Preliminary study of Toxocara canis Recombinant C-type Lectin as a suitable antigen for serodiagnosis of human toxocariasis

Parmida Malekzade
University of Tehran
Seyed Hossein Hosseini
University of Tehran
Mahsa Shahbakhsh
University of Tehran
Parviz Shayan
University of Tehran
Mohammad Zibaei
Alborz University of Medical Sciences
Shahram Jamshidi
University of Tehran
Elham Rismani
Pasteur Institute of Iran
Abdorreza Naser Moghadasi
Tehran University of Medical Sciences
Mohammad Akrami
Tehran university of medical sciences
Fateme Jalousian (jaousian_f@ut.ac.ir)
University of Tehran

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Abstract

C-type lectin (CTL) is the main protein part of the secretory-excretory product, secreted by secondary stage *Toxocara canis* larvae. Considering the antigenic characteristics of the protein, overlapping regions were identified as the highly potent MHC-I and MHC-II binding epitopes. Furthermore, several distinct regions were distinguished as the linear and non-linear B cell epitopes. To use this protein as a candidate for serological diagnosis, the recombinant pET-32a(+) plasmid containing the 660 bp sequence of *Toxocara canis* C-type lectin gene was synthesized and successfully cloned and expressed in Escherichia coli (DE3+). Subsequently, the expression of the insert was confirmed by SDS-PAGE and Western blot using an anti-His monoclonal antibody. Fifty-six serum samples were collected from pet owners. Forty-four samples were negative and twelve samples were positive performed with a commercial ELISA kit. The same samples were also tested using dot blot and western blot analysis prepared with recombinant CTL antigen (rCTL). Interestingly, the results achieved by dot blot and western blot analysis generated with rCTL showed that two negative samples determined by ELISA tests were positive in the mentioned tests, The other samples had comparable results as well as the ELISA test. A comparison of the test results of 56 human sera showed that the results were the same between the three test methods and the Kappa coefficient was calculated 1(p-value=7.25) and 0.9(p-value=1.29). It was suspected that the rCTL is more sensitive than the whole antigen secreted by *Toxocara canis*. In conclusion, this antigen could be further evaluated within the supplementary studies to improve the toxocariasis diagnostic kits.

Introduction

Toxocara spp. round worms of dogs and cats respectively, are zoonotic parasitics which cause infect in a large number of mammals and is a major burden for public health worldwide (Wickramasingh etal. **2009**). Human can become infected if they contact with dirt and ingest dirt containing *Toxocara* species eggs. Although rare, infection can also occur through eating undercooked or raw meat from infected paratenic hosts including chickens, cows, lambs, and pigs (Azizi et al. 2007). In the paratenic hosts including humans, the larvae can migrate through the tissues and cause visceral larva migrans (VLM), ocular larva migrans (OLM), neurological syndrome or neurotoxocariasis (NT) and covert toxocariasis (CT) (Eslahi et al. 2020). The diagnosis of human toxocariasis is mainly based on clinical, epidemiological, and laboratory data, which include imagining exams, blood exams, eosinoplilia, total IgE level, and serological tests (Zibaei et al. 2010). Enzyme-linked immunosorbent assay (ELISA) and Western blot (WB) are two methods that are available for the immunodiagnosis of toxocariasis, both using *Toxocara* excretory-secretory (ES) antigens (Zibaei et al. 2016; Borhani etal. **2017**). ES Ags are provided from infective larvae culture medium *in vitro* that contain lectins, musins and enzymes (Maizles **2013**). One improvement for enhancing diagnostic tests is to substitute the ES antigens with recombinant proteins (Meshgi et al. 2018). Numerous *in silico*-based investigations propose diverse antigens as appropriate candidates for diagnostic test design (Etebar etal. 2013). The performance of three recombinant antigens from *T. canis*, covering TES-26 and *Toxocara canis* C – type lectin1 or TES-32 and TES-120 were tested *in vitro*, along with detecting specific IgG4 antibodies. Whereas none of these

antigens alone showed 100% specificity, the combination of all three antigens recognized all positive samples and had a 2–4% cross-reaction with other parasitic infections. (Maizels 2013). Currently, the purified recombinant proteins are evaluated for their characteristics by ELISAs(Fathi et al. 2018). The purpose of this preliminary study was to introduce a recombinant protein, *T. canis*-rCTL, as a suitable antigen for laboratory diagnosis of human toxocariasis.

Materials And Methods:

Structure analysis of *T.canis*-C type lectin

The amino acid sequences of this protein were collected from the NCBI database with the sequence accession number AOV81587.1 (GeneBank mRNA: KU852582.1) (Etebar et al. **2018**), and aligned with the sequences of other parasitic helminthes through blast tools.

The protein sequence was loaded into NetSurfP-2.0 to predict the secondary structure elements from the primary sequence (Klausen etal. **2019**). The full-length 3D structure of protein was generated using using IntFOLD server (**https://www.reading.ac.uk/bioinf/IntFOLD/**). . The pipeline provides an integration of several methods: IntFOLD6-TS, for prediction of 3D structure, ModFOLD8, for scoring of model accuracy self-estimate (ASE), and DISOclust, for disorder prediction. The IntFOLD6-TS method works via multiple target-template alignments and threading methods of LOMETS package (McGuffin etal. **2019**; McGuffin etal. **2021**). Predicted model was evaluated by PROCHECK in terms of the stereo-chemical quality (Laskowski etal. **1993**).

Prediction of antigenicity of *T.canis*-C type lectin

The antigenic capability of a protein to trigger an immune response was evaluated by ANTIGENpro and Vaxijen v2.0 servers (Magnan etal. **2010**; Doytchinova etal. **2007**). ANTIGENpro evaluates the antigenic potential of protein using the sequence-based predictor with the overall accuracy of 75.51% in a prediction threshold of 0.5. Vaxijen predicts antigens using an independent method of sequence-alignment with the accuracy of 70% to 89%.

The potential of presenting epitope candidates of the protein sequence to T cells were evaluated based upon the processing of peptides in the cell using proteasomal cleavage/TAP transport/MHC class I combined predictor from IEDB database (Peters etal. **2003**; Tenzer etal. **2005**). The possible epitopes presenting by MHC-II was assessed using MHC II-NP (naturally-processed MHC II ligands). MHC II-NP extracts peptides considering the C- and N-terminus cleavage motifs and calculates the cleavage probability score. The continuous and discontinuous candidate epitopes presenting to antibodies were predicted based on the protein 3D structure using ElliPro (Ponomarenko etal. **2008**).

Cloning of *T.canis*-C type lectin in expression vector pET32a

Recombinant plasmid, pET32a were constructed with 660bp of *T.canis* C-Type lectin released in GenBank, with Accession number KU852582.1 (Etebar et al. **2018**). This sequence was used for codon

optimization gene sequence and gene synthesis by Generay Biotech Company (GENERAY Company, China). Recombinant Plasmids pET32a was used as cloning and reproduction vector. *E. coli Top10* (Embryonic Stem Cell Technology Co., Iran) strain was used as the source of Plasmid DNA and *E. coli BL21 (DE3)* (Bon Yakhteh Company, Iran) was used as host strains for the expression of rCTL. The recombinant plasmid pET32a/CTL was extracted from transformed *E. coli Top10* using MBST plasmid extraction kit (MBST, Iran), then rpET32a/CTL was introduced into competent *E. coli BL21 (DE3)* by *Calcium chloride heat-shock* transformation (Chang et al., 2017) and incubated overnight with shaking in Luria–Bertani medium (LB) containing 100mg/ml ampicillin according to the instructions of Shahbakhsh et al (2021) (Shahbakhsh et al. **2021**). Colony PCR and DNA sequencing (Bioneer, Korea) was used to confirm the desired construction of recombinant plasmid Using T7 promotor 5'TAATACGACTCACTATAGGG3' and T7 terminor 5'CTAGTTATTGCTCAGCGGT3' (Sinaclon, Iran).

Purification of recombinant *T.canis*-C type lectin

After overnight incubating at 37°C, 50 mL LB medium containing 100 µg/mL ampicillin is inoculated with 2.5 mL of overnight culture of *E. coli* BL21 (DE3) and grown at 37°C until an optical density 600 nm reached on 0.5-0.6. Expression of the recombinant protein was induced in the presence of 200 µL, IPTG (Sigma Company, USA) at a final concentration of 1 mM after 6h. The expression of rCTL was analyzed by SDS-PAGE using a 12% *polyacrylamide gel* electrophoresis and Coomassie Blue R-25099 staining (Laemmeli **1970**). The His tagged rCTL was purified by affinity chromatography *using* 1.5 ml *Ni-NTA spin columns* (Qiagen, Hilden, Germany) under *denaturing conditions according* to *manufacturer instructions* and then purified rCTL *lyophilized* and stored at -80 °C until use.

Human Serum samples collection

A total of 56 sera were collected from pet owners. Serum samples were collected from two distinct sources between the years of 2019-2021. Twenty-five sera were collected from pet owners referring to the Teaching and Research Hospital, Faculty of Veterinary Medicine, University of Tehran, Iran, All 25 petowners, were referred to Noor Pathobiology Medical Laboratory (Tehran, Iran) and diagnosed by ELISA commercial kit (Novagen, Germany). On the other hand, 31 pet owners' serum samples obtained from Department of Parasitology and Mycology, , Alborz University of Medical Sciences, Iran. All sera were stored at −30 °C until use.

SDS-PAGE and Western blot analysis

The amount of 25 µL (1.1 µg/µl) was loaded in a sample well of a *12%. SDS-PAGE mini gel* and after the end of electrophoresis, protein bands were trans blotted onto a 0.45-µm nitrocellulose membrane (Bio-Rad, Germany) in Tris-glycine buffer at 45 V for 180 minutes. The membrane was blocked with 3% nonfat skim milk in TBS-T (PBS pH 7.5 with 0.05% Tween 20 (Merck, Germany) for 1 hour. After three times washing with wash buffer (pH 7), the membrane incubated with mouse monoclonal antibody (mab) anti-6xHis (Sigma Company, USA) diluted to 1:1000 in PBS with 0.05% Tween 20 (pH 7.5) for 1 hour. All

incubation steps were performed at room temperature (18ºC) for 1 h under slight shaking followed by three washes with PBS- 0.05% Tween 20 (pH 7.2). Finally, the positive reaction was developed by using DAB (3, 3'-diaminobenzidine) (Sigma Company, USA) as substrate under visual observation within 8 minutes.

All 56 positive and negative sera were subjected to 0.45-µm nitrocellulose membrane (Bio-Rad, Germany) strips to observe the 41 kDa band of rCTL, staining in the positive samples and the absence of the band in the negative samples on the strips.

Indirect DOT-ELISA assays

In this study, the potential of rCTL for serodiagnoss of human toxocariasis was evaluated by indirect dot-ELISA and the results compared with the results of commercial ELISA kits, IgG antibody (Novagen, Germany) for all similar samples. The dot ELISA test was performed exactly in the previous step, except that the experiment were completed with one microliter of 0.19 µg/µL recombinant antigen rCTL, human sera at a dilution of 1:100 were added and *HRP conjugated goat* anti-human IgG (Razi Biotech, Iran) *at a 1:5000 dilution was manipulated as a* second antibody.

Statistical analysis

Cohen's kappa(k) was calculated to compare the agreement between the Dot-Elisa, Western blot and Commercial Elisa kit (Novagen, Germany) included overall, positive and negative percent agreement. The kappa coefficient range between 0 and 1 is defined as follows, a kappa value ≤0.40 denotes poor agreement, a value between 0.40 and 0.75 denotes acceptable agreement, and a value ≥0.75 denotes excellent agreement (Fleiss **1981**). *P-value* <0.05 regarded as statistically significant.

Results

Sequence alignment results (NCBI, BLAST) of *T.canis* C-type lectin amino acids revealed 63% similarity with *Anisakis* spp., 40% with *Dracunculus medinensis*, 32% with *Gongylonema pulchrun* and 58% with *Ascaris suum*. The *results highlighted* that there is no similarity in the regions of epitopes, so it is expected that these epitopes do not cross-react with antibodies produced against other parasitic infections. Based upon these results, *T.canis* recombinant C-type lectin was choosed for serological diagnosis of human toxocariasis.

Evaluation of secondary and tertiary structure

The secondary structure of protein was assessed by NetSurfP-2.0, which predicts the structural features by integrated deep learning. The structure was composed of two alpha helixes in the middle of the sequence, several scattered beta strands and mostly coils. Several residues (1-25 and 60-80) showed the probability of disordering (Figure **1.A**). The predicted model was visualized by PyMol software and depicted in Figure **1.B**. The predicted model was with a global quality score of 0.5045, where a score greater than 0.4 indicates a more confident model with higher similarity to the native structure. The

probability of an incorrect model is calculated as a p-value that represents the consistency of the global score. The predicted model was determined with a medium p-value of 2.918E-2 ($p < 0.05$). Model evaluation showed that 82.8% of residues were located in the most favored region of the Ramachandran plot (Figure **1.C**).

Prediction of presenting epitopes to T and B cells

The sequence of protein was scanned against a panel of the most frequently occurring alleles (Supplementary) using the IEDB recommended prediction method (NetMHCpan). The proteasome cleavage score is calculated as logarithms of the total cleavage sites from the C-terminus. The TAP score indicates the binding affinity of a peptide to TAP, where the higher value is interpreted as higher transport rates of a peptide. The MHC binding score presents –log (IC50) values that higher values associate with higher predicted efficiency. The sum of the proteasomal cleavage, TAP transport and MHC binding values determines the total score, which predicts the ratio of the amount of peptides presented by MHC molecules on the cell surface. Since IC50 less than 50 nM determines peptides with high binding affinity, the outputs were filtered for high affinity epitopes (Table **1**).

Table 1

Evaluation of the MHC I antigen processing

Allele	Position	Peptide	Proteasome Score	TAP * Score	MHC Score	Total Score	IC50 (nM)
HLA-A*68:01	68-77	TAAPGVTTTR	0.97	0.67	-0.93	0.71	8.6
HLA-A*68:02	85-94	WTPFNNNCYI	1.23	0.26	-1.66	-0.17	45.7
HLA-A*23:01, HLA-A*24:02	92-101	CYIASLPGRF	1.17	1.29	-1.1	1.36	12.7
HLA-B*58:01	94-103	IASLPGRFLF	1.32	1.11	-1.48	0.95	30.4
HLA-B*58:01	110-119	CTQTGSRVVW	1.83	0.32	-1.66	0.5	45.2
HLA-A*33:03, HLA-A*68:01	133-142	NFVNSFALGR	1	0.79	-1.59	0.2	38.5
HLA-A*68:01, HLA-A*31:01	144-153	VTRYWIGVNR	1.17	0.67	-1.46	0.38	28.9
HLA-A*23:01, HLA-A*24:02	146-155	RYWIGVNRQF	1.37	1.4	-0.99	1.78	9.7
HLA-A*68:01	208-217	TTPQGFVCKR	1.04	0.57	-1.25	0.37	17.7

* Transporter associated with antigen processing (*TAP*)

In prediction of naturally processed MHC II binding epitopes, the higher value of cleavage probability score and the lower amount of the percentile score indicates the greater chance of peptide presenting by MHC II molecules. The top ranked peptides derived from the protein sequence are summarized in Table **2**.

Table 2

Evaluation of the MHC II antigen processing

Position	Peptide	Cleavage probability score	Cleavage probability percentile rank	Peptide length
86-99	TPFNNNCYIASLPG	1.23746	0	14
70-83	APGVTTTRPRACPP	1.04149	0.05	14
70-84	APGVTTTRPRACPPN	1.02085	0.09	15
164-177	SPVIFSNWRPSQPD	0.94017	0.14	14
164-178	SPVIFSNWRPSQPDG	0.90274	0.18	15

In identification of the linear and non-linear B cell epitopes using the 3D structure of protein, the predicted epitopes are ranked based on PI (Protrusion Index) value. In calculating the PI value, the three-dimensional shape of the protein is approximated by a number of ellipses and the percentage of amino acids inside and outside the ellipse is determined. Candidate epitopes with larger PI values have greater solvent accessibility. The default score in ElliPro is 0.5. Linear peptides with scores above the threshold are ranked in Table **3**. Furthermore, four distinct regions constituting of residues as discontinuous B cell epitopes are depicted in Figure 2 and mentioned in Table **4**. The schematic view of the top ranked epitopes of MHC I, MHC II and B cells is depicted on the sequence of proteins (Figure **3**).

Table 3

Evaluation of the linear B cell epitopes

Position	Peptide	Length of peptides	Score
171-185	WRPSQPDGCCGSNVT	15	0.8
26-65	GIFQVCVNNVCVANNQGCNPPCVAPQVCVAPMCVAPPPAA	40	0.765
97-105	LPGRFLFNQ	9	0.666
137-145	SFALGRGVT	9	0.638
190-196	NYANFLG	7	0.623
78-85	PRACPPNW	8	0.615
200-211	DAPCGSLFTTPQ	12	0.612
124-129	TVGNFG	6	0.584

Table 4

Evaluation of the discontinuous B cell epitopes

Epitope number	Residues	Number of residues in epitope	PI score
1	N17, N22, N23, C25, G26, I27, F28, Q29, V30, C31, V32, N33, N34, V35, C36, V37, A38, N39, N40, Q41, G42, C43, N44, P45, P46, C47, V48, A49, P50, Q51, V52, C53, V54, A55, P56, M57, C58, V59, A60, P61, P62, P63, A65, T68, A69	45	0.717
2	L97, P98, G99, R100, F101, L102, N104, Q105, D108, W109, T111, Q112, T113, G114, S115, R116, A139, L140, G141, R142, G143, V144, T145, R153, Q154, F155, G156, Q157, W171, R172, P173, S174, Q175, P176, D177, G178, C179, C180, G181, S182, N183, V184, T185, N190, Y191, A192, N193, F194, L195, G196, Q197, D200, P202, C203, G204, S205, L206, F207, T208, T209, P210, Q211	62	0.684
3	A80, C81, P82, P83, N84, W85	6	0.644
4	Q122, T124, V125, G126, N127, F128, G129, N133, S137, F138, F168	11	0.52

Table 5

Comparison of results from the Dot-Elisa, Western blot and Commercial Elisa kit (Novagen, Germany) employed for the detection of IgG antibody against pet owners toxocariasis

Method		Western blot		Total	Kappa value	*P-value*
		Positive(+)	Negative(-)			
Dot-Elisa	+	14	0	56	1	7.25
	-	0	42			
commercial ELISA*	+	12	2		0.9	1.29
	_	2	42			
Total		14	44			

* Commercial Elisa kit (Novagen, Germany).

Discussion

The present study provides preliminary results of antigenic potential of *T.canis*- rCTL. These evidence is crucial since *Toxocara* spp. are distributed worldwide(Zibaei and Sadjjadi 2017). Toxocariasis is more common in children. In Iran nfection is more reported in children aged 11–14(Zibaei and Sadjjadi 2017). Diagnosis of human toxocariasis is conventionally based on clinical symptoms and serological diagnosis. ELISA and WB are the most reliable methods for diagnosing the specific antibodies against toxocariasis in human serum.C-type lectin contains a domain with the characteristics of host calcium binding lectins(C-type)(Loukas etal. 1999). *T.canis*-CTL was chosen as the most component of *Toxocara canis* excretory-secretory antigens and stage-specific protein, express in infective larvae however not in adult worms (Etebar et al. 2018). *T. canis*-C Type lectin is a calcium- dependent protein and its carbohydrate recognition domains (CRD) play an important role in the interaction of parasites with their hosts, CRDs of *T.canis*-C Type lectin are additionally showed 33–34% similarity to mouse and human C – type lectins (Etebar et al. 2018). In a phylogenetic tree, its sequence is found close to the species Estrongilidea(Etebar et al. 2018). In the present studies, a partial sequence of *T. canis*-C Type lectin with 660 bp in length which encoded 219 amino acids was evaluated in terms of antigenic potential. In bioinformatics study, the secondary and tertiary structure of proteins were examined for access to appropriate information for the determination of antigenic epitopes. In the 3D model, 82.2% of residues were located in the most favored region of the Ramachandran plot. The final model showed the overall

quality score higher than the threshold. Due to the antigenic and non-allergenic character of the protein, the sequence and the tertiary structure of the protein were used to identify T and B cell antigenic epitopes. Overlapping regions throughout the sequence were identified as epitopes presentable by MHC classes I and II. Also, regions that can be presented to B cells as epitopes were identified continuously and discontinuously throughout the protein sequence.In this study, the prepared recombinant C-Type lectin, expressed in *E. coli BL21 (DE3*) and purified by the nickel column via histidine tags, was able to maintain its immunogenicity. It is significant that two serums were positive with *T. canis*-rC Type lectin that were negative with ELISA commercial kit (Novagen, Germany). Subsequently, Western blot confirmed the positive results Western blot also confirmed the positive results. The negative test results were completely reliable with the commercial ELISA kit (Novagen,Germany) outcomes. Concordance assessment between the different assays showed. excellent agreement between the teqchniques, Choen's Kappa for assessing the level of agreement was calculated 1 between western-blot and dot-ELISA and 0.9 between western-blot and the commercial ELISA kit (Table 5). Preliminary results in this study showed that the recombinant antigen is more sensitive in recognizing infected samples. As yet, several recombinant antigens have been studied for the serological diagnosis of toxocariasis, and research on diagnostic antigens is in progress. A 26 kDa recombinant protein (Gems et al. 1995), which was a fraction of excretory-secretory antigen, was assessed by ELISA for recognizing IgG but the sensitivity was very low so the results were disappointing (Gems et al. 1995). TES-26 or TC- PEB1 (Maizels 2013), TES32 or TC-CTL1 (Zhan et al. 2015),TES-120 or TC- MUC1 and TES-30 (Santos et al. 2018) T. *canis* TES-30 and TES-120 recombinant proteins appeared serodiagnostic potential in sheep, horses and cattle through indirect ELISA (Santos et al. 2019). There are the recombinant antigens that have been presented and considered within the determination of toxocariasis. Recombinant 30 kDa antigen was also arranged and assessed with 11 sera of toxocariasis patients and appeared 100% sensitivity and 97.9% specificity (Yamasaki et al., 2000). Recombinant 30 kDa antigen with 26 cases of toxocariasis positive serum appeared sensitivity of 92.3% and specificity of 89.6% (Norhaida et al. 2008). Recombinant antigens of 120 kDa were also constructed and compared with recombinant antigens of 26 and 30 kDa, the results showed that with increasing molecular weight, the sensitivity increases and obviously the specificity decreases(Mohamad et al. 2009). Recombinant proteins are an effective tool for the determination of toxocariasis in humans with the choice of appropriate antigen.Commercial ELISA kit are, frequently, based on the *T. canis* larvae excretory-secretory antigens. The sensitivity and specificity of excretory-secretory antigens are satisfactory. These antigens are more suitable for screening tests (Zibaei et al. 2016). Utilization of excretory-secretory antigens from *T. canis* larvae increases the specificity of the ELISA, as well as, a positive ELISA results can be confirmed by WB, which is more specific when lower molecular weight bands, from 24 trecombino 35KDa are considered (Magnaval et al. 2001). Utilize of recombinant antigens was an important step in improving diagnostic tests (Fathi et al. 2016).DOT- ELISA with acceptable sensitivity and specificity could be a great strategy for assessment since it requires less amount of antigen, is cost-effective, and it is a quick test (Kordafshari et al. 2015). Dot-ELISA, as a modified ELISA approach, primarily based totally on the recombinant antigens has been used for the recognition of parasitic diseases such as EPC1 for hydatid cyst (Kordafshari et al. 2015) as well asWestern blot analysis of recombinant P23 showed that it could be recognized by the positive *C. parvum* serum, Due to

the simple handling and equipment, dot blot analysis with P23 could be recommended for calves screening against cryptosporidiosis(Shayan etal. 2008). To determine the specificity and also sensitivity of *T. canis*-rC Type lectin accurately and correctly, it is essential to test the number of more positive sera as well as sera infected with other parasitic diseases with this antigen.

Conclusion

This study provides baseline data on *T.canis*-rCtype lectin in Iran.

Although the results of this preliminary study were confirmed by *in silico*, *in vivo* and laboratory methods, however future studies on factors that make T.canis-rC type lectin as an acceptable antigen must also be proved.

Abbreviations

T.canis: *Toxocara canis*; *T.canis*-rCtype lectin: *Toxocara canis* recombinant C type lectin; TC-CTL: *Toxocara canis* C type lectin; TES: *Toxocara* excretory-secretory; WB: western blot; EPC1 antigen: *Echinococcus* protoscxolex C1.

Declarations

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Availability of data and materials: Datasets for this research are included in Etebar et al. **(2018)**

Authors'contributions: Seyed Hossein Hosseini, Fateme Jalousian, and Parviz Shayan designed the study, coordinated the study, and conceptualized it. Parmida Malekzadeh did Field work.Mohammad Zibaei and Shahram Jamshidi collected samples. Parmida Malekzadeh, and Mohammad Akrami collected data laboratory and analyzed laboratory data. Elham Rismani, Abdorreza Naser Moghadasi data curation and analysis. Mahsa Shahbakhsh and Fateme Jalousian did a Literature review and drafting of the manuscript. All authors reviewed the manuscript.

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Consent to participate: Consent has been obtained

Consent for publication: all authors have read and agreed to its content

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Figures

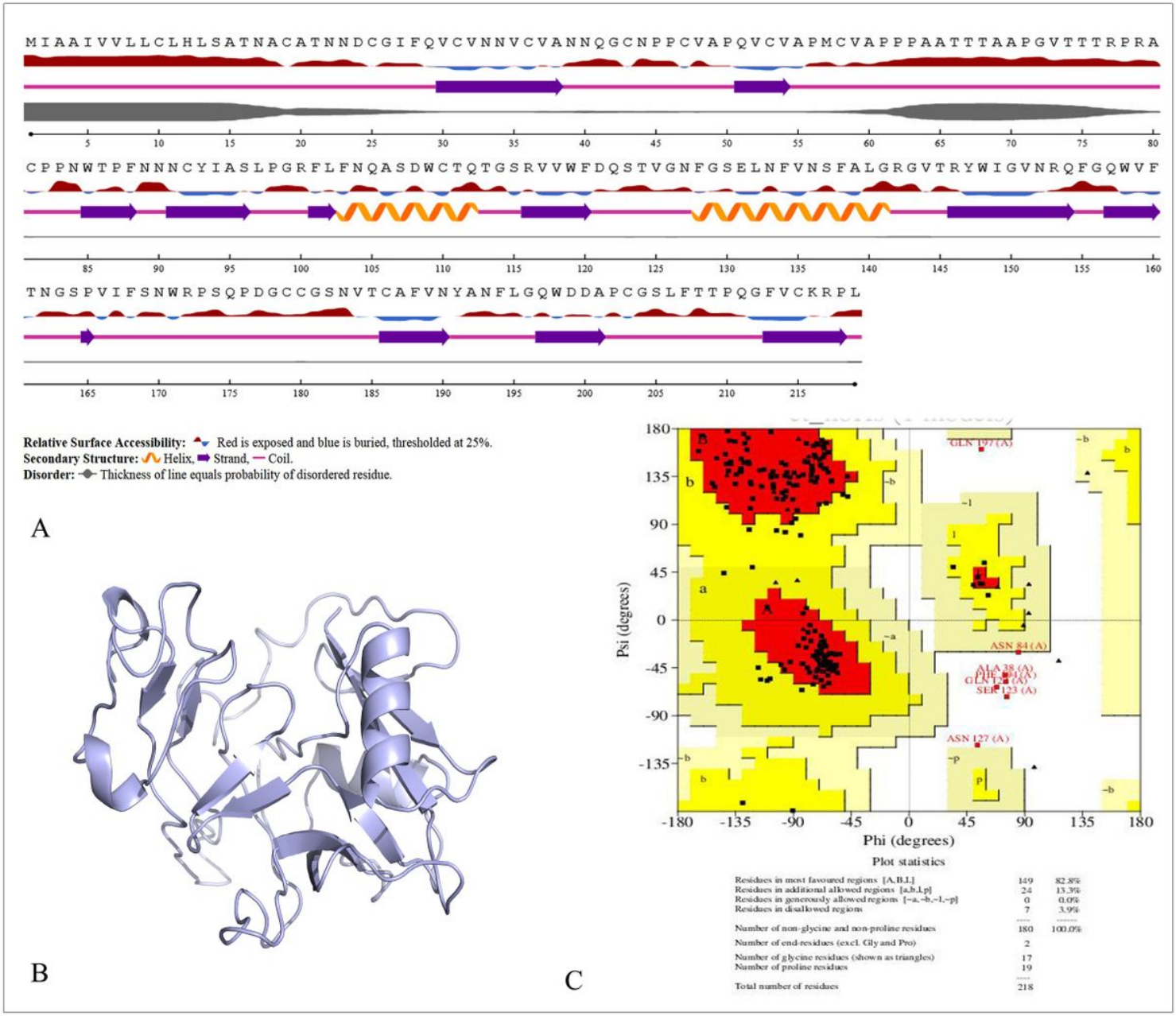

Figure 1

Evaluation of secondary and tertiary structure of *T.canis*- C type lectin. A) Secondary structure by NetSurfP-2.0, B) Cartoon view of 3D model, and C) Ramachandran plot.

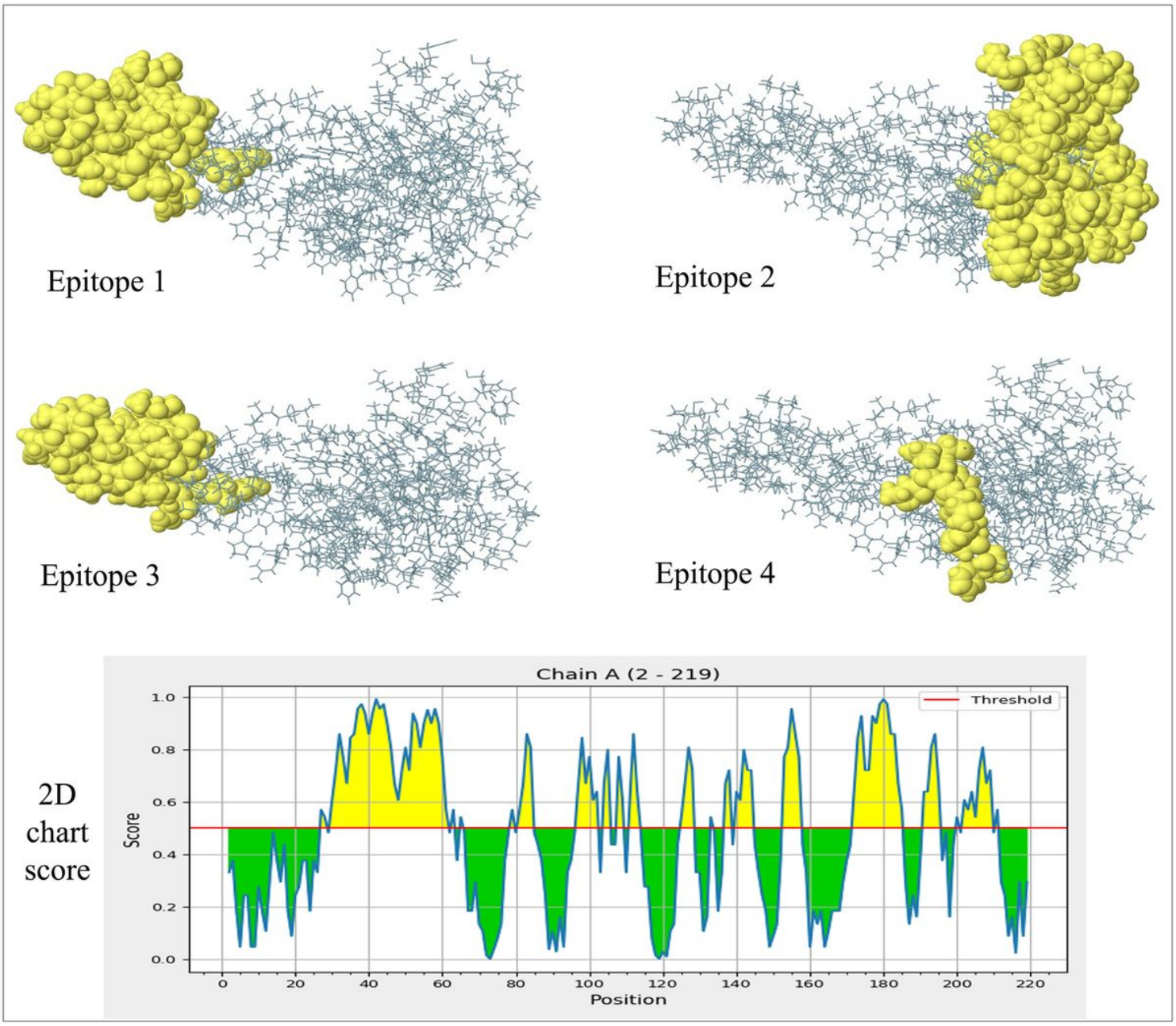

Figure 2

2D score chart and 3D structure mapping of the predicted discontinuous B cell epitopes.

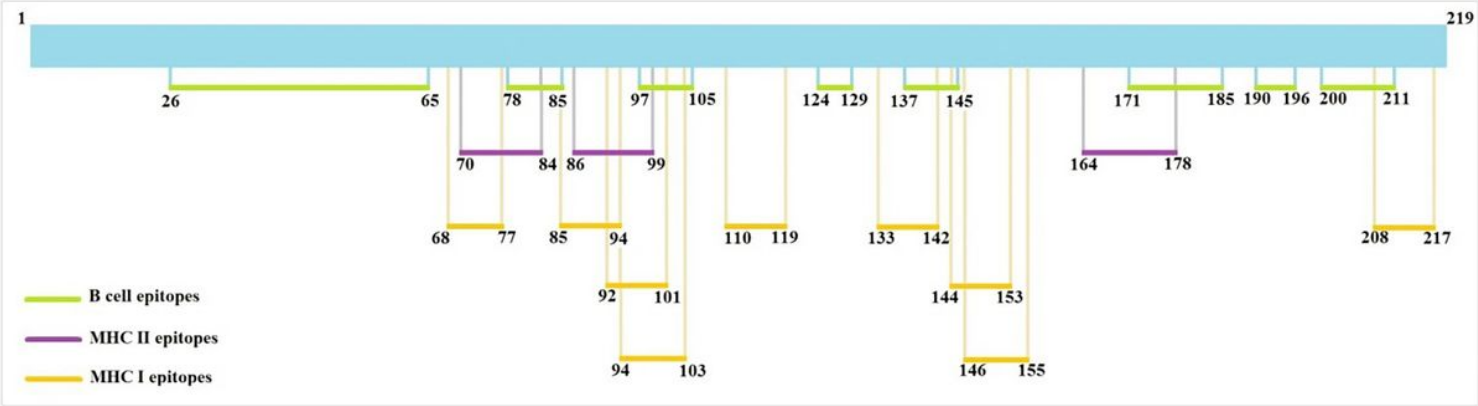

Figure 3

The schematic view of the top ranked epitopes of MHC I, MHC II and B cells on the sequence of protein.

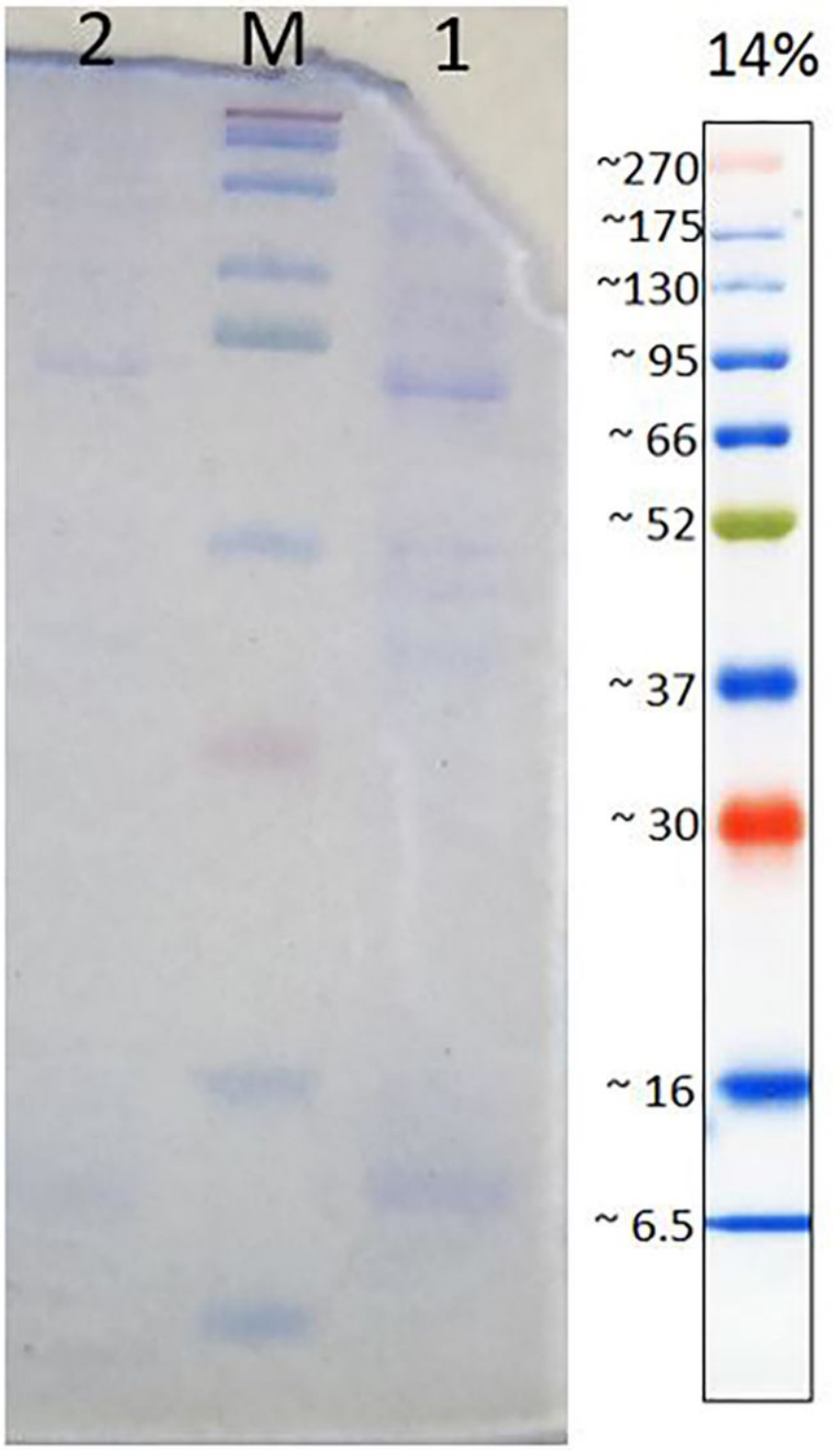

Figure 4

12% Sodium Dodecyl sulfate (SDS-PAGE) analysis of the purified *T.canis*- recombinant Ctype lectin, Lane1:purified *T.canis*-C type lectin 3 hours after induction with 1 mM of IPTG., Lane2: Pre staining Protein weight marker 6.5 to 270 KDa (Biolegend, USA), Lane3: purified *T.canis*-C type lectin 6 hours after induction with 1 mM of IPTG.

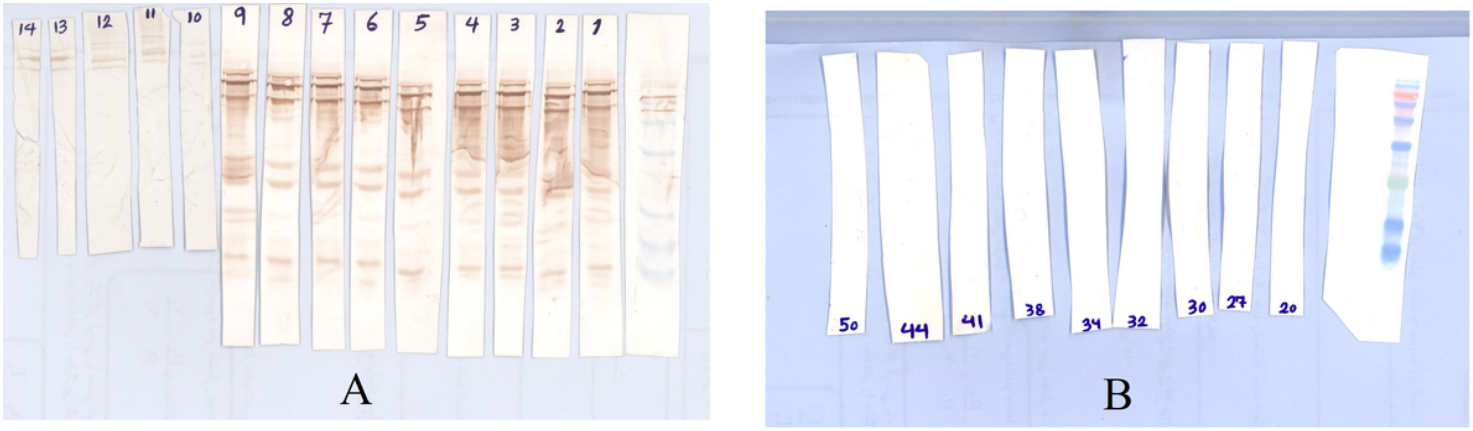

Figure 5

Western blot of the *T.canis* recombinant C- Type lectin(with 41KDa molecular weight) with the positive sera of human toxocariasis, Lane1-14: various positive sera of petowners, Lane 15: prestained protein marker 6.5 to 270 KDa (Biolegend, USA)(**A**), the different negative sera of petowners (**B**).