The Korean Journal of Parasitology
Vol. 40, No. 3, 113-117, September 2002

A seroepidemiological survey for toxocariasis in apparently healthy residents in Gangwon-do, Korea

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Abstract: We investigated the sero-prevalence of toxocariasis among healthy Korean adults in 1999. A total of 314 sera from normal inhabitants in Whachon-gun, Gangwon-do, Korea was examined for specific antibody levels against excretory-secretory products of second stage larvae of *Toxocara* (TES). The presence of cross-reactions with other helminthiases such as cysticercosis, paragonimiasis, sparganosis or clonorchiasis was also checked by specific IgG ELISA. Sera showing positive reaction against TES were also tested by IgG immunoblot and by IgE ELISA. Out of 314 subjects, 16 was found to be positive by TES IgG ELISA and immunoblot, among whom 12 were also positive by TES IgE ELISA. Among the 16 seropositive samples, two sera showed positive reaction against *Paragonimus* and sparganum antigen, respectively. These results inferred that cross-reactions were negligible between toxocariasis and other helminthiases. Toxocariasis seroprevalence among Korean rural adults was detected to be approximately 5%.

Key words: *Toxocara canis*, toxocariasis, cross-reactions, ELISA, serology, Korea

INTRODUCTION

In Korea, a 28 year-old female who complained of impaired vision in her right eye was found to be positive by *Toxocara* excretory-secretory (TES) IgE enzyme-linked immunosorbent assay (ELISA) and by immunoblot detecting specific anti-*Toxocara* IgG (Park et al., 1999). Furthermore, five cases of uveitis were serologically diagnosed as ocular toxocariasis (Park et al., 2000). Korean ophthalmologists had earlier reported three clinically diagnosed cases of ocular toxocariasis (Chang et al., 1982; Choi et al., 1987; Lee et al., 1987). A patient with hypereosinophilic syndrome involving liver and stomach was reported to be caused by human toxocariasis (Choi et al., 2001). Since toxocariasis in dogs and cats was known to be prevalent in Korea (Cho et al., 1981; Huh et al., 1993), it is highly likely that many more cases of toxocariasis involving eye, liver, lung or brain should be detected by applying of accurate serologic tests such as ELISA and immunoblot. However, toxocariasis is usually a benign self-limiting disease, and many healthy subjects display residual antibodies. The seroprevalence of this helminthiasis among the target population should therefore

• Received 24 June 2002, accepted after revision 20 August 2002.
• This study was supported by Hallym University, Korea (2001).
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be evaluated. In order to address this issue, samples of inhabitants from a Korean rural area were tested for toxocariasis and also for cysticercosis, paragonimiasis, sparganosis and clonorchiasis, in order to assess possible cross-reactions.

MATERIALS AND METHODS

Collection of sera

In 1999, 314 sera were collected from apparently healthy adults residing in Whacheon-gun, Gangwon-do, Korea and stored at -70°C until use.

Preparation of *Toxocara* excretory-secretory (TES) antigen

Eggs were recovered from uterus of *Toxocara canis* adult female. They were incubated in 0.5 % formalin for one month. If live larvae were seen in the egg, the eggshells were broken by a homogenizer. Live larvae were collected using a Baermann apparatus. The larvae were kept in and were stored in culture medium (RPMI 1640 buffered to pH 7.2 with 23mM L-glutamine and 100μg/ml gentamicin) at 37°C in a CO_2 incubator. The supernatant was removed weekly, concentrated by ultrafiltration, and used as *Toxocara* excretory-secretory (TES) antigen. Protein concentration of the TES antigen was measured using BCA protein assay reagent kit (Pierce, Rockford, IL, USA).

TES IgG ELISA and immunoblot

Micro-titer plates (Costar-Corning, Cambridge, CA, USA) were coated overnight at 4°C with 200 μl of TES-Ag solution in carbonate buffer (pH 9.6) containing 2.5 μg/ml proteins. Each serum was diluted at 1:50 in phosphate buffered saline containing 0.05% Tween 20 (PBS/T, pH 7.4), and incubated for 2 hr. Peroxidase conjugated anti-human IgG (Cappel, West Chester, PA, USA) was diluted at 1:1,000 and incubated for 2 hr. The substrate for color reaction was *o*-phenylenediamine. The reaction was stopped with 4 M H_2SO_4. Absorbance at 490 nm was recorded with a micro-titer plate reader (Bio-Rad M3550, Richmond, CA, USA). Cut-off absorbance value was 0.25 OD after calibration using proven positive sera, and also after comparison with immunoblot results.

For immunoblot analysis, the TES-Ag preparations were separated by 10 % SDS-PAGE and transferred to a nitrocellulose membrane (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) in a semi-blotter (Hoffer, San Francisco, CA, USA). The strips were then incubated with sera diluted at 1:100. An alkaline phosphatase-conjugated anti-human IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) diluted at 1:3,000 was used to detect the immuno-reaction. Nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate (Sigma Co. St. Louis, MO, USA) were used as enzyme substrates, and positive reactions were ascertained by the presence of 4 lower molecular weight bands at 24, 28, 30, and 35 kDa, and of 3 higher molecular weight bands of 132, 147 and 200 kDa, respectively (Magnaval et al. 1991 & 1992).

TES IgE ELISA

ELISA detecting specific anti-*Toxocara* IgE (sIgE ELISA) was performed using a TES Ag (Magnaval et al., 1992).

IgG ELISA for other helminth infection

To check any other helminth infection in collected sera, IgG ELISA was performed for cysticercosis, paragonimiasis, sparganosis and clonorchiasis using its specific antigen as previously described (Kim et al., 1984).

Statistical analysis

χ^2-test, regression and Pearson product-moment correlation was performed using statistical package, dBSTAT 3.0 (Kim, 2000).

RESULTS

Out of 314 subjects, 16 sera (5.1%) showed positive reaction against TES antigen by IgG ELISA. Numbers of positive female and male were 10/165 (6.1%) and 6/149 (4.0%), respectively (p=0.5745). Age distribution was 0/13 in 30-49 year-old class, 4/63 in 40-49, 3/83 in 50-59, 9/105 in 60-69, 0/40 in 70-79, and 0/9 in 80-99. The age was not available for one subject. Titers of sera against TES are

Table 1. Distribution of serum IgG ELISA titers against *Toxocara* excretory-secretory (TES) antigen in healthy adults residing in Whacheon-gun, Gangwon-do.

O.D. range	No. of inhabitants	%
0~0.050	0	0
0.051~0.100	8	2.5
0.101~0.150	85	27.1
0.151~0.200	180	57.3
0.201~0.250	25	8.0
0.251~0.300	2	0.6
0.301~0.350	5	1.6
0.350~	9	2.9
Total	314	100

presented in Table 1. Among the 16 positive subjects, 12 were also positive by TES IgE ELISA, one was positive against *Paragonimus* antigen and another against sparganum antigen. More serological informations on *Toxocara* seropositive samples are summarized in Table 2. In IgG immunoblot, 14 sera were strongly reacted and 2 sera showed to be week positive (Data not shown). In order to assess the effect of antibody titer of other helmintic infections on TES IgG ELISA value, regression equation was calculated as below:

$$Y = 0.15743\ X_1 + (-0.01785)\ X_2 + 0.10184\ X_3 + (-0.01029)\ X_4 + 0.15682$$

Table 2. Detailed serological information on 16 positive sera detected by TES IgG ELISA.

No.	Age	Sex	TES IgG ELISA	*Stronglyoides ratti*, IFA[a]	*Ascaris suum* IgG ELISA[b]	TES IgE ELISA[c]	*Toxocara* immunoblot	reaction with other helminths
1	40	F	0.356	N[d]	0.11	21	positive	
2	48	F	0.357	N	0.18	4	positive	
3	49	F	0.397	20	0.94	45	positive	
4	50	F	0.295	N	0.39	8	weakly positive	
5	59	F	0.322	N	0.81	17	positive	
6	60	F	0.295	20	0.25	79	positive	
7	68	F	0.52	N	N	1	positive	
8	68	F	0.353	20	0.73	9	positive	*Paragonimus* (0.42)[e]
9	69	F	0.302	N	N	10	positive	
10	69	F	0.392	N	N	4	positive	
11	44	M	0.338	N	0.72	5	positive	
12	54	M	0.380	N	N	5	weakly positive	
13	60	M	0.352	N	N	1	positive	
14	60	M	0.304	N	N	902	positive	sparganum (1.32)[e]
15	62	M	0.304	N	1.06	35	positive	
16	64	M	0.383	N	N	383	positive	

[a]cut-off value: 20, IFA: immunofluorescent assay
[b]cut-off value: 0.550
[c]cut-off value: 5 TU (*Toxocara* Unit)
[d]Non-detectable
[e]O.D. value by ELISA

Table 3. Correlation coefficient(r) among IgG ELISA titers against TES and other helminth antigen.

Variable	*Clonorchis*	Cysticercus	*Paragonimus*	Sparganum	TES
Clonorchis	1.0000				
Cysticercus	0.2526[a]	1.0000			
Paragonimus	0.3934[a]	0.3068[a]	1.0000		
Sparganum	0.3177[a]	0.1286[a]	0.2420[a]	1.0000	
TES	0.1883[a]	0.0485	0.1641	0.0388	1.0000

[a]$p < 0.05$, Number: 307 Degree of freedom: df = 304, Significance (0.05): t = 1.9678

Determinant Coefficient (r^2) = 0.04679

Y = IgG Antibody titer against TES
X_1 = IgG Antibody titer against *Clonorchis sinensis*
X_2 = IgG Antibody titer against Cysticercus
X_3 = IgG Antibody titer against *Paragonimus westermani*
X_4 = IgG Antibody titer against sparganum

The results of the correlation study are shown in Table 3. Regression and correlation analysis were done with only 307 sera, since serum samples of 7 subjects were not enough for the analysis. Correlation coefficient between TES and other helminthes were insignificant ($p > 0.05$, Cysticercus, *Paragonimus*, sparganum) or very low 0.1883 ($p < 0.05$, *Clonorchis*).

DISCUSSION

The data presented herein suggested that the seroprevalence in rural Korean adults might be approximatley 5%, even though this seroepidemilogical survey was not a randomized study. This result implied that the rate would be certainly higher if children were included, since toxocariasis is usually a pediatric disease. Results are variable depending on the other countries: 17.7% (59/333) in rural area, and 2.1% (4/186) in urban area in Chengdu Sichuan, China (Luo et al., 1999); 19.6% in Malaysia (Hakim et al., 1991); 25.6% of 519 6-13 year-old children in Iran (Sadjjadi et al., 2000); 5.8%-36.0% in Czech Republic (Uhlikova, 1998); 20.7% out 1,025 inhabitants in Poland (Hermanowska-Szpakowicz, 2001); and 2-5% and 14.2-37% of urban and rural areas adult, respectively in Midi-Pyrenees Region, France (Magnaval et al., 1994). The variation of seroprevalence may indicate probably different exposures to *Toxocara* or different criteria for positivity.

Most of the results obtained by TES IgG ELISA were between 0.100 and 0.250, and consistent with those of immunoblot (Tables 1 & 2). Out of 16 positive sera, 12 subjects were suspected to be in the active stage of the disease, since they were also positive by sIgE ELISA. Moreover, subjects No. 14 and 16 could be atopic. With the subjects No. 8 and No. 14, TES IgG ELISA results were most likely due to cross-reactions with *Paragonimus* and sparganum infection, respectively (Table 2).

The results in Table 3 showed that the correlation between results by TES IgG ELISA and from other helminthiases was very weak, suggesting a possibility of cross reaction. The regression equation was also calculated to identify if the titers to other helminthiases had any effect to the titers to TES. The regression coefficients was very low with 0.04679. Therefore, there is little possibility that serum titer to other helminthiases affected the titer to TES. However, there is a possibility that helminthiasis other than toxocariasis influenced on the results of TES IgG ELISA, because of its high antibody load, as seen in the positive inhabitants No. 8 and 14. In this study, we could not check the cross reaction with anisakiasis, trichinosis, or fascioliasis. However, the 7 band pattern of immunoblot can rule out the possibility of cross reaction with those helminthiases.

None of the 16 positive subjects had complained of any clinical sign consistent with systemic toxocariasis till year 2001. A half of them was screened for ocular involvement by an ophthalmologist and no specific symptom could be found. *Paragonimus* positive person was screened with Chest P-A without any lesion in the lung. Regarding the clinical status, the positive subjects may be distributed as follows: the subjects No. 2, 7, 10 and 13 (IgG positive, IgE negative,) could be classified as past infections, the subjects No. 1, 3, 4, 5, 6, 9, 11, and 12 (IgG positive, IgE positive) could be considered to be currently infected without any clinical symptom, the subjects No. 14 and 16 probably had an associated atopic status, and patients No. 8 and 15 had a concurrent helminthiasis.

In conclusion, the sero-positive rate of TES among apparently healthy adults in rural area was estimated to be approximately 5% in Korea. Furthermore, since serological cross-reaction between toxocariasis and other helminthiases was little, detection of specific antibody levels by TES IgG ELISA would be a good measure for serological screening of toxocariasis.

ACKNOWLEDGMENTS

We thank Mr. Yong-Sang YANG, in the Gangwon Branch of the Korea Association of Health, Chunchon 200-092, Korea, for helping us collecting sera.

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