**Explor Anim Med Res,**
**Vol.12, Issue - 2, 2022, p. 149-159**
**DOI : 10.52635/eamr/12.2.149-159**

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

***Research Article***

# DESIGNING OF MULTI-EPITOPE VACCINE CONSTRUCT EMPLOYING IMMUNO-INFORMATICS APPROACH TO COMBAT MULTI-TICK SPECIES INFESTATIONS

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*Received 25 April 2022, revised 15 August 2022*

**ABSTRACT: Ticks have an exceptional ability to parasitize diverse animal and human lives and cause direct losses by transmitting a wide variety of pathogens to their host. For the management of the tick a vaccine using mid-gut antigen, Bm86 was developed which provided a variable degree of efficacy against different strains of the cattle tick, *Rhipicephalus microplus*. Contrary to a single antigen-based vaccine, it is expedient to use multi-epitopes vaccine targeting multiple functions of ticks. To develop a universal tick vaccine against multi-species, multiepitopic construct using immunodominant conserved tick proteins with B and T-cell epitopes were designed employing various immuno-informatics techniques. The 3D structure of the final construct was predicted, refined, and validated by different computational techniques. Molecular docking of the construct was carried out with TLR-2 receptor to predict efficient cell mediate the immune response. The findings manifest that the multi-epitopic construct might be active against multi-tick species infestations, a problem faced by many tropical and subtropical countries.**

**Key words:** Multi-tick species, Immuno-informatics, Multi-epitopic vaccine.

## INTRODUCTION

In the wake of modernization, endless human-driven changes in the microcosm and macrocosm have catalyzed the emergence, re-emergence, and spread of a myriad of new zoonotic diseases. As an analogy, nearly 30 arthropod-borne human diseases are well recognized and many of them have emerged over the past years (WHO 2020). Quite often, most such pathogens propagate unnoticed in enzootic tick-vertebrate cycles in wild natural conditions but may pose significant morbidity and mortality when spilling over to domestic animals or humans (Jahfari and Sprong 2016). Undoubtedly, ticks and tick-borne diseases pose a major "One Health" issue for humans and animals, worldwide, as they can disseminate an extensive array of pathogens causing anaplasmosis, babesiosis, Lyme disease borreliosis, Rocky Mountain spotted fever, Crimean-Congo hemorrhagic fever, Kyasanur forest disease, Tickborne encephalitis (TBE), etc. and incite direct damage to its host (Kumar *et al*. 2020, Zhao *et al*. 2020). However, the concept of one health has not been suitably implemented due to insufficiency or the absence of transdisciplinary coordination in many developing countries. The problem is handled independently by animal and human health professionals and thus the magnitude of the problem has been further multiplied and reached a very alarming stage were at least 70% of emerging and re-emerging diseases are either zoonotic (spread from animals to humans) or vector-borne (carried from infected animals to others through insects).

However, single recombinant antigens are seldom sufficient against complex parasites like ticks. The Bm86 antigen, from the gut epithelium of *R. microplus* showed an appreciable but variable degree of efficacy (0 to 91%) in various geographical areas (de la Fuente *et al*. 2007). Practically, almost all single antigen vaccine using native antigen and their recombinant form is inefficacious to combat the infection (Willadsen 2008). Hence, contrary

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to a whole (single) antigen-based vaccine, it is expedient to use multi-epitope vaccines that have considerable advantages such as safety, specificity, and cost-effectiveness.

To address this affliction, the implication of protein derived subunit vaccine that includes short but remarkably immunogenic peptides seems promising as ticks are too complex to be used as a whole vaccine. Although pioneering technologies and a huge database are available, it is yet to be fully explored in tick vaccine development. A comprehensive understanding of tick immunity might give a leg up to developing highly successful vaccines. For instance, TGE (tick salivary gland extract) or saliva is reported to have immune-modulatory, anti-hemostatic, and vasodilatory bioactive compounds possessing potent pharmacological activity which are released to temper and exempt host immune response for their co-survival with the host (Bowman *et al*. 1997). Surprisingly, these chemicals subdue the pro-inflammatory response of macrophages by suppressing cytokine release and dramatically alter the proliferation of B and T lymphocytes (Ferreira and Silva 1998). Moreover, multiple components from the saliva of different ticks have been found to modulate host natural killer (NK) cells and complement system activation (Oliveira *et al*. 2010). Moreover, it has been recorded that dismantling the regular migration, maturation, and differentiation of dendritic cells (DCs) *in vitro/in vivo* by inhibiting IL-1, TNF-∝ and by propagating Th-2 polarised state is characterized by pronounced IL-4 and IL-10 level and diminished IFN-γ and IL-12 level (Hannier *et al*. 2003). The DCs are proficient antigen-presenting cells and chief enablers of T-cell activation thus, their modulation provided an appropriate opportunity to ticks for evading the host's immune responses that settle up with the host for the unimpeded meal and pathogen transmission (Cavassani *et al*. 2005). On this account, any halt in the precedent accord between the host and tick could result in a feasible tick control strategy. Here we propose to design multiepitope peptides against multi-tick species using CD8+ T cell epitope in combination with B and HTL epitopes from different antigenic proteins in an attempt to subvert this immunomodulatory state to the pro-inflammatory state. The multiepitopic peptides predictably have the potential to elicit strong cellular and humoral responses in hosts to combat multi-tick species infestation. Very similar initiatives pursuing a strategic approach to discover the epitope ensemble vaccine against SARS-CoV-2019 (Bibi *et al*. 2021), Ebola virus (Ullah *et al.* 2020), *Acinetobacter baumannii* (Solanki and Tiwari 2018), *Leishmania*, Onchocerciasis and *Plasmodium falciparum* have been reported (Khatoon *et al.* 2017, Shey *et al*. 2019, Damfo *et al*. 2017).

## MATERIALS AND METHODS

To design multi-epitopic peptide construct, immunodominant peptides were preferentially picked based on our foregoing laboratory experiments. A series of web servers were used to evaluate various interactions of the proposed MEV model with potential receptors with their physiochemical and immunological parameters. A summary of the general workflow is illustrated in the schematic diagram (Fig. 1).

### Retrieval of target protein sequences

In the preliminary experimental set, for designing multi-epitope peptides, firstly the amino acid sequences of Boophilin (PDB id: 2ODY), (CRPTI_RHIMP) Protease inhibitor carrapatin (UniProt id- P81162) and Rms-17 (the secretory serpin plays a pivotal role in the tick feeding process and pathogen transmission) [NOV41407 (Porto Alegre strain, Brazil).

Supplementarily, to ensure the phylogenetic relatedness of the amino acid sequences, target proteins from other members of the related tick family were also retrieved. To enhance the immunogenicity activation-associated secreted protein-1 of *Onchocerca volvulus* (A0A044VD00), a TLR-2 agonist was chosen as an adjuvant.

### Prediction of linear B-cell epitope

The B-cell epitopes are crucial for vaccine design as they stimulate the humoral immune response. Two different servers, BepiPred-2.0 (https://services.healthtech.dtu.dk/service.php?BepiPred-2.0) and ABCpred (https://webs.iiitd.edu.in/raghava/abcpred/ABC_submission.html) were used to predict B-cell

**Table 1. Presenting statistics of interaction of MAP construct with TLR2 (Cluspro 2.0 server)**.

| | Cluspro 2.0 (Weighted Score) | | | |
|---|---|---|---|---|
| **Docking Receptor (S)** | **with Balanced score** | **energy Electrostatic score** | **energy Hydrophobic** | **VdW+Elec** |
| Bovine TLR-2 | 99 mem -1051 | 97 members -1047.9 | 119 members -1090.1 | 126 members –275.5 |

epitopes of varying lengths (Jespersen *et al*. 2017, Saha and Raghava 2006).

**Prediction of IFN-γ inducing epitope**

Interferon-gamma (IFN-γ) is a pro-inflammatory cytokine that plays a potent immunostimulatory role. It is accredited as one of the crucial links between adaptive and innate immune systems. Apart from increasing the level of response to MHC antigens, it is one of the major activators for macrophages and Natural Killer (NK) cell Prediction of IFN-γ epitopes for the designed peptide was accomplished using IFN epitope server (http://crdd.osdd.net/raghava/ifnepitope/scan.php) (Dhanda *et al*. 2013).

**Construction of multi-epitope peptide sequence**

From the immunoinformatics prediction, a vaccine sequence was constructed using highly conserved epitopes. Thereafter, by introducing consecutive GPGPG linkers, the different epitopes were combined as a linear peptide. Likewise, in an attempt to amplify the immunogenicity activation-associated secreted protein-1 of *Onchocerca volvulus*, a TLR-2 agonist was integrated as an adjuvant (UniProt accession no. A0A044VD00) at the N-terminus exerting a linker (EAAAK).

**Prediction of immunogenicity and non-allergenic property of the construct**

VaxiJen V2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) tool was used to predict the antigenicity of the protein. Antigenicity had been predicted by an alignment-free approach based on auto cross-covariance (ACC) transformation of protein sequences into uniform vectors of principal amino acid properties with an input of the final protein sequence as a plain format.

**Table 2. Presenting statistics of interaction of MAP construct with TLR2 (HADDOCK 2.4 server).**

| Receptor | TLR-2 |
|---|---|
| HADDOCK score | -36.1 +/- 9.5 |
| Cluster size | 9 |
| RMSD from the overall lowest-energy structure | 0.4 +/- 0.2 |
| Van der Waals energy | -35.1 +/- 3.6 |
| Electrostatic energy | -329.1 +/- 21.8 |
| Desolvation energy | -7.3 +/- 3.6 |
| Restraint's violation energy | 721.4 +/- 72.4 |
| Buried Surface Area | 1577.0 +/- 55.7 |
| Z-Score | -2.2 |

AllerTOP v2.0 (https://www.ddg-pharmfac.net/AllerTOP/method.html) and AllergenFP (https://ddg-pharmfac.net/AllergenFP/) were used to predict multi-epitope vaccine allergenicity (Dimitrov *et al*. 2014a, Dimitrov *et al*. 2014b).

**Evaluation of physicochemical properties**

The physiochemical parameters such as amino acid composition, theoretical pI, instability index, *in vitro* and *in vivo* half-life, aliphatic index, molecular weight, and grand average of hydropathicity (GRAVY) were assessed using the web server ProtParam (http://web.expasy.org/protparam/)

**Prediction of secondary structure**

The e-services aided by PSIPRED, and Raptor-X property were utilized (McGuffin *et al*. 2000, Kallberg *et al*. 2014). The RaptorXProperty web server (http://raptorx.uchicago.edu/StructurePropertyPred/predict/) predicts the secondary structure properties of the protein chain through a template-free approach.

**Prediction of tertiary structure**

The input sequence was processed by the RaptorX server which enables anticipation of 2D and 3D protein structures coupled with DISO and ACC values.

**Refinement of the tertiary structure**

The 3D model of the multi-epitopic peptide was later subjected to refinement by GalaxyRefine server (http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE) progressively in two stages.

Structural changes concerning the initial model are expressed in the form of different scores like GDT-HA, RMSD, MolProbity. The Clash score, Poor rotamers and Rama favored are the three different elements of MolProbity score (Heo *et al*. 2013).

**Validation of tertiary structure**

After selecting 3D structure model, another crucial step is the validation of the tertiary structure for the identification of any potential error. ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php) and Molprobity server (http://molprobity.biochem.duke.edu) were used for tertiary structure validation. The protein structural analysis (ProSA web) is a freely accessible tool used for protein structure prediction and refinement for theoretical and experimental proteins (Wiederstein and Sippl 2007).

The molprobity server was used to attain the Ramachandran plot for the protein structure (Davis *et al*. 2007).

**Prediction of linear and discontinuous B cell epitope**

Linear and conformational epitopes are formed by the close vicinity of the amino acid residues which were very distantly located in the native linear chain, though; owing to the conformational folding of tertiary protein structure they get aligned into the neighborhood. For the prediction of discontinuous B-cell epitopes in the validated protein structure, Ellipro (www.iedb.org) web tool was used.

**Molecular docking of the constructs with bovine TLR-2**

The type and level of molecular interaction of the antigenic molecule to that of the immune receptor characterize the degree of generated immune responses and thus identification of active sites of the immune receptor is very crucial. In the present study, freely accessible bio-informatics tools named COPORT and SPPIDER were used for the prediction of active sites in TLR-2 and construction. For the prediction of the aforesaid molecular interactions / probable immunogenic trigger, structural complex-based docking of 'designed multi epitopic peptide - TLR-2' receptors was performed. The docking tools work on different algorithms to derive a near-native structural computation. Hence, we preferred to use two different best-rated servers to validate the results. The basis for using two docking servers was: Haddock 2.4 (https://wenmr.science.uu.nl/haddock2.4/) and ClusPro 2.0.

**Molecular dynamics simulation (MDS)**

In an attempt to ascertain the composite atomic motions of diverse proteins and nucleic acid molecules, a molecular dynamics study is done. In this experiment, a molecular dynamics simulation study was conducted for the designed vaccine construct recruiting the iMODSweb-server (http://imods.Chaconlab.org/).

**Codon optimization and in-silico cloning**

Codon optimization contributes immensely when codon expression and usage of the host organism differs significantly from that of the parent organism. It's evident that degenerate genetic code provides freedom to multiple codons of encoding single amino acid; codon adaptation becomes essential for improving the expression rate and quality of recombinant protein in *E. coli* (strain K12). In this experiment we used Java Codon Adaptation Tool (JCat) server (http://www.prodoric.de/JCat) which

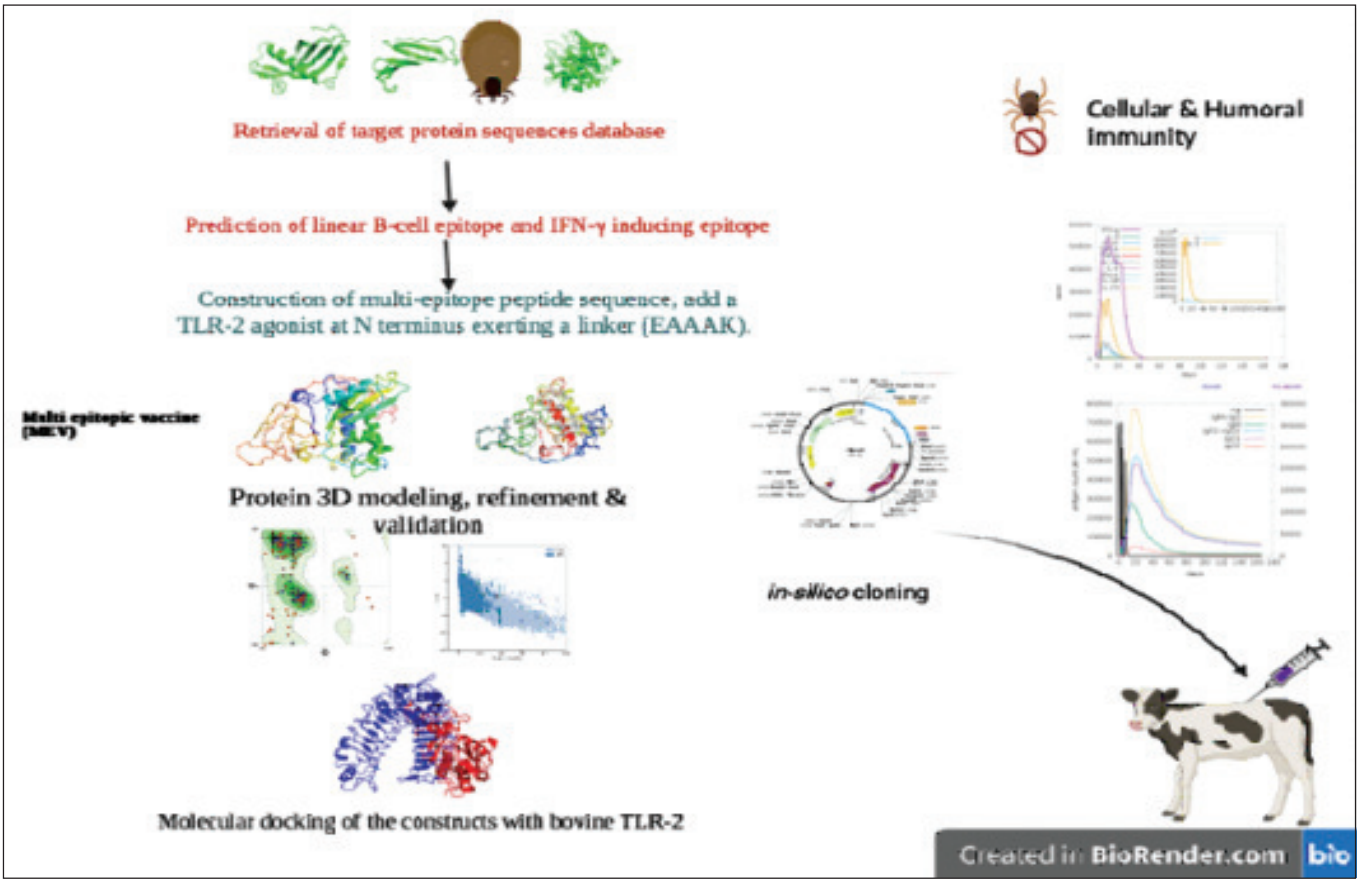


**Fig. 1. Brief summary of general workflow is illustrated in the schematic diagram (Created with biorender.com).**

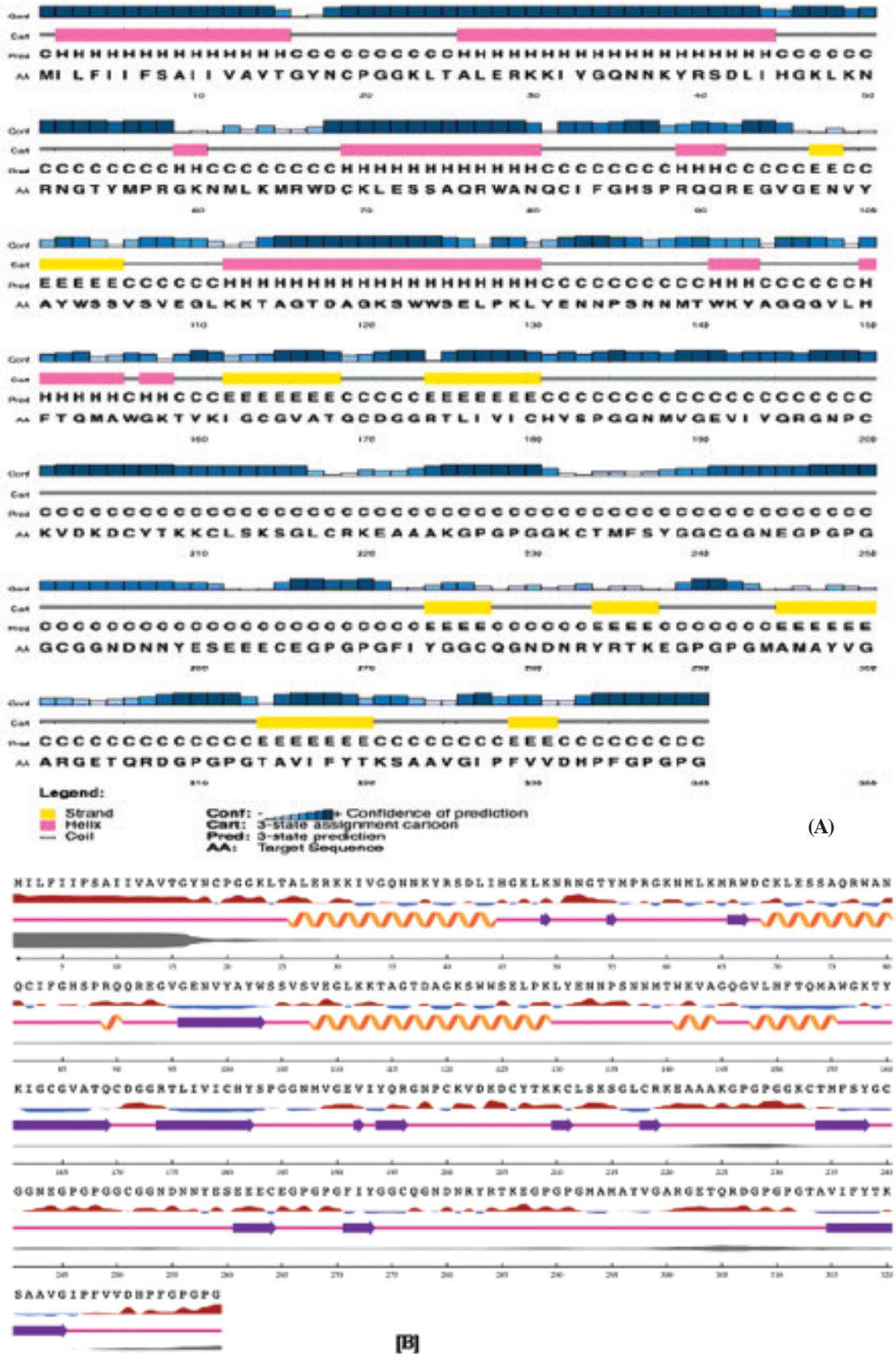


**Fig. 2. (A) Secondary structure prediction of vaccine constructs using the PSIPRED server having (23.5%) alpha-helices, (13.23%) beta-strands, and (63.2%) coils. (B) Surface accessibility of the final vaccine prediction using RaptorX Property server. The Red color represents the exposed residues (57%), and the blue colour represents the buried residues(27%).**

provides CAI values (Codon Adaptation Index) and GC contents within a range of 0.8-1 and 30-70%, respectively, to determine the degree of protein expression (Grote *et al*. 2005).

Later, the optimized codon sequence of the designed multi-epitope construct was cloned *in E. coli* plasmid vector pET-30a (+). Further, two restriction sites, NdeI and HindIII were inserted before (N terminus) and after (C-terminus) in the appraised sequence, respectively. Finally, the expression in the vector was confirmed using SnapGene software (www. Snapgene.com).

### Immune simulation

C-IMMSIM server (http://kraken.iac.rm.cnr.it/C-IMMSIM/index.php?page=1) was used to evaluate the immune stimulation and obtain an immune response profile by in silico simulation. Vaccine injection without LPS was selected and three doses of the vaccine were adjusted at 45 eight-hour-long steps that equal to a 15 days interval.

## RESULTS AND DISCUSSION

After meticulously screening a large number of epitopes using BepiPred-2.0 web and ABCpred servers, 35 B-cell epitopes based on their properties like non-allergenic, antigenic, peptide physiobiochemical properties, etc. were selected. Finally, three epitopes were selected based on their conservancy, exposed nature, and absence of transmembrane helices (TMHMM Server v. 2.0).

IFN-γ epitopes were predicted from all the short-listed epitopes using the IFN epitope. A total of 5 INF-γ inducing epitopes were used for the final construct.

From the above-predicted epitopes, a sequence was constructed using linkers. With the addition of GPGPG linkers, the different epitopes were combined as linear peptides. Three B cell epitopes and 5 INF-γ were selected to design the construct. After integration of adjuvant the final construct was found antigenic (0.706), non-allergenic, soluble, and non-toxic.

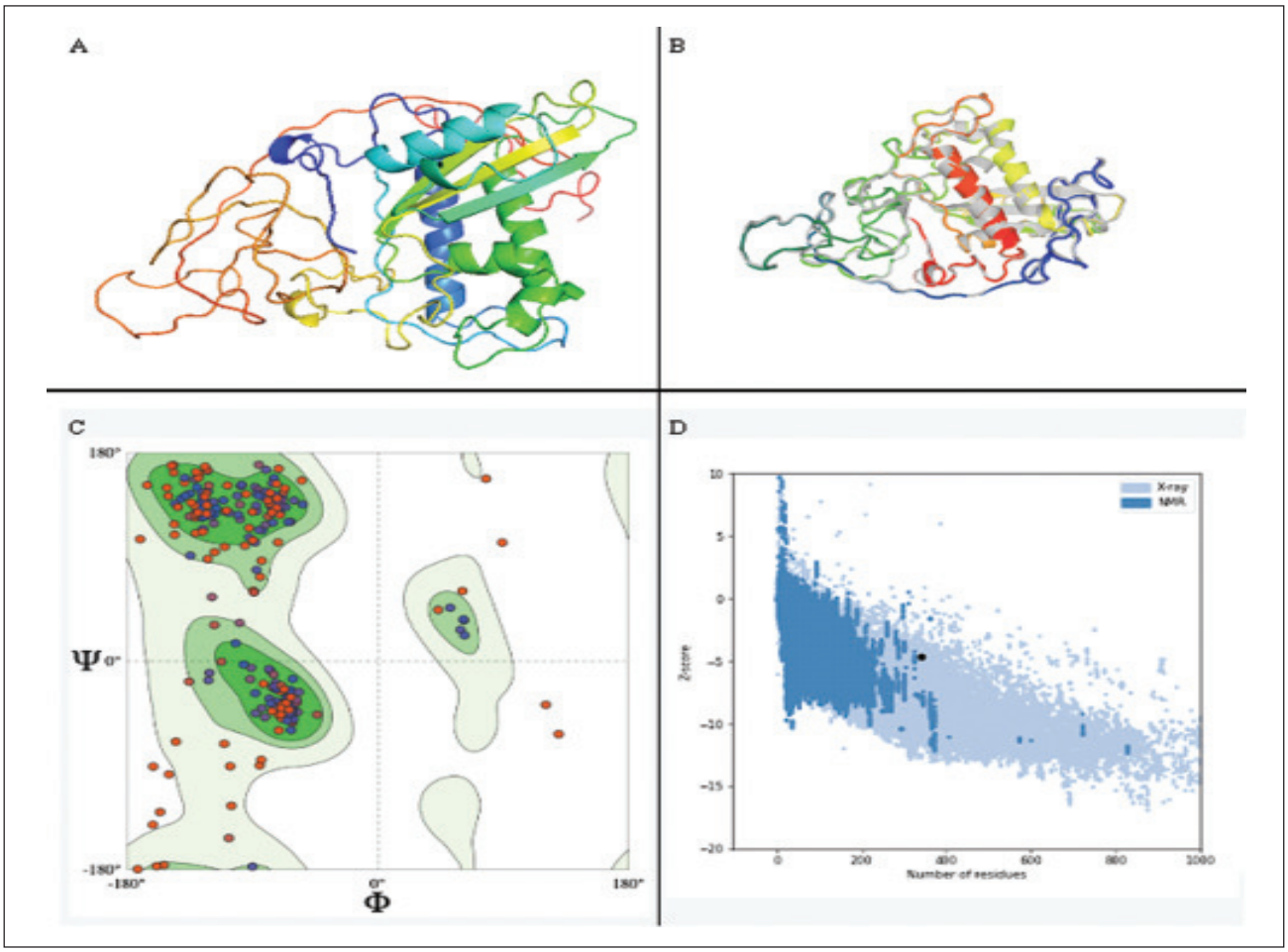


**Fig. 3. Protein 3D modeling, refinement, and validation.** [(A). The 3D model of a multi-epitope vaccine was obtained on the Phyre2 server following homology modeling. (B). Refinement: superimposition by the GalaxyRefine server of a refined 3D structure (colored) on a 'crude model' (gray). (C). Validation: Ramachandran plot analysis showing 90.53% in favored, 6.51% in allowed, and 2.96% in disallowed regions of protein residues and (D) ProSA-web, with a Z score of - 4.65].

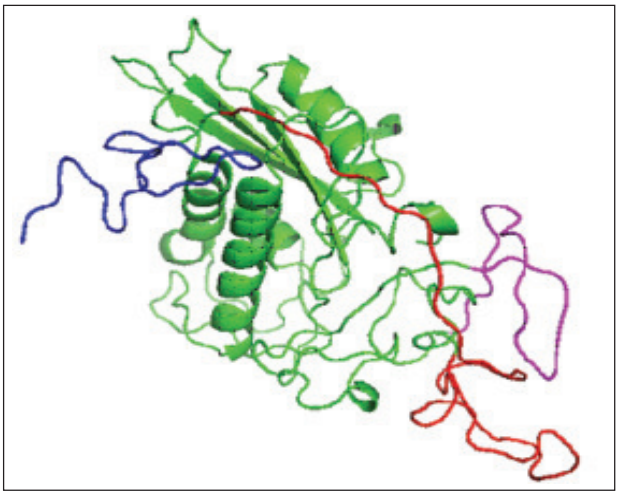

**Fig. 4. Linear B-cell epitopes prediction for the final subunit vaccine construct**. [Three linear B- cell epitopes have been shown in Blue (312-340), Red (266-309) and Magenta color (224-246)].

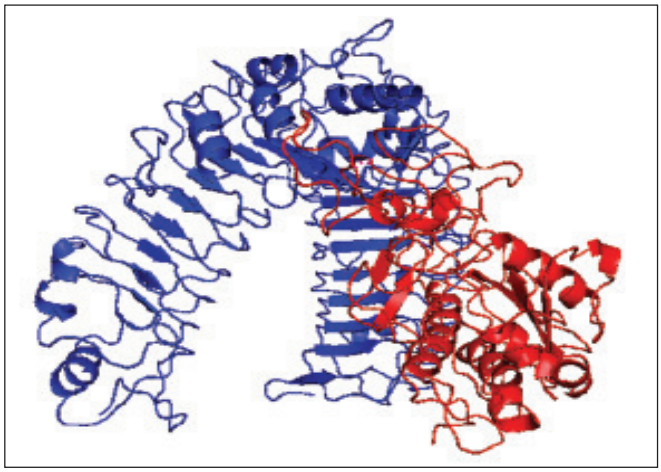

**Fig. 5. Docked complex of subunit vaccine construct with Bovine TLR (PDB ID: 2Z7X: A chain).**

The development of multiantigen cross-protective ticks vaccine is targeted in some laboratories with variable efficacy. Imamura *et al* (2006) reported that when two different serpins (RAS-1 and RAS-2) derived from *R. appendiculatus* were combined, it gave a significant protective immunity against ticks. In another vaccination trial using a multi-antigen vaccine consisting of four salivary antigens (Rm239, Rm76, Rm39, and Rm180) specific antibody production was efficiently stimulated against two antigens only, Rm239 and Rm76, conferring as the efficacy of 73.2% against *R. microplus* (Maruyama *et al.* 2017). In an alternative pathway, in the ongoing consideration, an effort was made to design a multi-epitopic peptide construct comprising immunodominant peptides, selected from different salivary tick proteins which are preferentially picked based on solubility and exposed nature of that protein. Indeed, protein abundance is one of the key features for the

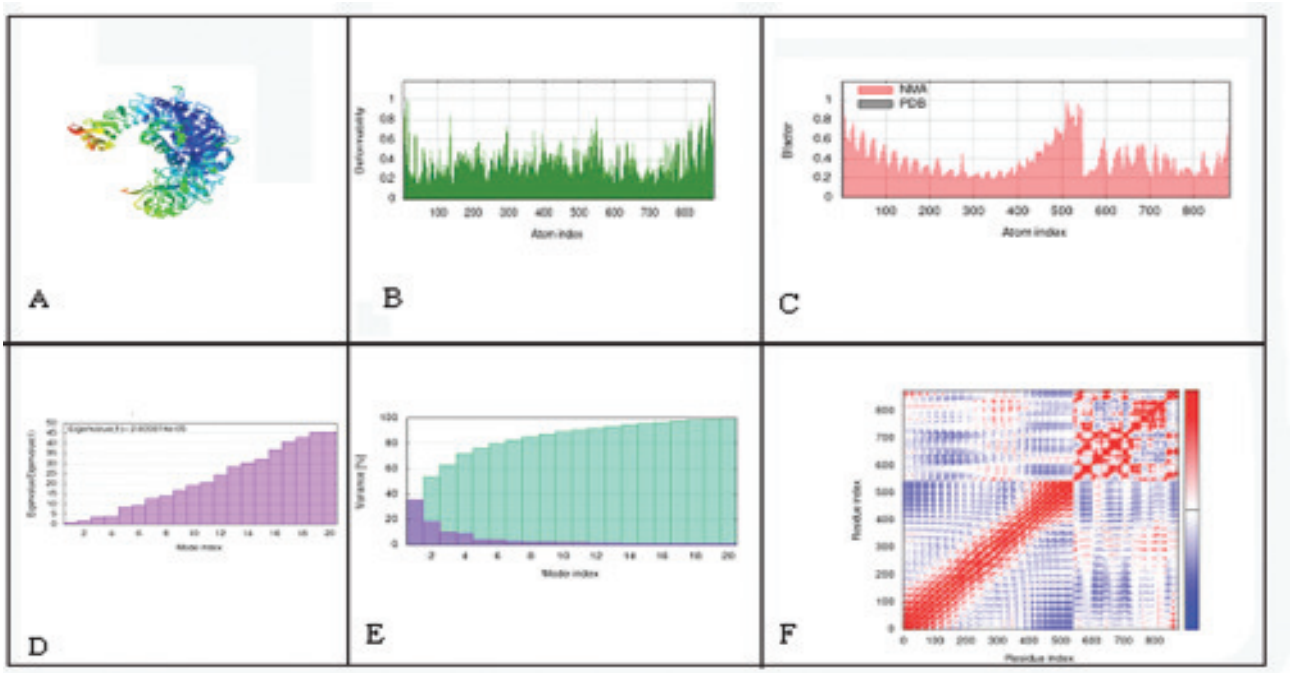


**Fig. 6. The results of molecular dynamics simulation of vaccine construct and TLR-4 docked complex.** [(A) NMA mobility, (B) deformability, (C) variance (red color indicates individual variances and green color indicates cumulative variances), (D) eigenvalues, (E) Bfactor, (F) co-variance map (correlated (red), uncorrelated (white) or anti-correlated (blue) motions) and (G) elastic network (darker gray regions indicate stiffer regions)].

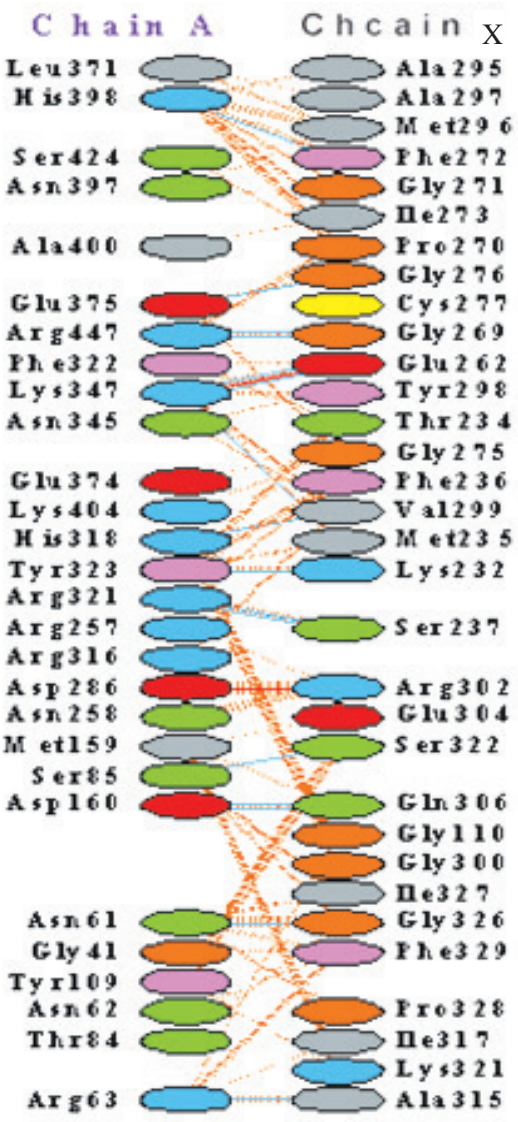


**Fig. 7. Schematic diagram of interactions between protein chains.** [Interacting chains are joined by coloured lines, each representing a different type of interaction, as per the key above. The area of each circle is proportional to the surface area of the corresponding protein chain].

selection of antigens, as this type of abundant protein is more likely to be processed and presented by MHC molecules to the T lymphocytes, and the protein abundance correlates with the peptide immunodominance (Petitdidier *et al*. 2019). The RmS-17, the saliva secretory serpin is involved in the immune evasion of the tick by inhibiting eosinophil, neutrophil-mediated inflammation, and platelet aggregation. A probable construct including multiple B and INF-γ epitopes from the selected tick proteins was designed and evaluated various physiochemical, and immunological characteristics and their interactions with potential receptors using a series of web servers.

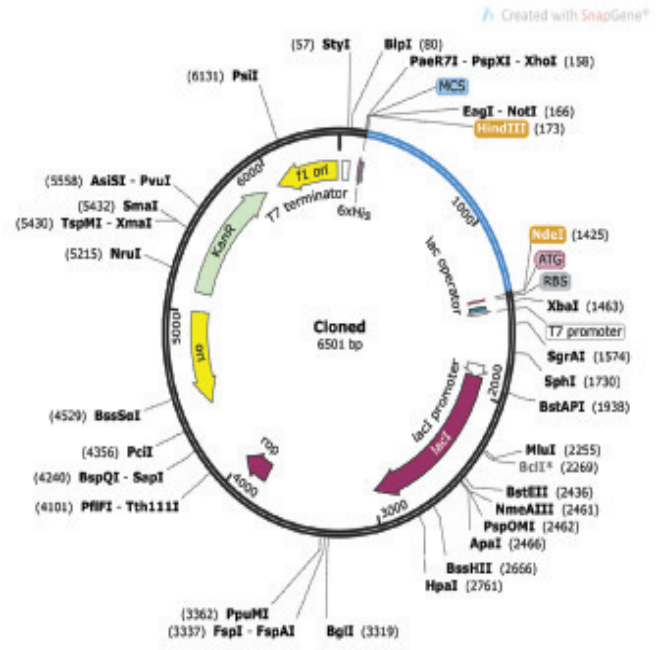


**Fig. 8. Final vaccine *in-silico* cloning into the pET30a (+) vector.** [Te codon sequence of the final vaccine is in red, which is a 1020 bp gene sequence generated by the JCat server. The pET30a (+) expression vector is in black. The codon sequence is inserted between HindIII (173) and NdeI (1425), forming a clone with a total length of 6285 bp. This image was created by SnapGene].

The final chimera was improved by employing specialized spacer sequences. Previously reported GPGPG linker was incorporated between our predicted epitopes to produce sequences with minimized junctional immunogenicity, therefore allowing the rational design of a potent multi-epitope construct.

It has been reported that tick salivary proteins can suppress T cell activities and generate Th2 immune responses that may induce immune suppression activity. In addition, tick saliva can also suppress interferon (IFN)-γ production by the T lymphocytes (Ramachandra and Wikel 1992). So, to combat such immune modulation events within a host, we have designed a chimeric vaccine incorporating different kinds of epitopes. Immuno-informatics analysis of the generated constructs indicated that it contains a significant number of high-affinity interferon-γ (IFN-γ) epitopes and linear B-cell epitopes.

The molecular weight of the final chimera construct was 36.49 kDa and it was predicted to be soluble upon expression and was consistent with its simulated immunogenicity. The theoretical pI of the predicted protein is 9.10 indicating that the construct is acidic. The predicted instability index (II) is 23.02 which specifies that the protein will be stable upon expression. The computed GRAVY and the aliphatic index showed that the protein

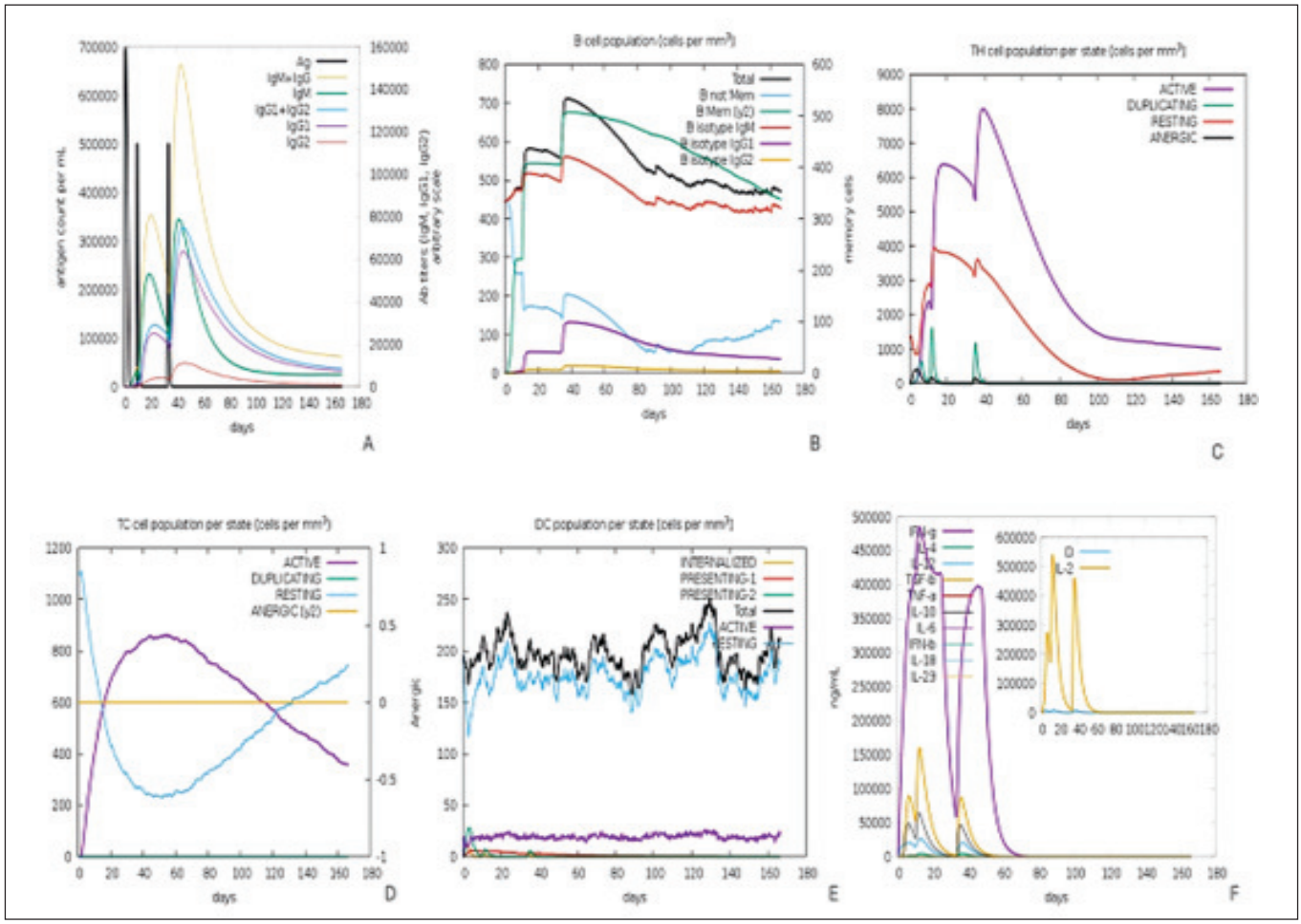


**Fig. 9. *In-silico* immune simulations in the host in response to MEV administration.** [A. Intermittent boosts in B-cell populations after secondary and tertiary doses. B. Number of B-cell types in three successive immune responses. C. Helper T-cell populations after three shots. D. Cytotoxic T-cell populations after three vaccine administrations. E. Dendritic cells per state. F. Generation of cytokines and interleukins at three different stages].

contains aliphatic side chains, symbolizing potential hydrophobicity. The secondary structure of the final construct analysis demonstrated that the protein consisted pre- dominantly of coils (63.2%), with 23.5% and 13.23% residues being alpha-helices and beta-strands, respectively (Fig. 2). In nature, the unfolded protein portion and alpha-helical coils have been reported to be one of the important forms of "structural antigens" (Corradin *et al.* 2007). The 3D structure of the final construct improved strikingly after the refinement with the GalaxyRefine server and showed desirable results on Ramachandran plot predictions. The Ramachandran plot shows that most of the residues are in the favored and allowed regions (90.5% and 6.5%) with very few residues (2.96 %) in the outlier region; a sign of the satisfactory model (Fig.3).

A recent study has reported the involvement of TLR-2 with tick infestation and blood histamine levels in bovine (Zhao *et al.* 2021). Stimulated dendritic cells generate lipopolysaccharide, the ligand of TLR-2, and ultimately weaken host immunity predisposing to tick infestation (Oliveira *et al*. 2010). To potentiate immune interaction between TLR-2 and the chimeric construct, a data-driven protein-protein docking analysis was performed and a TLR-2 ligand was used as an adjuvant with the designed chimera. Furthermore, molecular docking of the final construct with TLR-2 was performed in the ClusPro 2.0 and Haddock 2.4 servers to compare and validate the accuracy, stable protein-protein binding, and molecular interactions. It was found that the molecular docking was significant with negative energy values (Table-1 and 2, Fig. 5) of the top-ordered protein-protein docking complex. The lowest energy score of these complexes reflects the highest binding affinity between TLR and protein structure (Pandey *et al.* 2018). The molecular dynamic simulation results confirmed that the final construct can interact with TLR-2 suitably.

Using the C-ImmSim platform, we evaluated the vaccine construct's capacity to induce a significant

immunological response if it was given in a real-world circumstance. The findings demonstrated that after the primary immune response, the secondary and tertiary responses increased steadily. Antibodies (IgM+IgG, IgG1+IgG2, and IgG1) concentrations were found to have risen significantly (Fig. 9A). A considerable spike in levels of antibodies (IgM+ IgG, IgG1+IgG2 and IgG1 antibodies) was observed (Fig. 9A). The number of B-cells also increased noticeably, and IgG1 biotypes were seen along with considerable memory cell development (Fig. 9B). Active T-cells were seen to dramatically rise after getting a secondary and tertiary injection dose; however, they gradually reduced at later stages (Fig. 9C, D). According to Fig. 9E, levels of dendritic cells (DCs) and macrophages increased significantly following vaccine administration. Additionally, increased levels of cytokines (such as (IFN-γ) were observed (Fig. 9F). These findings suggest that the protein could stimulate a protective immunological response.

Further, the final requirement to authenticate *the in-silico* construct is an expression of the recombinant protein in a suitable host. Preferably, *Escherichia coli* (strain K12) expression systems have opted for the production of recombinant proteins followed by Codon optimization for achieving efficient recombinant protein expression within *the E. coli* expression system. The assessment values of the codon adaptability index (0.9731) and the GC content (56.14%) are complementing high-level protein expression in *the E. coli* system (Fig. 8). However, the experimental validation of the chimeric protein is needed to support the present study. A very scanty number of such studies have been carried out because of the limited availability of validated crystallographic protein structures (PDB) belonging to different tick species (Aguirre *et al*. 2018). To confront TTBDs in an efficient and environmentally friendly manner, new generation tick vaccine studies should be globally endorsed and explored embracing the "One Health" approach.

## REFERENCES

Aguirre AAR, Garcia MV, da Costa IN, Csordas BG, da Silva Rodrigues V *et al*. (2018) New records of tick-associated spotted fever group Rickettsia in an Amazon-Savannah ecotone, Brazil. Ticks Tick Borne Dis 9(4): 1038-1044.

Bibi S, Ullah I, Zhu B, Adnan M, Liaqat R *et al*. (2021) *In silico* analysis of epitope-based vaccine candidate against tuberculosis using reverse vaccinology. Scientific Reports 11(1): 1-16.

Bowman AS, Coons LB, Needham GR, Sauer JR (1997) Tick saliva: recent advances and implications for vector competence. Med Vet Entomol 11(3): 277-285.

Cavassani KA, Aliberti JC, Dias AR, Silva JS, Ferreira BR (2005) Tick saliva inhibits differentiation, maturation and function of murine bone-marrow-derived dendritic cells. Immunol 114(2): 235-245.

Corradin G, Villard V, Kajava AV (2007) Protein structure-based strategies for antigen discovery and vaccine development against malaria and other pathogens. Endocrine, Metabolic and Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine and Metabolic Disorders) 7: 259-265.

Damfo SA, Reche P, Gatherer D, Flower DR (2017) *In silico* design of knowledge-based *Plasmodium falciparum* epitope ensemble vaccines. J Mol Graph Model 78: 195-205.

Davis IW, Leaver-Fay A, Chen VB, Block JN, Kapral GJ *et al*. (2007) MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. Nucleic Acids Res 35(suppl_2): W375-W383.

de la Fuente J, Almazán C, Canales M, de la Lastra JMP, Kocan KM *et al*. (2007) A ten-year review of commercial vaccine performance for control of tick infestations on cattle. Anim Health Res Rev 8(1): 23-28.

Dhanda SK, Vir P, Raghava GP (2013) Designing of interferon-gamma inducing MHC class-II binders. Biol Direct 8(1): 1-15.

Dimitrov I, Bangov I, Flower DR, Doytchinova I (2014a) AllerTOP v. 2-a server for in silico prediction of allergens. J Mol Model 20(6): 1-6.

Dimitrov I, Naneva L, Doytchinova I, Bangov I (2014b) AllergenFP: allergenicity prediction by descriptor fingerprints. Bioinformatics 30(6): 846-851.

Ferreira BR, Silva JS (1998) Saliva of *Rhipicephalus sanguineus* tick impairs T cell proliferation and IFN-γ-induced macrophage microbicidal activity. Vet Immunol Immunopathol 64(3): 279-293.

Grote A, Hiller K, Scheer M, Münch R, Nörtemann B *et al*. (2005) JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. Nucleic Acids Res 33(suppl_2): W526-W531.

Hannier S, Liversidge J, Sternberg JM, Bowman AS (2003) Ixodes ricinus tick salivary gland extract inhibits IL-10 secretion and CD69 expression by mitogen-stimulated murine splenocytes and induces hyporesponsiveness in B lymphocytes. Parasite Immunol 25(1): 27-37.

Heo L, Park H, Seok C (2013) GalaxyRefine: Protein structure refinement driven by side-chain repacking. Nucleic Acids Res 41(W1): W384-W348.

Imamura S, Namangala B, Tajima T, Tembo ME, Yasuda J *et al*. (2006) Two serine protease inhibitors (serpins) that induce a bovine protective immune response against *Rhipicephalus appendiculatus* ticks. Vaccine 24(13): 2230-2237.

Jahfari S, Sprong H (2016) Emerging tick-borne pathogens: ticking on Pandora's box. Ecology and prevention of Lyme borreliosis 4:127-147. DOI : 10.3920/978-90-8686-838-4-9.

Jespersen MC, Peters B, Nielsen M, Marcatili P (2017) BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res 45(W1): W24-W29.

Källberg M, Margaryan G, Wang S, Ma J, Xu J (2014) RaptorX server: a resource for template-based protein structure modeling. In: Protein structure prediction (17-27). Humana Press, New York, NY.

Khatoon N, Pandey RK, Prajapati VK (2017) Exploring Leishmania secretory proteins to design B and T cell multi-epitope subunit vaccine using immunoinformatics approach. Sci rep 7(1): 1-12.

Kumar B, Manjunathachar HV, Ghosh S (2020) A review on *Hyalomma* species infestations on human and animals and progress on management strategies. Heliyon 6(12): e05675.

Maruyama SR, Garcia GR, Teixeira FR, Brandão LG, Anderson JM *et al*. (2017) Mining a differential sialotranscriptome of *Rhipicephalus microplus* guides antigen discovery to formulate a vaccine that reduces tick infestations. Parasites vector 10(1): 1-16.

McGuffin LJ, Bryson K, Jones DT (2000) The PSIPRED protein structure prediction server. Bioinformatics 16(4): 404-405.

Oliveira CJF, Carvalho WA, Garcia GR, Gutierrez FR, de Miranda Santos IK *et al*. (2010) Tick saliva induces regulatory dendritic cells: MAP-kinases and Toll-like receptor-2 expression as potential targets. Vet Parasitol 167(2-4): 288-297.

Pandey RK, Bhatt TK, Prajapati VK (2018) Novel immunoinformatics approaches to design multi-epitope subunit vaccine for malaria by investigating anopheles salivary protein. Scientific Reports 8(1): 1-11.

Petitdidier E, Pagniez J, Pissarra J, Holzmuller P, Papierok G *et al*. (2019) Peptide-based vaccine successfully induces protective immunity against canine visceral leishmaniasis. npj Vaccines 4(1): 1-9.

Ramachandra RN, Wikel SK (1992) Modulation of host-immune responses by ticks (Acari: Ixodidae): effect of salivary gland extracts on host macrophages and lymphocyte cytokine production. J Med Entomol 29(5): 818-826.

Saha S, Raghava GPS (2006) Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins 65: 40-48.

Shey RA, Ghogomu SM, Esoh KK, Nebangwa ND, Shintouo CM *et al*. (2019) *In-silico* design of a multi-epitope vaccine candidate against onchocerciasis and related filarial diseases. Scientific Reports 9(1): 1-18.

Solanki V, Tiwari V (2018) Subtractive proteomics to identify novel drug targets and reverse vaccinology for the development of chimeric vaccine against *Acinetobacter baumannii*. Scientific Reports 8(1): 1-19.

Ullah A, Sarkar B, Islam SS (2020) Exploiting the reverse vaccinology approach to design novel subunit vaccine against ebola virus. Immunobiology 20: 151949.

WHO (2020) https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases.

Wiederstein M, Sippl MJ (2007) ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res 35(Suppl_2): W407-W410.

Willadsen P (2008) Antigen cocktails: valid hypothesis or unsubstantiated hope? Trends parasitol 24(4): 164-167.

Zhao GP, Wang YX, Fan ZW, Ji Y, Liu MJ *et al*. (2021) Mapping ticks and tick-borne pathogens in China. Nat Commun 12(1): 1-13.

Zhao S, Yang M, Liu G, Hornok S, Zhao S *et al*. (2020) Rickettsiae in the common pipistrelle *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) and the bat soft tick *Argas vespertilionis* (Ixodida: Argasidae) Parasites Vectors 13(1): 1-6.

***Cite this article as:** Nandi A, Manisha, Solanki V, Tiwari V, Sajjanar B, Sankar M, Saini M, Shrivastava S, Bhure SK, Ghosh S (2022) Designing of multi-epitope vaccine construct employing immuno-informatics approach to combat multi-tick species infestations. Explor Anim Med Res 12(2): 149-159. DOI: 10.52635/eamr/12.2.149-159.