SEROEPIDEMIOLOGICAL STUDY OF THE PREVALENCE OF TOXOCARIASIS AMONG HEALTHY PERSONS IN SOME AREAS OF BULGARIA

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ABSTRACT

Background. Human toxocariasis is a zoonotic disease in which the human being is an accidental host. The broad distribution of its definitive hosts determines the presence of the disease worldwide. Demographic factors, the way and standard of living, the existence of domestic animals in the household and their infestation are important for the epidemiology of the infection and determine the differences in reported seroprevalence. Data on the prevalence of toxocariasis in our country are limited. In this regard, the aim of the present study is to determine the seroprevalence of this parasitic infection among healthy individuals, in three regions of Bulgaria (Plovdiv, Stara Zagora and Smolyan).

Material and Methods. Serum samples obtained from 247 individuals were tested by enzyme-linked immunosorbent assay (ELISA) for anti-Toxocara IgG antibodies.

Results. Positive results were found in 26 (10.5%) of them, which indicated the presence of an asymptomatic form of the disease in our country. We found different seropositivity rates in the studied districts of the country.

Conclusions. Performing immunological tests among various groups of the population is of great importance for the early detection of Toxocara infection, as well as for determining the main factors for the spread of the disease. Keywords: Toxocara, ELISA, seroprevalence.

INTRODUCTION

Toxocariasis is a helminthic zoonotic infection in humans caused mainly by canine (Toxocara canis) and feline (T. cati) nematode larvae (1). Infection occurs through the ingestion of invasive eggs from contaminated sources, or through the consumption of viscera from paratenic hosts containing Toxocara larvae (2). In humans, the parasite does not reach full development and remains in a larval stage in various organs, causing a variety of non-specific symptoms (3), therefore the disease often remains undiagnosed. Human infection can present itself with a wide range of clinical manifestations: from asymptomatic cases to the characteristic forms as visceral larva migrans syndrome (VLM), ocular larva migrans (OLM) syndrome, neurotoxocariasis and covert or common toxocariasis (4).

The identification of larvae in the human body is extremely difficult, so the main methods for the disease diagnosis are the serological ones (5, 6, 7). The primary examination of patients suspected for Toxocara infection, is performed with an ELISA test for detection of specific anti-Toxocara IgG antibodies. The latter is the recommended screening method due to its fast performance and relatively low cost (8). It is the main method for seroepidemiological studies as well, which provide data on the presence of hidden forms of this parasitic infection.

Toxocara-contaminated environment is the major risk factor for human invasion (9). Other factors include poverty, poor hygiene, gender and age, infestation of dogs and cats (10). In the scientific literature, there are differences in the data on the incidence of human toxocariasis due to the variety of diagnostic methods, the diversity in the study groups, geographical areas, lifestyle and standard of living (11). Environmental factors, such as climatic and soil conditions, also play an important role (12).
In Bulgaria, studies on the prevalence of toxocariasis are insufficient, and in this regard the aim of this study is to determine the seroprevalence of this parasitic infection among healthy individuals, living in three regions of Bulgaria (Plovdiv, Stara Zagora and Smolyan).

**MATERIALS AND METHODS**

**Patient samples:**
We examined serum samples from 247 individuals of different age groups from three regions of the country:
- Plovdiv District – serum samples obtained from 70 persons aged 25 to 79 years (average age 45 years); 55 women, 15 men.
- Stara Zagora District – serum samples from 46 persons aged 6 to 86 years (mean age 59 years), 23 women and 23 men.
- Smolyan District – serum samples from 131 persons aged 2 to 79 years (average age 43 years), 41 men and 90 women.

**Methods:**
Serum samples were tested for the presence of anti-Toxocara IgG antibodies with a commercial ELISA kit (R-Biopharm AG, Germany) according to the manufacturers’ instructions.

**RESULTS**
Of the tested serum samples from the Plovdiv region, three showed presence of specific anti-Toxocara IgG antibodies, and one had a borderline result (Fig. 1). The obtained seroprevalence was 4.3% (Fig. 2).

The values of the optical density in the ELISA of the examined samples vary from 0.063 (lowest value) to 0.765 (highest value), with an average of 0.238 (Table 1).

The examination of the serum samples from persons living in the Stara Zagora district, showed three positive and two borderline results (Fig. 1). The established seroprevalence in this area of the country was 6.5% (Fig. 2).

The obtained optical densities were from 0.065 to 1.064 (maximum value of the optical density), with an average value for the whole group - 0.238 (Table 1).

The largest number of people were examined in the Smolyan district (n = 131). We found presence of anti-Toxocara IgG antibodies in 20 serum samples and another 13 were with borderline results in ELISA (Fig. 1).

The highest seroprevalence was found in the Smolyan district, as it significantly exceeded that found in the other two studied regions (Plovdiv and Stara Zagora) (Fig. 2). The optical density of the examined samples of the Smolyan inhabitants was in the ranges from 0.064 (the lowest reported value) to 1.245 (maximum value) (Tabl. 1).

The total percentage of the spread of toxocariasis among healthy people (247) established by ELISA, for the three studied districts in Bulgaria – Plovdiv, Stara Zagora and Smolyan – was 10.5%.

A positive result was obtained in 26 individuals - 22 women and 4 men. The seropositivity in the studied women (168) from the three districts - 13.1%, significantly exceeded that obtained in men (79) - 5.06%.

Summarized data from the study are presented in Tabl. 2.

**DISCUSSION**
Toxocariasis is a parasitic disease with a widespread distribution around the world (4, 13). It is more intense in tropical and subtropical regions, and its transmission is linked to environmental conditions, rainfall, humidity and temperature, which are crucial for the egg development (14). Although to a lesser extent, it is also prevalent in countries in the temperate climate zone (3), with varying seropositivity rates reported across countries and in many of them these data are completely missing (13).

Risk factors for human infection include the number of local animal population and the prevalence of Toxocara spp. in them, soil contamination with Toxocara eggs and low socio-economic conditions. Older age, male gender, rural living, and data on raw liver consumption have been significantly associated with Toxocara-seropositivity (16).

The immunoscreening for toxocariasis conducted on 247 people from different age groups in three districts of the country - Plovdiv, Smolyan and Stara Zagora showed a positive result in 26 of the participants and an overall seropositive
The obtained percentage differed for the three regions, i.e. for the Plovdiv district it was 4.3%, for Stara Zagora – 6.5%, and the highest one was reported for the Smolyan district – 15.3%. The differences are probably due to climatic factors, eating habits, occupation, agricultural activities and the number of persons that have been examined.

According to a number of authors, toxocariasis is a disease spread mainly among children, which is due to their close contact with animals, playing in contaminated areas and still poorly developed hygiene habits, and more often affecting boys.

The previous two studies on the prevalence of toxocariasis in our country in healthy blood donors (n = 350) established 8.6% seropositivity (17), and 8% seropositivity (n = 50) (18) in ELISA. The highest seropositivity has been found in the Varna region (15%), which defines the disease as one of the most common parasitic zoonosis in that region (19).

Table 1. Optical density (OD) values (450 nm) among the studied healthy individuals by regions.

| Region   | OD mean | OD min. value | OD max. value |
|----------|---------|---------------|---------------|
| Plovdiv  | 0.238   | 0.063         | 0.765         |
| Stara Zagora | 0.238   | 0.065         | 1.064         |
| Smolyan  | 0.244   | 0.064         | 1.245         |

Table 2. Summarized data from the seroepidemiological study.

| Examined population region (number of examined persons) | Toxocara positive results | Toxocara seropositivity % | Seropositivity (n) | Seropositivity (%) |
|----------------------------------------------------------|---------------------------|---------------------------|-------------------|-------------------|
|                                                          |                           |                           | gender            | gender            |
|                                                          |                           |                           | male              | female            |
|                                                          |                           |                           | male              | female            |
| Plovdiv (70)                                            | 3                         | 4.3                       | 0                 | 3                 |
|                                                          |                           |                           | 0                 | 5.5               |
| Stara Zagora (46)                                       | 3                         | 6.5                       | 0                 | 3                 |
|                                                          |                           |                           | 0                 | 13                |
| Smolyan (131)                                           | 20                        | 15.3                      | 4                 | 16                |
|                                                          |                           |                           | 9.8               | 17.2              |
| Total (247)                                             | 26                        | 10.5%                     | 4                 | 22                |
|                                                          |                           |                           | 5.1               | 13.1              |
The infection is also diagnosed in elderly patients (22) and some authors point to frequent positive serological reactions in individuals over 60 years of age (23). There have been reports of more frequent involvement of males, but without a statistically significant difference in the prevalence of toxocariasis among females (24; 25). Our present results show higher prevalence of toxocariasis among the studied women (13.1%) compared to that found in men (5.06%). Similar results were reported by Cong et al. (2014), who observed significantly higher seropositivity among women in the studied healthy individuals, which is related to many factors as their greater involvement in the care of pets, more frequent contact with raw meat and vegetables and cooking at home. This, in their view, showed that women were at higher risk of *Toxocara* infection and that knowledge on the prevention of this disease needs to be expanded, especially for female groups (26).

Our study reveals that asymptomatic toxocariasis is a common infection among clinically healthy people in the studied regions of the country and this indicates that measures are needed to control the infection among its main animal hosts, to prevent environmental contamination and to improve the knowledge of the population on the mechanisms of infection.

**CONCLUSIONS**

Toxocariasis remains one of the most widespread and economically important zoonosis affecting humans. Detailed and up-to-date information is needed regarding its prevalence and risk factors which is essential for control of this disease.

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