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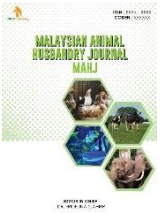

REVIEW ARTICLE

IN-SILICO MULTI-EPITOPE VACCINE CANDIDATE DESIGN AGAINST CHICKEN COCCIDIOSIS

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ABSTRACT

The economic burdens of coccidiosis pose a very significant challenge to commercial poultry farmers. Not only does it threaten human nutritional and pecuniary endeavors, but efforts to control the infection through hygienic measures and biosecurity are insufficient to contain it. Drug treatments using anti-coccidiosis agents are somewhat ineffective owing to frequent resistance concerns which prompted exploration into chemoprophylaxis as a more viable solution. However, challenges of the low immune response, side effects, and cost, are undermining factors in using the existing vaccines. By adopting an immunoinformatics-aided procedure, this study designed a potential broad-spectrum vaccine for chicken coccidiosis, mining from the various chicken *Eimeria* apical membrane antigens (AMAs) and other key sporozoite surface antigens (SSAs). Standard structural Bioinformatics tools were utilized to identify antigenic epitopes from the important proteins involved in the chicken *Eimeria* pathogenesis and conjugate them into a multi-epitope subunit vaccine. A potential broad-spectrum sub-unit vaccine construct consisting of 26 selected epitopes was designed through stringent analyses of immunological, physicochemical, structural, and molecular validations. While appropriate linkers were used for the conjugation, Beta defensin-3 adjuvant, and Padre Sequences were included to enhance the immune response. The resulting construct is a stable and promising vaccine candidate for further analysis and wet laboratory validation.

KEYWORDS

Chicken, Pathogens, Coccidiosis, Immunoinformatics, Epitope, Vaccine

1. INTRODUCTION

Chickens are undeniably one of the primary sources of animal protein for human consumption and are known for their high-quality protein products (Mesa-Pineda et al., 2021; Britez et al., 2023). Coccidiosis in chickens is an enteric pathogenic disease by *Eimeria* species that results in substantial global economic losses (Blake et al., 2020). As part of the Apicomplexan family, these parasites have a unique mechanism of invading host cells through membrane proteins aided by specialized organelles like micronemes which are crucial to their successful hosts' cell invasion (Suarez et al., 2017; Burrell et al., 2020). The *Eimeria* pathogens that cause chicken coccidiosis thrive in the intestinal epithelial cells after being transmitted through oocysts, leading to varying degrees of morbidity and mortality in poultry (Zaheer et al., 2022). These pathogenic species of *Eimeria* which include tenella, acervulina, praecox, among others, are known to cause chicken coccidiosis (Clark et al., 2017; Abbas et al., 2019). Coccidiosis pathophysiology begins primarily with the pathogen's invasion, which involves interactions between sporozoites and host cells, then migration to enterocytes, and subsequent proliferation. This leads to disruption of the normal mucosal cellular function resulting in increased permeability of the intestinal wall and poor nutrient absorption (Madlala et al., 2021). Consequential symptoms like fluid loss, diarrhea, weight loss, and intestinal hemorrhaging follow suit and most times culminate in death (Vrba et al., 2010). The severity and clinical outcomes depend on various factors like host-pathogen interactions, the species involved, the dose of infection, and the environment (Britez et al., 2023). Therefore, pathogenicity in chicken *Eimeria* infections varies from mild to severe (Tewari et al., 2011). Developing broad-spectrum vaccines that target various species of *Eimeria* pathogens in chickens is essential due to the negative impacts of coccidiosis on global economic and nutritional well-being. For many years, anti-coccidial drugs have been the primary method of control, but frequent cases of drug resistance have negatively affected its effectiveness (Zhang et al., 2012). Vaccines are considered the most effective method due to their efficacy and low likelihood of resistance development. However, the drawbacks of current vaccines include their limited broad-spectrum effectiveness, side effects, and costs, as most are from live and attenuated organism sources. The low efficiency associated with these vaccine preparations is primarily due to the antigenic variation among *Eimeria* species, driven by retro-transposons, resulting in limited cross-protection (Hinsu et al., 2018). Currently, there is rarely any chicken coccidiosis vaccine that provides protection against a wide range of *Eimeria* species. Immunoinformatics-designed multi-epitope vaccines are gaining recognition for their cost-effectiveness and flexibility in combating various pathogens. Subunit vaccines, unlike conventional ones, are safer because they do not contain infectious particles, reducing the risk of reinfection. Computationally optimized subunit vaccines are being recommended for disease prevention due to their overwhelming advantages over conventional vaccines (Sharma et al., 2021). Given the characteristic features of surface antigens as immune targets and the role of sporozoites in coccidiosis, surface proteins from sporozoite antigens were selected for this study. These proteins were chosen for their strong host-parasite interaction, ability to facilitate attachment and invasion, immune protection, and low variability (Vo et al., 2021). They include; the AMA-1 and other surface antigens like the Microneme and Immune-mapped proteins (MIC and IMP) (Liu et al., 2019; Wang et al., 2023). This in-silico

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study, therefore aimed at mining multiple viable epitopes of immunological interest from the various known chicken coccidiosis pathogens and designing a potential broad-spectrum vaccine construct via a stringent evaluation methodology.

2. METHODOLOGY

2.1 Target sequence Retrieval, Epitopes Prediction and Selection

Surface protein sequences from the known pathogenic chicken *Emeiria*, species were selected. This was followed by retrieval of sequences of immunological importance from UniprotKb repository in FASTA format. The retrieved sequences were subjected to standard immunoinformatics web tool of NetMHCI v 4.0, NetMHCII v2.3 and Immune Epitope Database (IEDB) for the various epitopes mining processes. At default thresholds, strong antigenic epitopes with relatively lower threshold were preferably selected on the basis of their major histocompatibility complex classes I and II binding abilities for the T-lymphocytes. Epitopes' percentile ranks and locations were considered for the Linear B-Lymphocyte mining process (Tarrahimofrad, et al., 2021; Khan et al., 2023).

2.2 Epitopes Screening

The selected predicted epitopes were subjected to screening pipelines to eliminate the poor immunologic and unsafe sequences by running them through antigenicity, allergenicity, immunogenicity and toxicity analyses. Antigenic potentials of the epitopes were analyzed using VaxiJen 2.0 web server whereas allergenicity and toxicity were evaluated with AllerTop 2.0 and Toxinpred web tools. The predicted HTL epitopes were further subjected to interleukin and cytokine inducing capacity evaluations with IFN-γ, IL-4 and IL-10 tools (Kumar et al 2021) for final selection. The selected CTLs, HTLs and LBC epitopes were shown in Tables 1, 2, and 3 respectively.

2.3 Vaccine Construct Design

The selected vaccine-fit epitopes of the linear B-lymphocytes and T-lymphocytes from the mined pool in conjunction with adjuvants and linkers were subjected to optimization process to design the potential vaccine construct shown in Figure 1 and 2 as primary and secondary structures respectively. Beta- defensing-3 and PADRE sequences were retrieved and used as adjuvants while EAAK, AAY, GPGPG and KK sequences were the linkers used. Linkers AAY and GPGPG were used separately for HTLs in different constructs while LBL epitopes were flanked by KK sequences. The connecting bond used for adjuvant-epitope linkage was the EAAK sequence (Madanagopal, 2023).

2.4 Vaccine Properties Prediction

The resulting construct was assessed for immunological, physicochemical and structural potentials (Bin-sayed et al., 2020). Double evaluations of the immunological parameters were performed. Antigenicity and allergenicity analyses were carried out with VaxiJen v2.0 / AntigenPro and AllerTop v2.0 / AllergenFP servers respectively. Toxicity evaluations were performed with ToxinPred2 and ToxDL web tools. The physicochemistry of the construct was analyzed with ExPASy-ProtParam web tool for quality and stability determination (Mahmud et al., 2021; Bashir et al., 2023). Key physiochemical factors like instability index, amino acid composition, aliphatic index, molecular formula, molecular mass, isoelectric potential (pI), extinction coefficient and the Grand Average of Hydropathicity (GRAVY), values were all analyzed (Pandey et al., 2021).

2.5 Vaccine Construct's Structural analysis

The secondary structure of the vaccine construct was predicted using the PSIPRED 4.0 server through the Psi-BLAST algorithm (McGuffin et al., 2000). For 3-D structure determination and modeling, the PHYRE 2.0 tool was employed (Roy et al., 2010). Refinement of the 3-D structures was conducted using GalaxyRefine web server, which uses CASP10 refining method, to assess structural stability (Kumar et al., 2023). The refined structures were then downloaded and the selected model was chosen based on overall quality assessments (Yang et al., 2022). Structural authentication of the vaccine constructs was done with Procheck and ProSA tools, which provided the Z-score and Ramachandran values, respectively (Wiederstein and Sippl, 2007). The construct's structural validation and molecular docking were also carried out. Chicken Toll-like receptor-15 (7-YLG) was used for docking the vaccine construct to assess their binding interactions. ClusPro v2.0 webserver which operates on PIPER algorithm was used for docking and data generation (Kozakov et al., 2017).

3. RESULTS

3.1 Epitopes Screening and Conjugation

A total of 26 epitopes consisting of 6 Linear B-lymphocytes, 7 Cytotoxic T-lymphocytes, and 13 Helper T-lymphocytes were conjugated to form the final construct which was consequently evaluated for antigenicity, allergenicity, and toxicity using two different web tools for each analysis, as detailed in

Table 1: Selected LBL Epitopes

S/N	Sequence	Length	Antigenicity	Allgen	Toxicity
1	DEEKEQNKAD	10	1.9332	None	None
2	QAEKAQEAAA	10	1.1195	None	None
3	DVETQQEQPG	10	1.7369	None	None
4	VRNGPSVDE	9	1.0819	None	None
5	GELCSAPAPTL	11	0.6525	None	None
6	DVGNTTQKTK	10	1.3775	None	None

Table 2: Selected HTL Epitopes

S/N	Allele	Sequence	Antigenicity	Allergenicity	Toxicity	Il-4	IL-10	IFN
1	HLA-DQA10201-DQB10402	RCAEMSYKTTASRNS	1.2121	None	None	+		
2	HLA-DPA10103-DPB10301	LLYRSRMRPAAKGDE	0.4939	None	None			+
3	HLA-DQA10201-DQB10402	KCAEMSFMTTAGKNS	0.6243	None	None	+		
4	HLA-DQA10501-DQB10201	NLTDEEVAEYDFEEL	0.8699	None	None	+		
5	HLA-DPA10201-DPB10501	QPIDANVYEALLKRQ	0.4900	None	None		+	
6	HLA-DQA10104-DQB10503	DEMEASFTLHHFAAP	0.6014	None	None	+		
7	DRB3_0101	LLDVMLVVDESGSIG	0.4638	None	None			+
8	HLA-DPA10103-DPB10301	DAALRLRRWDIIVSI	1.0522	None	None	+		

Table 2(cont): Selected HTL Epitopes								
9	HLA-DQA10103-DQB10603	.GTGITATAAAAETPA	1.0490	None	None	+		
10	HLA-DQA10201-DQB10202	GGASSFGGSTIDELA	0.7664	None	None			+
11	DRB1_0101	STCFRQGVGYKATEA	0.9143	None	None	+		
12	DRB1_0404	EANLLWTLPSENAEE	0.7075	None	None		+	
13	HLA-DQA10201-DQB10202	QEMGSEVETAEECQL	1.1766	None	None	+		

Table 3: Selected CTL Epitopes						
S/N	**Allele**	**Sequence**	**Allergenicity**	**Antigenicity**	**Immunogenicity**	**Toxicity**
1	HLA-A0301	MSYKTTASR	None	1.2632	-0.2075	None
2	HLA-C0401	ILDTEGSSL	None	1.1293	-0.06062	None
3	HLA-A0201	ILLASFVPA	None	1.0619	0.01323	None
4	HLA-A2601	ITLDAAGGY	None	1.1189	0.14205	None
5	HLA-C0303	NANLPHAYL	None	0.8173	0.03725	None
6	HLA-B3901	AQHGGRTCM	None	1.2861	0.12758	None
7	HLA-C0303	VSGGGGTAL	None	2.8448	0.16562	None

Table 4: Immunological and Physicochemical Analyses				
Parameters	**Tool**	**Value**	**Status**	**Remarks**
	Immunological			
Antigenicity	VaxiJen 2.0	1.0735	Very Antigenic	Very good
	AntigePro	0.9348	Very Antigenic	Very good
Allergenicity	AllerTop 2.0	None	Non-allergenic	Very good
	AllergenFP	None	Non-allergenic	Very good
Toxicity	ToxinPred2.0	0.0132	Non-toxic	Very good
	ToxDL	0.0005	Non-toxic	Very good
	Physicochemical			
Chemical formula	ProtParam	$C_{2236}H_{3542}N_{640}O_{728}S_{22}$	Balanced composition	Okay
No of amino acids	ProtParam	504	Moderate content	Good
Solubility	Protein-Sol	0.9784	Quite soluble	Good
Molecular weight	ProtParam	51743.89Da	Within normal range	Good
Theoretical pI:	ProtParam	5.74	Within normal range	Good
GRAVY Value	ProtParam	-0.570	Hydrophilic	Good
Extinction coefficient	ProtParam	36330 M^{-1} cm^{-1}	Within normal range	Good
Instability Index	ProtParam	38.07	Very Stable	Very good
Aliphatic index	ProtParam	56.92	Thermostable	Good
Half-Life	ProtParam	-1hr in-vitro in human reticulocyte. -30 min in-vivo in yeast. >10 hours in-vivo in E. coli,		Good

Table 5: GalaxyRefine Result showing 3-D model structures information						
Model	**GDT-HA**	**RMSD**	**MolProbity**	**Clash score**	**Poor rotamers**	**Rama favored**
Initial	1.0000	0.000	3.177	81.2	0.0	74.4
MODEL 1	0.9556	0.424	2.326	15.4	2.6	95.3
MODEL 2	0.9444	0.428	2.105	19.3	0.0	95.3
MODEL 3	0.9889	0.406	2.252	16.7	0.0	93.0
MODEL 4	0.9611	0.615	2.194	11.1	0.0	90.7
MODEL 5	0.9667	0.402	2.047	16.7	0.0	95.3

Table 6: Cluspro Result showingMolecular Docking information

Cluster	Member	Representative	Weighted Score
0	189	Center Lowest energy	-715.1 -918.3
1	149	Center Lowest energy	-733.0 -853.5
2	108	Center Lowest energy	-720.4 -883.4
3	74	Center Lowest energy	-720.1 -773.4
4	68	Center Lowest energy	-716.8 -845.5
5	67	Center Lowest energy	-805.3 -838.4

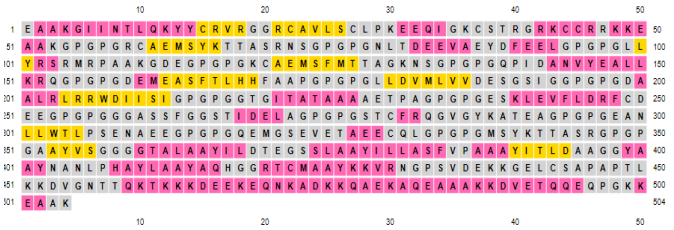


Figure 2: Primary structures of the Constructs

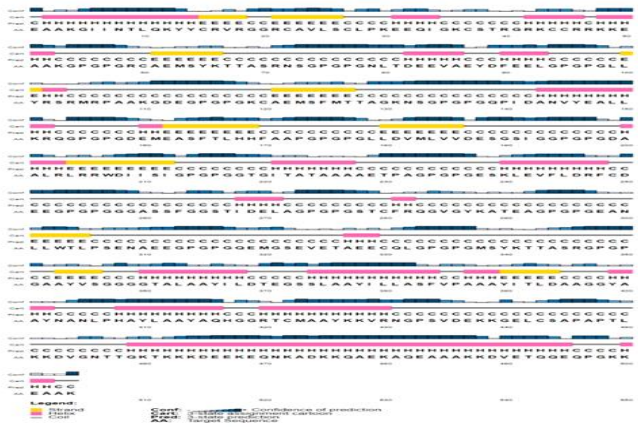


Figure 3: Secondary structures of the Constructs

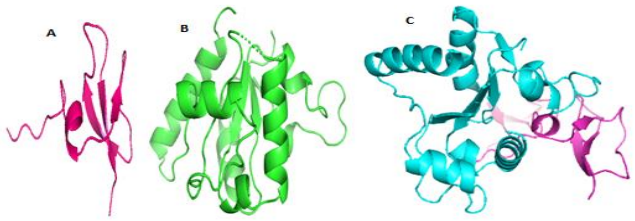
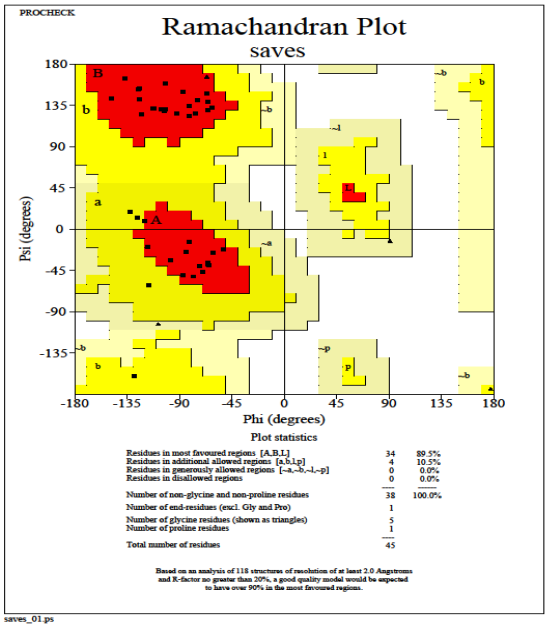


Figure 4: 3-D structures of A) Vaccine construct B) TLR-15 Receptor and C) Docked Vaccine-Receptor Complex



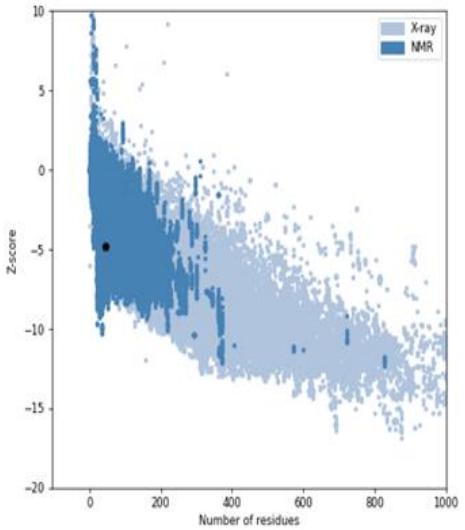


Figure 5: A) Ramachandran and B) Z-Score of the Constructs

4. Discussion

The development of prophylactic agents that can address the antigenic diversity challenge in chicken coccidiosis pathogenesis and overcome the low immunogenicity of current vaccines is necessary for the effective prevention of the disease. Advancements in structural and functional vaccinomics, which integrates 'Omics' technologies with reverse vaccinology, have significantly enhanced vaccine-likeness screening and development, especially against a variety of pathogens (Soltan et al., 2021). In-silico epitope mining and evaluation involve the use of computational tools to identify peptide sequences within antigens that can trigger immune responses.

In this study, mining and conjugation of potentially effective multi-epitope were carried out using several computational algorithms after which the final vaccine candidate was subjected to stringent immunological, structural, and physichochemical evaluations. The strong antigenicity, non-allergenicity, and non-toxicity of the construct were affirmed by the immunological analysis results. The physicochemical characterizations also revealed the excellent vaccine-likeness of the constructs as shown in Table 4. The molecular weights and instability indices of 51.743 kDa, and 38.07 which are less than 110 kDa and 40.0 respectively are in agreement with Kumar et al., (2023).

The values of 56.92 and -0.570 recorded for GRAVY and aliphatic index respectively indicate good thermo-stability and hydrophilicity, preferable for good water molecule interaction in conformity with Atapour et al., (2020) and Kumar et al., (2023). The construct exhibited good solubility, isoelectric points, and extinction coefficients values of 0.9784, 5.74, and 36330 M-1 cm-1 which also conform with Habib et al., (2023) and sarvmeili et al., (2024). The predicted half-life for the construct is 1 hour in-vitro, in mammalian reticulocytes, 30 minutes, and over 10 hours in-vivo in yeast and E coli respectively. This is good for further pre-clinical investigative analysis. The secondary structure determination and examination using PSIPRED identified regions consisting of helices, β-strands, and random coils.

From the five refined 3-D models generated by GalaxyRefine, model-3 was selected based on its Clash scores, GDT-HA, RMSD, Poor rotamer, and Ramachandran favored scores as shown in Table 5. The chosen refined model of interest was downloaded and validated using Prosa and Procheck web tools to produce its Z-score and Ramachandran plots as shown in Figure 5.0. Docking results from the ClusPro server demonstrated the binding stability and structural compactness of different conformations as a measure of the cluster sizes and binding energy values as shown in Table 6. The selection of Complex-' 0' as the best vaccine-receptor complex was primarily based on cluster size, as ClusPro ranks structures according to cluster population rather than solely energy values, in line with the approach of Desta et al. (2020).

5. Conclusion

Bioinformatics skills application in the exploration of immune components of living organisms has led to faster, cheaper, specific, and effective vaccine development process. Using computational optimization as a precursor to the wet lab validation process is an approach that is revolutionizing 'Vaccinology' in modern vaccine development. The results of in-silico guided mining and designing of potential broad-spectrum chicken coccidiosis vaccine construct from this current study produced a promising vaccine candidate based on vaccinomics evaluations. This research therefore provides a solid foundation for further molecular immunoinformatics analyses and wet lab validation using animal models for the development of an effective broad-spectrum chicken coccidiosis vaccine.

Authors' Contribution

O.C designed and carried out the in-silico experimentation while A.S performed the data analysis and proofreading.

Conflicts of Interest

"We hereby make a declaration no conflicts of interest regarding this work".

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