Abstract

Determination of professional activities, which are connected with higher risk of toxocariasis, is very important for well-timed diagnosis and prevention. That is why our research aimed to investigate the spread of toxocariasis in people, whose professional activities are related with forests.

Thirty-three people are included in the study – eight employees of Forest Research Institute at Bulgarian Academy of Sciences, Sofia, and 25 forestry workers from three different locations in South Bulgaria.

The specific parasitological diagnosis of toxocariasis is performed with ELISA and Western blot. Presence of anti-Toxocara IgG antibodies in ELISA was established in eleven forestry workers. This reveals very high seroprevalence of toxocariasis among this profession – 33.3%. Moreover, the examination of 15 sera (11 with positive and 4 with borderline ELISA results for toxocariasis) with Western blot showed the presence of two and more antigen bands in 11 samples (33.3%). These results show that professional activities in the forest could be defined as risk factor for development of toxocariasis.

Key words: toxocariasis, seroprevalence, ELISA, Western blot, total IgE

Introduction. Toxocariasis is helminthic zoonosis caused by the presence and migration of nematode larvae in human tissues – mostly Toxocara canis and

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T. cati (parasites in dogs and cats, respectively) [1, 2]. The disease is caused by ingestion of invasive eggs through dirty hands, soil, vegetables and fruits, water, contact with infected animal or consumption of uncooked meat from domestic paratenic animals. Infecting larvae hatch from parasitic eggs in the human body. The larvae migrate to different organs and cause disease with extremely polymorphic clinical picture – from asymptomatic to very severe organ damages, which depend on larvae number, localization and the immune response of the host [3]. The migrating larvae secrete and excrete highly immunogenic products, which cause Th2-type immune response with IL-4 and IL-5 production, IgE-antibodies synthesis and eosinophilia [4]. Some researches, revealed the correlation between Toxocara seropositivity and asthma, increased total IgE, eosinophilia and presence of allergen-specific IgE [5].

Due to very difficult identification of parasite’s larvae in the organism, the main methods of diagnosis still remain the serological tests ELISA and Western blot, which define specific IgG antibodies to the antigens, produced by Toxocara larvae [6].

The Toxocara seroprevalence varies widely from 2% [7] to 63% in some tropical countries [8] and these differences are due to lack of standardization, different methods of diagnosis and demographic specificity. In this connection detailed epidemiological information for most of the countries is still missing. The researches about toxocariasis risk groups in Bulgaria are also very limited. It is less known about Toxocara spp. presence in wild animals. That is why the aim of our study was to investigate the spread of toxocariasis in forestry workers in Bulgaria.

Materials. Thirty-three serum samples of people, whose professions are related with forests were examined – 8 employees of Forest Research Institute at Bulgarian Academy of Sciences, Sofia, and 25 forestry workers from three different locations in South Bulgaria – 6 from State Forestry, Maglizh; 10 from State Forestry, Poibrene, and 9 from State Forest Nursery, Plovdiv.

The participants are 28 men (85%) and 5 women (15%) aged between 18 and 87 years (average age 45 years) with no clinical signs of toxocariasis.

Methods. Specific Toxocara examination was performed through serological methods – ELISA and Western blot.

ELISA (R-Biopharm, Germany) test is used for determination of anti-Toxocara IgG antibodies in serum samples. The test reaction was accomplished according to manufacturer’s protocol and the results were presented in Sample index (SI). Samples with SI < 0.9 are accepted as negative, SI between 0.9–1.1 defines borderline samples and these with SI > 1.1 are accepted as positive.

All serum samples with positive or borderline result in ELISA were additionally examined with Western blot (LD BIO, France), according to manufacturer’s protocol. The presence of two low molecular antigen bands of size 24–35 kDa reveal specific anti-Toxocara IgG antibodies in the sample.

Determination of total IgE. Quantification of total IgE was performed
ELISA *Toxocara* positive results and ELISA SI mean values of the surveyed persons by gender, age and presence of specific allergic sensitization

<table>
<thead>
<tr>
<th>Positive results (N)</th>
<th>Mean SI (ELISA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male (28)</td>
<td>10</td>
</tr>
<tr>
<td>Female (5)</td>
<td>1</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
</tr>
<tr>
<td>&lt; 30 (5)</td>
<td>3</td>
</tr>
<tr>
<td>30–40 (10)</td>
<td>5</td>
</tr>
<tr>
<td>40–50 (6)</td>
<td>1</td>
</tr>
<tr>
<td>50–60 (7)</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 60 (5)</td>
<td>1</td>
</tr>
<tr>
<td>Specific allergic sensitization</td>
<td></td>
</tr>
<tr>
<td>Presence (20)</td>
<td>8</td>
</tr>
<tr>
<td>Absence (13)</td>
<td>3</td>
</tr>
</tbody>
</table>

with an ELISA kit (EUROIMMUN) according to the manufacturer’s instructions. Values above 100 IU/ml were considered elevated.

**Statistical data analysis.** The survey data were processed with statistical software GraphPad Prism 6.0 (GraphPad Software, Inc.).

**Results.** ELISA test for determination of anti-*Toxocara* IgG antibodies gave positive result in 11 people and 4 borderline results. The rest 18 examined people (54.5%) were negative for toxocariasis (Table 1). The total percentage of *Toxocara* seropositivity was 33.3%. Ten of 28 men (35.7%) and one of 5 women (20%) were positive.

The sample index values (SI) of people with positive ELISA vary in the interval from 1.27 to 3.80 (range 2.53 and average 2.26) of the whole group. Four of the ELISA results were borderline and vary in the area from 0.9 to 0.96 (the average SI value is 0.94).

The main part of *Toxocara*-positive ELISA results – 5 (46%) were people in the age group of 30–40 years old with average SI value 2.28 and people up to 30 years old – 3 (27.3%) with SI average value 2.29. The highest SI value was reported in a 36-year-old man – 3.80. No statistically significant differences (*p* = 0.858) were established in average SI values of different age groups. Detailed data about received SI of positive ELISA results, divided by sex and age, are presented in Table 1.

Most frequently *Toxocara*-positive results, according to the factor “workplace”, were reported in the group from State Forestry Poibrene – 5 of 10 examined (50%) and those from State Forestry Maglizh – 3 of 6 (50%). Three people (33.3%), who work at State Forest Nursery, Plovdiv, were also positive. None of the examined
Table 2

ELISA results and mean SI values of the surveyed persons according to the place of work

<table>
<thead>
<tr>
<th>Employees</th>
<th>ELISA positive</th>
<th>ELISA SI mean</th>
<th>ELISA borderline</th>
<th>ELISA negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest Research Institute, BAS</td>
<td>0 (0%)</td>
<td>–</td>
<td>1 (12.5%)</td>
<td>7 (87.5%)</td>
<td>8</td>
</tr>
<tr>
<td>Maglizh</td>
<td>3 (50%)</td>
<td>2.680</td>
<td>1 (16.7%)</td>
<td>2 (33.3%)</td>
<td>6</td>
</tr>
<tr>
<td>Poibrene</td>
<td>5 (50%)</td>
<td>1.944</td>
<td>1 (10%)</td>
<td>4 (40%)</td>
<td>10</td>
</tr>
<tr>
<td>Plovdiv</td>
<td>3 (33.3%)</td>
<td>2.353</td>
<td>1 (11.1%)</td>
<td>5 (55.6%)</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>11 (33.3%)</td>
<td>2.256</td>
<td>4 (12.1%)</td>
<td>18 (54.5%)</td>
<td>33</td>
</tr>
</tbody>
</table>

employees of Forest Research Institute, Bulgarian Academy of Sciences, Sofia, has positive ELISA result for toxocariasis (Table 2).

The examination of 15 sera (11 with positive and 4 with borderline ELISA results for toxocariasis) with Western blot showed the presence of characteristic antigen bands in 11 people (33.3%) – 10 men and 1 woman (Fig. 1). Three of W. blot positive results were of woodcutters from State Forestry, Maglizh, in the age group 30–40 years. Positive results were found also in 4 men from State Forestry, Poibrene, and in one 65-year-old woman and three men 60, 20 and 56 years old, respectively, from State Forest Nursery, Plovdiv (Fig. 2).

Quantification of total IgE showed that in 13 (39%) of the studied 33 forest workers the levels were above the limit of 100 IU/ml. In nine (69%) samples, an increase of total IgE was not associated with seropositivity to *Toxocara* spp. Only in four (31%) persons the positive ELISA and Western blot results are

![Fig. 1. Toxocara IgG W. blot results of surveyed employees engaged in forestry activities](image-url)
Fig. 2. Toxocara IgG W. blot positive results by age and place of work

Fig. 3. Comparison of the data for seropositivity to Toxocara spp. (ELISA for specific IgG and Western blot) to the data from determination of the total IgE accompanied by an increase of total IgE. In the remaining seven forest workers with proven seropositivity to Toxocara, levels of total IgE were not increased (Fig. 3).

**Discussion.** Toxocariasis is most risky for people, who often have a contact with contaminated environment. Most frequently reported risk factors are soil contact [⁹] and presence of cat or dog in the household [¹⁰], especially if the animal lives outdoor and eats other animals [¹¹]. Wild and wandering animals have significant importance for disease spread, as they are more infected [¹²]. They contribute to the circulation of the disease between wild nature, cities and
villages \cite{13}. The disease is also related with poor life conditions, poor hygiene, lack of education and low social and economic status \cite{14}.

Despite the fact that millions of people are predisposed or infected with \textit{Toxocara} spp., epidemiological information about the link between seropositivity and toxocariasis is still limited \cite{15}. The investigation of Woodruff et al. \cite{16} reveals significant disease spread (15.7\%) in dog breeders. Deutz et al. \cite{17} report 17\% seropositivity spread in the Australian hunters. Investigations in our country show 8.33\% seroprevalence in veterinary doctors and dog owners \cite{18}. Our researches, regarding people, whose professional activities are related with forests, revealed extremely high percentage of seropositivity – 33.3\% without any clinical signs of toxocariasis. This result is significantly higher than the percentage of \textit{Toxocara} seropositivity in healthy people from our country – 8.6\% \cite{19}.

The seropositive forestry workers were mostly men (10 from 28 examined) between 30 and 40 years old. The Western blot, which we performed on positive and borderline ELISA results, demonstrates the presence of antigen bands, characteristic for this disease in 11 examined people, which once again confirm the high level of seropositivity to \textit{Toxocara} in forestry workers.

Data analysis, regarding the levels of total IgE and their association with presence of anti-\textit{Toxocara} IgG antibodies in studied individuals, showed, that only in 4 (31\%) of blood samples the positive ELISA and Western blot results are accompanied by an increase of total IgE. Moreover, all these individuals were with elevated allergen-specific IgE against one or more inhalative and/or insect allergens \cite{20}. But in the remaining seven forest workers with proven seropositivity to \textit{Toxocara}, levels of total IgE were not increased. The information about the relation between \textit{Toxocara} seropositivity and allergy in literature is contradictory. Visceral larva migrans is often related with wheezing, but it is still unclear whether \textit{Toxocara} parasites predispose or induce asthma \cite{14}. There are studies, which claim, that allergic airway inflammation in mice could be complicated by experimental \textit{T. canis} infection, probably through increasing the levels of Th2-type immune response to inhalative allergens \cite{21}. Although there was no statistically significant difference, the average values of the specific anti-\textit{Toxocara} antibodies (SI), which we established in the persons with specific allergic sensitization, were higher (2.3\%) than the average values of people without sensitization and no elevated total IgE (1.88\%). On the basis of these data we can assume that the \textit{Toxocara}-invasion contributes to the higher sensitization of the forestry workers.

**Conclusion.** Determination of professional activities which are connected with higher risk of toxocariasis, is very important for well-timed diagnosis and prevention. The received high percentage of seropositivity in the studied group of forestry workers determines the professional duties in the forests as a risk factor for development of toxocariasis. This imposes a large and more focused searching and diagnosis of this parasitosis, especially in forestry workers, which are with higher risk of infection.
REFERENCES


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