SOCIALLY SIGNIFICANT ZOONOSES AND THE ENVIRONMENT

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Abstract

Zoonoses are diseases which are transmitted between vertebrate animals and humans and are endemic to certain regions with high morbidity.

The aim of the study is to conduct a sanitary parasitology testing of soil samples in a specific living quarter of Stara Zagora. The results have been associated indirectly with the morbidity of some parasitic zoonoses which are socially significant for Bulgaria.

Material and methods: data from official sources on the morbidity of cyst echinococcosis and toxocariasis were analyzed. A sanitary parasitological study of parasitic elements from 64 sites in 15 streets in a living quarter in Stara Zagora was performed.

Results: the annual ECDC analyses for the period 2006-2021 on the prevalence of cyst echinococcosis show that the cases of echinococcosis in Bulgaria vary between 30 and 50 % of operated patients in the EU/EEA. The cases of toxocariasis in humans in specific communities amount to 25% of the tested persons. The environmental investigation in several regions of Stara Zagora for zooparasitic elements through different periods manifests an increase of the relative share of the contaminated sites – between 68% and 92%. During the sanitary parasitological analysis, it was found that on the territory of 59 (92.2%) sites there were parasitic elements. Eggs of four parasitic genera were identified: *Toxocara* sp. – 22.3%) of samples, *Taenia* sp. – 6.5%, *Trichuris* sp. and *Ancylostoma* sp. - 1.6% and larvae of nematodes – 1.1% of samples. Parasitic zoonoses are a serious problem in communities, coinhabited by people and incorrectly kept pets or near populations of stray dogs and cats.

Key words: *parasitic zoonoses, environment contaminated with parasitic elements*

Introduction

According to the WHO definition, zoonoses are diseases transmitted naturally between vertebrate animals and humans [1]. They have a cosmopolitan distribution, and in some regions, among certain communities, they are endemic with high morbidity. Over 3 billion people globally suffer from one or more parasitic diseases [2,3]. Parasitic zoonoses spread over widening areas and infect a growing number of people, with certain communities facing a higher risk of contamination [4]. This is explained by multiple reasons, among which existing socio-economic problems, deteriorated sanitary conditions, cohabitation with incorrectly kept pets and populations of stray dogs and cats. Morbidity in these regions of the developing world is troublesome, but the share of zoonoses in regions with a higher living standard is also considerable [5,6]. Humans, through their insufficient health culture, due to the lack of health knowledge and behaviour in their everyday life, work and professional activity, may influence directly or indirectly the environment and turn it into a contamination hazard with pathogens of various origin. The environment, as a combination of abiotic and biotic components, is a leading

factor in the their spread and impact on humans, respectively human health [7]. The mechanisms of spread, transmission and the invasive stage of the parasite into the host organism can be the social factors in the epidemic process under specific economic, cultural and living conditions. These two types of factors (biological and social) are leading in parasitology [8]. Some of the most important socially significant parasitic zoonoses contain pathogens, transmitted from pets to humans [9,10]. For a number of pathogens, such as *Toxocara sp. Echinococcocus granulosus, Toxoplasma gondii, Giardia lamblia, Cryptosporidium sp.,* the transmission factors are found in the external environment: the soil, water, food, or animals [4,11]. In the presence of uncontrollably increasing populations of specific hosts – dogs and cats, contaminating the environment and susceptible population with risky behaviour and low health culture, the likelihood of transmission multiplies. Good understanding of biology and epidemiology of parasites and the risk factors leading to human infection is necessary for effective strategies of prevention [12]. The present study has been initiated in the context of the multisectoral One Health approach, which works gradually from local to global for the prevention and control of zoonoses [13].

The aim of this study is to conduct a sanitary parasitology testing of soil samples in a living quarter in the town of Stara Zagora, and associate the results indirectly with the morbidity of some socially significant parasitic zoonoses in Bulgaria.

Material and methods

In order to perform the sanitary parasitology testing, we took three soil samples on average from 64 sites in 15 streets in the Lozenets* quarter of Stara Zagora, by applying the Caldwell&Caldwell method [14]. The samples were taken in the beginning of December 2023. We collected soil from the superficial layer at a depth of 1-3 cm, at a weight of about 20 grammes. We used a classic protocol to test the soil samples for parasitic elements: homogenization, sedimentation, extraction, floatation, and microscopic identification. Five grammes from each sample were analyzed. The soil was homogenized in 10 ml 4% sodium hypochlorite and filtered through a wire sieve with 0.3 mm openings. The filtrate was centrifuged at 700 revolutions for 2 minutes, and the sediment was resuspended with 10 ml sodium dichromate solution (1,35 $mg.dl^{-1}$). The sample was recentrifuged at 500 revolutions for 3 minutes, after which sodium dichromate was added to a test-tube, until upper meniscus was formed. After 15 minutes, the floating material was removed with automatic pipette (from 2 to 200 µl) and was placed on a glass slide. For morphological identification of the parasitic forms (eggs, larvae, oocysts, and cysts) we performed light microscopy at an amplification of 400 x.

Results and discussion

The contamination of the environment, respectively the soil with zooparasitic elements is a serious public health problem. According to Wikipedia, the population of Lozenets quarter in Stara Zagora amounts to over 5000 people, predominantly from the Roma minority. About one-fifth of them constantly migrate to foreign destination, or the capital city of Sofia. The predominant part of the inhabitants are illiterate, uneducated, without a profession, and therefore permanently or temporarily unemployed. Their main occupation is seasonal trade, or activities related to maintaining hygiene in town. Varying number of stray dogs and cats freely roam the quarter, while the street hygiene is shockingly low.

In our study, the eggs identified in the soil samples are in the diagnostic and invasive stage of four genera of parasitic representatives. They are released in the environment through the feces of infested dogs and cats and represent a zoonotic risk for people. Out of the 64 samples taken from Lozenets quarter, 59 (92.2%) contained parasitic elements. From the studied 184 fractioned samples, in 61 (33.2%) we identified parasitic elements from the four genera: *Toxocara* sp. – in 41 (22.3%) samples, *Taenia* sp – in 12 (6.5%), *Trichuris* sp. and *Ancylostoma* sp. in 3 (1.6%) samples, and larvae of freely living nematodes – in 2 (1.1%) samples. In ten of the soil samples from the 15 streets, we noticed parasitic elements, from three streets – between 50 and 66.7% of the collected samples contained parasitic elements, from four streets – between 34.1 and 42.9%, and from three streets – between 16.7 and 31.0%. Only in the soil samples of five of the streets there were no eggs, or parasitic larvae. From one street, four parasitic genera were identified, from two streets – three genera, and from one street – two genera, and from five streets – only one genus. In two-thirds of the streets we found eggs of *Toxocara* sp. Four of the streets were contaminated with eggs of *Taenia* sp. and two – of *Ancylostoma* sp. Twenty-eight (43.4%) sites of collection of soil samples contained visible fecal waste, probably of animal origin. The eggs of *Taenia* sp., including those of *Echinococcus granulosus*, are morphologically identical and in terms of characterization it is impossible to differentiate their species. The fact that 12 soil samples from the streets of Lozenets quarter contain cestode eggs, does not exclude the possibility for some of them to belong to the species *E. granulosus.* In various studies, the relative share of the soil samples containing zooparasitic elements varies widely. A study in Stara Zagora found that 83.2% of the soil samples were contaminated, whereas 7.5% of them contained eggs of *Taenia* spp. [15]. Another study established 79.0% of the soil samples from sites of collection in Stara Zagora to be contaminated – playgrounds of kindergartens and nurseries, whereas four of them had eggs of *Taenia* spp. [16]. In a similar study in the Kardzhali region, 23.59% of the samples taken had parasitic invasive forms, of which 1% - eggs of *Taenia* spp. [17]. When testing the soil from different sites in Plovdiv area, it was found that around 2% of the samples contained eggs of *Taenia* spp. [18]. In 45% of the soil samples taken from different sites in Varna helminth eggs or larvae were identified, while 2.5% contained eggs of *Toxocara* spp. и *Taenia* spp. [19]. Cyst echinococcosis is a serious problem in human pathology. Its dynamic spread retains unfavorable trends, both for public health, and for the economy. The analysis of cyst echinococcosis showed a steady tendency for maintaining high levels of relative shares and morbidity in Bulgaria, compared to the overall EU/EEA cases [20, 21] Figure 1. and Figure 2.

The disease *Syndroma larva migrans oculi/visceralis (Toxocarosis)* is caused by the zoonotic parasites *Toxocara canis, T. cati* and *T. mistax*. Humans are a non-specific, random host, where the parasites do not complete their biological cycle, but remain in the L3 migration stage with visceral, ocular or CNS localization [22]. In humans who develop *Syndroma larva migrans oculi/visceralis* has not been reported or registered in the national or European registers. Information about the immune status, the number of cases, or levels of morbidity may be found in scientific publications, dissertations, or other scientifically reviewed topical materials. In a thorough and complex 18-year study from 2000 to 2017, from the included 2087 persons, 285 (13.45%) had antibodies against *Toxocara* sp. The calculated expenses for outpatient care amounted to 820.15 BGN [23]. Another five-year population study from 2016 to 2020 of toxocarosis in Northeast Bulgaria found that there was a seroprevalence of 18.5% [24]. Through PCR methods, a team from the National Centre for Infectious and Parasitic Diseases examined

the soil, sand, open water basins, and sediments from waste water treatment stations and found oocysts of *Toxoplasma gondii* in four of the samples, and per one positive for oocysts of *Cryptosporidium* spp. [25]. The varying degree of contamination and the diversity of parasitic genera present in the tested soils, directly depend not only on animal presence, the cohabitation of animals and humans, the hygiene level, climatic characteristics of the studied region, but also on the used diagnostic methods. Soil contamination varies in different geographical regions of the world. A number of studies emphasize that the contamination of the environment with *Toxocara* sp. eggs is a key risk factor for toxocarosis in humans. Despite the gravity of the problem, however, there is no exhaustive analysis of the published information. The calculated levels of spread in the different regions of WHO vary from 13% to 35%; West Pacific (15-58%), Africa (11-47%), South America (13-33%), Southeast Asia (3-49%), Near East and North Africa (11-24%), Europe (14-22%) and North and Central America (8-23%), [26]. The intense migration of people and animals, on the other hand, creates a real risk for extending the affected areas and transmission of zoopathogens to further locations. The impossibility to identify the larvae in the organism hinders the etiological diagnostics of the disease, for which mainly serological methods are used, such as ELISA and Western blot. The presence of crossed immunological reactions with other helminth disease and the long persistence of anti-*Toxocara* IgG antibodies necessitates the search for innovative diagnostic methods, some of which have already been developed [27].

The data from the report on the implementation of the National Program for the management of the population of stray dogs in the territory of the Republic of Bulgaria for the period January - December 2022 are disturbing. According to the data entered in VetIS for domestic and stray dogs in Bulgaria, their number in 2022 has increased and maintains an increasing trend. The population of stray dogs in the country in 2022 is still large - 35,020 which continues the alarming trend of the high incidence of parasitic zoonoses [27, 28].

Conclusion

The contaminated environment and the burden of parasitic zoonoses in people lead to a higher risk of invasion, significant healthcare costs for the infested persons, and serious economic loss for public health [6]. The zoonoses toxocarosis, and echinococcosis pose a severe problem in communities, where the cohabitation with incorrectly kept pets and near populations of stray dogs and cats is common [29]. It is imperative to conduct purposeful trainings of healthcare professionals working with contingents who are hazardous and vulnerable to zoonoses, in order to create a healthy behavioral culture. It is necessary to coordinate mutual and planned actions between human and veterinary specialists and persons, engaged with public health for the prevention of neglected parasitic zoonoses.

The authors and participants in this study declare no conflict of interests.

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Figure 1. Dynamics of confirmed cases of cystic echinococcosis (number) in the EUEEA and Bulgaria for the period 2006 - 2021

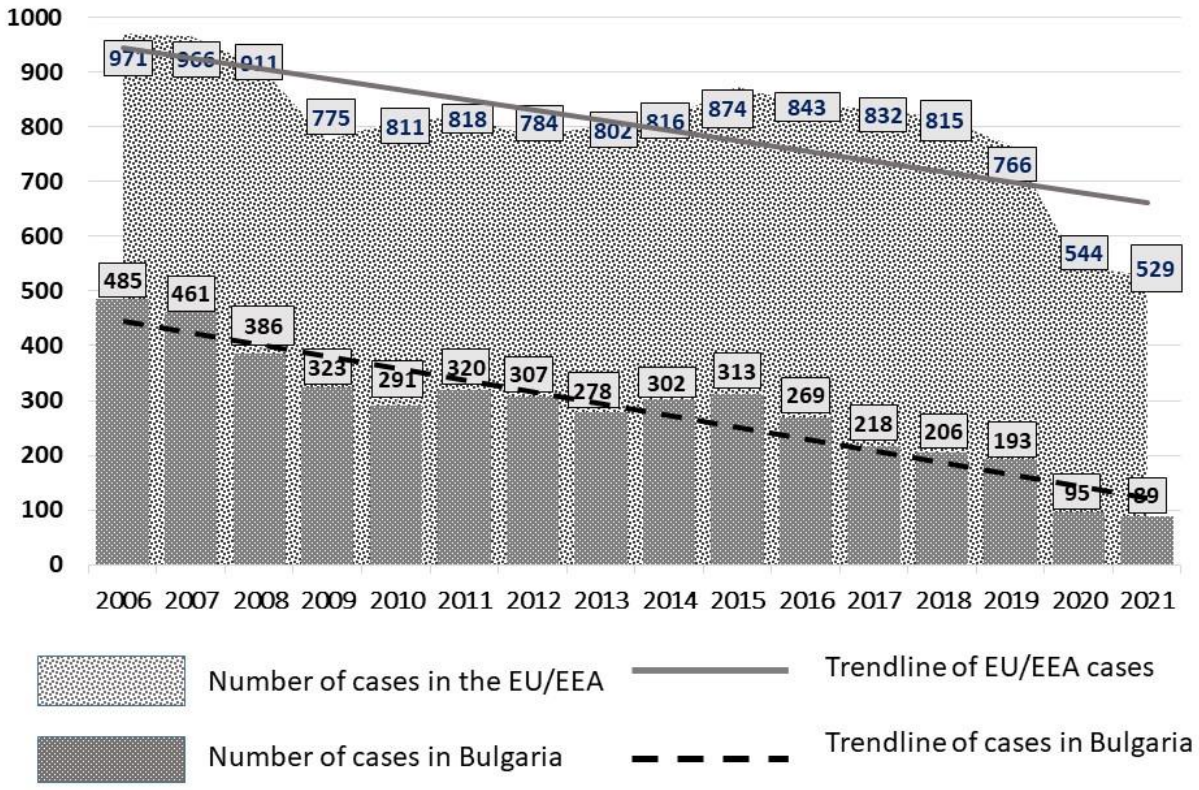

Figure 2. Dynamics of notification rate of cystic echinococcosis (per 100,000 population) in Bulgaria and the EUEEA during the period 2006-2021