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

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Abstract

The protein C-type lectin is secreted by the secondary-stage larve of *Toxocara canis* (*T. canis*). Its antigenic characteristics have been the subject of research. The recombinant pET-32a (+) plasmid containing the 660 bp sequence of *T. canis* C-type lectin gene was successfully synthesized, cloned, and expressed in *Escherichia coli* (DE3+). Serum samples were collected from 56 pet owners. Forty-four samples were negative and twelve samples were positive which were 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). The expression was confirmed by Sodium Dodecyl Sulfate (SDS-PAGE) and Western blot. The study results revealed that two samples, which tested negative in the ELISA analysis, were found to be positive when using the recombinant C-type lectin (rCTL) antigen in both dot blot and Western blot analyses. The overall test results showed a high level of agreement between the three methods, with Kappa coefficients of 1 and 0.9 for the comparisons. This indicates the potential of using recombinant CTL antigen, to detect positive samples that may be missed by E-S Antigen. The recombinant rCTL demonstrated higher sensitivity than the excretory-secretory Antigens, indicating its potential for improved serodiagnosis of human toxocariasis. The study proposes that the rCTL could be further evaluated in supplementary studies to improve and develop the diagnostic kits for toxocariasis.

Introduction

Toxocara spp. are zoonotic roundworms that commonly infect dogs and cats, causing a significant burden on public health worldwide (1). The two main species of *Toxocara* that affect

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humans are *T. canis* and *T. cati*. Humans can contract the infection if they come into contact with contaminated soil and ingest the soil. Although rare, infection can also occur through eating undercooked or raw meat from infected paratenic hosts including, chickens, cows, lambs, and pigs (2). 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; 3). The diagnosis of human toxocariasis is mainly based on clinical, epidemiological, and laboratory data, which include imagining exams, blood exams, eosinophilia, total IgE level, and serological tests (4). 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 (5, 6). ES Ags are provided from infective larvae culture medium *in vitro* that contains lectins, musins, and enzymes (7). One improvement for enhancing diagnostic tests is substituting ES antigens with recombinant proteins (8). Numerous *in silico-based* investigations propose diverse antigens as appropriate candidates for diagnostic test design (9). The performance of three recombinant antigens from *T. canis*, covering TES-26 and *T. 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, combining all three antigens recognized all positive samples and had a 2-4% cross-reaction with other parasitic infections. (7). Currently, the purified recombinant proteins are evaluated for their characteristics by ELISAs (10). The aim of this preliminary study was to propose *T. canis*-rCTL as a recombinant protein suitable 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) (11), and aligned with the sequences of other parasitic helminths through blast tools.

The protein sequence was loaded into NetSurfP-2.0 to predict the secondary structure elements from the primary sequence (12). The full-length 3D structure of the protein was generated using the IntFOLD server (https://www.reading.ac.uk/bioinf/IntFOLD). The pipeline provides an integration of several methods: IntFOLD6-TS, for the 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 the LOMETS package (13, 14). The predicted model was evaluated by PROCHECK in terms of the stereo-chemical quality (15).

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 (16, 17). ANTIGENpro evaluates the antigenic potential of protein using the sequence-based predictor with an overall accuracy of 75.51% in a prediction threshold of 0.5. Vaxijen predicts antigens using an independent method of sequence alignment with an accuracy of 70% to 89%.

The potential of presenting epitope candidates of the protein sequence to T cells was evaluated based upon the processing of peptides in the cell using proteasomal cleavage/TAP transport/MHC class I combined predictor from the IEDB database (18, 19). The possible epitopes presented by MHC-II were 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 (20).

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 (11). This sequence was used for codon optimization, gene sequence, and gene synthesis by Generay Biotech Company (China). Recombinant Plasmids pET32a were used as a 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 the host strain 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 and incubated overnight with shaking in Luria–Bertani medium (LB) containing 100 μg/ml ampicillin according to the instructions of Shahbakhsh et al. (21). Colony PCR and DNA sequencing (Bioneer, Korea) were 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, a 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 of 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 six hours. The expression of rCTL was analyzed by SDS-PAGE using a 12% polyacrylamide gel electrophoresis and Coomassie Blue R-25099 staining (22). 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 the lyophilized rCTL and stored at -80 °C until use.

Human serum sample collection

A total of 56 sera were collected from pet owners. Serum samples were collected from two distinct sources between 2019 and 2021. Twenty-five sera were collected from pet owners referred to the Teaching and Research Hospital, Faculty of Veterinary Medicine, University of Tehran, Iran. All 25 pet owners, were referred to Noor Pathobiology Medical Laboratory (Tehran, Iran) and diagnosed with ELISA commercial kit (Novagen, Germany). On the other hand, 31 pet owners' serum samples were obtained from the 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 12*%.* SDS-PAGE mini gel and after the end of electrophoresis, protein bands were transblotted 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 one hour under slight shaking, followed by three washes with PBS- 0.05% Tween 20 (pH 7.2). Finally, the positive reaction was developed 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 serodiagnosis of human toxocariasis was evaluated

by indirect dot-ELISA and the results were compared with the results of commercial ELISA kits IgG antibody (Novagen, Germany) for all similar samples. The dot-ELISA test was conducted with one microliter of 0.19 µg/µL recombinant antigen rCTL, human sera at a dilution of 1:100 was added and *HRP* conjugated goat anti-human IgG (Razi Biotech, Iran) at a 1:5000 dilution was manipulated as a second antibody.

Statistical analysis

In order to compare the agreement between the Dot-Elisa, Western blot, and Commercial Elisa kit (Novagen, Germany) Cohen's kappa(k) was calculated. This included overall, positive and negative percent agreement. The kappa coefficient ranges 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 (23). *P-value* <0.05 was 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 these parasitic infections. Based on these results, *T.canis* recombinant C-type lectin was chosen for serological diagnosis of human toxocariasis. The purified recombinant protein, with a molecular weight of 41 kDa, was subjected to SDS-PAGE analysis to verify its purity and molecular weight. The protein bands corresponding to the recombinant C-type lectin protein were then transferred onto a membrane and probed with sera antibodies for diagnostic purposes. This approach successfully identified and purified recombinant *T. canis* C-type lectin protein, which can be used for further studies in the immunodiagnosis of toxocariasis.

Evaluation of secondary and tertiary structure

The secondary structure of the protein was assessed by NetSurfP-2.0, which predicts the structural features by integrated deep learning. The structure is 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 had 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).

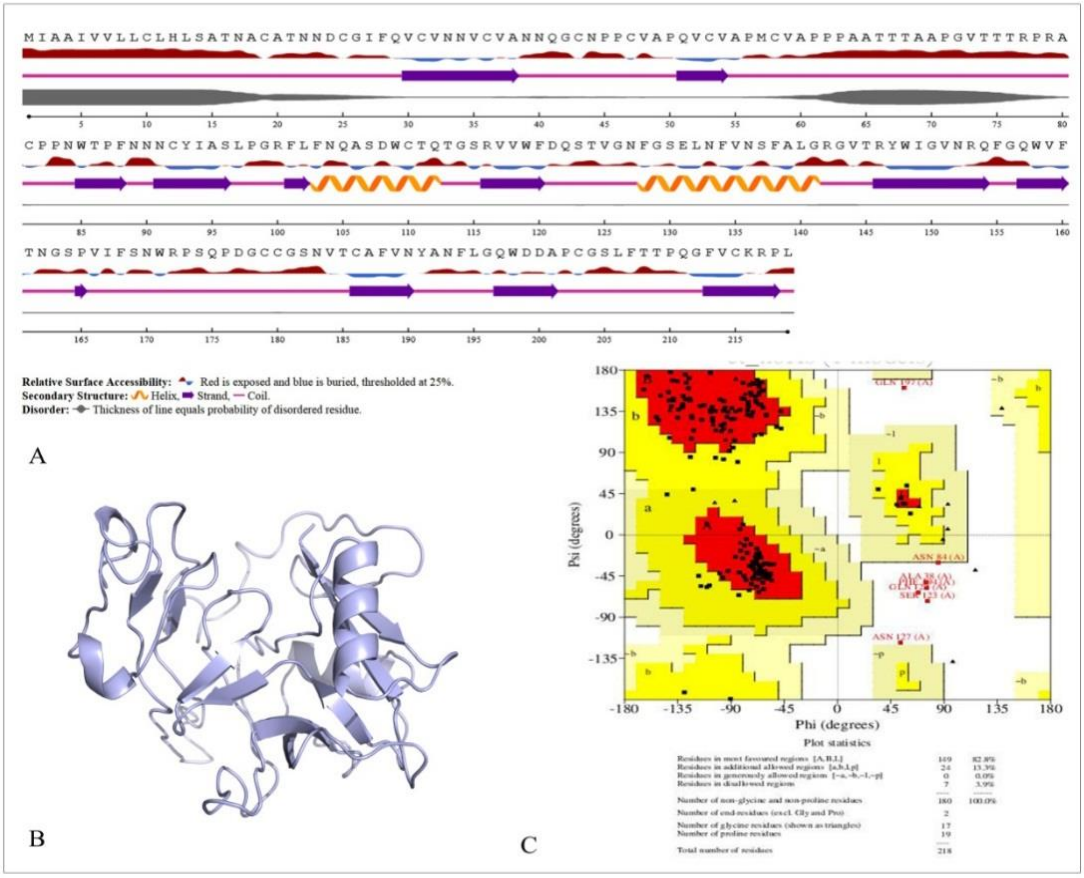

Fig. 1. Evaluation of secondary and tertiary structure of *T. canis*- C type lectin. A) Secondary structure by NetSurfP-2.0, B) Cartoon view of 3D model, and C) Ramachandran plot.

Prediction of presenting epitopes to T and B cells

The sequence of the 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 associated with higher predicted efficiency. The sum of the proteasomal cleavage, TAP transport, and MHC binding values determines the total score, which indicates the ratio of the number 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).

In the prediction of naturally processed MHC II binding epitopes, the higher value of the 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 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*)

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

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

In the identification of the linear and non-linear B cell epitopes using the 3D structure of the protein, the predicted epitopes are ranked based on PI (Protrusion Index) value. In order to calculate the PI

value, the protein's three-dimensional shape is approximated multiple ellipses. The percentage of amino acids inside and outside the ellipse is subsequently 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 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 shown on the sequence of proteins (Figure 3).

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

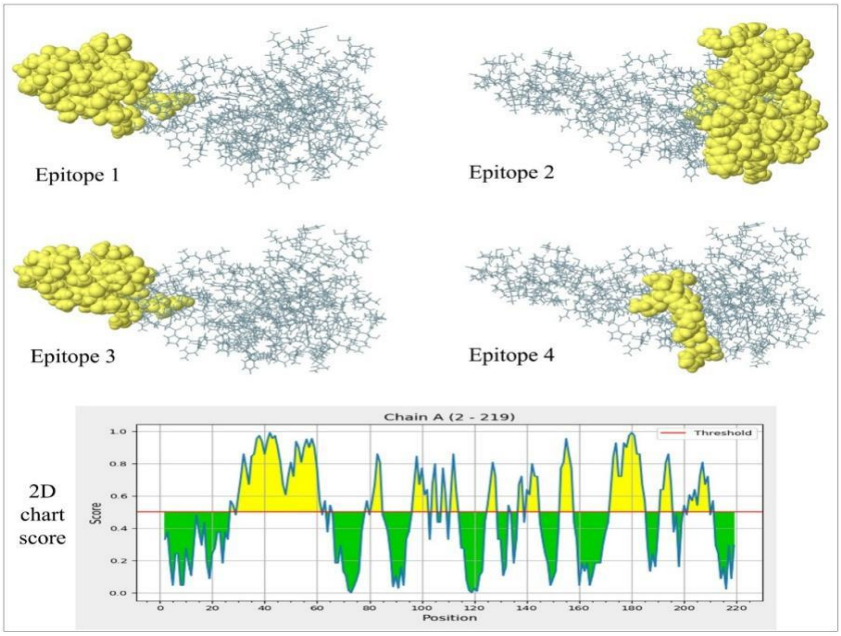

Fig. 2. 2D score chart and 3D structure mapping of the predicted discontinuous B cell epitopes.

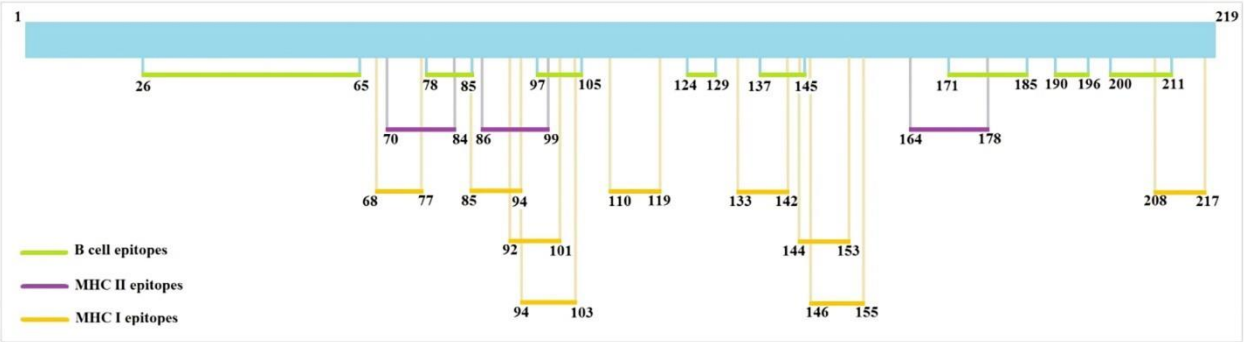

Fig. 3. The schematic view of the top-ranked epitopes of MHC I, MHC II, and B cells on the protein sequence.

The SDS-PAGE analysis of the purified *T. canis*-recombinant C-type lectin revealed a single band at 41 kDa, which corresponds to the recombinant C-type lectin protein 3 and 6 hours after induction with one mM of IPTG. This finding indicates the successful purification of the recombinant C-type lectin protein of *T. canis*, which can be used for further immunodiagnostic studies of human toxocariasis (Figure 4).

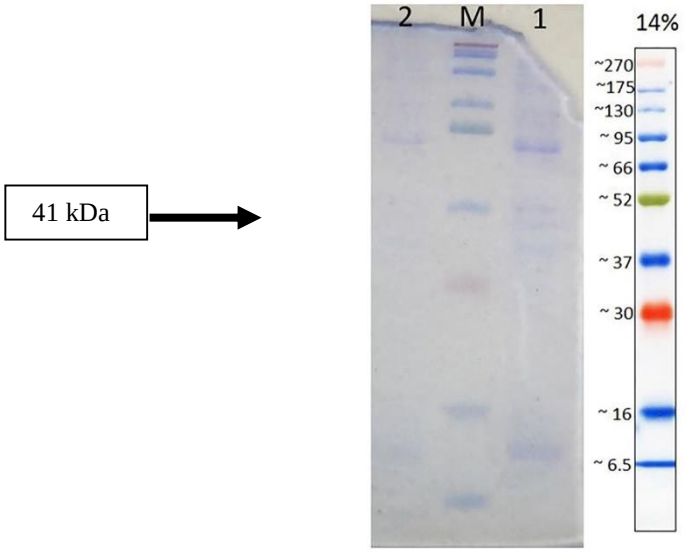

Fig. 4 The 12% sodium dodecyl sulfate (SDS-PAGE) analysis of the purified *T. canis*-recombinant C-type lectin revealed the following banding pattern. Lane 1: Purified *T. canis*-C type lectin 3 hours after induction with one mM of IPTG; Lane M: Pre-staining Protein weight marker 6.5 to 270 kDa (Biolegend, USA); Lane 2: Purified *T. canis*-C type lectin 6 hours after induction with one mM of IPTG. This analysis demonstrated the purification of the recombinant C-type lectin protein of *Toxocara canis*, with the band at 41 kDa, indicating the presence of the protein at both 3 and 6 hours after induction with IPTG.

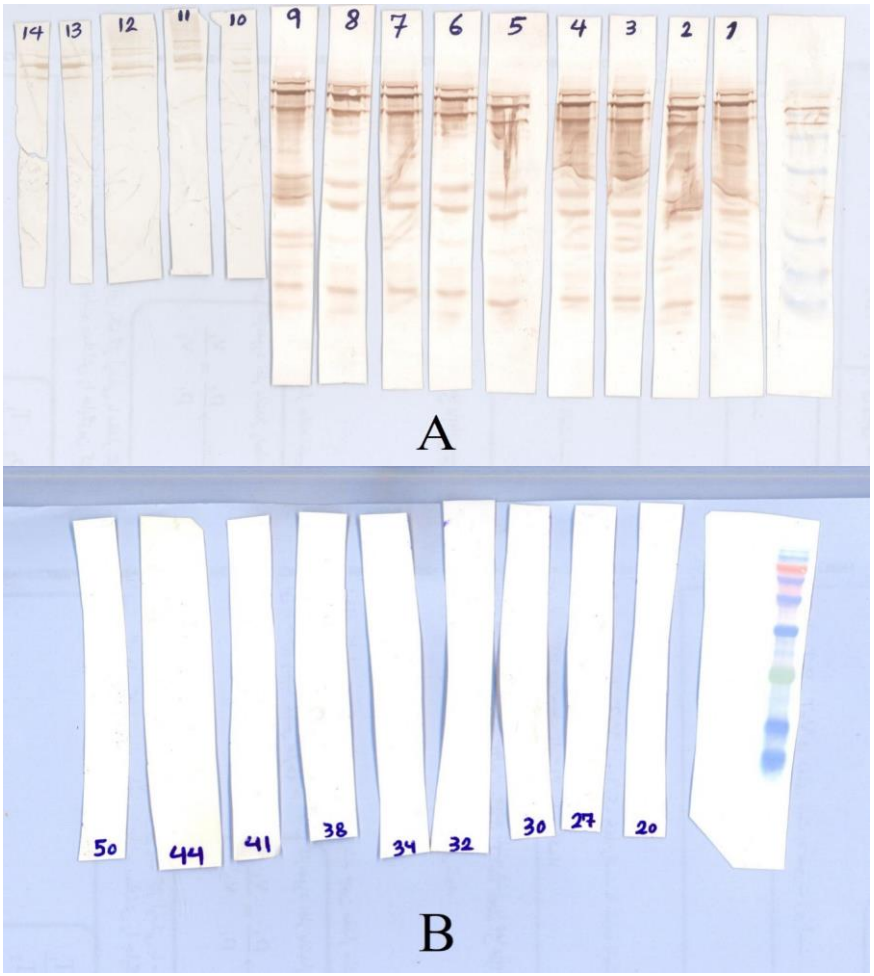

Fig. 5. Western blot of the *T. canis* recombinant C- Type lectin (with 41 KDa molecular weight) with the positive sera of human toxocariasis. Lanes1-14: various positive sera of pet owners; Lane 15: prestained protein marker 6.5 to 270 KDa (Biolegend, USA) (**A**), the different negative sera of pet owners (**B**).

Table 5. Comparison of the 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 kit*	+	12	0		0.9	**1.29**
	_	2	42			
Total		14	42			

* Commercial ELISA kit (Novagen, Germany).

Discussion

The recombinant C-Type Lectin (rCTL) showed potential as a suitable antigen for the serodiagnosis of human toxocariasis due to its higher sensitivity than the whole antigen secreted by *T. canis* (6). This suggests that the rCTL could be a more effective diagnostic tool for detecting human toxocariasis. Comparing the rCTL with other immunodominant antigens may provide insights into their relative effectiveness in diagnosing human toxocariasis (11). For example, a novel C-type lectin secreted by a tissue-dwelling parasitic nematode has been investigated (23). Comparing the rCTL with other antigens, such as this novel C-type lectin, can help determine which antigen is more suitable for diagnostic purposes. Diagnosing human toxocariasis, which can be caused by *Toxocara cati*, relies mostly on indirect means, particularly serology since the larvae are trapped in tissues and not readily detected. Diagnosing of toxocariasis is challenging, and the identification of the organism on microscopy is rarely achieved for practical reasons. However, several diagnostic methods are available, including serologic tests and secondary indicators of infection. Serologic tests, such as enzyme-linked immunosorbent assay (ELISA) and Western blotting, are commonly used for the diagnosis of toxocariasis. The best choice for serodiagnosis of the disseminated forms of toxocariasis, such as visceral larva migrans (VLM), relies upon the initial use of TES-ELISA, after which any positive result should subsequently be tested by Western blotting. However, ELISA assays have relatively poor sensitivity, approximately 80%. Other secondary indicators of infection include eosinophilia and hypergammaglobulinemia, along with serologic tests, which can contribute to the diagnosis of human toxocariasis. The present study provides initial findings on the antigenic potential of *T. canis*- rCTL. This evidence is crucial since *Toxocara* spp. is distributed worldwide (24). Toxocariasis is more common in children. In Iran, infection is more reported in children aged 11-14 (24). Diagnosis of human toxocariasis is conventionally based on clinical symptoms and serological diagnosis . The ELISA and WB are the most reliable methods for diagnosing the specific antibodies against toxocariasis in human serum. It is important to note that serologic tests can detect infections caused by both *T. canis* and *T. cati* but cannot differentiate between the two. Therefore, a combination of diagnostic methods and careful consideration of clinical symptoms and potential exposure to the parasites are crucial for an accurate diagnosis of human toxocariasis caused by *Toxocara cati* (*T. cati*) (4). It is necessary to evaluate the recombinant C-type lectin (rCTL) for serodiagnosis of *T.cati* in human toxocariasis and its potential to differentiate between *T. canis* and *T. cati* in human toxocariasis. Although, the rCTL has shown potential as a valuable antigen for the improvement of toxocariasis diagnostic kits, further studies are needed to determine its effectiveness in diagnosing *T. cati* infection in humans. Additionally, research has identified immunodominant antigens, including C-type lectins, for the laboratory diagnosis of toxocariasis, which can be used to develop more sensitive and specific diagnostic tools for *T. cati* infection in humans (5).

C-type lectin contains a domain with the characteristics of host calcium-binding lectins(C-type) (25**).** *T. canis*-CTL was chosen as the most important component of *T canis* excretory-secretory antigens and stage-specific protein, expressed in infective larvae but not in adult worms (11). *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 additionally showed 33-34% similarity to mouse and human C –type lectins (11). In a phylogenetic tree, its sequence is close to the strongilidea species (11). In the present studies, the antigenic potential of a 660 bp partial sequence of *T. canis*-C Type lectin, which encodes 219 amino acids, was evaluated. In the bioinformatics study, the secondary and tertiary structures 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 an overall quality score higher than the threshold. The protein's antigenic and non-allergenic nature allowed for the identification of T and B cell antigenic epitopes based on its sequence and tertiary structure. 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. Significantly, two serums tested positive with *T. canis*-rCType lectin but negative with the ELISA commercial kit (Novagen, Germany). The positive results were confirmed by Western blot analysis, which showed excellent agreement with the dot-ELISA assay (Cohen's Kappa = 1) and the commercial ELISA kit (Cohen's Kappa = 0.9). The negative test results obtained using the commercial ELISA kit (Novagen, Germany) were reliable. These findings suggest that the different assays used in this study are highly concordant and can be used identically to diagnose human toxocariasis (Table 5). Preliminary results in this study showed that the recombinant antigen is more sensitive in recognizing infected samples. Several recombinant antigens have been studied for the serological diagnosis of toxocariasis, and ongoing research on diagnostic antigens is still in progress. A 26 kDa recombinant protein (26), a fraction of excretory-secretory antigen, was assessed by ELISA for recognizing IgG but the sensitivity was very low so the results were disappointing (26). TES-26 or TC- PEB1 (7), TES32 or TC- CTL1 (27), TES-120 or TC- MUC1 and *T. canis* TES-30 and TES-120 recombinant proteins appeared serodiagnosis potential in sheep, horses and cattle through indirect ELISA (28-29). Recombinant antigens have been identified and considered in the diagnosis of toxocariasis. Recombinant 30 kDa antigen was also arranged and assessed with 11 sera of toxocariasis patients and appeared to have 100% sensitivity and 97.9% specificity (30). Recombinant 30 kDa antigen with 26 cases of toxocariasis-positive serum appeared sensitivity of 92.3% and specificity of 89.6% (31). 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 the specificity decreases (32). Recombinant proteins are an effective tool for the determination of toxocariasis in humans with the choice of appropriate antigens. Commercial ELISA kits frequently depend on the excretory-secretory antigens of *T. canis* larvae. The sensitivity and specificity of excretory-secretory antigens are satisfactory. These antigens are more suitable for screening tests (5). Utilization of excretory-secretory antigens from *T. canis* larvae increases the specificity of the ELISA, as well as, a positive ELISA result can be validated by Western blot, which exhibits greater specificity, particularly when considering lower molecular weight bands, ranging from 24 to 35 kDa (33). The Utilization of recombinant antigens was an important step in improving diagnostic tests (34). DOT-ELISA with adequate sensitivity and specificity could be an excellent assessment strategy due to its lower antigen requirement, cost-effectiveness, and rapid testing (35). 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 (35) as well as Western 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 (36). Although, the results of this preliminary study were confirmed by *in silico*, *in vivo*, and laboratory methods, future studies on the factors that make *T.canis*-rC type lectin an acceptable antigen must also be proven.

Conclusion

In conclusion, the recombinant C-Type Lectin (rCTL) demonstrated promise as a suitable antigen for the serodiagnosis of human toxocariasis due to its higher sensitivity compared to the excretory antigen secreted by *T. canis* and other antigens studied. Further research and comparison of rCTL with other antigens can provide valuable insights into the development of improved diagnostic kits for toxocariasis. In order to accurately determine

the specificity and sensitivity of *T. canis*-r C Type lectin, it is essential to test a large number of positive sera and sera infected with other parasitic diseases with this antigen. This study provides baseline data on *T. canis*-rC type lectin in Iran.

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Ethical approval

The study was approved by the Ethics Clearance Committee of Tehran University of Medical Sciences (No. IR.TUMS.REC.1400.033)

Conflict of interest

The authors declare no conflict of interest.

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