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Original Article

Exploring Adenylate Kinase 8 (ADK8) of *Echinococcus granulosus* as a Novel Immunogen against Cystic Echinococcosis: A Computational Perspective

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Abstract

***Background*:** Vaccination represents a promising strategy for preventing hydatidosis. We aimed to characterize the physicochemical properties of adenylate kinase 8 from *Echinococcus granulosus* (EgADK8) and identify potential B- and T-cell epitopes as vaccine candidates.

Methods: Multiple web-based servers were employed to predict immunogenic epitopes and evaluate the physicochemical, antigenic, structural, and subcellular characteristics of EgADK8.

Results: EgADK8 was predicted to be an extracellular protein with a molecular weight of 38.78 kDa. The protein showed no allergenic potential and exhibited high hydrophilicity, predicted thermal stability, and 36 potential post-translational modification sites. The secondary structure was mainly composed of alpha helices and random coils. The highest-quality three-dimensional model generated by the Robetta server achieved a confidence score of 0.79. Three independent prediction servers identified 16 linear and 9 conformational B-cell epitopes. Furthermore, two helper T lymphocyte (HTL) epitopes with predicted IFN-γ-inducing potential were identified after comprehensive screening. Additionally, five immunogenic human cytotoxic T lymphocyte (CTL) epitopes with predicted IFN-γ-inducing potential and three murine CTL epitopes were identified.

Conclusion: These findings provide preliminary computational evidence supporting further investigation of *Eg*ADK8 as a potential component of a multiepitope vaccine against CE. The main limitation of this study was its reliance on computational analyses. Therefore, experimental validation using the whole protein and/or selected epitopes, either individually or in combination with other antigens, is required.

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Introduction

Hydatidosis, also known as cystic echinococcosis (CE), is a major zoonotic parasitic disease caused by the metacestode stage of *Echinococcus granulosus*, representing a substantial public health concern (1, 2). In canids, the definitive hosts, adult tapeworms release gravid proglottids in the intestinal lumen, resulting in environmental contamination through fecal shedding of parasite eggs (3). The ingestion of parasite ova via contaminated forage by herbivores (e.g., cattle, sheep) and omnivores, including humans, leads to the development of chronic hydatid cysts, particularly in the liver and lungs (4, 5). Individuals affected by CE may suffer from impotence and disability (4, 6).

Current control strategies for CE rely mainly on public health education, improved hygiene practices during slaughter, and regular anthelmintic treatment of dogs in endemic regions (7). However, pharmacotherapeutic approaches have limitations such as drug resistance and the presence of drug residues in the dairy products of treated livestock (8, 9). Consequently, vaccination strategies are emerging as potentially more effective preventive measures (9-11). Various vaccine candidates have been investigated for their immunogenic properties and ability to provoke protective responses. A notable example is the oncospheral protein EG95, shown prophylactic efficacy against *E. granulosus* infections in multiple countries (12). Proteins expressed at different life stages of the parasite are considered promising vaccine candidates, as they may confer broader protection against multiple parasitic stages. Adenylate kinase 8 (*Eg*ADK8) is identified as a particularly advantageous candidate for vaccine development, due to its potential to provide comprehensive protection across different developmental stages of the worm. Its expression in strobilated worms, the germinal layer, and protoscoleces (PSCs) suggests its involvement in parasite development and highlights its potential as a vaccine target (13).

Conventional vaccinology traditionally relies on the use of killed or live-attenuated pathogens for vaccine development. Although this approach has been successful for several vaccines, it has important limitations, including biosafety concerns, lengthy development processes, complex manufacturing requirements, and high production costs (14, 15). In contrast, reverse vaccinology provides a computational approach that utilizes genomic and immunoinformatics analyses, including epitope prediction, to facilitate the rational design of epitope-based vaccines (16, 17).

Because EgADK8 is a multi-stage antigen and its predicted immunogenic potential, we sought to predict its immunogenic epitopes particularly, along with biochemical, antigenic, and structural characteristics using computational tools.

Materials and Methods

Amino acid sequence retrieval

The protein sequence corresponding to *Eg*ADK8 (Accession No.: KAH9284668.1) was acquired in FASTA format from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/).

Assessment of Antigenicity, Allergenicity, and Physico-Chemical Properties

Computational analysis of the protein's physicochemical parameters, including molecular weight (MW), amino acid composition, theoretical isoelectric point (pI), instability index, aliphatic index, grand average of hydropathicity (GRAVY), and estimated half-life, was performed using the ProtParam tool (18, 19). Antigenicity was predicted via the VaxiJen v2.0 server (threshold: 0.4; http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), while allergenicity was evaluated using AllergenFP v1.0 (88.9% accuracy; https://ddg-pharmfac.net/AllergenFP/) and AllerTOP v2.0 (85.3% accuracy; https://www.ddg-pharmfac.net/AllerTOP/). Protein solubility under native conditions was estimated using the ProteinSol server (https://protein-sol.manchester.ac.uk/) with a 0.45 threshold.

Post-translational modification (PTM) sites

Potential PTM sites were identified using specialized prediction tools. Phosphorylation sites (serine, threonine, tyrosine) were predicted using NetPhos 3.1. Acetylation and glycosylation (O- and N-linked) sites were analyzed using GPS-PAIL, NetOGlyc 4.0, and NetNGlyc 1.0, respectively. All tools were accessed via the DTU Health Tech portal (https://services.healthtech.dtu.dk/) and BioCuckoo repository (https://www.biocuckoo.org/).

Transmembrane domains and localization

Subcellular localization was predicted using DeepLoc 2.0 (https://services.healthtech.dtu.dk/services/DeepLoc-2.0/). Signal peptide regions and transmembrane helices were identified using the SignalP 6.0 and TMHMM 2.0, respectively, through the DTU Health Tech server suite, at https://services.healthtech.dtu.dk/services/.

Structural characterization

Secondary structure features, solvent accessibility, and disordered regions were evaluated using NetSurfP-3.0 (https://services.healthtech.dtu.dk/services/NetSurfP-3.0/). The tertiary structure was modeled via the Robetta server (https://robetta.bakerlab.org/), which employs automated homology modeling and provides confidence scores (C-score) ranging from 0 (low confidence) to 1 (high confidence).

Linear and non-linear B-cell epitopes

Linear B-cell epitopes were predicted using ElliPro, ABCpred, and SVMTriP (thresholds: default parameters; http://tools.iedb.org/ellipro/; http://crdd.osdd.net/raghava/abcpred/ABC_submission.html; http://sysbio.unl.edu/SVMTriP/prediction.php). Overlapping epitopes were further screened for antigenicity, allergenicity, and solubility. Discontinuous (non-linear) epitopes were analyzed using ElliPro with parameters set to 6 Å maximum distance and a minimum score of 0.5 (20).

Human leukocyte antigen (HLA)-associated epitopes

HLA class I and II binding epitopes (15-mer and 12-mer peptides) were predicted using the IEDB Analysis Resource, employing the NetMHCIIpan 4.1 EL algorithm and HLA reference dataset. Top-ranked epitopes were evaluated for cytokine-inducing potential: IFN-γ (via IFNepitope; https://webs.iiitd.edu.in/raghava/ifnepitope/predict.php) and IL-4 (via IL4pred; https://webs.iiitd.edu.in/raghava/il4pred/index.php). Antigenicity and immunogenicity were assessed using VaxiJen v2.0 and the IEDB class I immunogenicity tool, respectively. Allele population coverage prediction was also done using the IEDB population coverage tool (http://tools.iedb.org/population/).

Results

General properties

The *Eg*ADK8 protein exhibited a MW of 38,784.90 Da and a theoretical pI of 7.15, with an equal distribution of positively (n=46) and negatively (n=46) charged residues. Stability analysis predicted a half-life of 30 h in mammalian reticulocytes, with moderate instability (instability index: 42.46) under experimental conditions. Hydrophilicity was indicated by a GRAVY score of -0.239, while thermostability was supported by an aliphatic index of 98.02. Antigenicity prediction via VaxiJen v2.0 yielded a score of 0.5327 (threshold: 0.4), confirming its antigenic potential. No allergenic motifs or IgE epitopes were detected using AllergenFP and AllerTOP v2.0. Protein solubility, assessed via Protein-Sol, demonstrated favorable solubility with a score of 0.47 (threshold: >0.45).

Prediction of PTM sites

Analysis identified 34 phosphorylation sites (serine: 18, tyrosine: 12, threonine: 4) (Fig. 1), one N-linked glycosylation site (position 173), and one O-linked glycosylation site (position 204). No lysine acetylation sites were identified.

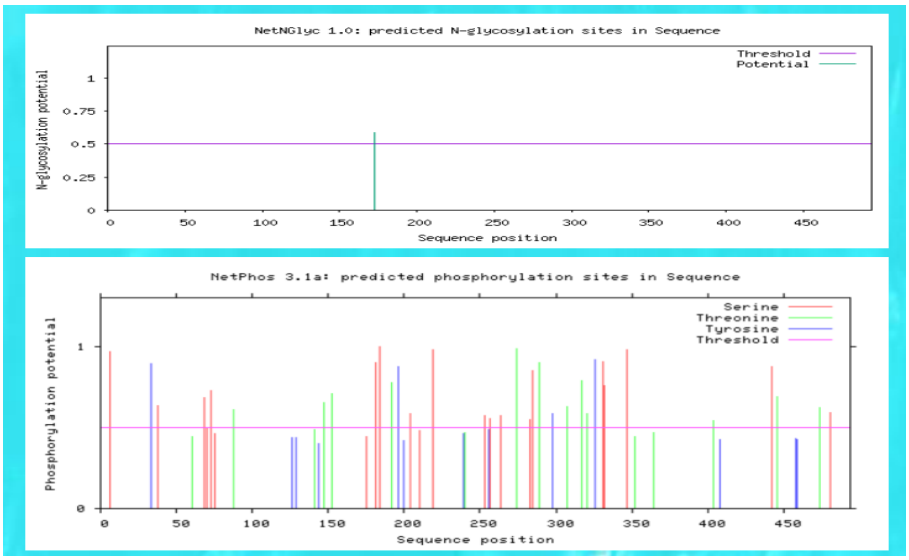


Fig. 1: Prediction of phosphorylation (serine, threonine, tyrosine) and N-glycosylation sites of the *Eg*ADK8 protein using NetPhos 3.1 and NetNGlyc 1.0 web servers, respectively. There existed 15 phosphorylation sites and a single N-glycosylation site in this protein

Transmembrane domains, signal peptide, and subcellular localization

SignalP-6.0 and TMHMM 2.0 analyses confirmed the absence of signal peptides and transmembrane helices, respectively. Subcellular localization prediction via DeepLoc 2.0 classified *Eg*ADK8 as a cytoplasmic protein with a confidence score of 0.7949.

Extrapolation of EgADK8 structures

Secondary structure analysis using NetSurfP-3.0 revealed a predominance of α-helices and random coils, with fewer extended strands. Surface accessibility analysis indicated that most residues were solvent-exposed, with no significant disordered regions (Fig. 2). Tertiary structure modeling via the Robetta server, employing the RoseTTAFold homology modeling algorithm, generated a high-confidence 3D model (C-score: 0.79). Model 1 was selected based on minimal error estimates (Fig. 3).

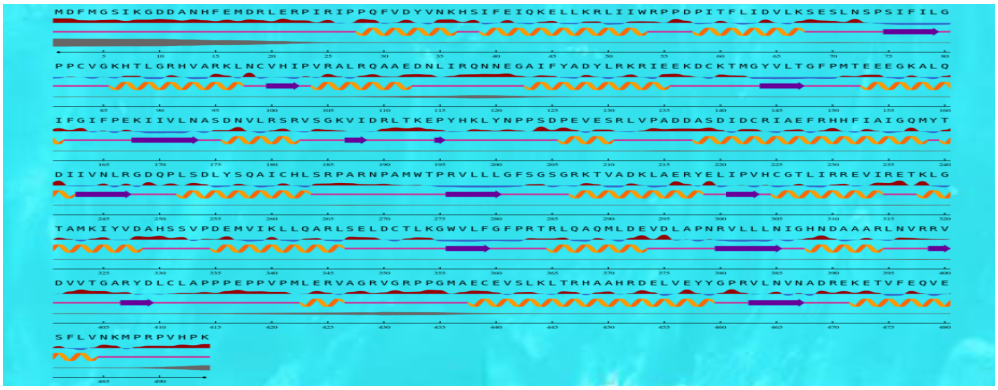


Fig. 2: NetSurfP-3.0 showed that helices and random coils are dominant within *Eg*ADK8 protein. Helices (orange zigzag); Strands (violet arrow); Coils (pink line). Red peaks: exposed residues; Blue reverse peaks: not-exposed residues; Thickness of the gray line indicates to possible disordered areas

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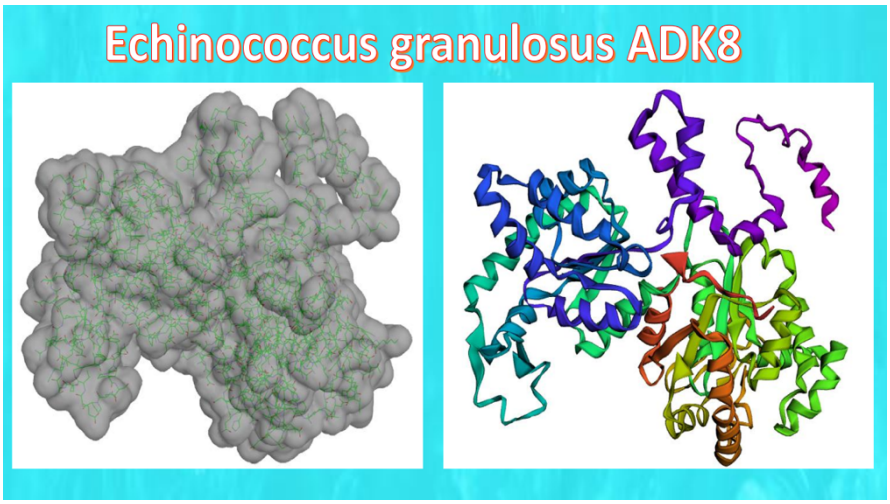


Fig. 3: 3D structure of the *Eg*ADK8 protein as ribbon structure (right) with surface topology (left), predicted by the Robetta server (C-score: 0.79)

B-cell epitopes prediction

The ABCpred web server (threshold: 0.8) identified ten linear B-cell epitopes. Cross-validation using SVMTriP and ElliPro yielded 16 overlapping epitopes, shared at least between two web servers, of which nine exhibited antigenicity (VaxiJen score >0.45), solubility (PepCalc), and non-allergenic properties (Table 1). ElliPro further predicted nine discontinuous epitopes with varying lengths and scores: 1) 6 residues (0.986), 2) 7 residues (0.982), 3) 8 residues (0.913), 4) 132 residues (0.709), 5) 87 residues (0.689), 6) 5 residues (0.604), 7) 4 residues (0.597), 8) 10 residues (0.565), and 9) 3 residues (0.506) (Fig. 4).

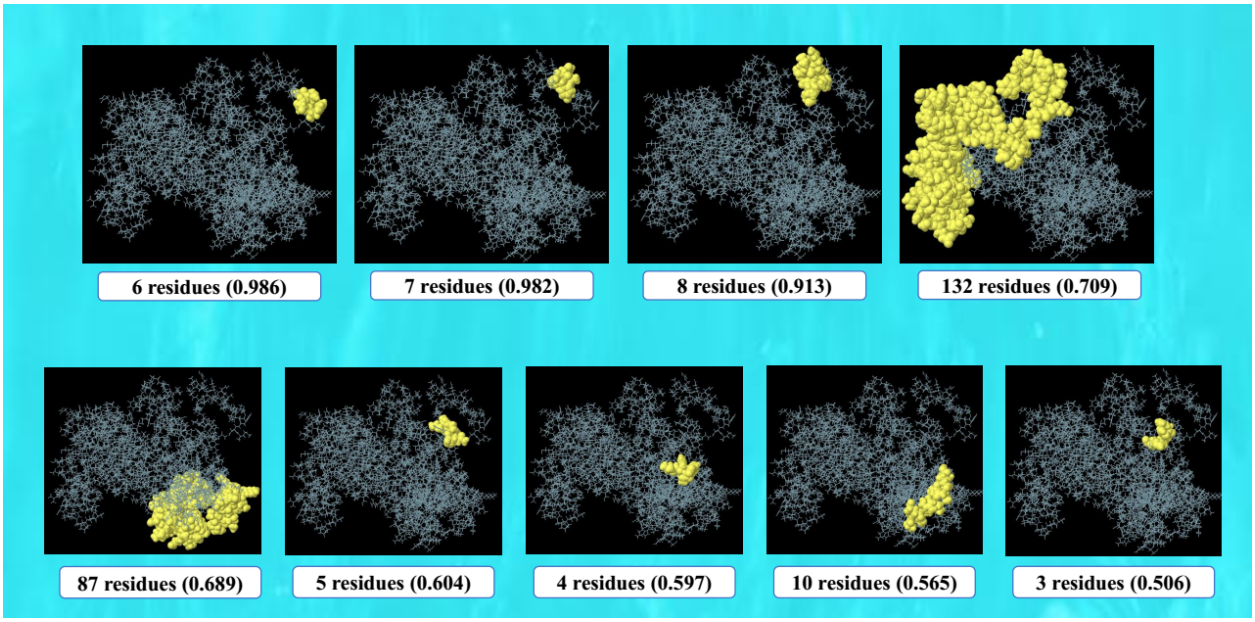


Fig. 4: Non-linear B-cell epitopes of the *Eg*ADK8 predicted by ElliPro with respective residues and prediction scores in parentheses

Table 1: Common linear B-cell epitopes discovered by three web servers (ABCpred, SVMTriP, and ElliPro). Asterisks indicate to the potential antigenic, soluble, and non-allergenic epitopes

Epitope No.	Epitope Sequence	Antigenicity (VaxiJen server)	Allergenicity (AllergenFP server)	Solubility (PepCalc server)
1	AGRVGRPPGMAECEVS	0.5105	No	Good
2	RIEEKDCKT	1.1284	Yes	Good
3	EGAIFYADYLRKRIE	0.9755	No	Good
4	LIIWRPPDPITFLID	1.0826	No	Poor
5	TKEPYHKLYNPPSDPE	0.1157	No	Good
6	PVPMLERVAGRVGRPP	-0.0628	No	Good
7	MGSIKGDDANHFEMDR	0.5702	No	Good
8	DAHSSVPDEM	0.8381	No	Good
9	LVPADDASDIDCRIAE	1.0267	No	Good
10	REVIRETKLGTAMKIY	0.9049	No	Good
11	GHNDAAARLNV	0.4143	No	Good
12	NHFEMDRLERPIRIPP	0.2750	Yes	Good
13	GMAECEVSLKLTR	0.3060	No	Good
14	NEGAIFYADYLRKRIE	0.7102	No	Good
15	ADDASDIDCRIAEFRH	1.1666	No	Good
16	ASDNVLRSRVSG	0.7965	No	Good

Epitopes associated with mouse and human MHC molecules

Among the top 20 predicted HTL epitopes, three demonstrated IFN-γ induction potentials, while seven lacked IL-4 induction capacity. A single epitope (GKVIDRLTKEPYHKL) exhibited dual non-IL-4-inducing and IFN-γ-inducing properties (Table 2). For murine HTL epitopes, one candidate (VEYYGPRVLNVNADR) showed IFN-γ induction without IL-4 activation (Supplementary Table 1). Five immunogenic human CTL epitopes (HTLGRHVAR$_{17-25}$, IWRPPDPITF$_{52-61}$, RPIRIPPQF$_{22-30}$, RPPDPITFL$_{54-62}$, QIFGIFPEK$_{20-28}$) were identified with IFN-γ induction capacity (Table 3). Three murine CTL epitopes (IIWRPPDPITFL$_{51-62}$, IWRPPDPITFLI$_{52-63}$, FEMDRLERPIRI$_{15-26}$) were also predicted (Supplementary Table 2). The world population coverage for HTL and CTL epitopes-alleles compositions predicted in the present study was 83.42% and 77.6%, respectively. Endemic areas, particularly East Africa, Oceania, and South America, were covered mostly by the HTL epitopes, with 71.77%, 82.02%, and 92.32%, correspondingly (Fig. 5).

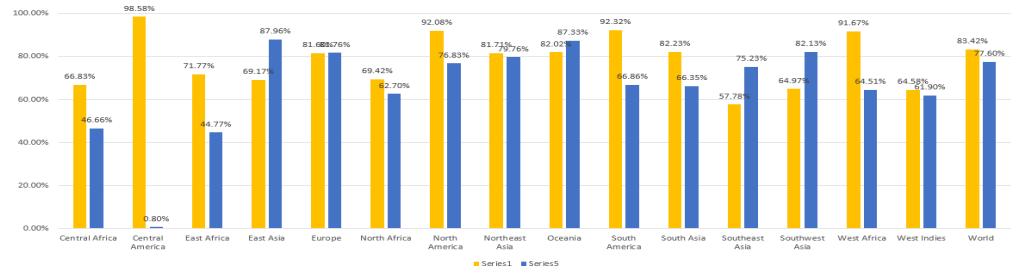


Fig. 5: HLA allele coverage regarding HTL epitopes-alleles (series 1) and CTL epitopes-alleles (series 2) compositions predicted in the present study, using the IEDB population coverage analysis tools; a moderately high population coverage was predicted for ADK8's epitopes, particularly regarding HTL epitope allele compositions in endemic areas, including South America (92.32%), Oceania (82.02%), and East Africa (71.77%). The world coverage for HTL and CTL epitopes was 83.42% and 77.6%, respectively

Table 2: HTL-associated epitopes for *Eg*ADK8 using HLA reference set and screening

Protein	Alleles from HLA reference set	Start-End	HTL epitope	Percentile rank	Antigenicity	IFN-γ inducing	IL-4 induction
***Eg*ADK8**	HLA-DRB1*15:01	452-466	RDELVEYYGPRVLNV	0.04	-0.0147	Negative	**Negative**
	HLA-DRB1*15:01	451-456	HRDELVEYYGPRVLN	0.06	-0.0453	Negative	**Negative**
	HLA-DRB1*07:01	473-487	ETVFEQVESFLVNKM	0.06	-0.2198	Negative	Positive
	HLA-DRB1*07:01	472-486	KETVFEQVESFLVNK	0.06	-0.1393	Negative	Positive
	HLA-DRB1*12:01	184-198	SGKVIDRLTKEPYHK	0.07	0.3397	Negative	**Negative**
	HLA-DRB3*01:01	4-18	MGSIKGDDANHFEMD	0.07	0.8655	Negative	Positive
	HLA-DRB3*01:01	3-17	FMGSIKGDDANHFEM	0.08	1.0916	Negative	Positive
	HLA-DRB1*12:01	185-199	GKVIDRLTKEPYHKL	0.09	0.2283	**Positive**	**Negative**
	HLA-DRB1*04:01	193-207	KEPYHKLYNPPSDPE	0.09	0.1178	Negative	Positive
	HLA-DRB3*01:01	2-16	DFMGSIKGDDANHFE	0.1	1.0654	Negative	Positive
	HLA-DQA1*05:01/DQB1*02:01	4-18	MGSIKGDDANHFEMD	0.11	0.8655	Negative	Positive
	HLA-DRB3*01:01	5-19	GSIKGDDANHFEMDR	0.11	0.8399	**Positive**	Positive
	HLA-DRB1*15:01	320-334	GTAMKIYVDAHSSVP	0.14	0.7808	Negative	**Negative**
	HLA-DQA1*03:01/DQB1*03:02	459-473	YGPRVLNVNADREKE	0.15	0.7043	Negative	Positive
	HLA-DQA1*03:01/DQB1*03:02	460-474	GPRVLNVNADREKET	0.17	0.6232	Negative	Positive
	HLA-DPA1*02:01/DPB1*14:01	321-335	TAMKIYVDAHSSVPD	0.17	0.8229	**Positive**	Positive
	HLA-DRB1*12:01	183-197	VSGKVIDRLTKEPYH	0.17	0.1861	Negative	**Negative**
	HLA-DRB1*04:01	192-206	TKEPYHKLYNPPSDP	0.17	0.1364	Negative	Positive
	HLA-DRB3*01:01	322-336	AMKIYVDAHSSVPDE	0.18	1.1658	Negative	Positive
	HLA-DRB1*15:01	453-467	DELVEYYGPRVLNVN	0.18	-0.1014	Negative	**Negative**

Table 3: CTL-associated epitopes for *Eg*ADK8 using HLA reference set and screening

Protein name	MHC-I allele	Start	End	Peptide	Percentile rank	Immunogenicity	IFN-γ inducer
***Eg*ADK8**	HLA-A*68:01	9	17	TVADKLAER	0.01	-0.0939	Negative
	HLA-B*40:01	17	25	AECEVSLKL	0.01	-0.16764	Negative
	HLA-B*40:01	36	44	VEYYGPRVL	0.02	0.08544	Negative
	HLA-A*01:01	10	18	VADKLAERY	0.01	-0.06903	Negative
	HLA-A*32:01	14	22	RIAEFRHHF	0.01	0.32237	Negative
	HLA-B*40:01	35	43	RETKLGTAM	0.04	-0.12768	Negative
	HLA-A*31:01	17	25	HTLGRHVAR	0.01	0.16905	**Positive**
	HLA-A*24:02	28	36	MYTDIIVNL	0.01	0.32086	Negative
	HLA-A*24:02	52	61	IWRPPDPITF	0.01	0.141	**Positive**
	HLA-B*07:02	22	30	RPIRIPPQF	0.04	0.1374	**Positive**
	HLA-B*44:03	14	22	EEGKALQIF	0.03	-0.19834	Negative
	HLA-B*15:01	47	55	RQNNEGAIF	0.01	0.23157	Negative
	HLA-B*07:02	54	62	RPPDPITFL	0.01	0.23436	**Positive**
	HLA-A*23:01	28	36	MYTDIIVNL	0.04	0.32086	Negative
	HLA-A*23:01	52	61	IWRPPDPITF	0.01	0.141	Positive
	HLA-B*44:03	17	25	AECEVSLKL	0.02	-0.16764	Negative
	HLA-A*11:01	20	28	QIFGIFPEK	0.03	0.36104	**Positive**
	HLA-A*68:01	36	44	ETKLGTAMK	0.02	-0.0812	Negative
	HLA-A*68:01	57	65	MVIKLLQAR	0.06	-0.26994	Negative
	HLA-A*26:01	54	62	TVFEQVESF	0.07	0.05265	Negative

Discussion

This study investigated *Eg*ADK8, a protein expressed consistently across parasitic stages (e.g., PSCs and strobilated adults), as a potential immunogen, with special focus on its immunogenic epitopes.

The physicochemical and structural

characteristics of the *Eg*ADK8 protein were systematically evaluated using bioinformatics tools. Employing the ExPASy ProtParam server, the primary sequence analysis revealed a protein composed of 344 amino acids with a MW of approximately 38.78 kDa, indicating a size compatible with antigenic proteins (21). The theoretical pI of 7.15 classifies *Eg*ADK8 as a neutrally charged molecule under physiological conditions, while an instability index of 42.46 suggests moderate stability profile (22). Further thermodynamic profiling via the aliphatic index (98.02) may forecast a propensity for structural resilience across fluctuating thermal environments, advantageous for maintaining conformational integrity during vaccine formulation and storage. The GRAVY score of -0.239 gives computational evidences of hydrophilicity nature of *Eg*ADK8, enhancing solvent interactions and epitope accessibility for immune recognition (23). Complementary solubility analysis using the Protein-Sol server yielded a score of 0.47, predictive of favorable aqueous solubility, a critical attribute for efficient recombinant protein expression and downstream purification processes. Antigenic potential was quantitatively assessed via the VaxiJen v2.0 platform, which assigned *Eg*ADK8 a score of 0.5327, exceeding the default pathogen-derived threshold (0.45), thereby confirming its capacity to provoke adaptive immune responses *in silico*. Rigorous allergenicity screening, conducted across multiple predictive algorithms (AllerTOP v2.0, AllergenFP v1.0, and AlgPred), consistently classified *Eg*ADK8 as non-allergenic, indicating a low predicted risk of allergenicity (24). Collectively, these computational insights, spanning biochemical stability, solubility, antigenicity, and safety profiles, preliminarily underscore *Eg*ADK8 for subunit and multi-epitope vaccines; However, experimental studies are required to validate these predictions.

PTMs, such as acylation, phosphorylation, and glycosylation, are enzymatic alterations to synthesized proteins, influencing their functionality, localization, and stability (25). PTM prediction may provide useful insights into protein processing and assist in selecting appropriate heterologous expression systems, particularly when recombinant proteins require specific modifications for proper folding or stability (26, 27). Computational analysis using NetPhos 3.1 identified 34 putative phosphorylation residues within the *Eg*ADK8 sequence, comprising 18 serine, 4 threonine, and 12 tyrosine residues. Additionally, a solitary O-linked glycosylation site and one N-linked glycosylation motif were detected. The presence of these PTMs emphasizes the necessity of employing eukaryotic expression platforms to preserve *Eg*ADK8's functional epitopes during recombinant production. Subcellular localization predictions via the DeepLoc server classified *Eg*ADK8 as cytoplasmic, consistent with its putative metabolic roles. Intracellular proteins can be processed and presented through MHC pathways, and may become accessible to antigen-presenting cells following parasite turnover or cellular damage. Therefore, *Eg*ADK8 was selected based on its predicted immunogenic epitope repertoire for future multi-epitope vaccine development rather than its surface exposure (28, 29).

Secondary structural analysis, facilitated by NetSurfP-6.0, revealed a predominance of α-helices and random coils, with a relatively low proportion of β-strands. These elements are stabilized by hydrogen bonding between amino hydrogen and carboxyl oxygen atoms contributing to protein folding and stability(30). The predicted distribution of secondary structural components suggests a well-organized protein fold; however, experimental studies are required to confirm its structural stability and antigenic properties (31). The abundance of α-helices and β-turns may enhance structural rigidity, thereby promoting stable interactions with immunoglobulins during humoral immune responses. For tertiary structure elucidation, the Robetta server generated a high-confidence (score: 0.79) 3D model of *Eg*ADK8 using RoseTTAFold, a deep learning framework that integrates sequence, residue-residue

proximity, and spatial geometry through a multi-track neural network. This integrative approach ensures atomic-level accuracy in predicting fold dynamics, essential for epitope mapping and structure-based vaccine design (32).

Effective immunity against CE involves the activation of adaptive immune mechanisms, particularly cellular (e.g., cytotoxic T-lymphocyte responses) and humoral (e.g., antibody-mediated) mechanisms. Computational vaccinology provides a rational framework for antigen selection and prioritization of potential epitopes (33). To identify potential B-cell epitopes within *Echinococcus granulosus Eg*ADK8, a consensus approach integrating ElliPro (linear epitopes), ABCpred (machine learning-based predictions), and SVMTriP (tripeptide similarity scoring) was employed. This consensus methodology revealed 16 overlapping linear epitopes, among which nine showed favorable predicted antigenicity (VaxiJen score >0.5), hydrophilicity, and non-allergenic properties. Among these, "AGRVGRPPGMAECEVS" (VaxiJen: 0.5105), "EGAIFYADYLRKRIE" (0.9755), and "ADDASDIDCRIAEFRH" (1.1666), exhibited the highest predicted antigenicity scores. These epitopes also showed favorable predicted physicochemical properties, suggesting their potential suitability for further immunological evaluation. Additionally, nine conformational B-cell epitopes were predicted, critical for stabilizing antigen-antibody interactions and potentiating neutralizing humoral responses. For T-cell immunity, epitope screening targeted HLA allelic variants and murine MHC molecules to prioritize high-affinity ligands. Using immunogenicity thresholds and cytokine induction profiles (IFN-γ > IL-4), two HTL epitopes, "GKVIDRLTKEPYHKL" (murine MHC-II) and "VEYYGPRVLNVNADR" (human MHC-II), were identified as potent IFN-γ inducers, indicative of Th1-polarized immunity. Further analysis predicted five human CTL epitopes (e.g., HTLGRHVAR$_{17–25}$, IWRPPDPITF$_{52–61}$) and three murine CTL epitopes (e.g., IIWRPPDPITFL$_{51–62}$, FEMDRLERPIRI$_{15–26}$), selected based on MHC binding affinity and immunogenicity scores.

An overall high population coverage was predicted for the HTL epitope–allele compositions identified in this study, with estimated coverages of 92.32%, 82.02%, and 71.70% in South American, Oceanian, and East African endemic regions, respectively. However, variations in population coverage among different geographical regions may reflect the considerable diversity and distribution patterns of HLA alleles worldwide. Regions exhibiting relatively lower coverage may contain populations with distinct HLA polymorphisms or underrepresented HLA variants, which could potentially reduce the recognition and presentation of vaccine-derived epitopes in certain individuals. Therefore, these differences should be considered during vaccine development, as limited HLA representation may influence the breadth and consistency of immune responses at the population level. For CTL epitope-allele combinations, a moderate population coverage was predicted, suggesting that MHC-I-restricted immune responses may be more influenced by HLA class I variability across populations. Overall, the predicted global population coverage for MHC-I and MHC-II allele compositions was estimated at 77.6% and 83.42%, respectively. The selected epitope sets have broad applicability; however, incorporation of additional epitopes with complementary HLA-binding profiles and validation in diverse endemic populations may further improve vaccine coverage and reduce potential disparities associated with HLA diversity. These findings collectively facilitate the development of a multi-epitope vaccine construct incorporating *Eg*ADK8-derived epitopes, either as a standalone candidate or in synergy with antigens from other parasite life stages.

In total, the computational results presented here provide a preliminary basis for focusing on *Eg*ADK8 epitopes. As an example, predicted HTL epitopes with IFN-γ-inducing capacity (*e.g.*, VEYYGPRVLNVNADR$_{456-470}$ in human) may

contribute to Th1-mediated immunity through macrophage activation and may enhance mechanisms associated with parasite control. Similarly, five human CTL epitopes (e.g., HTLGRHVAR$_{17-25}$, RPPDPITFL$_{54-62}$) coincided with MHC-1 binding motifs reported in protective vaccines for intracellular pathogens, suggesting their potential for further evaluation in human vaccine constructs. The predicted physicochemical properties of EgADK8, including a high aliphatic index, negative GRAVY value, and lack of predicted allergenicity, suggest favorable characteristics for vaccine development. In addition, the presence of multiple predicted PTM sites may influence protein structure and function, although their potential immunological relevance requires experimental validation.

This study met several limitations: 1) the identified B-cell and T-cell epitopes were predicted using computational algorithms, and differences among prediction tools may have influenced epitope selection; 2) although structural modeling was performed, molecular dynamics simulations were not conducted to evaluate the stability of the predicted protein structures or epitope conformations; 3) docking analyses with immune receptors, including MHC molecules and Toll-like receptors, were not performed, limiting the functional assessment of the predicted epitopes; 4) immune response simulations were not carried out to estimate the potential immunogenicity of the selected epitopes; 5) HLA diversity was only partly addressed by coverage of supertypes, and no attempt was made to assess predicted murine and human CTL (cytotoxic T-lymphocyte) epitopes for binding to particular HLA allele types that are predominant in endemic regions, which may dampen possible real-world vaccine effectiveness in genetically diverse populations; 6) protein/epitope conservation analysis was not done and could be investigated in future studies; and 7) all findings are based on *in silico* analyses and require experimental validation to confirm antigen processing, HLA binding, immunogenicity, and protective efficacy.

Conclusion

This study identified *Eg*ADK8 as a promising source of immunogenic epitopes for future multi-epitope vaccine development against *E. granulosus*. Computational analyses predicted favorable physicochemical characteristics, low allergenic potential, and multiple B-cell and T-cell epitopes with desirable immunological properties, supporting its potential for further investigation. Nevertheless, these findings are based entirely on *in silico* predictions and should be interpreted with caution. Future studies should validate the predicted epitopes through in vitro and in vivo experiments, including immunogenicity assessment, protective efficacy evaluation, and optimization of vaccine formulations, before considering their application in multi-epitope vaccine design.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Availability of supplementary data

All supplementary data are accessible via sending email to the corresponding author based on reasonable application.

Supplementary Table 1. HTL-associated epitopes for *Eg*ADK8 using some mouse MHC-II alleles with subsequent screening.

Supplementary Table 2. CTL-associated epitopes for *Eg*ADK8 using some mouse MHC-I alleles with subsequent screening.

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