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In silico prediction of vaccine and diagnostic epitopes in *Bartonella henselae* MopA, VceA, and LemA proteins

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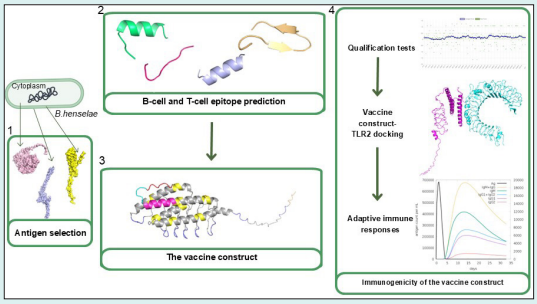
Abstract

Introduction: *Bartonella henselae* represents a significant zoonotic threat for humans and has diagnostic challenges. In addition, any commercial vaccine isn't currently available.



Methods: In this study, bioinformatics and immunoinformatics analyses were used to identify the vaccine and diagnostic epitopes of *B. henselae* MopA, VceA, and LemA proteins. Initially, the physicochemical properties, secondary structures, and antigenicity of the MopA, VceA, and LemA proteins from *B. henselae* were evaluated. Then, B and T-cell epitopes were predicted, and further analyzed in terms of antigenicity, allergenicity, solubility, and homology to the human proteome to determine suitable candidates for the final vaccine construct. The normal mode and molecular docking analyses were performed between the final vaccine construct and the TLR2 receptor. Finally, the immune response against the final vaccine construct was predicted.

Results: *In silico* analyses identified many B and T-cell epitopes with promising immunological and physicochemical properties. Among these, several epitopes showed high antigenicity scores (EINARVNQI, RAANALTVK, SVQIDTRTK and TSGQVISEDVGIKLE). In addition, the newly designed vaccine construct containing epitopes from MopA, Vce, and LemA proteins was found to interact with TLR2 and may stimulate adaptive immune responses.

Conclusion: The present study provides a comprehensive *in silico* epitope mapping of MopA, VceA, and LemA proteins of *B. henselae*. The identified epitopes and the vaccine construct can be candidate antigens for the development of serological tests and peptide vaccines. However, experimental studies are needed to validate their diagnostic performance and immunogenic potential.

Introduction

Bartonella is a genus of alpha-proteobacteria within the order *Rhizobiales* and the family of Bartonellaceae.[1,2] There are 38 identified species of *Bartonella* and approximately half are known to infect mammals.[3] The most clinically significant species are *B.* henselae, *Bartonella bacilliformis,* and *B. quintana.*[4] The primary reservoir for *B. henselae* is domestic cats and it causes cat-scratch disease in humans. *B. henselae* is transmitted in nature primarily through the cat flea (*Ctenocephalides felis*), which acquires the bacteria while feeding on the blood of infected cats. It is localized in flea intestines or cat red blood cells and can be transmitted to humans through flea bites or via cat scratches and bites contaminated with flea feces.[5] Cats positive for *B. henselae* are mostly asymptomatic but infection in humans can range from mild to potentially fatal. Immunocompromised individuals, particularly those with HIV/AIDS may show serious symptoms such as bacillary angiomatosis and endocarditis.[6]

In addition, *B. henselae* is considered within the scope of the One Health framework due to its zoonotic

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nature and the close interaction between humans, cats, and flea. There isn't any commercial vaccine against *B. henselae* and the most effective method of prevention is maintaining strict flea control in cats.[4] Early and accurate diagnosis is essential for effective treatment, but diagnosis is challenging due to the non-specific clinical symptoms.[7] Although diagnosis is mostly based on clinical symptoms, laboratory testing is required for definitive confirmation. However, the absence of a widely accepted gold-standard test significantly complicates the definitive diagnosis of infections.[8] Culture, molecular and serological methods (IFA and ELISA) are used for diagnosis but, their sensitivity and specificity are variable. Therefore, new diagnostic methods with high sensitivity and specificity are needed.[9] Recent studies have shown that recombinant *B. henselae* proteins used as antigen in serological assays have shown potential to increase the accuracy and validity of these tests.[10,11] In particular, in silico chimeric protein designs incorporating *B. henselae* GroEL protein, 17-kDa antigen, Pap31, SucB, P26, and BadA proteins have enhanced the accuracy of serological tests.[12] In addition to these proteins, MopA, a GroEL homolog protein, VceA, LemA, TrwE, TrwG, VirB2, VirB8, VirB10, and ParA proteins were identified as potential antigenic targets for serological diagnosis and vaccine development based on protein microarray screening data.[13] In addition to recombinant proteins, peptides predicted as epitope regions using immunoinformatics tools can also be used as antigens in ELISA to improve the sensitivity and specificity of diagnostic tests. Currently, these immunoinformatics tools are frequently used due to their high accuracy, low cost, and ease of use, and their time efficiency. Furthermore, advances in genome sequencing and the availability of vaccine development tools that predict many important parameters for next-generation vaccine design, such as vaccinology-related software and computational/statistical methods, have greatly facilitated the overall process.[14] Accordingly, in this study, the MopA, VceA, and LemA proteins were analyzed using *in silico* methods to identify novel epitope regions for potential use in vaccine research or in the development of new serological tests for humans, as these proteins were among the most sero-reactive antigens identified in a proteome-wide microarray analysis.[13]

Materials and Methods

Variation analysis

Protein sequences of *B. henselae* MopA, VceA, and LemA proteins were obtained from the NCBI (Accession numbers: MopA: CAF28126.1, VceA: CAF28044.1 and LemA: CAF27587.1). BLASTp analysis was performed to identify potential polymorphisms within each protein. For each analysis, the specified accession number was used as the reference sequence, and *B. henselae* was selected as the target organism. Default BLASTp parameters were applied. Sequence variation was assessed by examining amino acid substitutions across the aligned sequences using NCBI MSA Viewer version 1.26.0.

Prediction of physico-chemical properties, transmembrane regions, antigenicity, solubility, signal peptide and secondary structures

Expasy ProtParam,[15] TMHMM server v2.0,[16] Vaxijen v2.0,[17] Protein-Sol,[18] Signal-BLAST,[19] GOR IV[20] and PSIPRED 4.0[21] online tools, were used to predict physico-chemical properties, transmembrane regions, antigenicity, solubility, signal peptide and secondary structures of the analyzed proteins, respectively. In addition, the same tools, except for Signal-BLAST, were used for the vaccine construct derived from the epitopes selected in this study. During Signal-BLAST analysis, four different detection modes were used, and the presence of a signal peptide was accepted when all of them generated a positive result.[19]

Prediction of B-cell epitopes

Linear B-cell epitopes in the MopA, VceA, and LemA proteins were predicted by BCEPRED[22] whereas non-linear epitopes (Uniprot ID; MopA: O33963, VceA: X5M8G9 and LemA: X5M4A9) were predicted by ElliPro and BepiPred 3.0 online servers.[23] During the prediction of linear B-cell epitopes, peptides, those that have hydrophilicity, accessibility and exposed surface properties, were selected. Next, for each predicted B-cell epitope, additional analyses were conducted. Accordingly, the toxicity, antigenicity, allergenicity and solubility of the epitopes were predicted using ToxinPred,[24] the VaxiJen v2.0 and VaxiGen,[25] AllerTOP v2.1,[26] and PepCalc,[27] respectively. As a result, epitopes characterized as soluble, antigenic, non-allergenic and non-toxic as well as non-similar to the human proteome were considered B-cell epitopes. Predicted epitopes were screened against the human proteome using NCBI BLASTp analysis with *Homo sapiens* selected as the target organism. The top BLASTp hit for each epitope was evaluated, and epitopes with E-values > 0.01 and sequence identity < 70% were considered to have no significant similarity to human proteins and were retained for further analysis. This approach was also applied to the selection of T-cell epitopes. The predicted epitopes were visualized using PyMOL on three-dimensional protein structures generated by AlphaFold to assess their structural positions and potential surface accessibility.

Prediction of T-cell epitopes

MHC-I and MHC-II epitopes were predicted by NetMHCpan 4.1 EL method using the Next-Generation IEDB Tools.[28] MHC-I epitope prediction was performed using twelve HLA class I super-type, and MHC-II epitope prediction was performed using seven HLA class II reference set. Among the epitopes predicted as MHC-I (EL percentile < 0.5) and MHC-II (EL percentile < 2) epitopes, those characterized as soluble, antigenic, non-toxic, and non-allergenic as well as non-similar to the human proteome (E-value > 0.01 and identity < 70%) were considered MHC-I or MHC-II epitopes. The IEDB population coverage tool was used for population coverage analysis.

Design of the multi-epitope vaccine construct

The multi-epitope vaccine was constructed using the selected B and T-cell epitopes.[29] During the construction of multi-epitope vaccine, AAY and GPGPG linkers were used between B and T-cell epitopes, respectively. Also, a T-helper peptide called the PADRE sequence linked to RS-09 peptide (APPHALS) with a linker (PPGVS) at its N-terminus was added to the N terminus of the vaccine construct whereas a 6x His-Tag was added to the C-terminus.[30]

Refinement and validation of the vaccine construct

The GalaxyRefine server[31] was used to refine the predicted 3D structure of the vaccine construct generated by the AlphaFold 3.0 server.[32] The quality of the refined models was evaluated based on various structural parameters, including the MolProbity score, clash score, rotamer analysis, and Ramachandran plot. In addition, the final selected model was also validated using ERRAT and Verify3D through the SAVES server.

Docking and normal mode analysis

A docking analysis was applied to show the potential interaction between the refined vaccine construct and the human TLR2 receptor (PDB ID: 2Z7X) using HADDOCK.[33] Then, the docked vaccine-TLR2 complex were evaluated by normal mode analysis using the iMODS online server.[34]

Immunogenicity of the vaccine construct

The C-ImmSim 10.1 server with default parameters[35] was used to show the immunogenic potential of the vaccine construct.

Codon optimization and in-silico cloning

Codon optimization for *Escherichia coli* was conducted using a codon optimization tool. With SnapGene 2.0.1 software, the codon optimized gene encoding the vaccine protein was inserted into the pET28a(+) vector.

Results

Variation analysis

BLASTp results showed no variation for MopA (CAF28126.1) and VceA (CAF28044.1) proteins among the available *B. henselae* sequences in the NCBI database. However, a single amino acid substitution (I24T) was detected in the LemA protein sequence (CAF27587.1) compared with *B. henselae* sequences in the NCBI database.

Prediction of physico-chemical properties, transmembrane regions, antigenicity, solubility, signal peptide

When the physico-chemical properties of MopA, VceA, and LemA proteins were analyzed their molecular weights were found to be 57.6 kDa, 39.2 kDa, and 24.5 kDa. The theoretical isoelectric point (p*I*) values ranged from 5.10 to 9.44. Among the three proteins analyzed, MopA protein contained a higher proportion of negatively charged amino acid residues, whereas VceA and LemA proteins were enriched in positively charged residues. The predicted *in vivo* half-life in *E. coli* for all proteins was greater than 10 hours. The instability index of proteins ranged from 26.45 to 39.33 and all of the proteins were predicted to be stable. The aliphatic index ranged from 92.15 to 103.8. Negative GRAVY scores were predicted for all proteins, and each was also predicted to be antigenic and soluble. Transmembrane regions were identified in LemA and VceA proteins, whereas no such regions were detected in MopA protein. In addition, none of them contained signal peptides (Table 1).

Secondary structure

Secondary structure analysis has shown that alpha helices, extended strands, and random coils are distributed in varying proportions in the MopA, VceA, and LemA proteins. The dominant secondary structure in all three proteins is the alpha helix, and the proportions and locations of extended strands, alpha helices and random coils are presented in Table 2 and Fig. 1.

Table 1. Physico-chemical, antigenicity and transmembrane properties of the MopA, VceA, and LemA proteins

Properties	MopA	VceA	LemA
Amino acids	547	353	214
Molecular weight	57625.19	39169.12	24505.21
Theoretical p*I*	5.10	9.44	8.66
Negatively charged residues (Asp + Glu)	78	36	19
Positively charged residues (Arg + Lys)	66	46	21
Estimated half-life (*E. coli*)	> 10 hours	> 10 hours	>10 hours
Instability index	26.45/Stable	29.48/Stable	39.33/Stable
Aliphatic index	101.32	103.80	92.15
GRAVY	-0.041	-0.157	-0.198
Antigenicity	0.5253	0.7004	0.6199
*Solubility	0.66/Soluble	0.564/Soluble	0.592/Soluble
Number of predicted TMHs	0	1	1

*Solubility prediction was performed by removing the transmembrane domain predicted by the TMHMM server v2.0.

Prediction of B-cell epitopes

Among the predicted B-cell epitopes, those that have hydrophilicity, accessibility, and exposed surface properties, were selected and analyses of antigenicity, toxicity, allergenicity, solubility, and similarity to the human proteome were performed. Accordingly, two different epitopes (KEVKFGREARERLLRG and APQSDVDDAKSAYE) met all the selected criteria and were considered as B-cell epitopes (Supplementary file 1, Table S1).

Prediction of T-cell Epitopes

IEDB Next-Generation tools predicted many MHC-I and MHC-II epitopes for selected alleles. Accordingly, a total of 8 MHC-I epitopes meeting all selected criteria were found among the three proteins analyzed. Four of these were derived from MopA and four from VceA (Table S1). For the MopA protein, the EINARVNQI epitope predicted for the HLA-B*08:01 and HLA-A*26:01 allele showed the highest antigenicity value (1.6135) while for VceA, SVQIDTRTK (HLA-A*03:01) had the highest antigenicity (1.6781). A total of 5 MHC-II epitopes met all the selected criteria among the three proteins analyzed.

Table 2. Secondary structure of MopA, VceA, and LemA proteins

Properties	MopA	VceA	LemA
Alpha helix	339 (61.97%)	187 (52.97%)	134 (62.62%)
Extended strand	45 (8.23%)	56 (15.86%)	17 (7.94%)
Random coil	163 (29.80%)	110 (31.16 %)	63 (29.44%)

Three of these were derived from MopA, and two from VceA (Table S1). Any LemA related epitopes did not met the selected criteria. Among the MHC-II epitopes predicted for MopA protein, TSGQVISEDVGIKLE predicted for HLA-DRB3*01:01 allele showed the highest antigenicity value (1.4163). Among the MHC-II epitopes predicted for VceA protein, KKIAIKANQVVKKDD showed the highest antigenicity (0.9072) and was predicted for three alleles (HLA-DRB5*01:01, HLA-DRB3*02:02 and HLA-DRB1*15:01). According to the Population Coverage Analysis Tool, the selected epitopes associated with MHC-I and MHC-II alleles showed population coverages of 35.82% and 34.44%, respectively.

Physico-chemical, antigenicity, allergenicity, and solubility predictions of the vaccine construct

The selected epitopes were determined to be located on the surface of the proteins (Fig. S1). These selected epitopes were combined with linker sequences to create a potential vaccine construct (Fig. 2). Using Expasy ProtParam, it was predicted that the molecular weight of the vaccine construct was 28.28 kDa, the estimated pI value was approximately 9.33, and the negatively charged (Asp + Glu) residue score was 30, and the positively charged (Arg + Lys) residue score was 37. The estimated half-life in *E. coli* was predicted to be approximately > 10 hours. The vaccine construct was stable, with an instability index of 16.57. In addition, the GRAVY score and aliphatic index were -0.544 and 67.20, respectively. The antigenicity value was 0.9283, and the vaccine construct

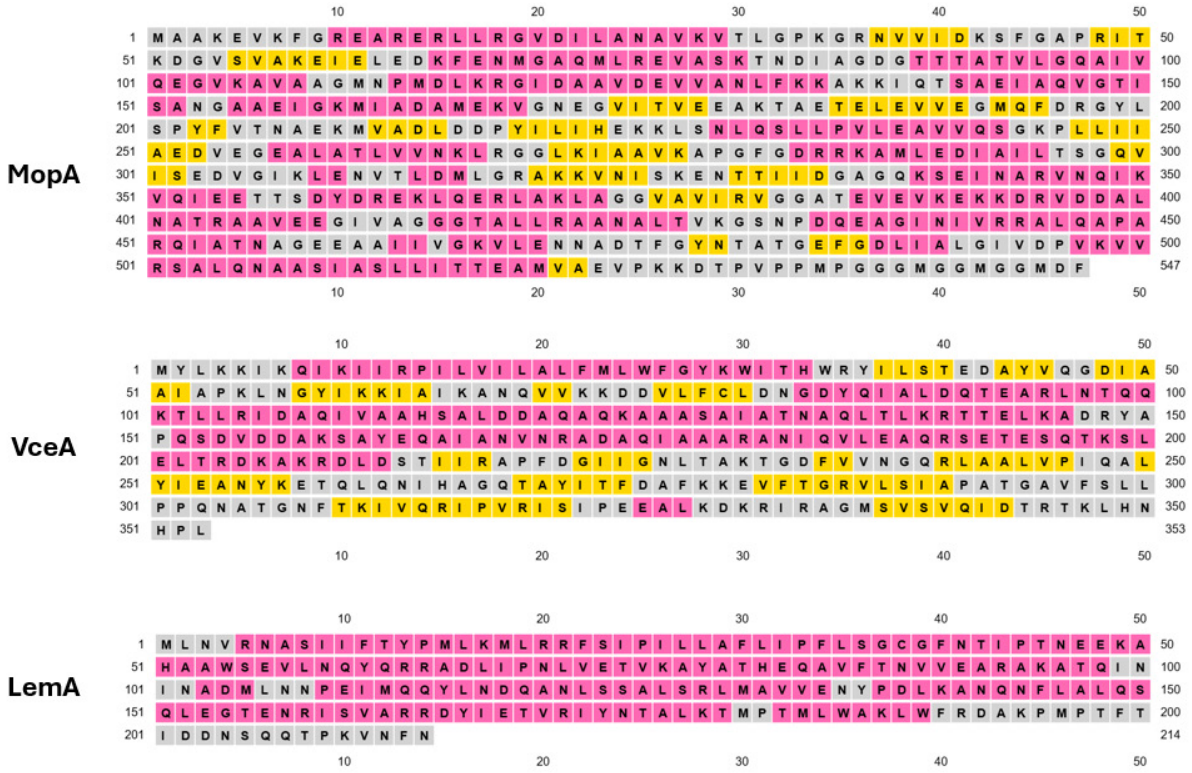

Fig. 1. Secondary structures including alpha helix (pink), beta strand (yellow), and coil residues (gray) are shown.

did not show allergenic properties and was soluble with a score of 0.678 (Table S2).

Tertiary structure prediction, refinement and validation

The 3D tertiary structure model of the vaccine construct was obtained using AlphaFold and then, the refined models generated by GalaxyWEB were evaluated based on structural quality parameters, and Model 4 was used for subsequent analyses due to its favorable MolProbity score (1.180), low clash score (3.9), absence of poor rotamers, and high Ramachandran favored value (98.9%). The structural quality of the refined protein was evaluated using ERRAT and Verify3D. Accordingly, the model showed an overall quality factor of 96.10% by ERRAT and a 3D-1D residue score of 83.03% by Verify3D.

Docking and normal mode analyses

Molecular docking analysis showed a significant binding score of -129.7 using HADDOCK. According to the results of normal mode analysis, an eigenvalue of 5.297671×10^{-7} was obtained. In addition, deformability and B-factor profiles indicated the stability of the docked model with localized regions of flexibility. Covariance and elastic network analyses showed the correlated motions within the docked model (Fig. 3).

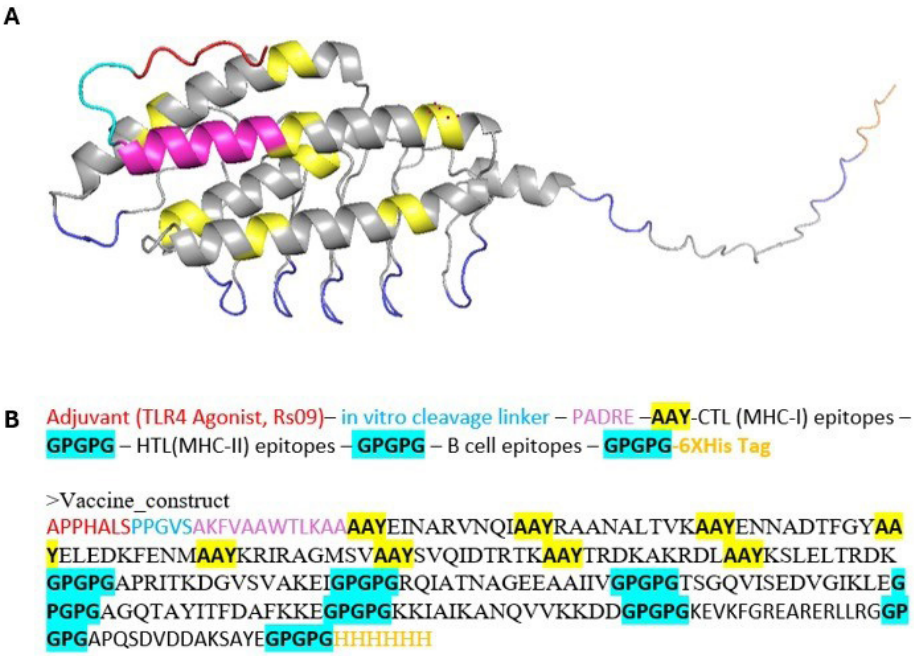

Fig. 2. A) 3D structure of the vaccine construct B) The amino acid sequence of the vaccine construct.

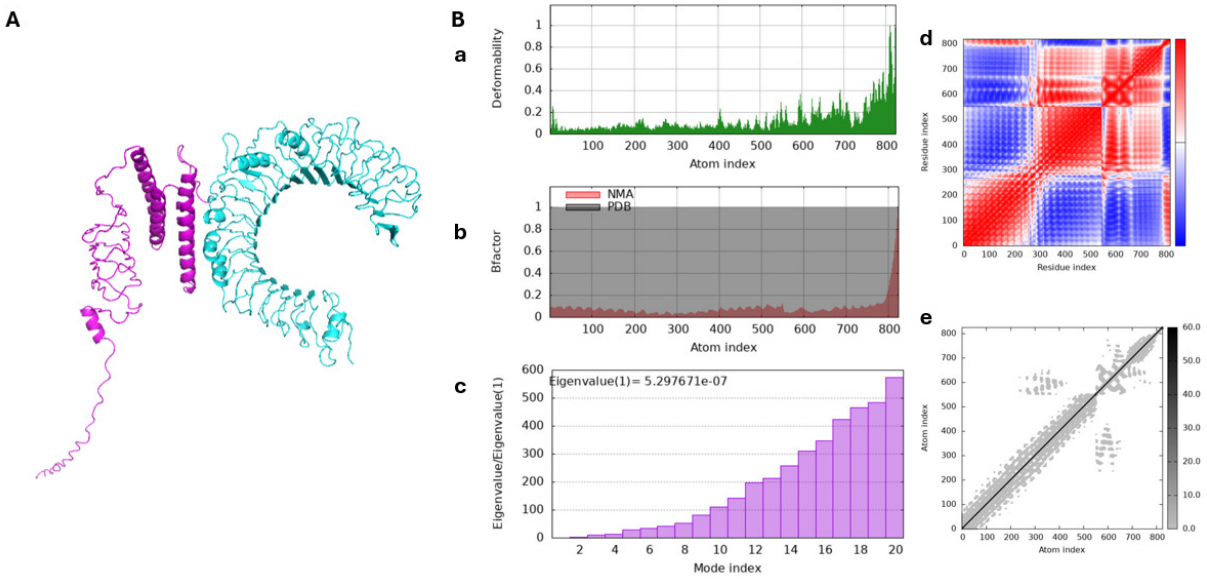

Fig. 3. A) Representation of the docked complex of the vaccine construct (blue) with the TLR2 (purple). B) Normal Mode Analysis (NMA) of the vaccine construct and TLR2 complex: (a) Main-chain deformability profile, (b) B-factor values, (c) Eigenvalue of the complex, (d) Covariance matrix, (e) Elastic network model.

Simulation of the specific vaccine immune responses

The *in silico* immune simulation performed via the C-IMMSIM server showed that the injected antigen (Ag) peaked at approximately 700,000 counts/mL within the first 2 days and was completely cleared by day 5. The humoral immune response was characterized by an increase in B-cell populations, stabilizing at approximately 500 cells/mm³ after day 5, with a dominant production of IgM and IgG1 antibodies. The cellular immune profile showed a marked Th1 dominance, with Th1 counts expanding to approximately 45000 cells/mm³. This activation was supported by high levels of IFN-γ production, peaking near 400000 ng/mL, and a coordinated increase in IL-2 levels (Fig. 4).

Codon optimization and in-silico cloning

The final vaccine construct (813 bp) was inserted into the pET28a(+) vector, resulting in a recombinant plasmid of 6148 bp with a GC content of 48.83% (Fig. 5).

Discussion

Bartonella henselae causes cat-scratch disease which is characterized by severe clinical symptoms in immunocompromised patients.[36] The wide range of clinical presentation of *B. henselae*, the difficulty in isolating the pathogen, and the limitations of current diagnostic methods represent a significant obstacle in both clinical practice and research. However, diagnostic studies using recombinant proteins as antigen in serological assays show enhanced sensitivity and specificity values.[9] For example, a recombinant chimeric protein (designed from BadA, Pap31 and GroEL) expressed in *E. coli* BL21 cells and purified by affinity chromatography has been reported to show high sensitivity (up to 90.9%) when tested with serum samples collected from humans infected with *B.* henselae.[12] A different study reported that combining GroEL and 17-kDa proteins as ELISA antigens showed a sensitivity of 82.8% and a specificity of 83.9% for the serological detection of *B. henselae* in humans.[11] In addition to the use of recombinant proteins as antigen in ELISA, synthesized peptides predicted to be epitopes by advanced immunoinformatics methods can also be used as antigens in serological tests to increase their sensitivity and specificity. Based on this, the present study aimed to identify B-cell and MHC I/II epitopes of the MopA, VceA, and LemA proteins, which have previously been reported to have antigenic potential,[13] using *in silico* methods to identify novel antigens for use in vaccine and serodiagnostic studies. Accordingly, any major differences in physicochemical properties were not observed among the analyzed proteins. All of them were predicted to be antigenic, soluble, and to have negative GRAVY values, showing that they are hydrophilic and can form strong interactions with water molecules.[37] However, MopA and VceA proteins may be better vaccine candidate proteins than LemA protein because MopA protein has a higher molecular weight (57.6 kDa) and does not contain transmembrane domains, while VceA protein has a higher

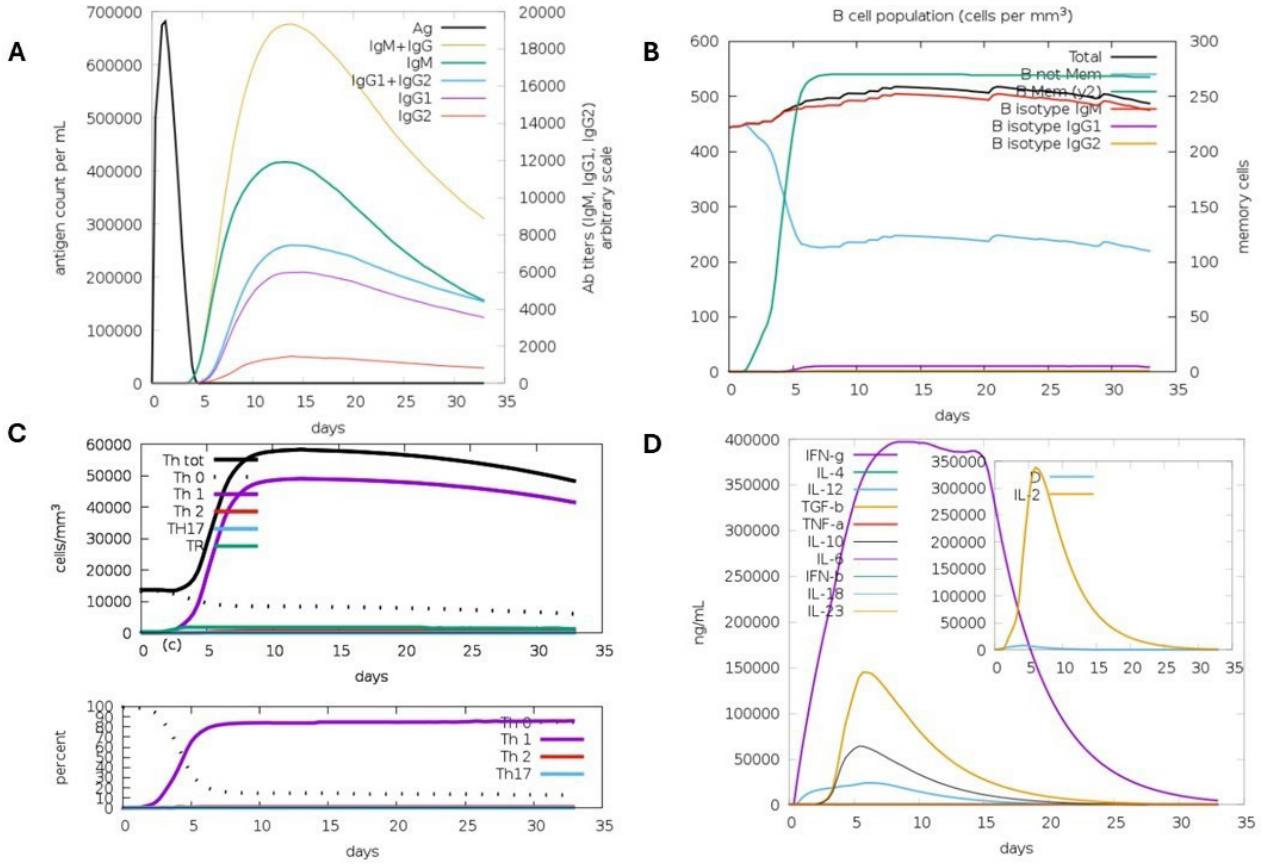

Fig. 4. *In silico* immune response results of the vaccine construct.

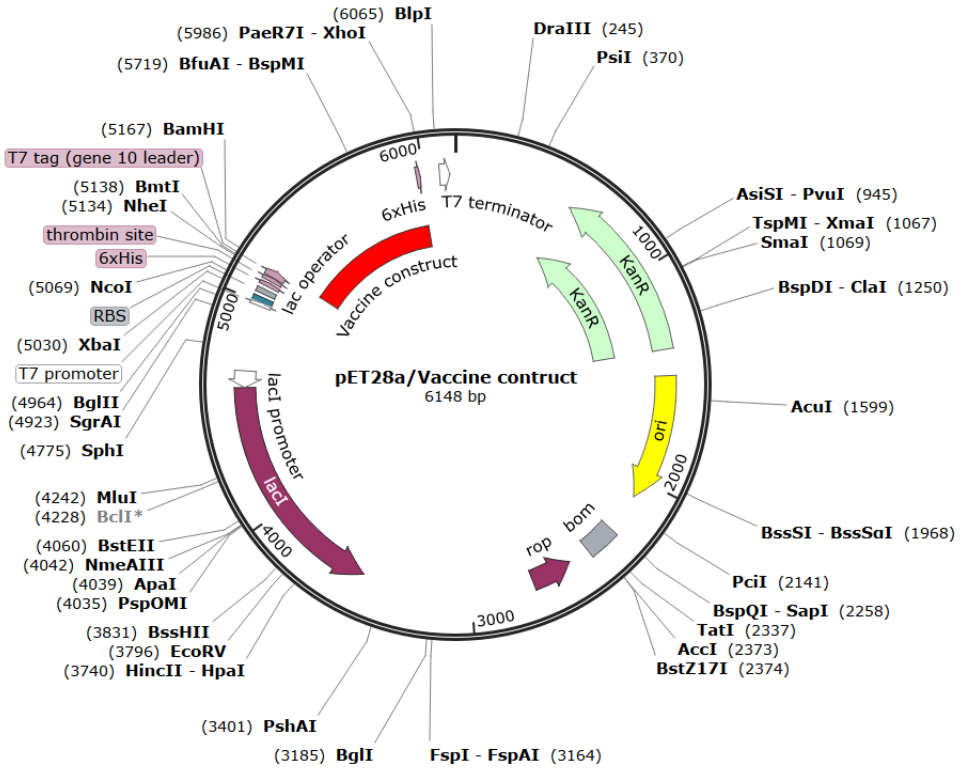

Fig. 5. pET28a(+) vector containing the final vaccine construct.

antigenicity value (0.7004). In this study, B-cell and MHC I/II epitopes were predicted among proteins analyzed. Among the B-cell epitopes, APQSDVDDAKSAYE was notable due to its high antigenicity values (0.8763). In addition, the SVQIDTRTK epitope from the VceA protein, predicted as an MHC-I epitope with the highest antigenicity value (1.6781), may be a promising candidate for vaccine research rather than for the development of serological tests, as MHC-I epitopes are associated with $CD8^+$ T lymphocyte responses (Table S1). Among the MHC-II epitopes, a MopA protein-derived epitope (TSGQVISEDVGIKLE) with the highest antigenicity value (1.4163) may be another candidate for vaccine research, as B lymphocytes are professional antigen-presenting cells via MHC-II and generate antibody responses with the help of $CD4^+$ T lymphocytes (Table S1).

Currently, *in silico* approaches are frequently used for epitope prediction.[38-43] After high-purity synthesis as peptides, these epitopes discovered in this study can be used as antigens, particularly in ELISA, for the diagnosis of *B. henselae* infecting animals and/or humans. The use of peptides as antigens in serological assays is considered more advantageous than the use of recombinant proteins, as recombinant proteins may contain cross-reactive epitopes that reduce assay specificity.[44] Moreover, peptides can be designed and produced more easily and at lower cost compared with other antigen sources, such as lysates or recombinant proteins of targeted pathogen.[45] To date, peptide-based ELISA has not been used for the diagnosis of *B. henselae*, except in a chimeric design containing peptides derived from different proteins.[12] In our previous studies, peptide-based ELISA has been used for the diagnosis or serotyping of other pathogens (such as *Toxoplasma gondii* and Maedi–Visna virus) and has generated comparable results to those obtained using lysate antigens or recombinant.[46-48] Based on previous studies in which advanced *in silico* approaches were applied for peptide prediction and subsequent evaluation of serodiagnostic performance yielded promising results, the epitopes predicted in this study are expected to be suitable for ELISA based diagnosis of *B. henselae* in humans or animals, and may also have potential for the development of peptide-based vaccines against *B. henselae*.

In addition to the discovery of epitope regions of analyzed proteins, in this study, the vaccine construct was created using selected epitopes, linker sequences and an immunostimulatory adjuvant. Physicochemical analysis of the vaccine construct revealed that it has a structural stability and is highly soluble in water. Structural modeling and validation analyses were performed to assess the reliability of the predicted 3D structure of the vaccine construct. Accordingly, the obtained results indicate that the refined vaccine construct has a reliable 3D structure due to its low MolProbity and clash scores, absence of poor rotamers, high Ramachandran favored value, an overall quality factor of 96.10% and a Verify3D score of 83.03%. TLR2, a component of the innate immune system, contributes to the development of host immune responses by recognizing various pathogenic ligands.[49,50] Based on this, the human TLR2 receptor was selected for

molecular docking and normal mode analyses with the vaccine construct. The docking and normal mode analyses shows that the vaccine construct is capable of interacting with human TLR2. However, molecular docking and normal mode analyses are computational approaches and do not show the efficacy of vaccine construct. Therefore, the predicted TLR2 interaction can be interpreted as a preliminary hypothesis. The *in silico* immune simulation performed using C-IMMSIM suggests that the vaccine construct may induce both humoral and cellular immune responses. The observed antigen clearance, B-cell activation, and IgM/IgG1 production indicate a potential antibody-mediated response, while the predominance of Th1 cells and elevated IFN-γ and IL-2 levels suggest a possible cell-mediated immune response. However, these findings are based on *in silico* results and therefore, experimental validation is necessary. It has also been reported that the use of *in silico* tools involves significant limitations, such as algorithmic bias and data sparsity.[51]

In a previous study, a multi epitope vaccine against *Echinococcus granulosus* was designed using bioinformatics analysis. Antibodies (IgG and IgE) and IL-4 were detected in the serum samples of dogs and sheep vaccinated with this multi-epitope vaccine. In addition, a reduction in the total number of hydatid cysts in sheep and high protection in dogs were reported.[52] These results suggest that multi-epitope vaccines designed by bioinformatics analysis can have potential in experimental vaccine studies.

Conclusion

In this study, MopA, VceA, and LemA proteins of *B. henselae* were analyzed using *in silico* approaches for epitope discovery. Although numerous epitopes were predicted, several were identified as particularly promising candidates for use in vaccine development or diagnostic applications. In addition, the newly designed vaccine construct containing epitopes from MopA, Vce, and LemA proteins was found to interact with TLR2 and stimulate adaptive immune responses, suggesting its potential as a vaccine candidate against bartonellosis. However, experimental validation was not performed in this study because binding assays between TLR2 and the vaccine construct, immunogenicity analyses, serological confirmation tests, and *in vivo* functional studies were not conducted. These are the limitations of this study, and the findings need to be evaluated in future experimental studies.

Authors' Contribution

Conceptualization: Mervenur Güvendi, Cemal Ün.
Investigation: Mervenur Güvendi, Hüseyin Can, Aysu Değirmenci Döşkaya, Muhammet Karakavuk, Mert Döşkaya, Adnan Yüksel Gürüz.
Methodology: Mervenur Güvendi, Hüseyin Can.
Supervision: Cemal Ün, Adnan Yüksel Gürüz, Mert Döşkaya, Aysu Değirmenci Döşkaya, Muhammet Karakavuk, Hüseyin Can.
Validation: Mervenur Güvendi, Cemal Ün.
Visualization: Mervenur Güvendi.
Writing–original draft: Mervenur Güvendi.
Writing–review & editing: Cemal Ün, Hüseyin Can, Muhammet Karakavuk, Mert Döşkaya, Aysu Değirmenci Döşkaya, Adnan Yüksel Gürüz.

Competing Interests

The authors declare no competing interests.

Declaration of AI-Assisted Tools in the Writing Procedure

The authors confirm that any content translated or language-checked using AI (ChatGPT by OpenAI) has been reviewed by them, and that they bear full responsibility for the final text.

Ethical Approval

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Supplementary files

Supplementary file 1 contains Tables S1-S2 and Fig. S1.

References

1. Álvarez-Fernández A, Breitschwerdt EB, Solano-Gallego L. *Bartonella* infections in cats and dogs including zoonotic aspects. *Parasit Vectors* **2018**; 11: 624. doi:10.1186/s13071-018-3152-6
2. Deng H, Le Rhun D, Buffet JP, Cotté V, Read A, Birtles RJ, et al. Strategies of exploitation of mammalian reservoirs by *Bartonella* species. *Vet Res* **2012**; 43: 15. doi:10.1186/1297-9716-43-15
3. Dias CM, do Amaral RB, Perles L, dos Santos Muniz AL, Rocha TF, Machado RZ, et al. Multi-locus Sequencing Typing of *Bartonella henselae* isolates reveals coinfection with different variants in domestic cats from Midwestern Brazil. *Acta Trop* **2023**; 237: 106742. doi:10.1016/j.actatropica.2022.106742
4. Breitschwerdt EB. Bartonellosis, One Health and all creatures great and small. *Vet Dermatol* **2017**; 28: 96-e21. doi:10.1111/vde.12413
5. Massei F, Gori L, Macchia P, Maggiore G. The expanded spectrum of bartonellosis in children. *Infect Dis Clin North Am* **2005**; 19: 691-711. doi:10.1016/j.idc.2005.06.001
6. Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB. *Bartonella* spp. in pets and effect on human health. *Emerg Infect Dis* **2006**; 12: 389-94. doi:10.3201/eid1203.050931
7. Regier Y, Rourke FO, Kempf VA. *Bartonella* spp. - a chance to establish One Health concepts in veterinary and human medicine. *Parasit Vectors* **2016**; 9: 261. doi:10.1186/s13071-016-1546-x
8. Blagova B, Yanev N. Human *Bartonella* infection: a review of literature. *J IMAB* **2021**; 27: 3759-64. doi:10.5272/jimab.2021272.3759
9. Cardoso TL, Gonçalves JM, Hartwig DD. *Bartonella henselae*: a challenging diagnosis with a bright future. *J Microbiol Methods* **2025**; 239: 107296. doi:10.1016/j.mimet.2025.107296
10. McCool TL, Hoey JG, Montileone F, Goldenberg HB, Mordechai E, Adelson ME. Discovery and analysis of *Bartonella henselae* antigens for use in clinical serologic assays. *Diagn Microbiol Infect Dis* **2008**; 60: 17-23. doi:10.1016/j.diagmicrobio.2007.07.017
11. Ferrara F, Di Niro R, D'Angelo S, Busetti M, Marzari R, Not T, et al. Development of an enzyme-linked immunosorbent assay for *Bartonella henselae* infection detection. *Lett Appl Microbiol* **2014**; 59: 253-62. doi:10.1111/lam.12286
12. Gonçalves JM, Cardoso TL, de Freitas SB, Woloski R, Neto A, da Silva Pinto L, et al. In silico analyses and design of chimeric proteins containing epitopes of *Bartonella henselae* antigens for the control of cat scratch disease. *Appl Microbiol Biotechnol* **2022**; 106: 8079-91. doi:10.1007/s00253-022-12269-3
13. Vigil A, Ortega R, Jain A, Nakajima-Sasaki R, Tan X, Chomel BB, et al. Identification of the feline humoral immune response to *Bartonella henselae* infection by protein microarray. *PLoS One* **2010**; 5: e11447. doi:10.1371/journal.pone.0011447
14. Salemi A, Pourseif MM, Omidi Y. Next-generation vaccines and the impacts of state-of-the-art in-silico technologies. *Biologicals* **2021**; 69: 83-5. doi:10.1016/j.biologicals.2020.10.002
15. Wilkins MR, Gasteiger E, Bairoch A, Sanchez JC, Williams KL, Appel RD, et al. Protein identification and analysis tools in the ExPASy server. *Methods Mol Biol* **1999**; 112: 531-52. doi:10.1385/1-59259-584-7:531
16. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting

transmembrane protein topology with a hidden Markov model: application to complete genomes. *J Mol Biol* **2001**; 305: 567-80. doi:10.1006/jmbi.2000.4315
17. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinformatics* **2007**; 8: 4. doi:10.1186/1471-2105-8-4
18. Hebditch M, Carballo-Amador MA, Charonis S, Curtis R, Warwicker J. Protein-Sol: a web tool for predicting protein solubility from sequence. *Bioinformatics* **2017**; 33: 3098-100. doi:10.1093/bioinformatics/btx345
19. Frank K, Sippl MJ. High-performance signal peptide prediction based on sequence alignment techniques. *Bioinformatics* **2008**; 24: 2172-6. doi:10.1093/bioinformatics/btn422
20. Garnier J, Gibrat JF, Robson B. GOR method for predicting protein secondary structure from amino acid sequence. *Methods Enzymol* **1996**; 266: 540-53. doi:10.1016/s0076-6879(96)66034-0
21. Buchan DW, Moffat L, Lau A, Kandathil SM, Jones DT. Deep learning for the PSIPRED Protein Analysis Workbench. *Nucleic Acids Res* **2024**; 52: W287-93. doi:10.1093/nar/gkae328
22. Saha S, Raghava GP. BcePred: prediction of continuous B-cell epitopes in antigenic sequences using physico-chemical properties. In: Nicosia G, Cutello V, Bentley PJ, Timmis J, eds. *Artificial Immune Systems*. Berlin: Springer; **2004**. p. 197-204. doi:10.1007/978-3-540-30220-9_16
23. Clifford JN, Høie MH, Deleuran S, Peters B, Nielsen M, Marcatili P. BepiPred-3.0: Improved B-cell epitope prediction using protein language models. *Protein Sci* 2022;31:e4497. doi:10.1002/pro.4497
24. Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R, Raghava GP. In silico approach for predicting toxicity of peptides and proteins. *PLoS One* **2013**; 8: e73957. doi:10.1371/journal.pone.0073957
25. Dimitrov I, Zaharieva N, Doytchinova I. Bacterial immunogenicity prediction by machine learning methods. *Vaccines (Basel)* **2020**; 8: 709. doi:10.3390/vaccines8040709
26. Dimitrov I, Bangov I, Flower DR, Doytchinova I. AllerTOP v.2--a server for in silico prediction of allergens. *J Mol Model* **2014**; 20: 2278. doi:10.1007/s00894-014-2278-5
27. Lear S, Cobb SL. Pep-Calc.com: a set of web utilities for the calculation of peptide and peptoid properties and automatic mass spectral peak assignment. *J Comput Aided Mol Des* **2016**; 30: 271-7. doi:10.1007/s10822-016-9902-7
28. Yan Z, Kim K, Kim H, Ha B, Gambiez A, Bennett J, et al. Next-generation IEDB tools: a platform for epitope prediction and analysis. *Nucleic Acids Res* **2024**; 52: W526-32. doi:10.1093/nar/gkae407
29. Pourseif MM, Moghaddam G, Naghili B, Saeedi N, Parvizpour S, Nematollahi A, et al. A novel in silico minigene vaccine based on CD4 + T-helper and B-cell epitopes of EG95 isolates for vaccination against cystic echinococcosis. *Comput Biol Chem* **2018**; 72: 150-63. doi:10.1016/j.compbiolchem.2017.11.008
30. Majidiani H, Pourseif MM, Kordi B, Sadeghi MR, Najafi A. TgVax452, an epitope-based candidate vaccine targeting *Toxoplasma gondii* tachyzoite-specific SAG1-related sequence (SRS) proteins: immunoinformatics, structural simulations and experimental evidence-based approaches. *BMC Infect Dis* **2024**; 24: 886. doi:10.1186/s12879-024-09807-x
31. Ko J, Park H, Heo L, Seok C. GalaxyWEB server for protein structure prediction and refinement. *Nucleic Acids Res* **2012**; 40: W294-7. doi:10.1093/nar/gks493
32. Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, et al. Addendum: accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* **2024**; 636: E4. doi:10.1038/s41586-024-08416-7
33. Honorato RV, Trellet ME, Jiménez-García B, Schaarschmidt JJ, Giulini M, Reys V, et al. The HADDOCK2.4 web server for integrative modeling of biomolecular complexes. *Nat Protoc* **2024**; 19: 3219-41. doi:10.1038/s41596-024-01011-0
34. López-Blanco JR, Aliaga JI, Quintana-Ortí ES, Chacón P. iMODS: internal coordinates normal mode analysis server. *Nucleic Acids Res* **2014**; 42: W271-6. doi:10.1093/nar/gku339
35. Rapin N, Lund O, Bernaschi M, Castiglione F. Computational immunology meets bioinformatics: the use of prediction tools for molecular binding in the simulation of the immune system. *PLoS One* **2010**; 5: e9862. doi:10.1371/journal.pone.0009862
36. Peláez Bejarano A, Sánchez Del Moral R, Guisado-Gil AB. *Bartonella henselae* encephalopathy in a paediatric patient: a case report and treatment review. *J Clin Pharm Ther* **2020**; 45: 840-4. doi:10.1111/jcpt.13178
37. Droppa-Almeida D, Franceschi E, Padilha FF. Immune-informatic analysis and design of peptide vaccine from multi-epitopes against *Corynebacterium pseudotuberculosis*. *Bioinform Biol Insights* **2018**; 12: 1177932218755337. doi:10.1177/1177932218755337
38. Can H, Köseoğlu AE, Erkunt Alak S, Güvendi M, Döşkaya M, Karakavuk M, et al. In silico discovery of antigenic proteins and epitopes of SARS-CoV-2 for the development of a vaccine or a diagnostic approach for COVID-19. *Sci Rep* **2020**; 10: 22387. doi:10.1038/s41598-020-79645-9
39. Can H, Erkunt Alak S, Köseoğlu AE, Döşkaya M, Ün C. Do *Toxoplasma gondii* apicoplast proteins have antigenic potential? An in silico study. *Comput Biol Chem* **2020**; 84: 107158. doi:10.1016/j.compbiolchem.2019.107158
40. Koçkaya ES, Can H, Yaman Y, Ün C. In silico discovery of epitopes of gag and env proteins for the development of a multi-epitope vaccine candidate against Maedi-Visna virus using reverse vaccinology approach. *Biologicals* **2023**; 84: 101715. doi:10.1016/j.biologicals.2023.101715
41. Dülek Ö, Mutlu G, Koçkaya ES, Can H, Karakavuk M, Değirmenci Döşkaya A, et al. Computational identification of monkeypox virus epitopes to generate a novel vaccine antigen against Mpox. *Biologicals* **2024**; 88: 101798. doi:10.1016/j.biologicals.2024.101798
42. Köseoğlu AE, Can H, Güvendi M, Erkunt Alak S, Değirmenci Döşkaya A, Karakavuk M, et al. Molecular characterization of *Anaplasma ovis* Msp4 protein in strains isolated from ticks in Turkey: a multi-epitope synthetic vaccine antigen design against *Anaplasma ovis* using immunoinformatic tools. *Biologicals* **2024**; 85: 101749. doi:10.1016/j.biologicals.2024.101749
43. Güvendi M, Can H, Yavuz İ, Özbilgin A, Değirmenci Döşkaya A, Karakavuk M, et al. In silico identification of *Leishmania* GP63 protein epitopes to generate a new vaccine antigen against leishmaniasis. *PLoS Negl Trop Dis* **2025**; 19: e0013137. doi:10.1371/journal.pntd.0013137
44. Pandey S, Malviya G, Chottova Dvorakova M. Role of peptides in diagnostics. *Int J Mol Sci* **2021**; 22: 8828. doi:10.3390/ijms22168828
45. del Valle Benitez Betancourt A, Silva TL, de Freitas Oliveira DK, Nicolau-Junior N, Garcia JL, Fujiwara RT, et al. A structural in silico analysis of novel epitopes from *Toxoplasma gondii* proteins for the serodiagnosis of toxoplasmosis. *Int J Mol Sci* **2025**; 26: 4689. doi:10.3390/ijms26104689
46. Can H, Köseoğlu AE, Erkunt Alak S, Güvendi M, Ün C, Karakavuk M, et al. Molecular prevalence and subtyping of *Blastocystis* sp. isolates in stray cats of İzmir, Turkey: first report of "ST4 allele 42" in cats. *Pol J Vet Sci* **2021**; 24: 217-23. doi:10.24425/pjvs.2021.137656
47. Koçkaya ES, Can H, Yaman Y, Kandemir Ç, Taşkın T, Karakavuk M, et al. Newly developed peptide-ELISA successfully detected anti-IgG antibodies against Maedi-Visna virus in sheep. *Vet Immunol Immunopathol* **2024**; 274: 110806. doi:10.1016/j.vetimm.2024.110806
48. Yavuz İ, Karakavuk M, Kandemir Ç, Can H, Erkunt Alak S, Köseoğlu AE, et al. An ELISA using a *T. gondii* GRA6-derived peptide as antigen successfully detected ovine toxoplasmosis. *Acta Parasitol* **2026**; 71: 20. doi:10.1007/s11686-025-01208-7
49. Albiger B, Dahlberg S, Henriques-Normark B, Normark S. Role of the innate immune system in host defence against bacterial infections: focus on the toll-like receptors. *J Intern Med* **2007**; 261: 511-28. doi:10.1111/j.1365-2796.2007.01821.x
50. Mukherjee S, Karmakar S, Babu SP. TLR2 and TLR4 mediated host immune responses in major infectious diseases: a review. *Braz J Infect Dis* **2016**; 20: 193-204. doi:10.1016/j.bjid.2015.10.011
51. Pourseif MM, Baradaran Hosseini SA, Khoshraftar SH, Omidi Y. The role of bioinformatics algorithms in modern biopharmaceutical design: progress, challenges, and future perspectives. *Bioimpacts* **2025**; 15: 33072. doi:10.34172/bi.33072
52. Pourseif MM, Moghaddam G, Nematollahi A, Khordadmehr M, Naghili B, Dehghani J, et al. Vaccination with rEGVac elicits immunoprotection against different stages of *Echinococcus granulosus* life cycle: a pilot study. *Acta Trop* **2021**; 218: 105883. doi:10.1016/j.actatropica.2021.105883