COMPARISON OF THE SENSITIVE SCREENING KIT WITH TWO ELISA SETS FOR DETECTION OF ANTI-*TOXOCARA* ANTIBODIES

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Abstract. Three serological tests (ELISA NOVUM, ELISA PU and ToxocaraCHEK) were compared using excretory-secretory (ES) antigen of second stage larvae of *Toxocara canis* on two sets of sera from patients aged 1-15 years, living in town and in the country, suspected of having larval toxocarosis. Of 60 serum samples examined for the presence of anti-*Toxocara* IgM and IgG antibodies 35 (58.3%) were positive with ELISA NOVUM, 30 (50.0%) with ELISA PU and 37 (61.6%) with ToxocaraCHEK. A significantly higher seropositivity with all the tests was detected in group of patients from the village (test χ^2, $p < 0.005$) when compared with patients from the town. The highest sensitivity (100%) was detected for a rapid qualitative ToxocaraCHEK. Compared with this screening test, both ELISA sets showed higher specificity but a lower sensitivity. Consistent findings (+;-) with ELISA NOVUM and ELISA PU were 88.3%; ELISA NOVUM and ToxocaraCHEK 96.7% and ELISA PU and ToxocaraCHEK 86.7%. This comparison indicates the suitability of ToxocaraCHEK set for screening for its simplicity and rapidity in detecting anti-*Toxocara* antibodies.

INTRODUCTION

The maintenance of human larval toxocarosis in urban and rural ecosystems is possible by the circulation of agents of this helminthic zoonosis. Larval toxocarosis affects mostly children coming into frequent contact with the environment containing disseminated eggs of *Toxocara* spp. The more frequent and severe infections are reported in village children coming from dog- or cat-keeping families (Dubinský *et al*, 1995; Kinšeková *et al*, 1998). Visceral form of toxocarosis (VLM) occurs more frequently than its ocular form (OLM). The symptoms accompanying the visceral form such as fever, pneumonia, abdominal pains, geophagy, hepatomegaly, pharyngitis, arthritis are the most frequent conditions in children under 5 years of age. Also eosinophilia is reported in most patients (Roig *et al*, 1992; Rugiero *et al*, 1995; Kinčeková *et al*, 1999). In view of the unspecific symptoms of larval toxocarosis, its diagnosis is possible only on the basis of serological examination. The currently used ELISA test, however, requires special laboratory equipments and is rather time-consuming. For the screening examination of larval toxocarosis, Akao *et al* (1997) recommend a rapid and sensitive test, not requiring any supplemental instrument. Moreover, the results can be read directly from the test in only 3 minutes. Of 14 different human parasitoses diagnosed by this test, cross reaction was observed only in gnathostomosis while in others, largely tropical parasitoses, no cross reactions were detected. This test, named by the authors as ToxocaraCHEK kit, showed a high correlation with ELISA, immune blot and double gel diffusion sets.

Sera of patients from urban and rural areas were used to compare the *Toxocara* screening kit with the ELISA test used at Parasitological Institute SAS and with the *Toxocara canis* IgG/IgM ELISA set from NOVUM DIAGNOSTICA GMBH.

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MATERIALS AND METHODS

Antigens

ES antigen from *Toxocara canis* infective larvae was prepared by the method of de Savigny (1975). Protein concentration was measured according to Lowry *et al* (1951).

Serum samples

The serum samples used for assessing diag-

nostic sensitivities were taken, on the basis of eosinophilia and clinical symptoms of the disease, from patients at the age of 1-15 years suspected of larval toxocarosis, living in the country (30 samples) and in town (30 samples). Stools floatating examination of patients were negative. In addition, the sera from 15 healthy adult volunteers, aged 19-23, were used as negative controls. To examine the cross reactivity of tests, investigated also were clinically, parasitologically or histologically proven cases of parasitic infections caused by the following parasite species: *Toxoplasma gondii* (toxoplasmosis), *Trichinella britovi* (trichinellosis), *Echinococcus granulosus* (hydatidosis), *Fasciola hepatica* (fasciolosis), *Taenia solium* (cysticercosis), *Ascaris lumbricoides* (ascariosis) and *Toxocara canis* (toxocarosis).

Tests used

ToxocaraCHEK kit manufactured by EY Laboratories, Inc Hong Kong was used for the detection of antibodies in human serum. The principle of the test is the immobilization of captured larval ES antigen (0.5 μg/device) on the nitrocellulose membrane of the test device (Fig 1a). Antibodies to ToxocaraCHEK in the human sample are trapped by the immobilized antigen. The presence of bound antibodies is revealed by subsequent treatment with a protein A-colloidal gold conjugate which binds to form a red color on the membrane.

The assay protocol is very simple. Three drops of buffer solution [0.2% bovine serum albumin (BSA) in 0.1 M PBS containing 0.05% Tween 20, pH 7.2] were applied to the membrane. After the buffer was completely absorbed in absorbent placed in a plastic device (Fig 1d), one drop of serum sample diluted 1:20 in buffer solution and three drops of buffer were applied. After the final buffer was absorbed, two drops of protein A-colloidal gold conjugate were applied to the device. The final washing step was performed with three drops of buffer solution then three drops of washing solution [0.1 M PBS containing 0.05% Tween 20, pH 7.2 (PBS-T)]. Result can be read immediately. In case of negative result ToxocaraCHEK may be used repeatedly (at least twice).

The ELISA test practiced at Parasitological Institute SAS (ELISA PU) uses microtitration plates with bound ES antigen prepared from infective *T. canis* larvae by the method of de Savigny (1975). The further procedure has been modified after Mančal (1987). Microtiter plates (Nunc, Immuno Plate) were coated overnight at 4ºC with antigen containing 1 μg/ml protein diluted with 0.1 M carbonate buffer, pH 9.6. After washing plates 3 times with PBS-T serum samples, conjugate and substrate (100 μl per well) were gradually added. Every time they were incubated for 30 minutes at 37ºC and washed 3 times with PBS-T. Serum samples were diluted 1:100 in PBS-T containing 0.05% bovine serum albumin (BSA) and horseradish peroxidase-labeled swine anti-human IgG was used at a dilution of 1:10,000 in PBS-T containing 0.5% BSA. To visualize the test, o-phenylendiamine in 0.05 M citrate buffer, pH 4.7 containing 0.05% hydrogen peroxide was used as chromogen. The reaction was stopped after 20 minutes of incubation in the dark at room temperature by adding 50 μl of 2 M sulfuric acid and measured spectrophotometrically at 492 nm (Labsystem Multiscan PLUS). The optical density of each sample was calculated by the formula: $OD = OD_{sample} - OD_{blank}$. The blank value should be less than 0.25. The quadruple mean values of pooled negative control sera were considered as cut-off.

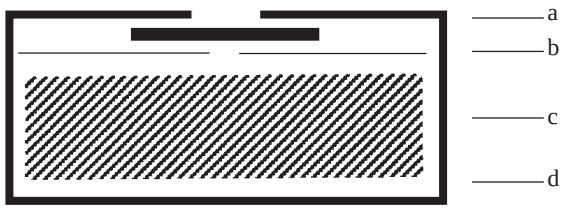

Fig 1–Schematic diagram of ToxocaraCHEK kit device: (a) ES antigen of *Toxocara canis* larvae (0.5μg/device) on nitrocellulose membrane; (b) plastic septum; (c) absorbent; (d) cover.

In using NOVUM TOXOCARA CANIS IgG/IgM ELISA (ELISA NOVUM) the instructions of producer protocol (NOVUM DIAGNOSTIKA GMBH, Germany) were followed. Sera were diluted 1:100 in sample solution. Diluted sample sera and controls were pipetted into microtiter strip wells as a solid phase coated with inactivated purified specific excretory-secretory *T. canis* antigen from the larval stage. Horseradish peroxidase conjugated anti-human IgG/IgM antibodies, TMB (3, 3′, 5, 5′-tetra-methyl-benzidine) solution and stopping solution (1.5 M sulphuric acid) were used. Absorption at 450 nm was read using an ELISA microtiter plate reader. Total incubation time was 30 minutes. Cut-off value (CO) was calculated by the producer protocol: CO = MN + 0.200 (MN - mean absorption value of negative control and a 0.200 - constant factor).

Table 1
Determination of cross-reactivity in two ELISA sets and ToxocaraCHEK kit at serum dilutions 1:2 and 1:20.

Infections	No. of patients	No. of serologically positive patients			
		ELISA NOVUM	ELISA PU	ToxocaraCHEK	
				1:2	1:20
Toxoplasmosis	5	0	0	1	0
Trichinellosis	5	0	0	1	0
Hydatidosis	5	0	0	0	0
Fasciolosis	2	0	0	0	0
Cysticercosis	3	0	0	0	0
Ascariosis	5	0	0	2	0
Toxocarosis	5	5	5	5	5

Statistical evaluation was done according to Reisenauer (1970). The sensitivity and specificity of the test was calculated according to Margolis (1983) and Knill-Jones (1987).

RESULTS

To evaluate the cross-reactions caused by other parasitic infections, ToxocaraCHEK test were performed at serum dilution 1:2 and 1:20. Four out of 25 sera from patients with other parasitosis as toxocarosis were positive at the serum dilution 1:2, but at the dilution 1:20 these positive reactions disappeared (Table 1).

Screening ToxocaraCHEK kit and two ELISA sets were compared. One of the ELISA sets (ELISA PU) determines only IgG and the other (ELISA NOVUM) both IgG and IgM subclasses of specific anti-*Toxocara* antibodies. For comparison we used two equally large sets of sera from suspected patients aged 1-15 years. Patients coming from urban areas showed a significantly lower seropositivity (test χ^2, $p < 0.005$) than those coming from rural areas (Table 2). The highest positivity was observed for ToxocaraCHEK test. For comparison, ELISA NOVUM had 96.7% sensitivity and 80% specificity. The lowest sensitivity (82.8% and 78.4%) but a high specificity (96% and 100%) was recorded for ELISA PU test. The ToxocaraCHEK recognized more positive sera (37 from 60) compared with the ELISA NOVUM (35 from 60) and ELISA PU (30 from 60), while with ELISA PU test more negative results were detected.

DISCUSSION

The diagnosis of human larval toxocarosis is intricate. Clinical symptoms tend to be unspecific. Elevated temperature, pneumonia, abdominal pains, etc, can also be observed in children with other diseases. Also blood and other laboratory tests are not unambiguous. Frequent eosinophilia (Kinčeková *et al*, 1998) also occurs with other parasitoses. Diagnosis requires a serological examination for the presence of specific anti-*Toxocara* antibodies. Acute toxocarosis in children has to be diagnosed promptly so that a special treatment be applied.

Many countries report the presence of anti-*Toxocara* antibodies in as much as 2 to 10% of healthy population (Genchi *et al*, 1990; Stürchler *et al*, 1986; Barriga, 1988; Caucanas *et al*, 1988; Ljungström and van Knapen, 1989; Cauchie *et al*, 1990). In some other countries, including the Slovak Republic (Havasiová *et al*, 1993) and the Czech Republic (Uhliková and Hübner, 1983), such antibodies occur in 15-20% of the healthy population.

Considering the fact that serological investigation is performed only by specialized laboratories, the confirmation of diagnosis would take several days. A rapid screening examination provided by ToxocaraCHEK makes this diagnosis possible in any laboratory within a very short time. The implementation of this test into routine practice would be very desirable. For its high sensitivity and sporadic cross reactions (Akao *et al*, 1997) this simple test appears to be suitable for practical use. The best agreement between the three compared assays in both the patient groups was observed

Table 2
Comparison of anti-*Toxocara* antibodies by two ELISA sets and ToxocaraCHEK kit.

		Sera of habitants from				
		Town		Village		Total
		+	-	+	-	
A. ELISA NOVUM						
ELISA PU	+	8	1	21	0	30
	-	2	19	4	5	30
Total		10	20	25	5	60
B. ELISA NOVUM						
ToxoToxocaraCHEK	+	10	1	25	1	37
	-	0	19	0	4	23
Total		10	20	25	5	60
C. ELISA PU						
ToxoToxocaraCHEK	+	8	3	21	5	37
	-	0	19	0	4	23
Total		8	22	21	9	60

a.ELISA PU: sensitivity 82.8%; specificity 96%; ELISA NOVUM: sensitivity 96.7%; specificity 80%; Consistent findings (+;-) 88.3%.
b.ToxocaraCHEK: sensitivity 100%; specificity 92%; ELISA NOVUM: sensitivity 94.6%; specificity 100%; Consistent findings (+;-) 96.7%.
c.ToxocaraCHEK: sensitivity 100%; specificity 74.2%; ELISA PU: sensitivity 78.4%; specificity 100%; Consistent findings (+;-) 86.7%.

between ToxocaraCHEK and ELISA NOVUM tests (96.7%). The second one detected both IgG and IgM anti-*Toxocara* antibodies, what permits to diagnose the early stage of this diseases. The less consistent findings between ELISA PU and ToxocaraCHEK (86.7%) may be caused by the fact that ELISA PU detects special IgG antibodies only. In such cases we may not rely on a screening examination alone but this should be complemented with a semi-quantitative ELISA.

ACKNOWLEDGEMENTS

This paper was partially supported by the Science Grant Agency VEGA, grant No. 2/5012.

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