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 \textit{Toxocara} spp. The more frequent and severe infections are reported in village children coming from dog- or cat-keeping families (Dubinsky et al., 1995; Kinseková 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, hepatomegal, 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; Kinceková 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 \textit{Toxocara} screening kit with the ELISA test used at Parasitological Institute SAS and with the \textit{Toxocara canis} IgG/IgM ELISA set from NOVUM DIAGNOSTICA GMBH.

MATERIALS AND METHODS

Antigens

ES antigen from \textit{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-
Detection of Anti-Toxocara Antibodies

Voll 31 No. 2 June 2000 395

Diagnostic 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 floating 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 microtiteration 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 4°C 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 37°C 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.

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.

<table>
<thead>
<tr>
<th>Infections</th>
<th>No. of patients</th>
<th>No. of serologically positive patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ELISA NOVUM</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Trichinellosis</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Hydatidosis</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Fasciolosis</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Cysticercosis</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Ascariosis</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Toxocarosis</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

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 NOVUM) determines only IgG and the other (ELISA PU) 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 $\chi^2$, $p < 0.005$) 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.
DETECTION OF ANTI-TOXOCARA ANTIBODIES

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

<table>
<thead>
<tr>
<th>Sera of habitants from</th>
<th>Town</th>
<th>Village</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>A. ELISA NOVUM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA PU</td>
<td>8</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>B. ELISA NOVUM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ToxoToxocaraCHEK +</td>
<td>10</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>C. ELISA PU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ToxoToxocaraCHEK +</td>
<td>8</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>22</td>
<td>21</td>
</tr>
</tbody>
</table>

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

- ELISA NOVUM: sensitivity 96.7%; specificity 80%; Consistent findings (+, -) 88.3%.
- ELISA PU: sensitivity 82.8%; specificity 96%; Consistent findings (+, -) 86.7%.

ACKNOWLEDGEMENTS

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

REFERENCES


Havasiová K, Dubinský P, Štefančíková A. A seroepidemiological study of human Toxocara infection in


