ABSTRACT

Toxocariasis is a tissue zoonosis caused by *Toxocara* spp. and the exposure to these geohelminths varies in different parts of the population in a particular region.

The purpose of the study is to reveal the seroprevalence of toxocariasis in North-eastern Bulgaria and to establish its demographic structure.

Material/Methods: Within the scope of a broad seroepidemiological study, 701 individuals from North-eastern Bulgaria were tested for anti-*Toxocara* antibodies (ELISA) between 2017 and 2020. The results were stratified and analysed by age, gender, and place of residence.

Results: The overall seroprevalence of human toxocariasis in North-eastern Bulgaria is 18.54%. Children (18.85%) and adults (18.30%) show similar levels of infection, but the stratified analysis by age in 5-years intervals revealed notable discrepancies. In younger children, the seroprevalence is higher than the observed for the entire population (0-4 years - 22.22%; 5-9 years - 22.22%), while in adolescents and adults is lower than average. In the elderly, the seroprevalence rises to its highest levels (60-64 years - 30.56% and over 65 years -31.67%). Although there are variations between males and females, gender is not a significant factor for the distribution of toxocariasis. Higher seroprevalence levels in residents of the smaller settlements of the region (children - 28.18%; adults -29.10%) correspond to a 2.5-fold increase in the odds of contracting toxocariasis in comparison to the ones living in Varna city.

Conclusion: The overall seroprevalence of toxocariasis in North-eastern Bulgaria is alarmingly higher than the levels reported from other parts of the country, placing this unknown infection in a leading position among the parasitic zoonoses. The place of residence is the most significant factor driving the uneven distribution of the disease.

Keywords: Age Distribution, Demographics, Seroepidemiology, Sex Distribution, Toxocariasis, Residence Characteristics,
tosis, and its distribution is still in the process of research. The first seroepidemiological study for the period 1997-2005 found overall seropositivity of 10.9% [4], and according to the latest publications, the overall prevalence for 2015-2017 in Bulgaria is 19.1% [5]. Our initial studies of the distribution of this parasitosis in children and adults with clinical symptoms from North-eastern Bulgaria revealed overall seropositivity for *Toxocara* IgGs of 13.38% [6].

Even though worldwide research on the seroprevalence of toxocariasis is proving its leading position among zoonotic helminthiases, the disease remains neglected, both epidemiologically and clinically [3, 4].

**MATERIALS AND METHODS:**

A comprehensive epidemiological study was conducted between 2017 and 2020 to establish the presence of specific anti-*Toxocara* antibodies in the population of North-eastern Bulgaria. A total number of 701 individuals (male: n=348 / female: n=363) participated in the research; of those 313 were children (age 2-18) and 388 adults (age 18-88). To assess the impact of the type of settlement on the risk of contracting toxocariasis, the results were further stratified in two groups – residents of Varna city (n=457) and residents of smaller settlements of North-eastern Bulgaria (n=244).

**Ethical approval:** The Commission for Ethics of Research - MU-Varna endorsed the design and protocol for the study. Trained medical specialists gave detailed information about the study’s purpose and procedures, and informed consent was obtained from all respondents or their parents and legal guardians in cases of children under 18 years of age. Information on the demographic parameters (age, gender, education), ethnicity, residency and other risk factors was gathered personally and anonymously from each responder in a questionnaire designed especially for this survey. No personal identifying information is presented in the study.

**Serology:** Screening for specific *Toxocara* IgGs was performed using a commercial kit for enzyme-linked immunosorbent assay (*ELISA* - *Ridascreen Toxocara IgG, R – Biopharm*). For verification of the obtained results, the sera with positive or borderline levels were subjected to additional testing with Western blot (LD BIO). Both tests were executed according to the specified manufacturer instructions.

**Data analysis:** The resultant prevalence by age, gender, and place of residence is expressed within the 99.9% confidence interval (CI) calculated by Clopper-Pearson’s exact method for binomial proportions. The size of the observed differences is represented as odds ratios (OR). Each OR is accompanied by its 99.9% asymptotic CI and the value of the exact probability (p-value) for the OR being equal to 1. The data were processed and analysed with the R language and environment for statistical computing [7].

**RESULTS:**

The overall seroprevalence stratified by age and gender in the studied population of North-eastern Bulgaria is presented in table 1.

**Table 1.** Seroprevalence of toxocariasis among children and adults of North-eastern Bulgaria.

|                | Male                  | Female                | OR (male/female) |
|----------------|-----------------------|-----------------------|-----------------|
|                | Tested | Positive | Seroprevalence % (CI) | Tested | Positive | Seroprevalence % (CI) |                 |
| Children       | 180    | 28       | 15.56 (10.59-21.69)    | 133    | 31       | 23.31 (16.10-30.52)    | 0.61 (0.34-1.08) |
| Adults         | 168    | 38       | 22.62 (16.27-28.97)    | 220    | 33       | 15.00 (10.56-20.42)    | 1.66 (0.99-2.80) |
| Total          | 348    | 66       | 18.97 (14.84-23.10)    | 353    | 64       | 18.13 (14.11-22.15)    | 1.05 (0.72-1.54) |

Specific *Toxocara* IgGs were detected (ELISA) and confirmed (Western Blot) in 130 individuals, which evaluates to a total seroprevalence of human toxocariasis of 18.54%. The seropositivity among children (under 18 years of age) is 18.85%, comparable to the levels registered in adults - 18.30%.

The analysis of the distribution by gender revealed a seroprevalence of 18.97% in males and 18.13% in females. Although the detailed assessment of the results showed some variations in the distribution by age and gender, the estimated relative chances of infestation (ORs) indicate that the observed differences are without statistical importance.

In order to establish the actual distribution of toxocariasis in the population of North-eastern Bulgaria, we further stratified the result by age in 5 years intervals (fig. 1). In children, the highest seroprevalence is registered in the two youngest age groups- 22.22% in the 0-4 and 5-9-year-old groups. An interesting observation is the increase of seropositivity in adults after 45 years of age with values above the average for the entire population. The highest levels were recorded among 60-64-year-olds (30.56%) and in seniors over 65 years (31.67%).
The type of settlement is an essential factor frequently associated with a higher risk for exposure and infection with infection with *Toxocara* spp. and other zoonotic parasites. In our research, we divided the studied population and the subsequent results into two groups – residents of the urbanised infrastructure in the district centre – Varna City, and residents of smaller settlements and rural areas - including little towns and villages. The seropositivity in children and adults in the two studied groups are presented in table 2. The stratification by age, as well as the overall results, show that the odds of toxocariasis in rural areas is significantly higher than in the city with $\text{OR} \geq 2.5$.

**Table 2. Seroprevalence of toxocariasis in North-eastern Bulgaria according to the type of residency.**

| Age Group | Varna city | Small settlements | OR (Varna city / Small settlements) |
|-----------|------------|-------------------|-----------------------------------|
| Test | Positive | Seroprevalence % (CI) | Test | Positive | Seroprevalence % (CI) | (CI) |
| Children | 203 | 28 | 13.79 (9.04-18.54) | 110 | 31 | 28.18% (20.02-37.56) | 2.45 (1.38-4.36) |
| Adults | 254 | 32 | 12.60 (8.51-16.69) | 134 | 39 | 29.10% (21.58-37.57) | 2.85 (1.67-4.82) |
| Total | 457 | 60 | 13.13(10.17-16.57) | 244 | 70 | 28.69% (23.10-34.80) | 2.66 (1.81-3.92) |

**DISCUSSION:**

This first seroepidemiological survey on the distribution of toxocariasis in the North-eastern region of Bulgaria revealed a relatively high seroprevalence of 18.54%. The findings are similar and comparable with the already published initial results from our previous studies [6,8], which shows that this level of seroprevalence in the region is a stable and permanent phenomenon.

The observed overall prevalence of human toxocariasis in North-eastern Bulgaria shows values higher than the data published in the first systematic analysis for the spread of this parasitosis in Bulgaria (seropositivity - 10.9%) for the period 1997-2005 [9]. The result is also exceeding the reported levels of countrywide seroprevalence of 13.7% showed in the latest comprehensive analysis of all officially registered cases of toxocariasis between 2000 and 2017 [10]. The higher seropositivity levels are observed when our stratified analysis by age and gender is compared with the results in similar demographic groups in several seropositivity studies in Bulgaria [4,10,11]. This shows that the increased seropositivity in the region is typical for all strata of the population and is not the result of random differences in any particular group.

Several independent factors could contribute to the discovered higher levels of seropositivity for toxocariasis in the North-eastern region of Bulgaria. The broad range of participants recruited in our survey allowed us to establish the prevalence of this zoonosis not only in the individuals with clinical signs but also to detect a vast number of hidden asymptomatic cases in patients within selected risk groups.
(minorities, professions with animal exposure) and the general population. The environmental conditions may also play a significant role in the observed above-average levels in the region. The milder and hotter climate in North-eastern Bulgaria is proven to be favourable for the better survivability of the *Toxocara* eggs in the soil [12]. Another factor facilitating the spread of the infection is the poor control of the populations of stray animals and inadequate breeding methods in the rural areas of the region [13,14].

The observed differences in the seroprevalence of toxocariasis by gender - 15.56% in boys versus 23.31% in girls and 22.62% in men versus 15.00% in women are not significant (table 2), and we can not attest that gender plays a role as a driving risk factor for the spread of the infection with *Toxocara* spp. in the studied population.

The majority of seroepidemiological studies on toxocariasis worldwide point out that children are at a higher risk of contracting toxocariasis [1,2]. Our initial results established an almost identical relative share of the infection in children (18.85%) and adults (18.30%), but the additional stratified analysis of the seroprevalence by age revealed notable discrepancies (fig.1). In childhood, most affected are young children (0-9 years of age – 22.22%). This is probably due to several behavioural traits - undeveloped personal hygiene; playing outdoors with soil and sand; more frequent contact with small animals; onychophagia, geophagia, and others [1,15]. Seroprevalence in adolescents and adults (up to 44 years) drops below the average, probably due to the improved personal hygiene and other behavioural factors reducing the risk of exposure. After 45 years, the relative share of the infection rises above the mean, reaching alarmingly high levels of 1/3 affected in the population over 60 years. This results in more than twofold increase of the odds of contracting toxocariasis after reaching age 60 and above, in comparison to the chances in the younger ages - OR over 60 years/under 60 years =2.30 (1.42-3.72). These statistically significant differences could be explained by the constant and accumulating exposure over time to the parasitised environmental factors or hosts, as a similar trend is observed in another cosmopolitan zoonosis – toxoplasmosis [16]. The latest systematic analysis of the seropositivity for toxocariasis in Bulgaria showed similar age distribution - highest seroprevalences in children aged 0-4 years and in adults aged 45-49 years, but as aforementioned with lower levels of an infestation than the ones discovered in the North-eastern region [10].

Our research confirmed the global trends that the type of residence is one of the leading risk factors for the spread of toxocariasis (Table 2). The seroprevalence in the inhabitants of the smaller settlements in the region (28.69%), compared to those living in Varna city (13.13%), determine 2.66 times higher odds for infestation with *Toxocara* spp. Furthermore, the impact of the type of settlement is relevant in comparison by age in both children - 28.18%: OR= 2.45 and adults - 29.10%: OR= 2.85, which proves that the residents of rural settlements are under a significant and continuous risk for infestation with this parasitic zoonosis. This trend is observed in the generalised 17-year analysis of the spread of toxocariasis in our country [10]. Similar to ours are the results in another recently published report on the spread of toxocariasis - seropositivity of 17.35% for those living in cities and of 33.33% for the residents of villages [5]. The observed results testify for the growing socioeconomic inequalities between the large urbanised cities and smaller rural settlements where several potentiating factors work simultaneously – low socioeconomic status, poverty and unemployment, lack of proper housing, undeveloped water and sewage infrastructure, the concentration of professions associated with agriculture, animal breeding and increased exposure to animal hosts and parasitised environment, lower level of education and healthcare.

Although the discovered seropositivity for toxocariasis in North-eastern Bulgaria is higher than the average for Bulgaria, those results are comparable to the levels registered in other European countries and do not reach the alarming trends found in the poorest regions of the developing world [1–3].

**CONCLUSIONS:**

This seroepidemiological study of the demographic characteristics of the seroprevalence of toxocariasis in the Northeast region, Bulgaria, reveals a high level of parasitism among the population in the region, comparable and exceeding the latest summary data for the country, which puts toxocariasis as one of the leading zoonotic infections. Statistically significant and alarmingly higher seropositivity was found in two risk groups – young children up to 9 years and adults over 60 years, which is associated with increased exposure to a contaminated with invasive elements environment, connected with improper hygiene and frequent contact with soil, sand, and hosts.

Our study confirms that the worldwide tendency for higher seropositivity in the rural regions is pertinent to the North-eastern parts of Bulgaria due to closer contact and coexistence with sources of infection (dogs and cats) as well as lower social, economic status, poorer living conditions, communal infrastructure, problematic access to healthcare and education and others. Early enforcement of proper hygiene habits in children and sustaining them through adulthood, washing of vegetables and fruits, improvement of living conditions and health culture, and the efficient control of stray and pet dogs and cats would contribute to a significant limitation of the spread of toxocariasis in the country and the region.

Due to the exceptional variety of non-specific clinical symptoms of toxocariasis and the established high prevalence, which includes many asymptomatic (covert) cases, it is necessary to increase the knowledge of different medical specialists for this zoonosis in order to be included in the differential diagnosis of a wide range of diseases.

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