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An Immunoinformatic Approach to a Protease-Resistant Epitope Design for a Giardiasis Vaccine

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Abstract

Giardiasis, a pervasive waterborne parasite infection caused by *Giardia lamblia*, with an estimated 200 million annual infections and substantial morbidity (Mohamed et al., 2019 and Hajare et al., 2022). Current therapies face growing issues with drug resistance, highlighting the urgent need for a new vaccine. This study employs computational methods to predict immunogenic, protease-resistant, surface-exposed epitopes on the alpha-1 giardin protein, essential for vaccine efficacy. B-cell and T-cell epitopes were predicted using tools like IEDB Bepipred and Vaxign. ExPASy was used as a stringent filter to validate protease stability against trypsin and pepsin (Cohen, 1973). Epitopes were also assessed for conservation across *Giardia* strains (NCBI BLAST) and surface exposure using SWISS-MODEL and RCSB visualization tools. Epitopes passing the B-cell filter but failing other criteria were modified via a mutagenesis protocol. The methodology was reapplied for the mutated sequence to optimise scores across all assessment criteria. Three of 34 predicted epitopes were identified as viable after passing all assessment criteria. A chi-square test comparing observed and expected viable epitopes yielded a statistically significant p-value of 9.37x10^-8, demonstrating that the selection method was highly effective and not due to chance. The identified epitopes serve as a validated blueprint for a to mitigate the global impact of giardiasis.

Keywords
Immunoinformatics, giardiasis, vaccine, mutagenesis and epitopes.

1. Introduction
Giardiasis, caused by the intestinal protozoan *Giardia lamblia*, is a global health challenge and one of the most common intestinal parasites worldwide (Mohamed et al., 2019). Its impact on physical and cognitive development is profound particularly among children in resource-limited nations (Jenikova et al., 2011). As a leading waterborne pathogen, *Giardia* contaminates water sources, perpetuating infection in areas with inadequate sanitation (Nazer, 2019). Although morality is low, giardiasis causes persistent illness and severe dehydration, highlighting the need for effective prevention. Prior immunoinformatic studies solely focus on identifying immunogenic surface proteins. This study focuses on identifying protease-resistant epitopes on the alpha-1 giardin to support development of a stable, affordable vaccine for high-risk underserved populations.

1.1 Objectives - To computationally identify protease-resistant, conserved B-cell and T-cell epitopes on the Giardia lamblia alpha-1 giardin protein predicted to be surface-exposed, thereby advancing a multi-epitope vaccine strategy capable of inducing Immunoglobulin A (IgA) and Immunoglobulin G (IgG) antibody responses.

2. Literature Review
Giardiasis poses a major global health challenge, potentially contributing to up to 500,000 deaths worldwide, predominantly affecting children in developing regions with limited sanitation and contaminated water supplies

(Mohamed et al., 2019; Hajare et al., 2022). The disease's morbidity extends beyond acute illness to long-term effects on physical growth and cognitive development (Jenikova et al., 2011). Current treatments including metronidazole face challenges from drug resistance, treatment failures, and side effects, underscoring the need for alternative preventive measures (Loderstädt & Frickmann, 2021 and Mørch et al., 2008).

Alpha-1 giardin, a highly conserved protein present in over 97% of *Giardia lamblia* strains, has emerged as a promising vaccine candidate due to its immunogenic properties and proven protection in animal models (Jenikova et al., 2011; Davids et al., 2019). However, previous studies have insufficiently addressed the critical factor of protease resistance necessary for vaccine efficacy, especially for oral administration where digestive enzymes like trypsin and pepsin degrade many protein epitopes (Cohen, 1973). Combining immunoinformatics and protease cleavage analysis enables identification of stable, surface-exposed epitopes eliciting B lymphocyte (B-cell) and T lymphocyte (T-cell) responses.

3. Methods

An in-sillico approach was adapted to maximise cost-efficiency. An epitope-centric approach focused on alpha-1 giardin regions predicted to induce memory lymphocytes targeting the parasite. Multiple validated machine learning models predicted immunogenicity, structural integrity, surface exposure, and conservation of candidate epitopes. These models will also be responsible for assessing the stability of the mutated alpha-1 giardin sequence.

3.1 Data Collection

3.1.1 Protein Sequence Identification:

The complete alpha-1 giardin protein sequence was retrieved from the NCBI Protein Database in FASTA format.

3.1.2 Epitope Prediction:

B-cell Epitopes: The IEDB B-cell Epitope Prediction tool was utilizing Bepipred 2.0 linear epitope prediction tool; candidates with scores >0.75 were selected. Parker Hydrophilicity and Faus & Chapman flexibility scores further filtered epitopes, where higher positive values indicated greater surface exposure and conformational mobility, respectively.

T-cell Epitopes: Vaxign was employed for T-cell Major Histocompatibility Complex (MHC) Presenting Class I and Class 2 binding predictions. Epitopes demonstrating a statistically significant binding affinity (p-value < 0.05) were considered viable. Candidates similar to human proteins were excluded to reduce autoimmunity risks. Adhesion probability was also assessed with low scores indicating non-adhesion.

3.1.3 Conservation Assessment

National Centre of Biotechnology information (NCBI) Basic Local Alignment Search Tool (BLAST) assessed the conservation of the alpha-1 giardin amino acid among the Giardia strains. Analysis of the top 25 alpha-1 giardin strains confirmed the sequence uniformity, minimizing immunogenic loss risk.

3.1.4 Protein Visualization

Selected epitopes were mapped onto the 3D alpha-1 giardin protein via SWISS-MODEL. Resulting PDB files were analysed in Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB) visualization tools to identify and confirm surface-exposed epitopes, categorized as "Yes" (visibly on surface), "Low Yes" (partially blocked), "High Yes" (isolated), or "No" (embedded). All "yes" categories were deemed viable.

3.1.5 Protease Cleavage Validation

Protease stability was assessed using the Expert Protein Analysis System Peptide Cutter tool to predict the trypsin and pepsin cleavage sites. Epitopes predicted as intact ('Non-Cleaved') were deemed viable; those showing cleavage were excluded.

3.1.6 Rational Mutagenesis and Validation

Epitopes failing initial screens for immunogenicity or stability underwent rational amino acid substitutions (mutagenesis) to enhance immunogenicity, flexibility, or structure. Mutated sequences were analyzed through the immunoinformatics pipeline, including epitope prediction and protease cleavage. Overall mutated protein stability was assessed using MolProbity.

4. Results and Discussion

4.1 Numerical Results

Table 1: Epitopes with hydrophilicity scores >0.75 (IEDB Bepipred) were analyzed for B-cell response potential using Park Hydrophilicity and Faus-Chapman flexibility scores.

Table 1. B-Cell Epitope Scores for the Amino Acids on Alpha-1 Giardin Sequence

Amino Acid Sequence	Start Pos.	End Pos.	IEDB Bepipred score	Parker Hydrophilicity Score	Faus and Chapman score
KELPDD	47	53	0.945	4.586	1.191
EDWHETA	105	111	0.844	3.164	0.919
ASRPGSPED	149	157	1.664	5.219	1.169
VPSAEYRP	181	188	1.007	2.857	1.064
NDKGDEKR	240	247	1.007	7.214	1.214

Table 2. Corresponding T-Cell p-values for Each Epitope Sequence

Amino Acid Sequence	Start Pos.	End Pos.	T-Cell MHC 1 Analysis (p-value)	T-Cell MHC II Analysis (p-value)
KELPDD	47	53	0.0102	0.0247
EDWHETA	105	111	0.0464	0.0244
ASRPGSPED	149	157	0.000348	0.0141
VPSAEYRP	181	188	0.00127	0.00123
NDKGDEKR	240	247	N/A	N/A

Table 2: Vaxign results identified epitopes with significant T-cell MHC class I and II binding affinity ($p < 0.05$); NDKGDEKR did not meet the threshold.

Overall, these results have identified 4 epitopes (KELPDD, EDWHETA, ASRPGSPED) that have a high probability of inducing a B and T lymphocyte response. Specifically, their high scores compared to the 0.75 score threshold demonstrate a high probability of initiating a B-cell response. Likewise, the low p-values of all 4 epitopes compared to threshold indicate these epitopes will successfully stimulate both T-cell MHCs. By contrast, epitope sequence NDKGDEKR showed promising scores during the B-cell validation yet did not meet the p-value threshold for both T-Cell MHCs suggesting the protein sequence may be too positively-charged (due to the very high Parker Hydrophilicity score), making inclusion in mutagenesis protocol to add more flexible negatively-charged amino acids to increase likelihood to stimulate T-cell response.

Through inputting the FASTA sequence on Vaxign, the alpha-1 giardin showed no human protein homology, minimizing autoimmunity risk. Furthermore, adhesion probability scored low (0.064), indicating non-adhesive properties. Lastly, analysis of the top 25 strains revealed >95% amino acid sequence conservation in alpha-1 giardin epitopes, suggesting minimal immunogenic loss risk.

Table 3. NCBI BLAST Analysis of Conservation

Amino Acid Sequence	Start Pos.	End Pos.	Conservation in top 25 Strains	Certainty (%)
KELPDD	47	53	100%	>97%
EDWHETA	105	111	100%	>97%
ASRPGSPED	149	157	72%	>97%
VPSAEYRP	181	188	100%	>97%

Table 3: Table Showing the percentage conservation of the epitope sequences on the top 25 strains of alpha-1 giardin on the different *Giardia lamblia* protozoan types, with their associating percentage certainty in the conservation.

The high conservation of each epitopes is promising, thus allowing sound recognition of epitopes on vaccines to sequences on alpha-1 giardin protein on the *Giardia lamblia.* Concerns may arise from the slightly poor conservation of the ASRPGSPED epitope sequence suggesting immunoglobulins may not identify less prominent giardia lamblia strains; which may give rise to horizontal gene transfer between parasites to favouring omission of ASRPGSPED sequence from the surface protein. Though, this potential effect is minimised due to the high conservation of the other epitope sequences and the use of a multiprotein vaccine approach (Table 4).

Table 4. Qualitative Assessment of the Surface Exposure of Epitope Sequence

Amino Acid Sequence	Start Pos.	End Pos.	Surface Exposed?
KELPDD	47	53	Yes
EDWHETA	105	111	Yes
ASRPGSPED	149	157	High Yes
VPSAEYRP	181	188	Low Yes

Key:
Yes - Epitope is visibly on the surface
No - Epitope is embedded in protein
Low Yes - Epitope is slightly embedded in protein but mostly on surface
High Yes - Epitope is singled out and is almost separate from the rest of protein.

Qualitative findings directly support the findings on Table 1 & 2. Specifically, the high surface exposure is consistent with the very strong Parker Hydrophilicity scores, further validating the high probability of the epitopes inducing an immune response. An exception to these findings is the comparatively surface exposure of the epitope sequence VPSAEYRP, which is consistent with this comparatively lower hydrophilicity score compared to the other surface very surface-exposed epitopes, indicating a need for a mutagenesis protocol to include more positively charged amino acids within the protein sequence.

Table 5. Protease Cleavage Validation through Expert Protein Analysis System Peptide Cutter

Amino Acid Sequence	Start Pos.	End Pos.	Cleaved by Pepsin and Trypsin?	Amino Acids Lost
KELPDD	47	53	Yes	48 and 55
EDWHETA	105	111	No	N/A
ASRPGSPED	149	157	No	N/A
VPSAEYRP	181	188	No	N/A

Table 5: Table showing the result from the expert protein analysis system peptide cutter tool showing if any amino acid from the epitope sequence was cleaved from the alpha-1 giardin sequence.

These results imply that the epitope sequences "EDWHETA, ASRPGSPED, VPSAEYRP" will not be hydrolysed through the injection of the alpha-1 giardin protein into the gastrointestinal system. Supporting the study's objective in ensuring the identified will indefinitely induce an immune response as they will not be hydrolysed by the most common proteases. Though, the sequence "KELPDD" was cleaved indicating that a slight decrease in the overall positive charge of this sequence will decrease cleavage probability (Vita et al., 2018).

Table 6. B-Cell Epitope Scores for the Amino Acids on Mutated Alpha-1 Giardin Sequence

Amino Acid Sequence	Start Pos.	End Pos.	IEDB Bepipred score	Parker Hydrophilicity Score	Chou and Fasman score
QLPDD	48	53	0.5216	3.814	1.217
VPSAGGR	180	187	0.4551	3.229	1.169
NDGGDQH	239	246	0.5079	6.643	1.361

Table 6: Mutated epitopes with hydrophilicity scores >0.75 (IEDB Bepipred) were reassessed for B-cell potential by Parker Hydrophilicity and Chou & Fasman flexibility scores. Notably, QLPDD replaced HQPLDD due to higher scores, although the histidine (H) residue was retained for structural integrity.

Table 7. Corresponding T-Cell p-values for Each Mutated Epitope Sequence

Amino Acid Sequence	Start Pos.	End Pos.	T-Cell MHC 1 Analysis (p-value)	T-Cell MHC II Analysis (p-value)
HQLPDD	47	53	0.000808	0.000204
VPSAGGR	180	188	0.019700	0.013500
NDGGDGH	239	246	0.049400	0.021400

Table 7: A table showing the vaxign results for the mutated epitopes with the corresponding p-values to show statistical likelihood of initiating a T-Cell Macrophage class 1 and 2, with a threshold of $p<0.05$.

Table 8. Qualitative Assessment of the Surface Exposure of Mutated Epitope Sequence

Amino Acid Sequence	Start Pos.	End Pos.	Surface Exposed?
QELPDD	47	53	Yes
VPSAGGR	180	187	Yes
NDGGDGH	239	246	Yes

The replacement of the lysines with glycine and histidine in "NDKGDEK" to reduce cleavage probability by slightly reducing the epitopes overall positive charge while using the histidine to maintain the flexibility (Kellogg, Leaver-Fay and Baker, 2010). These changes are consistent with the decrease in Parker hydrophilicity scores from 7.214 to 6.643 and the score increase in the Faus & Chapman test from 1.214 to 1.361. Thus, making this mutated epitope sequence far more likely to induce a T-cell & B-cell response.

Key:
Yes - Epitope is visibly on the surface
No - Epitope is embedded in protein
Low Yes - Epitope is slightly embedded in protein but mostly on surface
High Yes - Epitope is singled out and is almost separate from the rest of protein (Table 8).

Table 9. Protease Cleavage Validation through Expert Protein Analysis System Peptide Cutter for Mutated Epitopes

Amino Acid Sequence	Start Pos.	End Pos.	Cleaved by Pepsin and Trypsin?
QELPDD	47	53	No
VPSAGGR	180	187	No
NDGGDGH	239	246	No

The replacement of glutamic acid and tyrosine to glycine in "VPSAEYR" to increase flexibility and reduce the overall negative charge proved to be effective and was consistent with the results in Table 1 & 6, noting the increase in the Faus & Chapman score from 1.064 to 1.169. These changes were clearly successful by the increase in qualitative surface exposure from a low yes to a yes. Increasing immunogenicity of the epitope sequence (Table 9).

The replacement of lysine to glutamine in "KELPDD" to reduce reactivity proved to be very effective in preventing the cleavage of this epitope sequence by trypsin and pepsin (Zhang, 2008). This allowed the new mutated sequence to adequately induce an immune response without being hydrolysed, allowing for stronger artificial immunity development.

Table 10. MolProbity Scores Analysing the All-Atom Contacts in the Mutated FASTA Sequence

All-Atom Contacts	Clashscore, all atoms:	3.01		98th percentile* (N=1784, all resolutions)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.			
Protein Geometry	Poor rotamers	20	8.23%	Goal: <0.3%
	Favored rotamers	205	84.36%	Goal: >98%
	Ramachandran outliers	5	1.71%	Goal: <0.05%
	Ramachandran favored	277	94.86%	Goal: >98%
	Rama distribution Z-score	-1.11 ± 0.43		Goal: abs(Z score) < 2
	MolProbity score^	2.14		68th percentile* (N=27675, 0Å - 99Å)
	Cβ deviations >0.25Å	2	0.72%	Goal: 0
	Bad bonds:	1 / 2406	0.04%	Goal: 0%
	Bad angles:	30 / 3238	0.93%	Goal: <0.1%
Peptide Omegas	Cis Prolines:	0 / 8	0.00%	Expected: ≤1 per chain, or ≤5%
Low-resolution Criteria	CaBLAM outliers	7	2.4%	Goal: <1.0%
	CA Geometry outliers	1	0.34%	Goal: <0.5%
Additional validations	Chiral volume outliers	0/339		
	Waters with clashes	0/0	0.00%	See UnDowser table for details

Overall, the data presented supports the development of the mutated epitopes as there were low clashes between atoms and a high overall MolProbity score (Table 10). One important factor to note is the poor geometric scores due to the increased hydrophilicity of the original epitopes to increase immunogenicity likelihood though, it is not a crucial factor of this mutagenesis protocol as the synthetic epitope sequences will be small; making geometrical imbalances and stress on bond angles insignificant as they can easily fold toward their function.

3.2 Visual Results

Figure 1: A Bepipred 2.0 linear epitope prediction graph showing the calculated scores for each amino acid along the alpha-1 giardin polypeptide sequence. The yellow highlighted regions indicate the amino acid sequences that were identified as potential linear B-cell epitopes compared to the set threshold of 0.350.

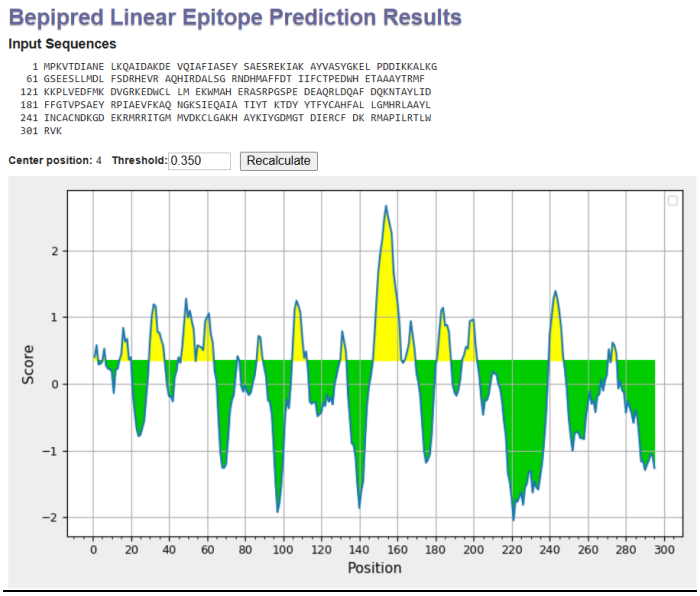

Figure 1. Graph showing the B-Cell Epitope Scores of Different Amino Acids along the Alpha-1 Giardin Polypeptide Sequence

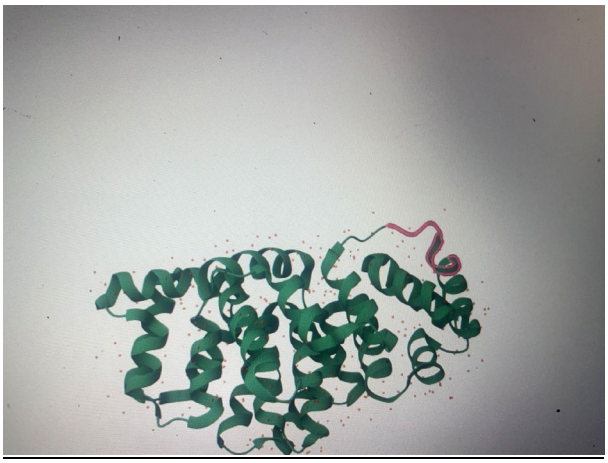

Figure 2. Highlighted Image of Epitope Sequence ASRPGSPED taken from RCSB

Figure 2: 3D model of the sequence of the alpha-1 giardin polypeptide using a PDB file created through SWISS-MODEL and inputted into RCSB. The pink highlighted part is the ASRPGSPED epitope sequence.

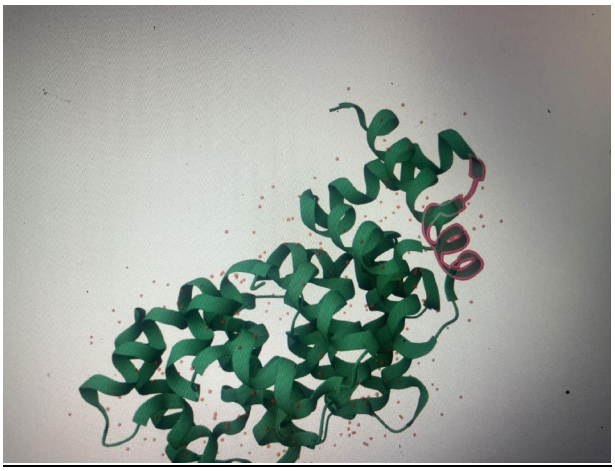

Figure 3. Highlighted Image of Epitope Sequence KELPDD taken from RCSB

Figure 3: 3D model of the sequence of the alpha-1 giardin polypeptide using a file created through SWISS-MODEL and inputted into RCSB. The pink highlighted part is the KELPDIKKA epitope sequence.

Figure 4. Highlighted Image of the Epitope Sequence VPSAEYRPI taken from RCSB

Figure 4: 3D model of the sequence of the alpha-1 giardin polypeptide using a PDB file created through SWISS-MODEL and inputted into RCSB. The pink highlighted part is the VPSAEYRPI epitope sequence. The top of the screen shows the mouse highlighting the specific amino acid sequence.

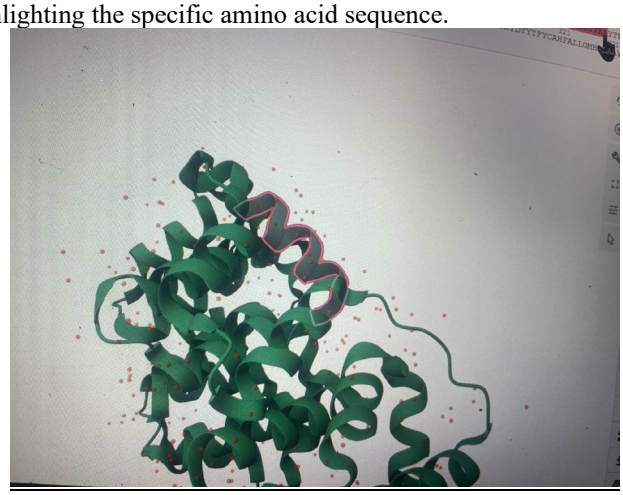

Figure 5. Highlighted Image of Epitope Sequence EDWHETA Taken from RCSB

Figure 5: A 3D model of the sequence of the alpha-1 giardin polypeptide using a PDB file created through SWISS-MODEL and inputted into RCSB. The pink highlighted part is the TPEDWHETTA epitope sequence. The top of the screen shows the mouse highlighting the specific amino acid sequence.

3.3 Proposed Improvements

Future research should include additional common gastrointestinal proteases beyond trypsin and pepsin to better validate the suitability of epitopes for giardiasis treatment. Employing molecular dynamics simulations to further assess the structural stability of the mutated epitope is also recommended, especially given the suboptimal MolProbity scores observed. These approaches will reduce the risk of unexpected alterations during preclinical testing and expand the repertoire of epitopes capable of eliciting effective immune responses, thereby improving therapeutics prospects for giardiasis patients.

4. Validation

4.1 Statistical Analysis

Objects of Analysis: The full set of predicted B-cell epitopes on the alpha-1 giardin protein (Total = 34 epitopes) were used.

Success Criteria (Dependant Outcome): An epitope was classified as 'viable' only if it simultaneously strictly met all predefined criteria including; high predicted immunogenicity (strong B-cell scores and <0.05 for T-cell values), surface-exposed, high conservation (>95%), and predicted complete cleavage resistance to trypsin and (both) pepsins. All mutated epitopes will have to meet the same criteria besides the conservation testing.

4.2 Success Rate Calculation: The proportion of viable (protease-stable, surface-presenting, immunologically promising) epitopes was quantified. The success rate was calculated as:

$$Success\ Rate\ (\%)\ =\ \frac{Total\ Number\ of\ Predicted\ Epitopes}{Number\ of\ Viable\ Epitopes}\times\ 100$$

4.3 Margin of Error (MoE) and 95% Confidence Interval (CI) (Frost, 2022): A 95% CI for the observed success rate was computed to estimate the true population proportion. The formula used was:

$$Margin\ of\ Error\ =\ Z\ \times\ \sqrt{\frac{p(1-p)}{n}}$$

$$Confidence\ Interval\ =\ p\ \pm MoE$$

Where Z is 1.96 corresponding with the confidence interval for 95%, p is the success rate, and n is the total number of epitopes existing on the sequence.

4.4 Chi-square Test

To statistically assess whether the observed cleavage pattern differed significantly from random expectation, a chi-square ($\chi 2$) test was performed. The null hypothesis posited an equal probability (50%) of epitopes being viable (failing all epitope assessments) observed counts (O) were compared against expected counts (E).

$$X^2\ =\ \frac{(O-E)^2}{E}$$

The p-value was determined using the CHISQ.DIST.RT function in Google Sheets (with 1 degree of freedom) using the sum of the variance values (Bobbitt, 2021).

Z = 1.96; corresponds to the 95% confidence level,

p = 0.08824 is the sample proportion, and

n = 34 is the sample size. This yielded a margin of error of approximately 9.5%.

Therefore, the 95% confidence interval (CI) for the true proportion of viable epitopes was determined as:

$$(p-MoE\ ,p\ +\ MoE)\ =\ (0,18.4\%)$$

The lower bound is truncated at 0, since proportions cannot be negative. This interval reflects the range in which the true success rate is expected to lie with 95% confidence.

Table 11. Chi squared Calculation

Category	Observed	Expected (null hypothesis)	(O-E)^2/E
Viable	3	17	17
Non-Viable	31	17	11.5

The sum of the last column was used to calculate the variance value (*X^2),* which was found to be 28.5 (Table 11). Additionally the Chi-Squared test was carried out using the Chisq.DIST.RT(*X^2 , 1),* on google sheets. This function yielded a p-value of 9.37x10^-8 which was far below the statistical significance threshold of p<0.05, showing notable statistical significance when stimulating an immune response when infected with *Giardia lamblia*.

5. Discussion

The stringent multiparameter screening yielded a low success rate of 8.8% (95% CI: 18.4%), consistent with the scarcity of highly conserved, immunogenic epitopes. A chi-square test produced a significant p-value of 9.37 × 10^-8, confirming the non-random selection of viable epitopes. The epitopes EDWHETA, ASRPGSPED, and VPSAEYRP showed strong B-cell flexibility, hydrophilicity, and T-cell MHC I/II reactivity, supporting their immunogenic potential and aligning with in vivo data (Jenikova et al.). Surface exposure analyses further validated these findings (Table 4, Figures 1–3). Lack of homology to human proteins reduces autoimmune risk, a critical vaccine safety criterion. The low adhesion score reflects positively as it demonstrates that protein is not likely to bind or interact with tissue thus allowing full contact with immunoglobulins.

The epitopes displayed >95% sequence conservation across 25 G. lamblia strains (Table 3), and demonstrated in silico stability against trypsin and pepsin cleavage, essential for oral vaccine efficacy (Davids et al., 2019). Unlike prior in silico studies focusing broadly on surface proteins, this work identifies alpha-1 giardin epitopes with combined high B- and T-cell scores and protease stability, providing a reliable basis for peptide vaccine development that may reduce costs and improve therapeutic success.

Rational mutagenesis enhanced epitope features: HQLPDD (from KELPDD) restored protease resistance and improved T-cell affinity (p=0.000808 vs. 0.0102). VPSAEYRP's redesign to VPSAGGR increased surface exposure, enhancing B-cell accessibility despite marginally reduced immunogenicity scores (Tables 4 and 8). Post-mutagenesis MolProbity analysis confirmed overall protein structural integrity, though some scores marginally missed stringent thresholds due to MolProbity's strictness. These results suggest successful epitope optimization likely supportive of effective immune recognition.

6. Conclusion

To summarise, this study applied a stringent computational pipeline to identify five protease-resistant, conserved, surface-exposed epitopes (EDWHETA, ASRPGSPED, VPSAGGR, NDGGDGH, and QLPDD)as promising vaccine candidates warranting further preclinical validation.

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Biography

Evan Moloseni is a high school student at Hilton College aspiring to become a medical researcher. He received the medical research gold badge at the 2025 Regional Eskom Expo and multiple awards for his excellence in science and mathematics, including the Richard Strackbosch scholarship. Evan leads an interdisciplinary research team developing a DNA nanorobot with the FutureTech Foundation and collaborates on psychological research with Dr. Graber. Additionally, he completed a simulated internship optimizing protocols at LifeArc and serves as Administrative Director for Diversicode, focusing on neurodivergent children's psychological support. He plans to pursue an MD/PhD.