IN SILICO SCREENING OF ANTIGENS FOR A POTENTIAL VACCINE CANDIDATE AGAINST *Toxoplasma gondii* IN GOATS

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ABSTRACT
Toxoplasma gondii (T. gondii), an obligate intracellular parasite, is a significant zoonotic pathogen causing economic losses in livestock and public health risks. In goats, T. gondii infections can lead to reproductive disorders such as abortion and neonatal mortality, necessitating effective control measures. Vaccination is a promising strategy, but the development of vaccines against T. gondii remains challenging. This study, therefore, employed in silico approaches to identify potential antigens suitable for vaccine development against T. gondii in goats. A novel multiepitope vaccine was designed using immunoinformatics techniques targeting T. gondii MIC, ROP 8, 16 and 18 and GRA7 antigens, consisting of antigenic and non-allergenic T-cell epitopes and B-cell epitopes. These screened epitopes were fused together using AAY, GPGPG and KK linkers. The 50S ribosomal protein L7/L12 with the accession number (P9WHE3) was used as an adjuvant to enhance the performance of the vaccine. The tertiary model of the proposed vaccine candidate was predicted and validated to confirm the structural quality of the vaccine. The designed vaccine was highly antigenic (0.5183), with molecular weight of 41.02 kDa, instability and aliphatic index of 29.06 and 93.97, respectively; this classified the vaccine as stable. The GRAVY score of 0.119 showed that the vaccine is slightly soluble. Using immunoinformatics approaches, nine T-cell and six B-cell epitopes were found from the chosen goat toxoplasma antigens. Based on the in-silico experiment, the multi-epitope peptide-based vaccine was found to be both non-allergic and highly antigenic. Molecular docking, dynamics simulation, in silico codon optimization, cloning, expression, and vaccine built in silico/online immunological simulations should all be performed on the developed peptide-based vaccine to determine the protective efficacy of the vaccine construct against T. gondii infection as well as its safety.
Keywords: *Toxoplasma gondii*, Vaccine candidate, Immunoinformatics, Epitope prediction, Goats

INTRODUCTION

Goat as one of the earliest domesticated animals have a lengthy, rich history and are still valuable economically in a variety of industries (Swan *et al.,* 2021) particularly as the demand for goat products rises around the world. Disease control is one among the many difficulties the goat industry faces, numerous illnesses that can have a major impact on commerce and productivity, including toxoplasmosis, foot-and-mouth disease, and caprine arthritic can afflict goats (Fleming *et al*., 2020). Goats infected with *T. gondii* are quite common throughout the world and are thought to be a major contributor to reproductive problems (Dubey, 2010). *Toxoplasma gondii* (*T. gondii*), is an obligate intracellular parasite belonging to the Apicomplexan Phylum, which harbours a great portion of infectious disease-causing parasites. It is a globally distributed protozoan parasite that causes toxoplasmosis (Gao *et al*., 2018; Ducournau *et al*., 2020; Al-Malki, 2021). Toxoplasmosis is zoonotic and has been reported to be high in low-income countries (Al-Malki, 2021; Chu and Quan, 2021). Almost all warm-blooded animals are known to be infected with *T. gondii* (Ducournau *et al*., 2020). Hence, the present study aimed to identify antigenic T-cell and B-cell epitopes through immunoinformatics techniques and design a potentially cost-efficient peptide-based vaccine by exploring the *Toxoplasma gondii* antigens of goats.

MATERIALS AND METHODS

Toxoplasma gondii of goats' protein sequences retrieval

Using the National Centre for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/protein/), the amino acid sequences of *Toxoplasma gondii* isolated from the goat host were identified and obtained.

Identification of conserved sequences

To create conserved sequences with at least 15 amino acid residues, the sequences were collected in FASTA format and aligned using multiple sequence alignment (MSA) was carried out with the MEGA version 11((Thompson *et al*., 1994)

Antigenicity and transmembrane structural analysis

The identified conserved sequences were exposed to antigenicity testing using (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), where the target organism was a parasite and the selection criteria were set at a threshold of 0.4. The sequences that met the threshold of ≥0.4 were chosen and designated as likely antigens. These

antigens' transmembrane helix characteristics were evaluated further using (http://www.cbs.dtu.dk/services/TMHMM/).

Determination of T-cell binding epitopes

The conserved protein sequences that passed the transmembrane selection criteria were subjected to (https://services.healthtech.dtu.dk/service.php? which is a website that uses neural networks to produce possible nonamers in order to forecast the epitopes of cytotoxic T lymphocytes (CTL). The server's default settings, which include the A1 supertype and an epitope identification threshold of 0.75, were used to identify the nonamers. The $CD8^+$ epitopes from the IEDB's online resources were predicted using the discovered nonamers (http://tools.iedb.org/mhci). The prediction of $CD8^+$ epitopes were achieved using the Stabilized Matrix Base Method (SMM) and parameters including amino acid length of 9.0 residues, and IC50 values of epitopes <250. The helper T-cell (HTL/$CD4^+$) epitopes were identified using the SMM-align prediction method, allele length of 15 residues, and IC50 value ≤2 Percentile rank from the (http://tools.iedb.org/mhcii/). To determine which T-cell epitopes would likely be antigens, the projected T-cell epitopes were assessed for antigenicity below a threshold of 0.5. Using the IEDB's online tools, the identified $CD8^+$ T-cell epitopes were subjected to additional testing for conservation (http://tools.iedb.org/conservancy/)' (Higgs *et al*., 2006; Bui *et al*., 2006) and allergenicity usinghttps://webs.iiitd.edu.in/raghava/algpred/submission.html to filter out allergenic sequences (Dimitrov *et al*., 2013).

Prediction of B-cell epitopes

The prediction of B-cell epitopes was achieved using the ABCpred online service (https://webs.iiitd.edu.in/raghava/abcpred/ABC_s ubmission.html/), It uses an artificial neural network to recognize protein sequences that contain B-cell regions. The forecast was made using the server's default parameters. Using immunoinformatics approaches similar to those used for T-cell epitope analysis, the resulting shortlisted epitopes were examined to assess their antigenicity, conservancy, and allergenicity.

Design of Multi-epitope vaccine

AAY linkers for CD8+ epitopes, GPGPG linkers for CD4+ epitopes, and KK linkers for B-cell epitopes were used to join the final T- and B-cell epitopes found from all three antigens during the prediction stage, which was considered as possible vaccination candidates. The 50S ribosomal protein L7/L12 adjuvant (accession number P9WHE3) was attached to the N-terminal of the vaccine with an AAY linker. Adding these linkers and an adjuvant to the vaccine construct aided in the flexibility and improved stability of the tertiary structure or model of the proposed vaccine, while the adjuvant enhanced the immunogenicity of the designed vaccine (Onile *et al.,* 2020).

Assessment of antigenicity, Allergenicity, solubility, and physicochemical properties of vaccine construct

The designed Multiepitope vaccine construct sequence was exposed to Vaxijen v2.0 and AllergenFP 1.0 servers to predict the antigenicity and Allergenicity of the construct (Dimitrov *et al*., 2014). The auto-cross covariance (ACC) transformations and E-descriptors serve as the foundation for these Allergenicity classifier servers. Assessing the Allergenicity of a vaccination offers valuable information about its propensity to trigger an allergic response. The vaccine construct's solubility was assessed in further detail by the (https://scratch.proteomics.ics.uci.edu), which is an SVM-based tool used to accurately predict the solubility of protein sequence through ten-fold cross-validation (Dimitrov *et al*., 2014).

RESULTS

Protein sequences availability and conserved sequences identification

A total of 22 amino acid sequences belonging to *T. gondii three* antigens of Goat Microneme antigens (MIC), Rhoptry antigens (ROP 8, 16 AND 18), and dense granules antigens (GRA 7) were obtained from the NCBI, comprises of 6 MIC, 8 ROP 8,16 and 18 and 8 GRA 7 were subjected to multiple sequence alignment using Molecular Evolutionary Genetics Analysis (MEGA) version 11 and the conserved sequences were generated A total of 22 amino acid sequences belonging to three antigen of *T. gondii,*.

Antigenicity and transmembrane structural analysis

The generated conserved sequences were assessed for antigenicity, where out of the 22 conserved sequences, a total of 8 sequences for GRA7, 6 sequences for MIC and 3 sequences for ROP (8, 16 and 18) were found to be antigenic. Those sequences that passed the antigenicity testing were further tested for transmembrane.8 sequences for GRA 7, 2 sequences for MIC and 1 for ROP 8, 16, and 18 sequence passes the transmembrane helix analysis.

Determination of T-cell binding epitopes

A total of 3 CD8+ epitopes were detected to be antigenic in nature. 1 MIC antigen, 1 for ROP antigen and 1 for GRA antigen. All the CD4+ T-cell epitopes passed the conservancy and allergenicity analysis from the identified

peptides and were regarded as the final potential vaccine candidates as shown in Table 1. A total of 6 CD4+ epitopes (15 mer in length) were identified and considered for incorporation into the final vaccine construct (Table 2).

Table 1: CD8+ T-cell predicted epitope candidates that interacted with different MHC I alleles.

Epitope	HLA allels	**Percentile rank ≤ 3**	**Antigenicity**	**Allergenicity**
MIC				
LTDRNFNTS	HLA-A*01:01	1	1.0961	*Non-Allergen*
ROP				
WIVNGFFLY	HLA-A*01:01	1	2.5177	*Non-Allergen*
GRA				
VTDDNIYEE	HLA-A*01:01	2.9	0.6984	*Non-Allergen*

Table 2: CD4+ T-cell predicted epitopes candidates that are antigenic and interact with different MHC II HLA-alleles

Epitope(s)	**HLA allele(s)**	**Percentile rank ≤ 3**	**Antigenicity**	**Allergenicity**
MIC				
LLPPSLFLLINSSLV	HLA-DRB1*01:01	0.01	1.0431	*non-allergen*
LPPSLFLLINSSLVE	HLA-DRB1*01:01	0.01	0.8790	*non-allergen*
ROP 8				
VGTFFRRDIPAAALR	HLA-DRB1*04:01	0.20	0.7206	non allergen
GTFFRRDIPAAALRF	HLA-DRB1*04:01	0.20	0.7322	non allergen
GRA				
IFFALCVLGLVAAAL	HLA-DRB1*01:01	0.56	0.5747	non allergen
FALCVLGLVAAALPQ	HLA-DRB1*01:01	0.56	0.5276	non allergen

3.1.4 Prediction of B-cell epitopes*:* The prediction of B-cell epitopes was achieved where a total of 6 epitopes were detected and were both Antigen and non-Allergenic

Table 3: B-cell candidates

SEQUENCE	Score	Antigenicity Value	Allergenicity
MIC			
AGAITMLLTDRNFNTS	0.96	0.6288	Non Allergen
MVMPIMIGGFGNWLVP	0.93	1.1229	Non Allergen
ROP 8, 16 AND 18			
RATISYHRDRRTLMTF	0.89	0.7814	Non Allergen
KKMIQGVSDEGAGWIV	0.89	0.7190	Non Allergen
GRA			
AATASDDELMSRIRNS	0.93	1.0493	Non Allergen
NAGVDSKGTDDHLTTS	0.87	0.9684	Non Allergen

Design of Multiepitope vaccine

The final obtained antigenic, conserved, and non-allergenic T and B-cell epitopes from the three antigens were fused using flexible linkers. This resulted in a final predicted vaccine construct consisting of one (1) MIC + one (1) ROP 8,16 and 18 + one (1) GRA7 CD8+ epitopes and two (2) MIC + two (2) ROP 8,16 and + two (2) GRA7 CD4+ epitopes respectively and six (6) B-cell epitopes joined using AAY, GPGPG linkers and KK linkers, respectively, The adjuvant, 50S ribosomal protein L7/L12 (accession number: P9WHE3 was attached to the N-terminal of the vaccine construct with the aid of the EAAK linker. The attachment of an adjuvant to the designed vaccine construct enhanced the immunogenicity of the construct and composition of the vaccine, resulting in a multi-epitope with 395 amino acid residues.

A PRIMARY STRUCTURE OF THE FINAL VACCINE DESIGN

MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEFDVILE
AAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKEAAKL

TDRNFNTSAAYWIVNGFFLYAAYVTDDNIYEEGPGPGLLPPSLFLLINSSLVGPGPGLPPSLFLLINSSLVE GPGPGVGTFFRRDIPAAALRGPGPGGTFFRRDIPAAALRFGPGPGIFFALCVLGLVAAALGPGPGFALCV LGLVAAALPQKKAGAITMLLTDRNFNTSKKMVMPIMIGGFGNWLVPKKRATISYHRDRRTLMTFKKK KMIQGVSDEGAGWIVKKAATASDDELMSRIRNSKKNAGVDSKGTDDHLTTS

Figure 1. *The primary structure of the designed vaccine in one letter format of amino acid, Adjuvant (Yellow Highlight), Three CD8+ Epitope (Red) and linkers (blue), 6 CD4+ epitope (Black) and linker(green) B-cell epitopes (orange) and linkers (purple).*

Assessment of Antigenicity, Allergenicity and Solubility of vaccine construct

These assessments revealed the designed vaccine to be non-allergenic, antigenic with a Vaxijen score of 0.5132, the obtained solubility probability of 0.7086 as highly soluble. As regards solubility, the GRAVY (0.119) obtained for the designed vaccine protein was indicating the hydrophobic nature of the vaccine.

DISCUSSION

HTL (CD4+) is important in activating the plasma B-lymphocytes that make antibody, Additionally, it stimulates the CTL (CD8+) and macrophages that eliminate the infectious organisms or target antigen (Abass et al., 2021). According to research by Shamriz *et al*. (2016), in order to elicit the intended immunological response, epitopes must be detectable by MHC I and MHC II. The B-lymphocyte epitopes play a crucial role in immune system cell activation, humoral and antibody-mediated immunity induction. According to Dimitrov *et al.* (2013), who used computational screening for antigenic epitopes that are also non-allergenic, the predicted B-cell epitopes were shown to be both antigenic and non-allergenic. Peng *et al.* (2020) findings are in agreement with the final peptide-based vaccine design. Identified candidates with similar ratings as possible vaccine targets after evaluating a number of *T.* gondii antigens using technologies such as VaxiJen and solubility predictors. Using a criterion of 0.4 for VaxiJen, Taha *et al.* (2021) found several antigens that exceeded this value to be viable vaccination candidates.

It is acceptable for expression and purification because its molecular weight is within the range of the majority of recombinant subunit vaccines. According to research by Foroutan *et al.* (2019), *T.* gondii antigens that have been identified as potential vaccine candidates have comparable molecular weights. According to research on T. gondii vaccines by Naz *et al.* (2019), immunogenic proteins have appropriate folding and balanced hydropathicity to guarantee solubility while retaining enough hydrophobic areas to interact with cellular membranes. The vaccine construct's stability and solubility are impacted by hydrophilicity. A study by Shahid *et al.* (2021) found that somewhat hydrophilic vaccine candidates have better solubility for both experimental and clinical uses, supporting reasonable solubility with a GRAVY value of 0.119. Vaccines effectively recognize antigens by targeting their extracellular, hydrophilic areas. Research by Foroutan *et al.* (2019) highlights the importance of choosing proteins that are slightly hydrophilic because they are more likely to be exposed on the pathogen surface, increasing the possibility that they may elicit an immune response.

A Z-score of -6.42 for a developed Toxoplasma gondii vaccine indicates the protein model's structural evaluation, which generally shows how well the model fits within the anticipated range of energetics or structural quality. Wiederstein and Sippl's (2007) findings, which emphasized that acceptable Z-scores rely on the size of the protein and the database of known structures, are consistent with this score. A Z-score of -6 to -7 is regarded as appropriate for a 42 kDa protein.

CONCLUSION

Using immunoinformatics approaches, nine T-cell and six B-cell epitopes were found from the chosen goat toxoplasma antigens, based on the in-silico experiment, the multi-epitope peptide-based vaccine was found to be both non-allergic and highly antigenic. Molecular docking, dynamics simulation, in silico codon optimization, cloning, expression, and vaccine build in silico/online immunological simulations should all be performed on the developed peptide-based vaccine to determine the protective efficacy of the vaccine construct against *T. gondii* infection as well as its safety.

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