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Design of Innovative multiepitope mRNA-based vaccine against Theileria annulata infection in cattle using immunoinformatic and molecular modelling approaches

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Abstract

Theileriosis is a tick-borne disease that affects ruminants in tropical and subtropical areas and causes clinical signs ranging from mild to severe illness. *Theileria annulata* primarily infects cattle and cause severe economic losses in livestock. Chemotherapeutic agents are used to treat theileriosis in animals; however, these agents can not completely eradicate the pathogen from animals and promote the establishment of carrier status. Vaccination is an essential option for controlling and preventing theileriosis. This study aimed to design an effective vaccine against *Theileria annulata* infection in cattle using immunoinformatics and modeling approaches. For this purpose, *Theileria annulata* sporozoite surface antigen was selected as a target protein. Immunoinformatics tools were used to select non-homologous to host (cattle) and antigenic epitopes from the selected protein. The proposed multiepitope vaccine was generated by combining five cytotoxic T cells, six helper T cell epitopes, and four linear B-lymphocyte epitopes. Additionally, peptide RS09 was added as an adjuvant to increase the antigenicity of the proposed candidate. Immunoinformatics analysis of the immunogenicity and physiochemical profile of the proposed vaccine shows its antigenic (1.0120) and nonallergenic, stable, and hydrophilic. Furthermore, the docking study of bovine TLR4 with the proposed vaccine shows a strong interaction between the two molecules with low energy and high stability. The proposed vaccine can be a promising option against *Theileria annulata* infection in cattle; however, laboratory-based studies are needed to develop the vaccine candidate.

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Introduction

Theileriosis in cattle is a prevalent tick-borne disease in sub-tropical and tropical areas. Several species of *Theileria* are causing the disease in bovines, leading to high economic losses (1-3). *T. annulata*, *T. parva*, *T. mutans*, *T. tarurotragi*, *T. velifera*, and *T. sinensis* can infect cattle of different ages and cause the disease (4-6). *Theileria* spp. is unlike other Apicomplexa genera, such as *Plasmodium or Toxoplasma*, because of some unique features. *Theileria* exists free inside the cell and connects with the host microtubules, directly forming schizonts (6). The hard ticks of the genus Haemaphysalis, Rhipicephalus, and Hyalomma are the main vectors of the Theileria (3-8). *Theileria annulata* is a hemoprotozoan parasite that causes bovine tropical theileriosis (Mediterranean theileriosis), which highly infects cattle and water buffaloes and leads to economic losses and some health complications (9-11). The distinct

signs are fever, anemia, respiratory disorders, enlargement lymph node size, and emaciation with low milk production (4). Mostly, the untreated infected animals will die after 20-25 days. The epidemiology and genetic variety of *Theileria* species still need to be fully understood as other tick-borne microorganisms (12). Various isolates of *Theileria annulata* are different in their virulence in the same infected area. Thus, vaccination with attenuated parasitic cells is active only with local isolates but not heterologous (13). Also, this type of vaccine reduces the efficiency of drugs usually used against theileriosis (14,15). Moreover, in some cattle, live attenuated vaccines may cause transmission of the infection to other animals (16). Multiepitope vaccines are safe and effective compared with the old live attenuated vaccines available. They are also low-cost and easy to store (17). This vaccine can provide immunity against many microorganisms, viruses, and parasites (18-20). A multiepitope-based vaccine is a modern vaccine that depends on protein epitope prediction. It is a branch of reverse vaccinology that uses bioinformatics to predict selected protein epitopes to design the vaccine candidate, starting with genome sequencing and analysis (21). *Theileria annulata* sporozoite surface protein (TaSP) is a highly antigenic parasite surface membrane protein that can be used to diagnose tropical theileriosis (22). A designed experiment in Sudan resulted in 100% protection. However, these results cannot be the same in all countries because a live attenuated vaccine uses a local isolate (23).

The current study used immunoinformatic tools to design an effective multiepitope vaccine for *T.annulata* infection in cattle.

Materials and methods

The processes in which the mRNA multiepitope vaccine construes are presented in (Figure 1).

Ethical approval

The study and its design received ethical approval from the Medical Ethics Committee of Al-Qadisiyah University (Approval number 263/2024).

Sequence retrieval of protein

NCBI obtained the TaSP sequence. The TaSP protein and several epitopes were selected. The vaccine must be antigenic, nonallergic, and designed from virulence proteins for a good immune response. Including the peptide RS09 sequence in the main vaccine structure increased the antigenicity of the vaccine candidate.

Epitope selecting

The Immune Epitope Database (IEDB) web server (24) was used to select T and B cell epitopes. Default settings were used to select epitopes, except the target host was changed to a cow. High scores and overlapped epitopes were selected for further analysis. The IEDB conservancy analysis tool was used to check the conservancy of the predicted epitopes, and epitopes with 100% conservancy were selected for further analysis.

Figure 1: The schematic study workflow.

Bovine homology

The homology between predicted epitopes and all peptides against the Bovidae (taxid:9895) protein database was checked using the NCBI BLAST P. If the E-value is 0.5 or more, the epitope was considered non-homologous to bovine peptide.

Epitopes analysis

VaxiJen v2.0 (25) was used to check epitope antigenicity using the server's Default setting. Epitope allergenicity was checked with AllerTOP (26). Finally, the ToxinPred server was used to check the toxicity of the predicted epitope. Nontoxic, Antigenic, and nonallergic epitopes were combined to build the vaccine construct.

Vaccine assembly

The selected epitopes were fused using amino acid linkers (GPGPG, AAY, and KK). Amino acid linkers enhance the subunit vaccine's stability and flexibility and maintain the independent immunogenic activity of epitopes (27). Peptide RS09 is a TLR4 agonist that reports its ability to induce a robust immune response; therefore, RS09 was selected as an adjuvant and fused at the N terminal of the construct via the EAAAK amino acid linker. HBB and Rabbit beta-like globin were fused to the 3′ and 5′ ends of the mRNA vaccine construct as an untranslated region sequence (UTR) to increase the stability of the mRNA molecule (27).

Physiochemical and immunological character of the vaccine candidate

Multiepitope mRNA vaccine construct immunogenicity was predicted with VaxiJen v2.0, while Allergenicity was tested using the AllerTOP server. ProtParam was used to

predict some Physiochemical properties of the construct, such as theoretical isoelectric point (PI), molecular weight, hydropathicity index, and *in-vitro* and *in-vivo* estimated half-life (28).

Prediction of the construct Secondary structure

The Prabi server was used to build the Secondary structure of the modeled vaccine, such as the alpha helix, coils, and beta-turn (29).

Prediction of 3D structure, Refinement, and validation

The rosetta server (30) was used to build the 3D structure of the modeled vaccine. The model with a high score and good quality was chosen as a predicted structure for the construct. Then, the selected structure was refined with the Galaxy Refine web server (31). Ramachandran plot was analyzed by the SAVES v6.1 (32) to validate the refined candidate.

Docking of the modeled candidate to TLR4

The LZerD Web Server **(**33) was used to find a possible interaction between the vaccine candidate and the selected receptor. The structure of the bovine TLR4(Q9GL65) was downloaded from UniProt. Docking complexes were visualized using PyMOL software (educational version). The PDBsum web server (34) was used for further interaction between residues of the docking complex.

Codon optimization and mRNA structure

The VectorBuilder (35) is a web server that optimizes vaccine sequences' code for expression in the selected foreign host (cattle). The secondary structure of the structure was predicted using the RNA fold.

Molecular Dynamic simulation of vaccine candidate structure.

The iMODS confirmed the physical motion of atoms and molecules with the modeled vaccine-bovine receptor complex (36). The stability and movement of molecules within the structure over time were assessed using the web server; understanding the complex's dynamic behavior can help the researcher elicit an immune response.

Results

Sequence retrieval

Sequences of TaSP were obtained from NCBI. VaxiJen v2.0 was used to predict TaSP antigenicity. Results show that the selected protein is antigenic based on a threshold of 0.4, and the antigenic score of TaSP was 1.0811. Furthermore, the Allergenicity of TaSP was predicted with AllerTOP v. 2.0, and results showed that TaSP is probably non-allergen.

Prediction of epitopes

A short list of CTL epitopes of TaSP protein with high scores was predicted with the IEDB web server. As shown (Table 1), five antigenic, nonallergic, and conservative CTL epitopes were selected to construct the multiepitope mRNA-based vaccine. In the same manner, Six HTLs were selected from the primary shortlist. The selected HTL epitopes have a high antigenic score, are nontoxic and nonallergic to the host, and are conservative at a ratio of 100% (Table 2). Four antigenic and nonallergenic LBL epitopes with other properties that are summarized in (Table 3) were selected to construct the vaccine candidate.

Table 1: Cytotoxic T Lymphocyte epitopes used for constructing the mRNA vaccine candidate

No.	Epitope	Antigenicity	Toxicity	Allergenicity	Conservancy
1	EEEEEENKS	1.9611	Non	None	100%
2	AQGGVIIGA	1.6638	Non	None	100%
3	DFKPKPRRY	1.6128	Non	None	100%
4	AQPGVSSSS	1.0631	Non	None	100%
5	KTKGSEKKK	1.5407	Non	None	100%

Table 2: Helper T Lymphocyte epitopes used for constructing the mRNA vaccine candidate

No.	Epitopes	Antigenicity	Toxicity	Allergenicity	Conservancy
1	EGLFQKIKNKLLGSG	1.1544	Non	None	100%
2	RNAVTRQTDSISGPI	0.9470	Non	None	100%
3	KEGLFQKIKNKLLGS	0.9702	Non	None	100%
4	PRNAVTRQTDSISGP	0.6306	Non	None	100%
5	PRRYEGQGTDAVKLK	1.0573	Non	None	100%
6	KKEGLFQKIKNKLLG	0.6749	Non	None	100%

Table 3: Linear B lymphocyte (LBL) epitopes that are used for constructing the vaccine candidate

	Epitopes	Antigenicity	Toxicity	Allergenicity	Conservancy
1	TKGSEKKKELEE	1.685	No	None	100%
2	LQMVPHQKNLNG	0.8639	No	None	100%
3	INKKGTEDQDQTSGSGSKGTEGGSLRGQDLTEEE	1.4286	No	None	100%
4	KDVSEEHVGIGDLSDPSSRTPNAKPAEL	0.8182	No	None	100%

Construction of multiepitope mRNA vaccine candidate

The selected CTL, HTL, and LBL were fused with amino acid linkers GPGPG, AAY, and KK. As shown (Figure 2), Peptide RS09 was fused to the construct via the EAAAK liner at the N end. RS09 is an adjuvant that increases the vaccine's antigenicity and optimal induction of the host immune system. CTL epitopes linked with GPGPG, while AAY was used to link HTL epitopes and KK for LBL epitopes. Functional elements essential for translating the mRNA to protein within the host were included in the vaccine construct. The functional elements, including the Kozak sequence and signal peptide, were fused to the N terminal of the construct. The stability of the mRNA molecule is enhanced by adding human hemoglobin subunit beta HBB and Rabbit beta-globin as UTR at 3′ and 5′ end sequence of the vaccine construct, respectively. Finally, a modified cap structure (m7GCap) at the N terminal and a poly(A) tail and C terminal are included in the vaccine structure to increase stability and enhance the translation efficiency of the mRNA (Figure 2).

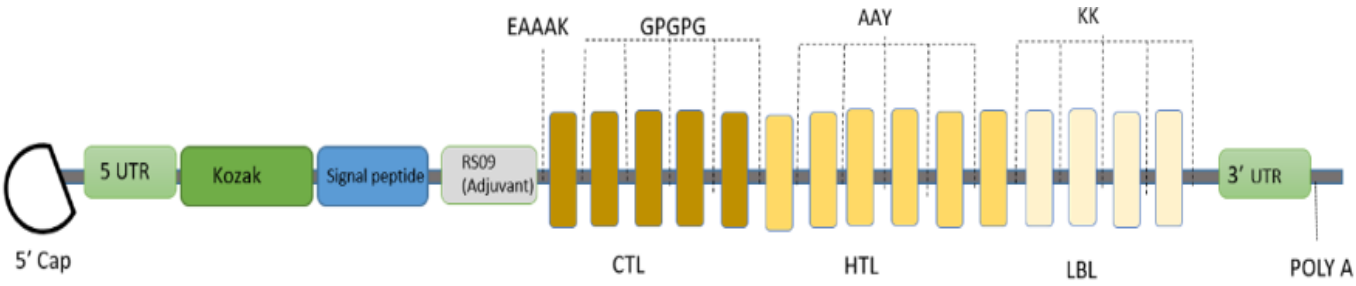

Figure 2: Final construction of multiepitope mRNA vaccine candidate. Kozak sequence, signal peptide, and adjuvant (peptide RS09) were fused to the N end of the vaccine candidate. The candidate consists of Five CTL epitopes (dark brown), six HTL epitopes (Gold), and four LBL epitopes (light gold). EAAK, GPGPG, AAY, and KK represent amino acid linkers that fused epitopes. Two UTR (HBB and anti-rabbit…..) were added to the vaccine construct's ٣′ and 5′ end sequence (green rectangle). cap structure (half circle) and poly A are included in the vaccine construct at the N and C terminals, respectively.

Immunological and Physiochemical properties of the vaccine candidate

ProtParam was used to predict the physiochemical properties of the modeled vaccine. The results showed that the Instability index of the vaccine candidate is 32.34, which indicates that the protein is stable and has an Appropriate molecular weight of 30371.94. The Grand average of hydropathicity is -0.855, meaning the constructed protein is hydrophilic and bear with a pI of 9.44. Furthermore, the estimated half-life of the construct is more than 20h in yeast cells, more than 10h in *E. coli*, and 4.4h in mammalian reticulocytes. Other Physicochemical properties of the vaccine construct are summarized in (Table 4). Some immunological properties of the construct were estimated using immunoinformatics tools. An estimate of vaccine antigenicity of the vaccine construct with the VaxiJen web server shows that the construct is highly antigenic with a high antigenic score of 1.0120. also, estimated Allergenicity and toxicity of the construct with AllerTOP and Toxinpred, respectively, show that the construct is nonallergenic and nontoxic to the host.

Prediction of secondary structure

The secondary structure was predicted with the Prabi web server. The protein consists of 291 amino acids. The percentage of random coil was 53.93%, while alpha helix was 40.21 and extended strand 5.84 of the total protein structure. Default settings of the web server were used to analyze the secondary structure of the construct. It is well documented that antibodies require an unfolded protein region to recognize protein. Overall, results revealed that the vaccine candidate has a wide region to bind to the host antibody (Figure 3).

3D structure, refinement, and validation

Three deaminations of the vaccine construct were predicted using the *trRosetta* web server, which relies on a similar structure in the PDB bank. The server predicts Five

models based on confidence score; one of the five models was selected as the predicted structure for the vaccine construct (Figure 4). Refinement of the selected structure was performed using the Galaxy web server. Out of the 5 refines models, model one was selected (Figure 4) because it has good structural properties, including the lowest RMSD (0.336), MolProbity score (1.548), Clash score (11.2) and Rama favored region of 52.2. Analysis of the Ramachandran plot with the PDBsum web server showed that 95.5% of residues were located in the favorite area, while 1% of residues were in the disallowed area, 3.5% of residues were located in the additional allowed area (Figure 4). ProA web was used to estimate the vaccine candidate's Z score (Figure 4). The estimated Z score is-5.95. The negative value of the Z score indicates high confidence in the structure. Overall, structural analysis of the vaccine candidate showed that it is stable, flexible, and valid.

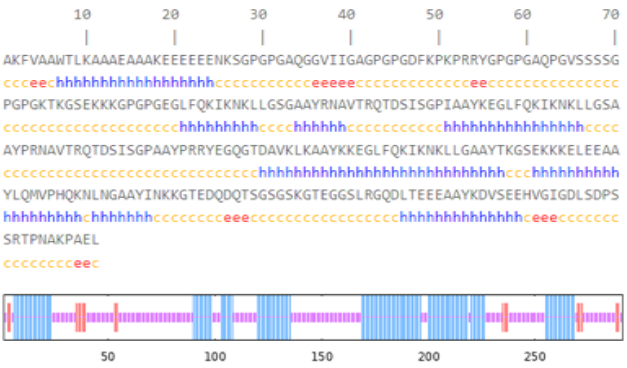

Figure 3: Secondary structure of the vaccine. The structure predicted with the Prabi web server. The Letters in orange represent random coil structure, the letters in blue represent alpha helix, and the red represents extended stand.

Table 4: Physicochemical and Immunogenic characteristics of the vaccine candidate

Properties of the construct	Measurement	note
Amino acids	291	Suitable
MW	30371.94	Appropriate
pI	9.44	Basic
Formula	C1329H2134N384O428S1	-------
half-life (*Escherichia coli*, in vivo)	>10 hours	-----
half-life (mammalian reticulocytes, in vitro)	4.4 hours	------
half-life (yeast cells, in vivo)	>20 hours	-----
Grand average of hydropathicity	-0.855	Hydrophilic
Instability index of vaccine	32.34	Stable
Antigenicity	None- allergen	Antigenic
Allergenicity	1.0120	Nonallergic
Toxicity	None	Nontoxic

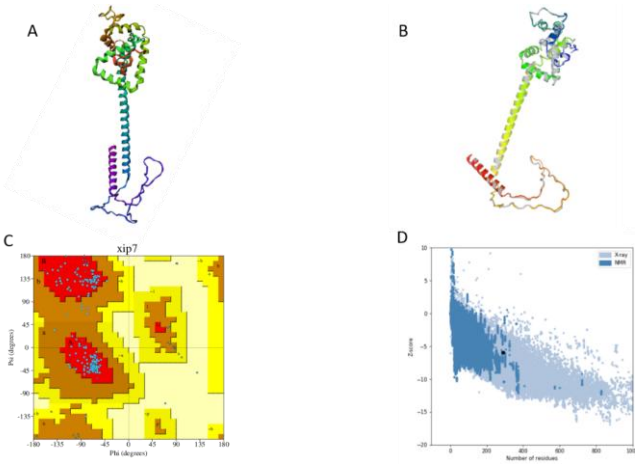

Figure 4: Modeling, refinement, and validation of the modeled vaccine. A: 3D structure of the vaccine candidate. B: Refined structure of the predicated model. C: validation of vaccine structure and analysis of Ramachandran plot. D: Predicated Z score of the modeled vaccine.

Interaction of the vaccine candidate with TLR4 and molecular dynamic simulation

LZerD web server was used to estimate the possible interaction between the vaccine candidate and bovine TLR4. The server provides 10 models for the complex. Model one was chosen as the predicted model (Figure 5). The selected model has a good Ranksume score (234) and other final model scores (GOAP score -134380.25, GOAP rank 8, DFIRE score 100331.42, DFIRE rank 42, ITscore-50573.19, IT rank 23). Complex stability (vaccine candidate with bovine TLR4) was estimated with iMODS server (Figure 5 B-F). In (Figure 5), the covariance matrix was represented. The plot shows the connection between amino acids, in which the correlated amino acids are present in red, the blue color represents anti-correlated residues, and white refers to uncorrelated residues. Furthermore, as shown in figure 5, the elastic network model was generated to show which residue pair is connected by springs, where the grey-colored area refers to a harder string. Overall, the docking complex is stable and has good intermolecular interaction. In (Figure 5), the peak of the deformity graph shows deformable regions of

the vaccine construct, where the graph shows residues with coiled shapes. Characterization and flexibility of the docking complex were performed via normal mode analysis (NMA) and B-factor graph (Figure 5). In the graph, comparisons were expressed between NMA and PDB of the vaccine construct. The low deformation index of the vaccine (Eigenvalues 2.6347) refers to the high stability of the vaccine–receptor complex (Figure 5). In figure 5, individual and accumulative variance of the vaccine–receptor complex model was represented in the graph. The green bar represents accumulative variance, while the blue represents individual variance. Overall, the results revealed that the vaccine-receptor complex is stable. The PDBsum webserver predicted the molecular interaction between the modeled vaccine and the bovine receptor. As shown in the (Figure 5), there is 1 salt bridge (red line), 4 hydrogen bonds (blue line), and 178 nonbonding contacts (yellow line) as a residue interaction across the interface of the vaccine candidate and Bovine TLR4 receptor. It is well documented that hydrogen bond plays an important role in the stability of the complex, for the presence of this type of bond in the docking complex reveals that the complex is stable and the mRNA multiepitope vaccine can be recognized by the immune system of the host. Together, these results suggest that the vaccine complex is stable and can be recognized by the immune system of the selected host. The vaccine may be a promising option for treat*ing theileriosis* in animal livestock.

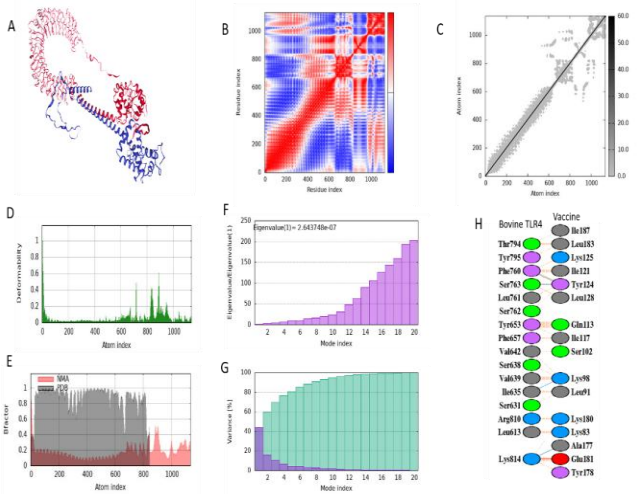

Figure 5: Visualization of fine docking of vaccine candidate with bovine TLR4 with molecular dynamic simulation. A: docking complex of vaccine construct and bovine TLR4. B: the covariance matrix of the vaccine–receptor complex. C: Elastic network model D: deformity graph. E: Graph of B-factor. F: Eigenvalues value the vaccine–receptor complex model. G: individual and accumulative variance of the vaccine–receptor complex model. H: prediction of the molecular interaction between the modeled vaccine and bovine receptor using The PDBsum webserver.

Codon optimization and mRNA structure

Codon optimized the reversed transcript sequence of the mRNA vaccine with the VectorBuilder® (35). The results showed that the GC content in the optimized sequence was 60.05%, while The Codon Adaptation Index (CAI) was 0.87. The result revealed that the modeled vaccine could be Transmitted to the selected host (cattle). RNA fold servers were used to predict the structural arrangement of the mRNA-modeled vaccine (Figure 6). The result showed that the free energy of the thermodynamic ensemble is -1365.30 kcal/mol. The centroid secondary structure has a minimum free energy of -1045.18 kcal/mol. These results revealed that the mRNA-modelled vaccine is stable and provided good insight into the thermodynamic robustness of the construct.

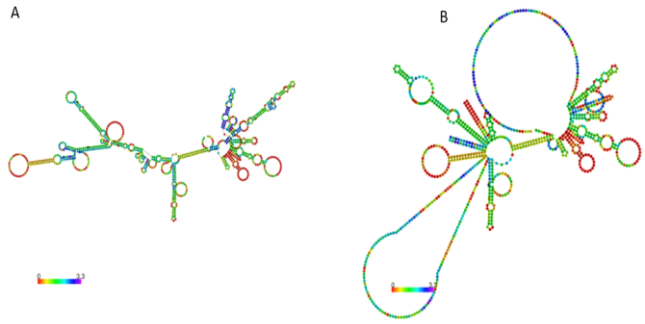

Figure 6: Structure of the mRNA-modelled vaccine. A: Ideal secondary structure configuration predicted by RNA fold server. B: The centroid secondary structure.

Discussion

Theileriosis is a critical tick-borne disease affecting a wide range of ruminants and causes severe economic losses to animal producers. The disease is widely documented in tropical and subtropical areas, and its prevalence is related to ticks—clinical signs of the disease range from mild to severe illnesses depending on the strain and susceptible host. Theileriosis can be diagnosed through the main clinical signs: fever, anemia, and superficial lymph node enlargement. PCR and CRISPR were also used Chemotherapeutic agents are widely used for the treatment of theileriosis. However, these agents can not completely eradicate the pathogen from animals and promote the establishment of carrier status. Furthermore, the Multi-strain of the parasite and its ability to infect a wide range of farm animals make controlling the disease difficult (37).

Several researchers recommend vaccination as a global strategy against theileriosis in farm animals. Live attenuated vaccines are efficient in control of the disease; however, several limitations were recorded while using this type of vaccine, including vaccination with attenuated parasitic cells is active only with the local isolates but not heterologous, and

live attenuated vaccine may cause transmission of the infection to the other animals furthermore this type of vaccine reduces the efficiency of drugs that normally used against theileriosis (38). During the COVID-19 outbreak, a new generation of vaccines, including mRNA code and reverse vaccinology, was developed to combat the outbreak. Reverse vaccinology associated with mRNA code was used to design a multiepitope vaccine against various infectious agents (39). A multiepitope mRNA vaccine against *Theileria annulata* based on TaSP protein was designed utilizing structural biology and immunoinformatics tools. TaSP is a highly antigenic protein that can induce the host's immune system. It also provides cross immune for other strains rather than *Theileria annulata* .

TaSP was chosen as a target protein to build the subunit vaccine candidate. Conserved, overlapped, and highly antigenic epitopes from the selected antigen were used to build the vaccine candidate. Immunogenicity was also enhanced using Peptide RS09 as an adjuvant. Peptide RS09 is a new class of adjuvant that can induce the immune system by binding with TLR4 and increasing antibody production (40). After assembling the vaccine candidate, several immunoinformatics tools were used to test the stability and immunogenicity of the construct. These test results show that the mRNA proposed vaccine is stable, immunogenic, and highly interactive with the immune receptor of the selected host. The proposed multiepitopes mRNA vaccine can be produced at a lower cost than other traditional vaccines. It is also easy to set the vaccine according to changes in the genome content of the pathogen. Results of several immunoinformatics tests of the proposed vaccine reveal that the vaccine might be a promising choice to treat and control theileriosis in cattle. Furthermore, the proposed vaccine is mRNA-based; therefore, there is no risk of insertional mutation that can occur in the host cell's genome. However, several lab studies are needed to evaluate the efficiency and safety of the proposed vaccine (41).

Conclusion

Multiepitope mRNA-based vaccines were designed against *Theileria annulata* infection in cattle. The modeled vaccine is based on the *Theileria annulata* sporozoite surface antigen and uses immunoinformatics and molecular modeling. Stability, good antigenicity, and potential interaction with the immune receptor made the modeled vaccine a promising option to treat and control theileriosis in farm animals. Furthermore, laboratory studies are needed to confirm the efficiency and safety of the modeled vaccine.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Ota N, Mizuno D, Kuboki N, Igarashi I, Nakamura Y, Yamashina H, Hanzaike T, Fujii K, Onoe S, Hata H, Kondo S. Epidemiological survey of *Theileria orientalis* infection in grazing cattle in the eastern part of Hokkaido, Japan. J Vet Med Sci. 2009;71(7):937–944. DOI: 10.1292/jvms.71.937
2. Adjou Moumouni PF, Aboge GO, Terkawi MA, Masatani T, Cao S, Kamyingkird K, Jirapattharasate C, Zhou M, Wang G, Liu M, Iguchi A. Molecular detection and characterization of *Babesia bovis*, *Babesia bigemina*, *Theileria* species and *Anaplasma marginale* isolated from cattle in Kenya. Parasit Vectors. 2015;8:1–4. DOI: 10.1186/s13071-015-1106-9
3. Kho KL, Amarajothi AD, Koh FX, Panchadcharam C, Nizam QN, Tay ST. The first molecular survey of theileriosis in Malaysian cattle, sheep, and goats. Vet Parasitol Reg Stud Rep. 2017;10:149–153. DOI: 10.1016/j.vprsr.2017.08.003
4. Bishop R, Musoke A, Morzaria S, Gardner M, Nene V. *Theileria*: intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. Parasitology. 2004;129(S1):S271–S283. DOI: 10.1017/S0031182003004748
5. Bishop R, Musoke A, Morzaria S, Gardner M, Nene V. *Theileria*: intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. Parasitology. 2004;129(S1):S271–S283.
6. Sivakumar T, Fujita S, Tuvshintulga B, Kothalawala H, Silva SP, Yokoyama N. Discovery of a new *Theileria* sp. closely related to *Theileria annulata* in cattle from Sri Lanka. Sci Rep. 2019;9:1–10. DOI: 10.1038/s41598-019-52512-y
7. Sivakumar T, Fujita S, Tuvshintulga B, Kothalawala H, Silva SS, Yokoyama N. Discovery of a new *Theileria* sp. closely related to *Theileria annulata* in cattle from Sri Lanka. Sci Rep. 2019;9(1):16132.
8. Mohamed SB, Alagib A, AbdElkareim TB, Hassan MM, Johnson WC, Hussein HE, Taus NS, Ueti MW. Molecular detection and characterization of *Theileria* spp. infecting cattle in Sennar State, Sudan. Parasitol Res. 2018;117:1271–1276. DOI: 10.1007/s00436-018-5775-0
9. Brown CGD. Dynamics and impact of tick-borne diseases of cattle. Trop Anim Health Prod. 1997;29:1–3S. DOI: 10.1007/BF02632905
10. Ahmed J, Yin H, Schnittger L, Jongejan F. Ticks and tick-borne diseases in Asia with special emphasis on China. Parasitol Res. 2002;88(1):51–55. DOI: 10.1007/s00436-001-0574-3
11. Elsify A, Sivakumar T, Nayel M, Salama A, Elkhtam A, Rizk M, Mosaab O, Sultan K, Elsayed S, Igarashi I, Yokoyama N. An epidemiological survey of bovine *Babesia* and *Theileria* parasites in cattle, buffaloes, and sheep in Egypt. Parasitol Int. 2015;64(1):79–85. DOI: 10.1016/j.parint.2014.10.002
12. Ullah K, Numan M, Alouffi A, Almutairi MM, Zahid H, Khan M, Islam ZU, Kamil A, Safi SZ, Ahmed H, Tanaka T. Molecular characterization and assessment of risk factors associated with *Theileria annulata* infection. Microorganisms. 2022;10(8):1614. DOI: 10.3390/microorganisms10081614
13. Taylor LH, Katzer F, Shiels BR, Welburn SC. Genetic and phenotypic analysis of Tunisian *Theileria annulata* clones. Parasitol. 2003;126:241–252. DOI: 10.1017/S0031182002002810
14. Khatri N, Nichani AK, Sharma RD, Khatri M, Malhotra DV. Effect of vaccination in the field with the *Theileria annulata* (Hisar) cell culture vaccine on young calves born during the winter season. Vet Res Commun. 2001;25:179–188. DOI: 10.1023/A:1006473307868
15. Mhadhbi M, Chaouch M, Ajroud K, Darghouth MA, BenAbderrazak S. Sequence polymorphism of cytochrome b gene in *Theileria annulata* Tunisian isolates and its association with buparvaquone treatment failure. PLoS One. 2015;10(6):e0129678. DOI: 10.1371/journal.pone.0129678

16. Maritim AC, Young AS, Lesan AC, Ndungu SG, Mutugi JJ, Stagg DA. Theilerial parasites isolated from carrier cattle after immunization with *Theileria parva* by the infection and treatment method. Parasitol. 1989;99(1):139–147. DOI: 10.1017/S0031182000061126
17. Kallerup RS, Foged C. Classification of vaccines. In: Foged C, Rades T, Perrie Y, Hook S, editors. Subunit vaccine delivery. USA: Springer; 2014. 15–29 p. DOI: 10.1007/978-1-4939-1417-3_2
18. Sominskaya I, Skrastina D, Dislers A, Vasiljev D, Mihailova M, Ose V, Dreilina D, Pumpens P. Construction and immunological evaluation of multivalent hepatitis B virus core virus-like particles carrying HBV and HCV epitopes. Clin Vaccine Immunol. 2010;17:1027–1033. DOI: 10.1128/CVI.00468-09
19. He L, Cheng Y, Kong L, Azadnia P, Giang E, Kim J, Wood MR, Wilson IA, Law M, Zhu J. Approaching rational epitope vaccine design for hepatitis C virus with meta-server and multivalent scaffolding. Sci Rep. 2015;5:12501. DOI: 10.1038/srep12501
20. e Silva RD, Ferreira LF, Hernandes MZ, De Brito ME, De Oliveira BC, Da Silva AA, de-Melo-Neto OP, Rezende AM, Pereira VR. Combination of in silico methods in the search for potential CD4(+) and CD8(+) T cell epitopes in the proteome of *Leishmania braziliensis*. Front Immunol. 2016;7:327. DOI: 10.3389/fimmu.2016.00327
21. Kanampalliwar AM, Soni R, Girdhar A, Tiwari A. Web-based tools and databases for epitope prediction and analysis: a contextual review. Int J Comput Bioinform In Silico Model. 2013;2:180–185. [available at]
22. Seitzer U, Bakheit MA, Salih DE, Ali A, Haller D, Yin H, Schnittger L, Ahmed J. From molecule to diagnostic tool: *Theileria annulata* surface protein TaSP. Parasitol Res. 2007;101:217–223. DOI: 10.1007/s00436-007-0685-6
23. Elati K, Nijhof AM, Mwamuye MM, Ameen V, Mhadhbi M, Darghouth MA, Obara I. Sequence polymorphisms in a *Theileria annulata* surface protein (TaSP) known to augment the immunity induced by live attenuated cell line vaccine. Transbound Emerg Dis. 2022;69(6):3350–3359. DOI: 10.1111/tbed.14687
24. Vita R, Mahajan S, Overton JA, Dhanda SK, Martini S, Cantrell JR, Wheeler DK, Sette A, Peters B. The immune epitope database (IEDB): 2018 update. Nucleic Acids Res. 2019;47(D1):D339–D343. DOI: 10.1093/nar/gky1006
25. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumor antigens, and subunit vaccines. BMC Bioinformatics. 2007;8:4. DOI: 10.1186/1471-2105-8-4
26. Saha S, Raghava G. Prediction of allergenic proteins and mapping of IgE epitopes in allergens. Protocol Exchange. 2007;1. DOI: 10.1038/nprot.2007.505
27. Chen X, Zaro JL, Shen WC. Fusion protein linkers: property, design, and functionality. Adv Drug Deliv Rev. 2013;65(10):1357–1369. DOI: 10.1016/j.addr.2012.09.039
28. Gasteiger E, Hoogland C, Gattiker A, Duvaud SE, Wilkins MR, Appel RD, Bairoch A. Protein identification and analysis tools on the ExPASy server. In: Walker JM, editor. The proteomics protocols handbook. USA: Humana Press; 2005. 571–607 p. DOI: 10.1385/1-59259-890-0:571
29. McGuffin LJ, Bryson K, Jones DT. The PSIPRED protein structure prediction server. Bioinformatics. 2000;16(4):404–405. DOI: 10.1093/bioinformatics/16.4.404
30. Roy A, Kucukural A, Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. Nat Protoc. 2010;5(4):725–738. DOI: 10.1038/nprot.2010.5
31. Heo L, Park H, Seok C. GalaxyRefine: protein structure refinement driven by side-chain repacking. Nucleic Acids Res. 2013;41(W1):W384–W388. DOI: 10.1093/nar/gkt458
32. Lovell SC, Davis IW, Arendall WB III, De Bakker PI, Word JM, Prisant MG, Richardson JS, Richardson DC. Structure validation by Cα geometry: ϕ, ψ and Cβ deviation. Proteins. 2003;50(3):437–450. DOI: 10.1002/prot.10286
33. Esquivel-Rodriguez J, Filos-Gonzalez V, Li B, Kihara D. Pairwise and multimeric protein-protein docking using the LZerD program suite. In: Kihara D, editor. Protein structure prediction. USA: Springer; 2014. 209–234 p. DOI: 10.1007/978-1-4939-0366-5_15
34. Laskowski RA. PDBsum 1: a standalone program for generating PDBsum analyses. Protein Sci. 2022;31(12):e4473. DOI: 10.1002/pro.4473
35. Mendia C, Peineau T, Zamani M, Felgerolle C, Yahiaoui N, Christophersen N, Papal S, Maudoux A, Maroofian R, Patni P, Nouaille S. Clarin-2 gene supplementation durably preserves hearing in a model of progressive hearing loss. Mol Ther. 2024;32(3):800–817. DOI: 10.1016/j.ymthe.2024.01.021
36. López-Blanco JR, Aliaga JI, Quintana-Ortí ES, Chacón P. iMODS: internal coordinates normal mode analysis server. Nucleic Acids Res. 2014;42(W1):W271–W276. DOI: 10.1093/nar/gku339
37. Al-Saadi M, Al-Sallami D, Alsultan A. Molecular identification of *Anaplasma platys* in cattle by nested PCR. Iran J Microbiol. 2023;15(3):433. DOI: 10.18502/ijm.v15i3.12904
38. Alawadi ZI, Alsultan A, Alsallami D, Alnomasy SF, Alqasmi M, Almufarriji FM, Alotaibi BS, Mazhari BB, Alenazy R. Designing a multiepitope mRNA-based vaccine against enterotoxigenic *Escherichia coli* infection in calves: immunoinformatics and molecular modeling approach. Open Vet J. 2024;14(6):1417. DOI: 10.5455/OVJ.2024.v14.i6.9
39. Abbi S, Qasim A. Impact of zinc supplementation on nutrients digestibility and blood mineral concentration during hot season of local growing lambs. Mesopotamia J Agric. 2024;52(1):79–93. DOI: 10.33899/mja.2024.146430.1364
40. Al-Shatty HR. The use of solar sterilization of soil prepared for forest seedling and its effect in inhibiting the growth of soil fungi and molecular diagnostics by using PCR technique. Mesopotamia J Agric. 2024;52(1):170–181. DOI: 10.33899/mja.2024.145974.1345
41. Hanoush NH. Honey as antibacterial agent used against *Bacillus* spp. isolated from locally produced juice. Mesopotamia J Agric. 2023;51(4):72–85. DOI: 10.33899/mja.2023.143201.1273

تصميم لقاح مبتكر متعدد المحددات المستضدية ومعتمد على الحمض النووي الريبوزي المرسال للوقاية من داء الثليريا الحلقية في الأبقار بالاستخدام تطبيقات المعلوماتية المناعية والنمذجة الجزيئية

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الخلاصة

داء الثليريا هو مرض ينقل بواسطة القراد ويصيب المجترات في المناطق الاستوائية وشبه الاستوائية. يسبب المرض علامات سريرية تتراوح بين المرض الخفيف الى الشديد. تصيب الثليريا الحلقية الماشية في المقام الأول وتسبب خسائر اقتصادية فادحة للمربين. تستخدم الأدوية الكيميائية لعلاج داء الثليريا في الحيوانات، لكن هذه الأدوية لا يمكنها القضاء تمامًا على العامل الممرض في الحيوان وأيضًا تساهم في تعزيز حالة الحيوانات الحاملة للمرض. يعد التطعيم أحد الخيارات المهمة للسيطرة على داء الثليريا والوقاية منه. هدفت هذه الدراسة إلى تصميم لقاح فعال ضد داء الثليريـــا الحلقية في الأبقار باســـتخدام المعلوماتيـــة

المناعية وأساليب النمذجة. لهذا الغرض، تم اختيار المستضد السطحي لبويغيات الثليريا الحلقية كبروتين مستهدف. تم استخدام مجموعة من أدوات المعلوماتية المناعية لاختيار المحداد المستضدية غير المتجانسة من البروتين المحدد. تم تصميم اللقاح متعدد المحددات المستضدية المقترح من خلال اختيار خمس محددات لخلايا الخلايا السامة التائية اللمفية، وستة محددات للخلايا اللمفية التائية وأربع محددات لخلايا باء اللمفية. بالإضافة إلى ذلك، تمت إضافة الببتيد ار اس ٠٩ كمعزز مناعي لزيادة الخواص المستضدية للقاح. أظهرت نتائج تحليل المعلوماتية المناعية للمناعة والخواص الفيزيائية والكيميائية للقاح المقترح أن درجه الاستضدادية كانت (١,٠١٢٠) وانه غير مسبب للحساسية، ومستقر ومحب للماء. علاوة على ذلك، أظهرت دراسة الالتحام مع الخلايا المشابهة للتول البقري مع اللقاح المقترح ارتباطا قويًا بينهما عند طاقة منخفضة وثبات عالي. بشكل عام، يمكن أن يكون اللقاح المقترح خيارًا واعدًا ضد داء الثليريا الحلقية في الأبقار، ولكن هناك حاجة إلى دراسات معملية وسريرية لتطوير اللقاح المقترح.