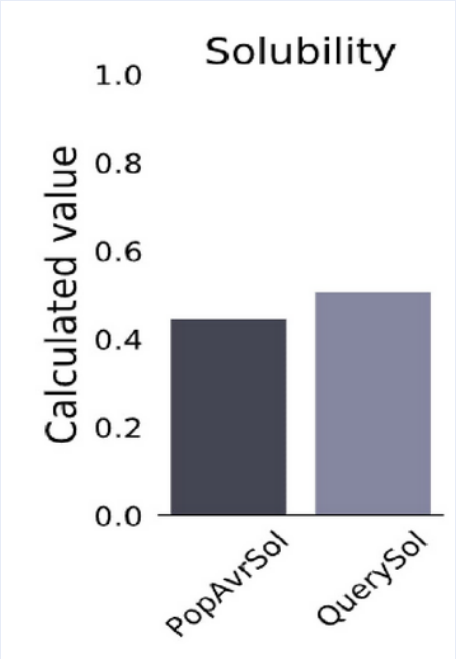

Figure 3: **Image showing the vaccine construct with thermo-stability and hydrophilic properties.**

on the vaccine tertiary construct[31]. Moreover, the Jmol viewer allows for the uptake and foresight of immune epitopes in a given protein sequence or structure. Conformational epitopes with protrusion index scores greater than 0.7 are shown in **Figure 5** a-c.

Molecular Docking

HDOCK predicted 10 models docking between the vaccine construct and TLR7. The rankings were based on the Docking Score, Confidence Score, and Ligand RMSD (Å) (**Figure 7** and **Table 7**).

Immune Response Incentive

The server C-ImmSim simulates immune findings indicated in a system of effective immune systems and an extended duration of the vaccine candidate. The vaccine design, as shown in Figure 8, produced a strong and consistent main and secondary immune response. The main immune response was defined by the level of the IgM antibodies after 4–5 days of antigenic exposure. The basic immune response was distinguished by enhanced B-cell generation and increased IgM and IgG1 + IgG2 expression and IgM + IgG antibodies. The advanced vaccine construct not only evoked a strong B-cell increase but also led to a multitude of memory B-cells. Specific interleukin concentration, a high level of IFN-γ for a long interval, CD4 activity, HTL count, and B-lymphocyte count were revealed.

DISCUSSION

It is important to note that the approach we took in this study is novel and different from the conventional approach already adopted for vaccine development for RVFV, with its aforementioned limitations, resulting in the construction of a safe, non-allergenic, and non-toxic subunit vaccine design that is capable of eliciting optimal immunity against RVF in ruminants and can be further developed into a vaccine for evaluation in ruminants.

RVF is a zoonotic illness spread by mosquitoes. It poses a lethal threat to humans and livestock in many sub-Saharan countries, such as South Africa, Egypt, Madagascar, Mauritania, and Senegal. The disease has been documented in the Arabian Peninsula as well as in the Middle East and India[39]. In animals, the disease has a high fatality rate, where it causes epizootics of abortion and fetal death and high mortality in neonatal ruminants. The virus also causes human infections, although with a lower mortality rate. The Smithburn live attenuated RVFV variant has been extensively adopted in different countries for prevention; however, like many live attenuated vaccines, it faces challenges with safety due to its reversal to virulence, characterized by vaccine-induced teratogenesis, abortion, and fetal death. As a result, it is not recommended for usage in developing nations[40]. Furthermore, the vaccine faces a challenge due to the risk of genetic reassortment[41]. Therefore, novel approaches to designing safe and effective vaccines against this disease are needed.

Advances in Whole-Genome Sequencing (WGS) and proteomics have increased our understanding of viral proteins, which are being used in vaccine design via a reverse vaccinology approach. The use of peptide vaccines has emerged as an outstanding alternative to conventional approaches to vaccine design[42]. Subunit epitope-based vaccines provide an excellent alternative when considering safety, practicability, and cost-effectiveness. Moreover, the potency and immunogenicity of these vaccines can be boosted through the selected engineering of target epitopes[43]. By applying recent developments in immunoinformatics and computational technologies, scientists can selectively construct vaccines with minimal side effects and allergenicity[44]. Consequently, some vaccines have been designed against various pathogens such as SARS-CoV-2, Ebola, hepatitis C, and cholera by applying this approach[45–48]. Our objective in this

study was to design a safe, non-allergenic, and non-toxic subunit vaccine that can elicit optimal immunity against RVF in ruminants.

We selected the M glycoprotein gene after immunoinformatics analysis and an extensive review of the literature on RVF vaccines. The M segment was chosen due to its high antigenicity and the presence of large extra-cytoplasmic segments. Furthermore, experimental vaccines developed using this protein have yielded good results. In different studies, RVFV vaccines developed using the glycoprotein showed excellent immune responses and demonstrated full protection against experimental heterologous challenge infections in sheep and cattle[49,50]. The vaccine was also shown to differentiate naturally infected from vaccinated animals (DIVA)[51,52]; in an immunological study, the pivotal role of the Gn and Gc segments in antibody production and the immune response against RVFV were also demonstrated. Furthermore, the M segments constitute surface proteins, so they have a great potential for inducing the production of neutralizing antibodies[53,54].

The glycoprotein gene was screened for linear B-cells, CD4 HTLs, and CD8 CTLs, which were all integrated into the eventual vaccine construct. Research by Dodd *et al.*[55] has shown the role B-cells and CD4 HTLs play in mediating viral clearance. Specifically, CD4 cells were shown to play major roles in maintaining robust IgG and neutralizing antibody responses, as well as controlling viral replication *in vivo*. CD4 activates B-cells and signals isotype class switching, affinity maturation, and antibody production through its effect on antibody-mediated immunity. It also mediates cytotoxicity by stimulating CD8 cells[55].

The final vaccine construct was made up of 11 epitopes, comprising six CTL peptides, three HTL peptides, and two linear B-cell peptides. All peptides had their physicochemical properties assessed to ensure that they were antigenic, non-allergenic, and non-toxic. The HTL peptides were also evaluated for their ability to induce IL-4 and IL-10. These peptides were linked with AAY and GPGPG linkers. Linkers are vital in vaccine design as they ensure that protein amino acid residues are situated in a conformation similar to their natural state. A 38-amino-acid-long *Bos taurus* beta-defensin was further added to increase the vaccine's immunogenicity with the help of a rigid EAAAK linker.

Analysis of the vaccine's antigenicity, allergenicity, and other physicochemical properties showed favorable results. The vaccine was found to be antigenic, non-allergenic, and non-toxic, indicating a favorable safety potential. Further physicochemical analysis indicated the vaccine to be stable, basic, and soluble.

The potency of the vaccine to induce an immune response was further evaluated by molecular docking analysis and immune simulation. Two toll-like receptors, TLR7 and TLR8, which have an affinity for viral RNA ligands, were selected. The vaccine construct had more binding affinity for TLR8, with a binding score of −324 than for TLR7, which had a binding score of −278. Furthermore, immune simulation with C-ImmSim showed a sustained immune response for up to 350 days after injection with 100 units. After 350 days, the IgG + IgM had dropped to a basal level of about 5000 antibody titers from a peak of about 30,000 that had been reached during the first 30 days of administration. There are three stages of immunity, which are innate or natural, adaptive or active, and passive immunity. Innate is a sort of general protection, passive immunity is a temporary immunity that lasts for a short time, and adaptive immunity develops throughout our lives, beginning when we are exposed to diseases or immunized against them. RVF is an evolving arboviral disease that requires concerted efforts for prevention and control. To this end, novel vaccines that can combine potency and safety are needed, both in animals and humans.

CONCLUSION

The vaccine construct designed in this study potentially possesses properties differing from those of conventional vaccines against RVF because the approach was different. The construct is safe, non-allergenic, and non-toxic, capable of eliciting optimal immunity against RVF in ruminants, and can be further developed into a vaccine for evaluation in ruminants.

In this study, we presented an immunoinformatics approach to developing a multi-epitope subunit RVFV vaccine for use in ruminants. The M glycoprotein gene was selected because of its high antigenicity and the availability of experimental evidence of its efficacy in vaccines. Six CTL, three HTL, and two B-cell epitopes were joined together in a chimeric construct and subjected to analysis. *In-silico* analysis demonstrated the immunogenicity and safety of the vaccine construct; however, further *in vitro* and *in vivo* analyses are required to validate the study.

ABBREVIATIONS

AAY: A type of linker used in the vaccine construct, **ANN**: Artificial Neural Networks, **BA**: Binding Affinity, **BoLA**: Bovine Leukocyte Antigen, **CAI**: Codon Adaptation Index, **CTL**: Cytotoxic T Lymphocytes,

DIVA: Differentiating Infected from Vaccinated Animals, **DRB1**: HLA-DR beta-1, ***E. coli***: *Escherichia coli*, **EAAAK**: A type of linker used in the vaccine construct, **ExPASy**: Expert Protein Analysis System, **GC**: Guanine-Cytosine, **GPGPG**: A type of linker used in the vaccine construct, **HTL**: Helper T Lymphocytes, **IEDB**: Immune Epitope Database, **IL**: Interleukin, **JCAT**: Java Codon Adaptation Tool, **MEGA**: Molecular Evolutionary Genetics Analysis, **MHC**: Major Histocompatibility Complex, **NCBI**: National Center for Biotechnology Information, **pET-28a (+)**: A type of expression vector, **ProSA-web**: Protein Structure Analysis-web, **RVF**: Rift Valley Fever, **RVFV**: Rift Valley Fever Virus, **SOPMA**: Self-Optimized Prediction Method with Alignment, **TLR**: Toll-Like Receptor, **TMHMM**: Transmembrane Hidden Markov Model, **WGS**: Whole Genome Sequencing

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AUTHOR'S CONTRIBUTIONS

Conceptualization and experimental design: EKO; data collection and analysis, ORT, FOT, APT, AMB, OIO, BVO, JIT; supervision and result interpretation: EKO, ORT; manuscript draft: EKO, ORT, IOA; formal analysis, review and editing: IOA. All authors commented on the previous versions of the manuscript, and read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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