

# Cross-reactivity of Toxocariasis with Crude Antigen of *Toxascaris leonina* Larvae by ELISA

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Roundworms of *Toxocara canis* and *Toxascaris leonina* are common gastrointestinal helminths of canids over the world. Humans are infected with *T. canis* larvae through ingestion of infective eggs in contaminated environments or larvae by consumption of raw or uncooked meat or livers. Recently, patients of clinically diagnosed toxocariasis are increasing and require correct diagnosis in Korea. The present study investigated serological cross-reactivity between crude antigens of *T. canis* (TCLA) and *T. leonina* (TLLA) larvae. We collected serum specimens from 177 toxocariasis patients who were clinically suspected in the Seoul National University Hospital and 115 healthy controls. An ELISA method for toxocariasis was used to evaluate diagnostic efficacy of TLLA for serodiagnosis of human toxocariasis. The IgG ELISA using TLLA gave 14 (14.3%) positives of 98 TCLA positive specimens among 177 suspected toxocariasis patients. Most of them showed high absorbances with TCLA. In conclusion, there is a partial cross reaction between serum specimens of toxocariasis and TLLA.

**Keywords:** Toxocariasis; *Toxocara canis*; *Toxascaris leonina*; Larvae; Crude Antigen; Serodiagnosis; Serum IgG; Cross-reaction

## INTRODUCTION

Toxocariasis is a cosmopolitan zoonotic parasitic disease caused by migrating larvae of *Toxocara canis* or *Toxocara cati* in human bodies. Humans become commonly infected by accidental ingestion of embryonated *Toxocara* eggs in soil or larvae in the liver or meat of mammals or birds. After the ingestion, the larvae do not grow to adults but migrate to various organs inducing human toxocariasis, which is commonly presented as covert (adult) or common (child) toxocariasis, and less frequently as visceral or ocular larva migrans (1). A seroepidemiological survey for toxocariasis in Korea reported seropositive rate of 5% in general population in 2002 (2), 68% toxocariasis in unknown eosinophilia patients in 2006 (3), and 45.5% among eosinophilia patients in Chungcheongnam-do in 2012 (4). Recently serum IgG antibody test recognized 5.9%, 10.0%, and 12.4% positive rates among 610 healthcare examinees by eosinophil counts, < 350/μL, 350-500/μL, and > 500/μL respectively (5). Contrary to *T. canis*, *Toxascaris leonina* does not infect humans although it is commonly found among dogs.

Differential diagnosis of toxocariasis by serology is practically required for a certain population with eosinophilia, about 4% of hematology examination in a tertiary hospital in Seoul (6). To diagnose toxocariasis, enzyme-linked immunosorbent assay (ELISA) is a common method using excretory-secretory product released by *T. canis* larvae (7, 8) or using crude antigen from

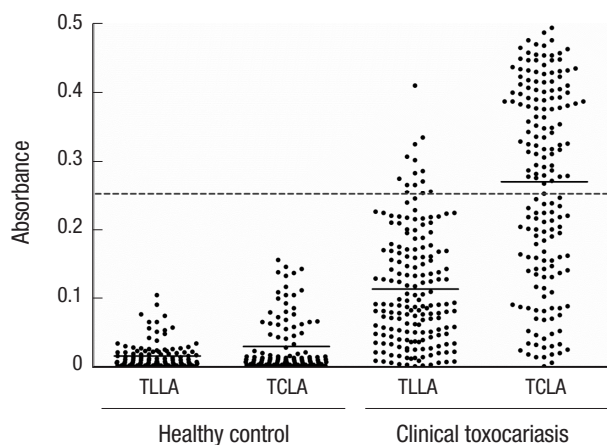
second-stage larvae of *T. canis* (TCLA) (9). The ELISA using TCLA showed 92.2% sensitivity and 86.6% specificity (9). To prepare *T. canis* larvae, the worms should be collected and identified in the laboratory by observing cephalic alae and egg morphology under a microscope. However, it is difficult to distinguish the two species, and it is possible that TCLA is contaminated with crude antigen of *T. leonina* larvae (TLLA).

In Korea, *T. canis* and *T. leonina* are commonly found in the intestine of canids. Surveys on *T. canis* in dogs showed 14.4% in the 1970s (10) and 0.9% in 2004 while that of *T. leonina* was 13% (11). Therefore, it is required to evaluate the serodiagnostic value of TLLA in toxocariasis. The present study investigated diagnostic values of TLLA for human toxocariasis by evaluating the cross-reactivity with TLLA.

## MATERIALS AND METHODS

### Isolation and cultivation of *T. canis* and *T. leonina* eggs

Live adult female worms of *T. canis* or *T. leonina* were obtained by anthelmintic medication of naturally infected dogs. The adult worms were transported to the laboratory and washed in sterile physiologic saline solution. Their uterus was dissected to collect fertilized eggs. The eggs were in vitro cultivated for embryonation of the L2 larvae. The live L2 larvae were collected as described previously (9).



**Fig. 1.** Distribution of ELISA absorbances of clinical toxocariasis and healthy control with TCLA and TLLA. TCLA, crude antigen of *Toxocara canis* larvae; TLLA, crude antigen of *Toxascaris leonina*. Horizontal bars represent the mean values.

### Preparation of TCLA and TLLA

Both TCLA and TLLA were made from the larvae of *T. canis* and *T. leonina* using a homogenizer in PBS at pH 7.2 (8.1 mM Na<sub>2</sub>PO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, and 136 mM NaCl). The larval homogenate was centrifuged at 13,000 rpm for 30 min at 4°C and the supernatant was cryopreserved at -70°C until needed. The protein concentration of the sample was estimated by the BCA™ Protein Assay Kit (Pierce, Rockford, IL, USA).

### Human serum samples

To evaluate the value of the two antigens for the diagnosing toxocariasis in Korea, we analyzed the assays with different groups of serum samples. A total of 292 serum samples were collected and used in this study. Among them, 177 were obtained from clinically diagnosed toxocariasis patients and 115 serum samples of negative controls from healthy individuals with no evidence of helminth infections.

### Development of ELISA

ELISA for the detection of antibodies in serum was performed by using both TCLA and TLLA. The wells of polystyrene, flat bottom, 96-well microtiter plates (Corning, Tewksbury, MA, USA) were filled with 100 µL of coating buffer containing TCLA or TLLA. The ELISA was performed as described previously (9).

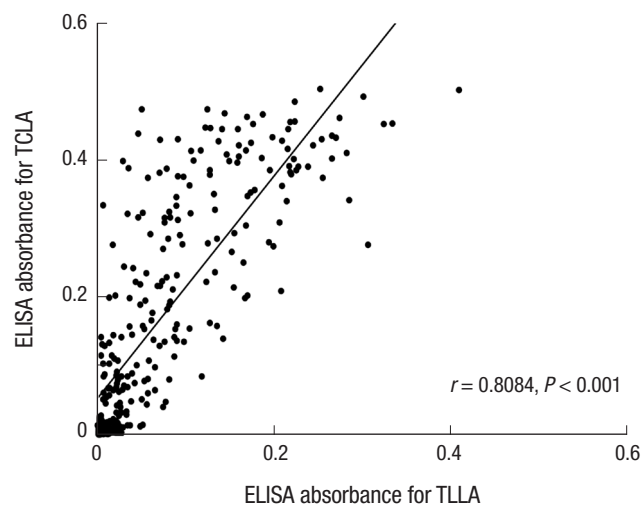
### Ethics statement

All experiments were conducted in accordance with an approved protocol by the institutional review board of the Seoul National University College of Medicine for using human materials (IRB No.: E-1205-009-408). The dogs were treated with levamisole tablets by their owners and the discharged worms were provided after oral agreement.

**Table 1.** Diagnostic value of *T. leonina* larval antigen by ELISA

Helminthiases	Total	No. of positive* serum	
		TLLA	TCLA
Healthy control	115	0	0
Clinical toxocariasis	177	14 (14.3%) <sup>†</sup>	98

\*Absorbance > 0.25; <sup>†</sup>Positivity of TLLA ELISA. TLLA, crude antigen of *Toxascaris leonina*; TCLA, crude antigen of *Toxocara canis* larvae.



**Fig. 2.** Correlation of ELISA absorbances between TCLA and TLLA. Data of total subjects (n = 292) were used for analysis. TCLA, crude antigen of *Toxocara canis* larvae; TLLA, crude antigen of *Toxascaris leonina*.

## RESULTS

### Determination of diagnostic values of TLLA ELISA

The mean absorbances of IgG ELISA using TCLA and TLLA from serum samples of clinically diagnosed toxocariasis and healthy control are presented in Fig. 1. Of the 177 clinical toxocariasis patients, 98 were positive by TCLA ELISA and 14 were positive by TLLA ELISA. The IgG ELISA using TLLA gave 14.3% (14/98) positivity for toxocariasis as shown in Table 1. These data exhibited low diagnostic value with TLLA.

### Cross-reactivity in ELISA between TCLA and TLLA

TLLA cross-reacted with 14 (0.291 ± 0.043) serum specimens of 177 total suspected toxocariasis patients, and most of them showed high absorbance with TCLA. The correlation analysis evaluated quantitative changes of ELISA absorbance with TCLA compared with the absorbance with TLLA, and the Spearman's correlation coefficient (Spearman's rho,  $\gamma$ ) was 0.8084. The ELISA absorbance with TCLA was correlated well ( $P < 0.001$ ) with those with TLLA (Fig. 2). These data exhibited high cross-reactivity of TLLA with serum of clinical toxocariasis.

## DISCUSSION

TLLA cross-reacted with serum antibodies of toxocariasis pa-

tients by ELISA. Its diagnostic sensitivity was 14.3% when the cut-off value was set at 0.25 same as with TCLA (9). The correlation of ELISA absorbance of the 292 total serum samples between TCLA and TLLA was analyzed. The Spearman's correlation coefficient (Spearman's rho,  $\gamma$ ) was 0.8084 ( $P < 0.001$ ), which indicated the two antigens might produce well correlated absorbance. In the present study, ELISA using TLLA may detect the samples of high absorbance with TCLA.

Since *T. leonina* does not induce larva migrans in human bodies, TLLA is not included in the antigen list of ELISA. Instead, most ELISA tests use TCLA because human toxocariasis is the target. However, TCLA cross-reacts frequently with many other helminthiases (9, 12-14). The cross-reaction is a major obstacle for serodiagnosis to be solved. This is the right reason why multi-antigen ELISA, which screens one serum with several antigens together, is required.

When the proper antigen is not enough, the ELISA may replace the antigen which cross-reacts mostly. This antigen replacement should be limited only within cross-reacting helminthiases of same taxon. In this context, it is plausible to make a serodiagnosis of human toxocariasis using TLLA. However, the present study confirmed diagnostic sensitivity of TLLA was too low to replace TCLA. The diagnostic sensitivity of ELISA may be lowered when the mixed antigen of the 2 species is used. Therefore, proper species identification of the ascarid nematodes from dogs is important in the process of antigen preparation.

The present study had some limitations. We had no human serum of *T. leonina* infection, and reverse cross-reaction between the 2 species was impossible. We could not confirm cross-reactivity of TLLA with other human helminthiases because of its limited amount. Further study on the components of the 2 antigens is recommended.

In conclusion, TLLA cross-reacts with serum of human toxocariasis but its diagnostic sensitivity is significantly low.

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## DISCLOSURE

The present authors have no potential conflicts of interest to disclose.

## AUTHOR CONTRIBUTION

Study design: Jin Y, Hong ST. Data collection: Jin Y, Shen C, Huh S. Data review: Jin Y, Choi MH, Hong ST. Manuscript writing: Jin Y, Choi MH, Hong ST. Manuscript approval: all authors.

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